PERGAMON

Psychoneuroendocrinology 28 (2003) 657–673

PNEC

www.elsevier.com/locate/psyneuen

Olanzapine's effects to reduce fear and anxiety and enhance social interactions coincide with increased progestin concentrations of ovariectomized rats

Cheryl A. Frye [a,b,c,*], Angela M. Seliga [a,b]

[a] *The University at Albany-SUNY, Department of Psychology, 1400 Washington Avenue, 12222 Albany, NY, USA*

[b] *The University at Albany-SUNY, Department of Biological Sciences, 1400 Washington Avenue, 12222 Albany, NY, USA*

[c] *The University at Albany-SUNY, The Center for Neuroscience Research, 1400 Washington Avenue, 12222 Albany, NY, USA*

Received 21 February 2002; received in revised form 4 June 2002; accepted 11 June 2002

Abstract

Administration of olanzapine, an antipsychotic drug, can dose-dependently increase the levels of progesterone's metabolite, 5α-pregnane-3α-ol-20-one (3α,5α-THP) in the brain, which may have anxiolytic effects. The purpose of this experiment was to investigate the effects of olanzapine administration on anxiety behavior and progestin levels. Ovariectomized (ovx) rats (N = 33) were administered olanzapine (IP: 5.0 or 10.0 mg/kg) or vehicle (saline buffered with acetic acid) and an hour later were tested for motor and anxiety behavior (n = 8 per group) or had tissue collected for measurement of progestin concentrations (n = 3 per group). Rats that were administered 5.0 or 10.0 mg/kg of olanzapine spent less time freezing in response to shock in the defensive burying task, spent more time on the open arms of the elevated plus-maze, and spent more time in social interaction with a conspecific than did vehicle-administered rats. Olanzapine (5.0 or 10.0 mg/kg, IP) significantly increased plasma and produced non-significant increases in whole brain concentrations of progesterone and 3α,5α-THP compared to that seen following vehicle administration. Together, these data are consistent with the hypothesis that olanzapine reduces fear, has anxiolytic effects, and may enhance social interaction in part due to increasing progestin concentrations.

* Tel.: +1-518-442-4836; fax: +1-518-442-4867.
E-mail address: cafrye@cnsunix.albany.edu (C.A. Frye).

doi:10.1016/S0306-4530(02)00049-5

Keywords: Neurosteroid; Non-genomic; Fear; Progesterone; Allopregnanolone

1. Introduction

Olanzapine (LY170053, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[2,3-b][1,5] benzodiazepine) is a novel "atypical" antipsychotic agent, with 5-hydroxytryptamine2 and dopamine D1/D2 antagonist activity and anticholinergic properties, that has demonstrated beneficial effects in a variety of treatment studies. Double-blind studies reveal that olanzapine may be as effective as traditional antipsychotics, such as haloperidol, to reduce schizophrenic symptoms (negative symptoms, positive symptoms, disorganized thoughts, impulsivity/hostility, and anxiety/depression; Davis and Chen, 2001; Conley and Mahmoud, 2001); however, olanzapine's therapeutic effects occur with negligible extrapyramidal side effects and akathisia. In a six-month, double-blind, placebo-controlled treatment study, olanzapine significantly improved affect, cognition, interpersonal relationships, and impulsivity of women with borderline personality disorder (Zanarini and Frankenburg, 2001). Acute olanzapine administration was also more effective than lorazepam at reducing agitation of patients with bipolar mania (Meehan et al., 2001). In an eight-week open label trial, olanzapine was also useful in alleviating the symptoms of combat-induced, post-traumatic stress disorder (Petty et al., 2001). Olanzapine may also reduce anxiety symptoms associated with neurodegenerative disorders, such as Alzheimer's (Mintzer et al., 2001) or Huntington's disease (Squitieri et al., 2001). Atypical antipsychotics are also being used increasingly to treat autism with encouraging results (Santosh and Baird, 2001). These findings suggest that olanzapine may be therapeutic in a broad range of situations.

Several studies demonstrate that olanzapine has behavioral effects in animal models which suggest it reduces fear and may attenuate anxiety. Olanzapine (0–10 mg/kg) had dose-dependent effects on male rats to reduce the acquisition of conditioned freezing (Inoue et al., 1996). In a conditioned avoidance paradigm, olanzapine inhibited the avoidance response of mice (Fu et al., 2000) and rats (Moore et al., 1992). Olanzapine to rats reversed the deficits in prepulse inhibition, and increased the startle reactivity, produced by social isolation due to individual housing from weaning until adulthood (Bakshi et al., 1998). Olanzapine also increased licking of an electrified water bottle in the Geller–Seifter conflict task, suggesting that it may have anxiolytic activity (Moore et al., 1992). Unlike typical antipsychotics, these behavioral effects are not associated with motor disturbances (Steinpreis et al., 1999; Stanford and Fowler, 1997; Trevitt et al., 1999).

Previous reports suggest that olanzapine, an atypical antipsychotic drug, may increase levels of the neurosteroid, 5α-pregnane-3α-ol-20-one (3α,5α-THP). Traditionally, olanzapine's therapeutic efficacy has been attributed to its actions to block about 43–80% of dopamine (type 2) and near saturation of serotonin (type 2) receptors (Kapur et al., 1998). However, administration of 5.0 or 10.0 mg/kg of olanzapine

to male (Marx et al., 2000) or female rats (Frye and Seliga, 2002) increases central 3α,5α-THP levels. These studies suggest that another possible mechanism by which olanzapine may have its therapeutic effects is through actions of 3α,5α-THP.

There is evidence that 3α,5α-THP, which can be metabolized from progesterone or synthesized de novo from cholesterol by glial cells, acts as a positive modulator of $GABA_A$/benzodiazepine receptor complexes (GBRs) to mediate anxiety behavior of rodents. First, endogenous levels of 3α,5α-THP are increased during proestrus (Frye and Bayon, 1999), when anxiety behaviors are decreased compared to those seen on other phases of the cycle or by male rats, which are characterized by comparatively lower 3α,5α-THP levels (Frye et al., 2000). Second, direct administration of 3α,5α-THP to rats produces anxiolysis in the elevated plus-maze task (Akwa et al., 1999; Bitran et al., 1991; Frye and Lacey, 2000; Laconi et al., 2001; Fernandez-Guasti and Picazo, 1999). Third, enhancing the production of 3α,5α-THP by administering a mitochondrial benzodiazepine receptor agonist, FGIN 1-27, also produces anxiolytic effects in the elevated plus-maze and shock-probe burying tasks (Bitran et al., 2000). Fourth, administration of picrotoxin, a GBR blocker, attenuates the anxiolytic effects of pregnenollone-induced 3α,5α-THP (Bitran et al., 1995). Based on these studies, it is evident that 3α,5α-THP can modulate anxiety behavior through actions at GBRs.

Previously in our laboratory, we investigated olanzapine's effects on progestin-facilitated socio-sexual behavior (Frye, 2001). The olanzapine regimen which elevates 3α,5α-THP concentrations also facilitates sexual receptivity of ovariectomized, estrogen-primed rats (Frye and Seliga, 2002). Notably, increased propensity for social interactions are typically seen with reduced anxiety. Therefore, we hypothesized that olanzapine will reduce fear, increase anxiolytic behavior, and enhance social interactions, and these behavioral changes will occur concomitant with endogenous changes in 3α,5α-THP levels.

2. Methods

These methods were pre-approved by the Institutional Animal Care and Use Committee at SUNY Albany.

2.1. Animals and housing

Female Long–Evans rats (N = 24), approximately 55 days of age, were obtained from the breeding colony at SUNY Albany (original stock from Taconic Farms, Germantown, NY) for behavioral testing. An additional 9 rats of the same age and from the same breeding colony were obtained for tissue collection. Rats were group housed (four per cage) in polycarbonate cages (45 × 24 × 21 cm) in a temperature- and humidity-controlled room (21 ± 1 °C) in the laboratory animal care facility. Rats were maintained on a 12/12 hour reversed light cycle (lights off 8:00 am) with continuous access to Purina Rat Chow and tap water in their home cages. All rats were ovariectomized (ovx) under Rompum (60 mg/kg; Bayer Corp., Shawnee Mis-

sion, KS) and Ketaset (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) anesthesia prior to behavioral testing or tissue collection.

2.2. Drug regimens

Olanzapine was generously provided by Eli Lilly, Inc. Dosages and vehicle (saline buffered with acetic acid) were based upon those previously reported to increase the production of 3α,5α-THP (Marx et al., 2000; Frye and Seliga, 2002). Olanzapine (IP: 0.0, 5.0, or 10.0 mg/kg) was administered and rats were tested or rapidly decapitated an hour later. Diazepam (1 mg/kg) was administered to an additional 8 ovx rats one hour prior to testing in the defensive freezing task. This was done to pharmacologically validate freezing behavior as a measure of fear and anxiety and to enable comparisons of olanzapine's effects. The diazepam regimen was based upon that previously utilized to produce effects in the conditioned defensive burying task (Treit et al., 1981).

2.3. Behavioral testing (experiment one)

Anxiolytic behavior was assessed using a battery of tests (Johnston and File, 1991; Pellow et al., 1985). Testing occurred in one of two brightly lit rooms adjacent to the animal housing facility. The anxiolysis tests were administered to each rat individually in the same order without any rest periods due to one or more of these tasks possibly influencing behavior on subsequent tasks. Any carry-over effects from previous tasks would therefore be uniform across all rats. In one room, rats were tested in the horizontal crossing, open field, elevated plus-maze, and holeboard tasks. Immediately following completion of holeboard in the first room, rats were tested in the social interaction, tail flick, paw lick, and defensive freezing tasks in the second room. For all of the non-automated tests, behavioral data were collected by an observer blind to the hypothesized results. Each of the behavioral tasks are described below.

2.3.1. Horizontal crossing

The horizontal crossing task was used to assess motor behavior (Brudzynski and Krol, 1997; Frye et al., 2000; Frye and Seliga, 2001). Rats were placed in a brightly lit $39 \times 39 \times 30$ cm Digiscan Optical Animal Activity Monitor (Accuscan Instruments Inc., Columbus, OH) while the total number of beam breaks that occurred in five minutes was mechanically recorded.

2.3.2. Open field

Behavior in the open field was utilized to assess anxiety and motor behavior (Frye et al., 2000; Frye and Seliga, 2001; McCarthy et al., 1995; Pellow and File, 1986). The open field arena ($76 \times 57 \times 35$ cm) has a 48-square grid floor (6×8 squares, 9.5 cm/side); an overhead light illuminates the central squares (all but the 24 perimeter squares were considered central). Rats were placed in the bottom left corner

of the open field and an experimenter recorded the number of central, peripheral, and outer squares the rat entered in 5 minutes.

2.3.3. Elevated plus-maze

The elevated plus-maze was used to assess anxiety and exploratory behavior (Dunn et al., 1998; Frye et al., 2000; Frye and Seliga, 2001; Pellow et al., 1985). The apparatus consisted of four arms, 49 cm long and 10 cm wide, elevated 50 cm off the ground. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. Rats were placed at the intersection of the open and closed arms of the maze and were observed for 5 minutes. The amount of time (secs) the rats spent on the open and closed arms and the number of open and closed arm entries were recorded. Rats were considered to be either in the closed or open arms of the maze and open arm time was recorded only when the rat had all four paws on the open arm.

2.3.4. Holeboard

Behavior in the holeboard task was used to assess exploratory behavior (File et al., 1985; Frye et al., 2000; Frye and Seliga, 2001; Zangrossi and File, 1992). An open field ($60 \times 60 \times 35$ cm) divided into 9 equal squares that has equally spaced holes (each 3.8 cm diameter) at the four intersecting grid lines was utilized. The incidence of head dips rats made into the holes were recorded by an experimenter for 5 minutes.

2.3.5. Social interaction

Social interaction was also utilized as an indication of anxiety and exploratory behavior (Cheeta et al., 2001; Frye et al., 2000; Frye and Seliga, 2001; Ge et al., 1997). The social interaction test was conducted in an open field ($60 \times 60 \times 35$ cm) similar to that used for the holeboard test. An ovariectomized, estrogen-primed female rat was placed in one corner of the open field and the experimental female was placed in the opposite corner and was left undisturbed for 10 minutes. The total duration of time (secs) that the experimental rat engaged the stimulus female (crawling over and under partner, sniffing of partner, following with contact, genital investigation of partner, tumbling, boxing and grooming) was recorded. Stimulus rats were conspecifics that were randomly selected and were not behaviorally tested in this study.

2.3.6. Tail flick

The tail-flick paradigm was utilized to assess nociception (D'Amour and Smith, 1941; Frye and Duncan, 1994, 1995; Frye et al., 2000; Frye and Seliga, 2001). Rats were placed on the platform of the tail-flick apparatus (San Diego Instruments, San Diego, CA) and their tails were smoothed above the radiant heat source. The mean latency of three tail-flick trials was used as an index of nociception (maximum latency 10 secs).

2.3.7. Paw lick

The paw-lick test was also utilized to assess nociception (Bardo and Valone, 1994; Frye et al., 2000; Frye and Seliga, 2001; Smythe et al., 1994). The paw-lick apparatus consisted of a slide-warming tray (Fisher Scientific, Swanee, GA model # 77) heated to 53 °C. Rats were confined to the tray by placing a clear, floorless, plastic chamber (28.5 × 17.5 × 12.5 cm) on top of the hotplate. The latency to lick the hindpaw was recorded (maximum latency 180 secs).

2.3.8. Defensive freezing

The defensive freezing test assessed behavior in response to a shock stimulus (Frye et al., 2000; Frye and Seliga, 2001; Gallo and Smith, 1993; Treit, 1985; Treit et al., 1981). The test apparatus (26.0 × 21.2 × 24.7 cm), constructed of clear Plexiglas, has an electrified probe (2.5 cm in diameter and 10.0 cm in height) in the right rear corner, 3.0 cm from the back wall and 2.5 cm from the right wall. The probe was extended 4.5 cm above wood-chip bedding, which was 5.0 cm deep from the bottom of the chamber. The probe was wrapped by two wires which were connected to a shock source (Lafayette Model A615B, Lafayette, IN) set to deliver 6.66 mA of unscrambled shock when the animal placed both forepaws on the pedestal. The experimenter terminated the shock and recorded the duration of freezing (defined as the animal remaining motionless), burying, and passive avoidance behaviors in response to the shock stimulus for 15 minutes.

2.4. Tissue collection (experiment two)

An additional 9 ovx rats (n = 3 per group), which were not behaviorally tested, were administered olanzapine (IP: 0.0, 5.0, or 10.0 mg/kg). These rats were rapidly decapitated and trunk blood was collected at the time analogous to behavioral testing for Experiment 1. Following centrifugation (4 °C at 3000 x g for 8 min), the serum was aliquoted and stored at –70 °C until radioimmunoassay for P and 3α,5α-THP.

2.5. Radioimmunoassay of plasma and brain progestins

Plasma and brain samples were assayed for P and 3α,5α-THP according to previously published methods (Finn and Gee, 1994; Frye and Bayon, 1998, 1999; Frye et al., 1998; Frye et al., 1996; Purdy et al., 1990; Purdy et al., 1990b). Briefly, P and 3α,5α-THP were extracted from plasma samples using diethyl ether. Progesterone was extracted from brain tissue homogenized with a glass/Teflon homogenizer in distilled water with diethyl ether. 3α,5α-THP was extracted from brain samples that had been homogenized with a glass/glass homogenizer and tissue tearer using a series of centrifugation and filtrations with 50% MeOH, 1% acetic acid. Assays were performed using tritiated steroids for P (NET- 208: specific activity = 47.5 ci/mmol) and 3α,5α-THP (NET-1047: specific activity = 51.3 ci/mmol) purchased from New England Nuclear, Boston, MA. The P antibody was from Dr. GD Niswender, Colorado State University, Boulder, CO (#337 used in 1:30,000 dilution). The 3α,5α-THP antibody was from Dr. Robert Purdy, Veteran's Medical Center,

La Jolla, CA (#X-947 and used at 1:5,000). Standard curves for P (range = 50 pg/ml–8000 pg/ml) and 3α,5α-THP (range = 10 pg/ml–4000 pg/ml) were prepared in duplicate. The standards were added to BSA assay buffer, followed by addition of the appropriate antibody and [^{3}-H] steroid.

Assay tubes were vortexed and incubated at 4 °C for 24 hours. Separation of bound and free was accomplished by the rapid addition of dextran-coated charcoal. Following incubation with charcoal, samples were centrifuged at 1200 g; the supernatant was pipetted into a glass scintillation vial with scintillation cocktail. Sample tube concentrations were calculated using the logit–log method of Rodbard and Hutt (1974), interpolation of the standards and correction for recovery. The intra-assay and inter-assay coefficients of variance for P were 9% and 10% and for 3α,5α-THP were 12% and 15%, respectively.

2.6. Statistical analyses

One-way analyses of variances (ANOVAs) were used to examine effects of olanzapine dosage on behavior. Where appropriate, ANOVAs were followed by Fisher's post-hoc tests and least squares mean comparisons between groups.

3. Results

3.1. Experiment 1: Olanzapine increases anxiolytic behavior but not motor behavior

3.1.1. Horizontal crossing

There were no significant differences in the total number of beam breaks in horizontal crossings task of rats that were administered 0.0 (738 ± 89), 5.0 (682 ± 84), or 10.0 (608 ± 120) mg/kg of olanzapine.

3.1.2. Open field

Rats that were administered 10.0 mg/kg of olanzapine made significantly ($F(2, 21) = 4.751$, $p = 0.02$) fewer total square entries (70 ± 13), than did rats administered 5.0 mg/kg olanzapine (124 ± 15), or vehicle (110 ± 10). Rats administered the higher dosage of olanzapine (10.0 mg/kg) also entered significantly fewer ($F(2, 21) = 4.801$, $p = 0.02$) outer squares in the open field (71 ± 7), than did rats administered 5.0 mg/kg olanzapine (104 ± 9), or vehicle (103 ± 10). Rats administered 5.0 mg/kg of olanzapine tended ($F(2, 21) = 2.774$, $p = 0.09$) to make more central entries in the open field (15 ± 4) than did rats administered vehicle (6 ± 2) but not 10.0 mg/kg olanzapine (9 ± 2)

3.1.3. Elevated plus-maze

Olanzapine-administered rats (5.0 and 10.0 mg/kg) spent significantly more ($F(2, 21) = 5.014$, $p = 0.02$) time on the open arms of the elevated plus-maze than did

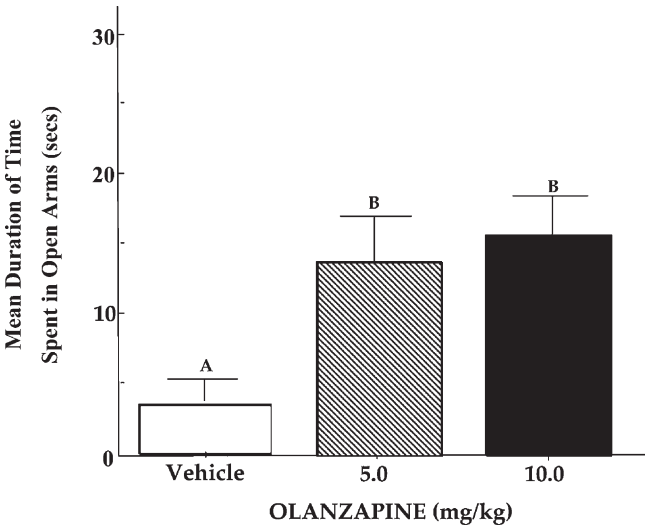

Fig. 1. Mean duration of time spent in the open arms (± SEM) of the elevated plus-maze following administration of vehicle (white bar), 5.0 (striped bar), or 10.0 (black bar) mg/kg of olanzapine. Different letters denote a significant difference at $p < 0.05$.

vehicle-administered rats (see Fig. 1). There was a significant decrease ($F(2, 21) = 5.014$, $p = 0.02$) in the duration of time spent in the closed arms by rats administered 5.0 (286 ± 3 secs) or 10.0 (285 ± 4 secs) mg/kg of olanzapine compared to vehicle (296 ± 1 secs). Olanzapine (5.0 or 10.0 mg/kg) significantly increased the percentage of time spent on the open arms ($F(2, 21) = 4.878$, $p = 0.02$) and the percentage of entries to the open arms ($F(2, 21) = 4.878$, $p = 0.02$). Notably, olanzapine did not have a significant effect on indices of general activity. For example, olanzapine had no significant effect on the number of closed arm entries or the total number of arm entries (see Table 1).

Table 1
Olanzapine's effects on performance in the elevated plus-maze task compared to vehicle administration (± SEM). ∗ indicates a significant difference from vehicle

Behavioral Measures	Olanzapine Dosage (mg/kg)		
	0.0	5.0	10.0
% Open Arm Time	1.38±0.35	4.79∗±1.00	5.05∗±1.18
% Open Arm Entries	11.39±30.23	24.27∗±4.72	30.23∗±5.32
Closed Arm Entries	5.38±0.42	4.63±0.53	4.00±0.50
Total Arm Entries	6.13±0.52	6.13±0.55	6.13±0.85

3.1.4. Holeboard

There was a tendency (F (2, 21) = 2.568, p = 0.10) for the 10.0 mg/kg dosage of olanzapine to increase the number of head dips (5 ± 1) compared to that made by vehicle rats (2 ± 1) but not rats administered 5.0 mg/kg of olanzapine (3 ± 1).

3.1.5. Social interaction

Olanzapine-administered rats (5.0 and 10.0 mg/kg) spent significantly more (F (2, 21) = 7.764, p = 0.003) time in contact with a conspecific in genital investigation, sniffing, crawling over and under, tumbling, boxing and grooming interaction time compared to vehicle-administered rats (see Fig. 2).

3.1.6. Tail flick

The 10.0 mg/kg dosage of olanzapine significantly decreased (F (2, 21) = 3.371, p = 0.0538) tail-flick latencies (3.2 ± 0.3) compared to that of vehicle rats (4.6 ± 0.5) but not rats administered 5.0 mg/kg of olanzapine (3.7 ± 0.4).

3.1.7. Paw lick

There were no significant differences between the groups in the latencies to lick the hindpaw. The paw-lick latencies for each group were vehicle (151 ± 14), olanzapine 5.0 mg/kg (157 ± 12), and olanzapine 10.0 mg/kg (167 ± 7).

3.1.8. Defensive freezing

Olanzapine-administered rats (5.0 and 10.0 mg/kg) spent significantly less (F (2, 21) = 5.960, p = 0.009) time freezing compared to vehicle-administered rats (see

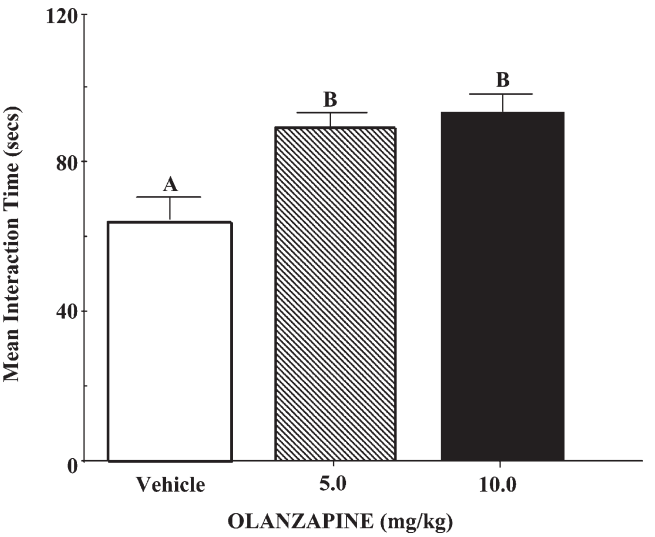

Fig. 2. Mean duration of interaction time (± SEM) in the social interaction task following administration of vehicle (white bar), 5.0 (striped bar), or 10.0 (black bar) mg/kg of olanzapine. Different letters denote a significant difference at $p < 0.05$.

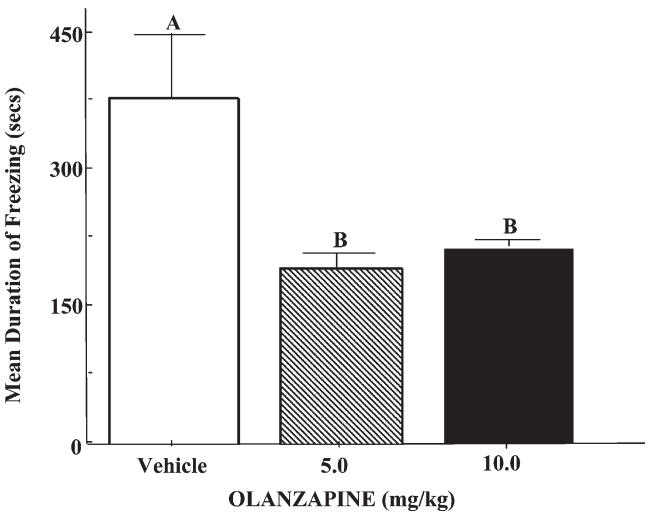

Fig. 3. Mean duration of freezing after touching the electrified prod (± SEM) in the defensive burying task following administration of vehicle (white bar), 5.0 (striped bar), or 10.0 (black bar) mg/kg of olanzapine. Different letters denote a significant difference at $p < 0.05$.

Fig. 3). Olanzapine produced a non-significant increase in burying ($F(2, 21) = 2.095$, $p = 0.15$) and a significant increase in the duration of time spent in passive avoidance of the shock probe ($F(2, 21) = 4.237$, $p = 0.03$) compared to vehicle. Administration of 1 mg/kg of diazepam produced a similar, albeit more salient, pattern of effects on defensive freezing (98.63 secs) and on passive avoidance of the probe as did olanzapine; however, this diazepam regimen did not increase the duration of time spent burying the probe (see Table 2).

3.2. Experiment 2: Olanzapine increases plasma and central progesterone and 3α,5α-THP concentrations

3.2.1. Progesterone

Plasma progesterone concentrations were significantly increased in olanzapine-compared to vehicle-administered groups ($F(2, 6) = 19.653$, $p = 0.002$) and whole

Table 2
Olanzapine's effects on performance in the defensive freezing task compared to vehicle administration (± SEM). ∗ indicates a significant difference from vehicle

Behavioral Measures	Olanzapine Dosage (mg/kg)			Diazepam (mg/kg)
	0.0	5.0	10.0	1.0
Defensive Burying	90.25±26.70	146.13±24.41	154.38±20.83	88.75±34.70
Passive Avoidance	432.5±55.95	571.00∗±21.80	544.63∗±14.98	712.63∗±33.70

Table 3
Progesterone concentrations (± SEM); n = 3 observations per group. ∗ indicates a significant difference from vehicle administration. # indicates a tendency for a difference from vehicle administration

		Olanzapine Dosage (mg/kg)		
Progesterone Concentration		0.0	5.0	10.0
	Plasma (ng/ml)	9.70±1.10	19.60∗±1.52	23.48∗±1.93
	Whole Brain (ng/g)	4.66±1.14	7.44#±0.53	7.09#±0.33

brain progesterone concentrations tended to be greater in olanzapine- vs. vehicle-administered rats ($F(2, 6) = 3.809$, $p = 0.08$) (see Table 3). Vehicle-administered rats had progesterone concentrations within the range previously reported for diestrus rats, while olanzapine-administration increased progesterone levels to that of proestrous rats (Butcher et al., 1974; Belanger et al., 1981; Frye and Bayon, 1998).

3.2.2. 3α,5α-THP

Plasma 3α,5α-THP concentrations were significantly increased in olanzapine- compared to vehicle-administered groups ($F(2, 6) = 32.636$, $p = 0.0006$) and whole brain 3α,5α-THP concentrations were non-significantly increased in olanzapine- vs. vehicle-administered rats (see Table 4). Vehicle-administered rats had 3α,5α-THP concentrations that were analogous to those previously reported for diestrous rats; however, olanzapine increased 3α,5α-THP levels to be commensurate with the range previously reported for estrous rats (Purdy et al., 1990; Frye and Bayon, 1999).

4. Discussion

The results of this experiment are consistent with our hypothesis that olanzapine administration reduces fear, produces anxiolysis, and enhances social interactions by increasing levels of neuroactive progestins. Olanzapine (5.0 or 10.0 mg/kg) reduced fear behavior, as indicated by less time spent freezing in response to an aversive shock compared to that seen for vehicle-administered rats. Rats that were adminis-

Table 4
3α,5α-THP concentrations (± SEM); n = 3 observations per group. ∗ indicates a significant difference from vehicle administration

		Olanzapine Dosage (mg/kg)		
3α,5α-THP Concentration		0.0	5.0	10.0
	Plasma (ng/ml)	2.78±0.68	16.05∗±1.93	16.77∗±1.15
	Whole Brain (ng/g)	1.74±0.29	3.72±1.36	4.39±1.67

tered 5.0 or 10.0 mg/kg of olanzapine exhibited less anxious behavior relative to the rats that received vehicle, as seen by their increased time spent on the open arms of the elevated plus-maze relative to vehicle control rats. Olanzapine also increased the duration of time rats spent in social interaction with a conspecific as compared to vehicle administration. These same dosages of olanzapine significantly increased plasma, and produced non-significant increases in whole brain, concentrations of progesterone and 3α,5α-THP compared to that seen following vehicle administration. Together, these data are consistent with the hypothesis that olanzapine reduces fear, has anxiolytic effects, and increases social interactions, in part due to olanzapine's actions to increases progestin concentrations.

The present findings provide additional support for olanzapine's alteration of affect. Acute administration of olanzapine has anti-depressive effects to reduce the duration of immobility in the Porsolt forced swim test (Nowakowska et al., 1999). Acute or chronic administration of olanzapine to male rats produces anxiolysis (Nowakowska et al., 1999). Our data extend these findings to demonstrate that olanzapine administration to female rats increases exploration of the open arms in the elevated plus-maze. Also, olanzapine administration significantly decreased the duration of time rats spent freezing in response to the shock stimulus. However, all of the data are not consistent with olanzapine producing anxiolysis. In the defensive freezing task, there was a non-significant effect for 5.0 or 10.0 mg/kg of olanzapine to increase the duration of time rats spent burying the shock probe.

In addition to altering anxiety, olanzapine enhances social interactions. Olanzapine administration increased the duration of time conspecifics engaged in social interactions. These results coincide with previous results from our laboratory that demonstrate that olanzapine enhances lordosis and decreases aggressive behaviors of estrogen-primed rats compared to that of control rats administered vehicle (Frye and Seliga, 2002). Olanzapine may also help overcome effects of social isolation. Olanzapine administration can reverse social withdrawal behaviors of adult rats that were reared in isolation as pups (Bakshi et al., 1998; Heidbreder et al., 2001). Similarly, phencyclidine-induced social isolation can also be reversed by olanzapine treatment (Corbett et al., 1995; Sams-Dodd, 1997). Together, these findings suggest that olanzapine enhances social behavior and can reverse the effects of social isolation, which may contribute to being more permissive of normative social interactions.

The present data suggest that olanzapine may alter pain sensitivity and motor behavior, in addition to its anxiolytic action. In the tail-flick task, 10.0 mg/kg of olanzapine significantly decreased the latencies for rats to withdraw their tails from a radiant heat source. This suggests that olanzapine may increase the sensitivity to pain. However, in the paw-lick task, there were no significant differences between olanzapine and vehicle administration and the opposite pattern of effects was observed for olanzapine to *increase* hind paw-lick latencies. It is possible that olanzapine reduced sensitivity of the rats' paws and this contributed to decreases in duration of time spent freezing, increases in time spent burying, and increases in time spent in passive avoidance of the shock probe in the defensive freezing task (see Table 2 and Fig. 3). Also, olanzapine may have produced effects in the defensive freezing task due to alterations in motor behavior. In the open field task, olanzapine (10.0

mg/kg) significantly decreased total square entries compared to vehicle administration. However, in subsequent tasks, olanzapine increased exploratory activity, as indicated by significant increases in open arm time in the elevated plus-maze, and a tendency to increase head dips in the holeboard task compared to vehicle administration. Olanzapine's opposite effects on different tasks of nociception and motor behavior may in part be due to carry-over effects between tasks. Counterbalancing the order of tests would have been preferable, as it would have addressed these concerns; however, many more animals would have been necessary to enable such an investigation.

The present findings confirm and extend the evidence for olanzapine's ability to induce progestin concentrations. Previous research has shown that 5.0 or 10.0 mg/kg of olanzapine (IP) increases central levels of 3α,5α-THP and serum progesterone levels in male rats compared to that produced by vehicle or 2.5 mg/kg of olanzapine (Marx et al., 2000). Our lab has previously shown that these same dosages of olanzapine increase central, whole brain and plasma concentrations of both 3α,5α-THP and progesterone in ovariectomized, estradiol-primed rats (Frye and Seliga, 2002).

Comparisons of the progestin levels produced by olanzapine in the present and previous studies suggest that there may be sex effects in olanzapine-induced progestin levels. Plasma progesterone levels of male rats administered 10.0 mg/kg of olanzapine were approximately 75 ng/ml (Marx et al., 2000). Female rats administered 10.0 mg/kg of olanzapine, in the present study, and our previous investigation, had peripheral progesterone levels between 21 and 32 ng/ml. Central 3α,5α-THP concentrations of female rats administered olanzapine in the present and previous experiments are comparable (3–6 ng/g) to 3α,5α-THP levels of male rats administered olanzapine (Marx et al., 2000). The increased levels of progesterone induced by olanzapine among male rats may be due to increased adrenal hormone production (Marx et al., 2000). Although we did not measure corticosterone levels of our female rats and consequently are unable to directly address this, olanzapine (5.0 and 10.0 mg/kg) did significantly increase corticosterone levels of male rats (Marx et al., 2000). Also in the present investigation, olanzapine had the most robust effects on plasma, rather than whole brain levels of progestins, which would be consistent with olanzapine increasing progestin concentrations due to activity on the adrenal cortex. Olanzapine's behavioral effects are presently unknown in hypophysectomized or adrenalectomized rats. Also, the few observations of olanzapine's effects on whole brain concentrations of progestins in the present study have large variability, making it problematic to conclude that olanzapine had central neurosteroidogenic effects that produced the behavioral effects observed. It would be an improvement over the current study to examine olanzapine's functional effects and changes in circulating, as well as central, neuroendocrine responses in the same animals.

The present results provide indirect evidence that gonadal hormones may enhance olanzapine's progestin-inducing effects. Plasma and whole brain progestin levels are slightly lower in this study than in our previous investigation of olanzapine's effects in estradiol-primed rats. Estrogen can induce progestin metabolism (Cheng and Karavolas, 1973); by comparison, co-administration of estrogen and olanzapine resulted in greater progestin concentrations than did olanzapine alone. Our previous study

also demonstrated that co-administration of olanzapine and progesterone had additive effects to increase progestin concentrations (Frye and Seliga, 2002).

In support of our hypothesis that olanzapine and gonadal hormones may have additive effects, there is evidence of sex differences in olanzapine's effects among people. Women taking the same dosages of olanzapine as their male counterparts have higher steady-state olanzapine concentrations (Kelly et al., 1999); the therapeutic threshold for women occurs at a much lower dosage than men (Perry et al., 2001). These differences may be explained, in part, by females being more sensitive to changes in progestins than are males. Based on the present and previous findings of olanzapine's progestin-enhancing effects and the evidence of sex differences, it would be interesting to directly investigate the differential sensitivities of male and female rats in response to olanzapine administration.

Olanzapine, like other therapeutic drugs (e.g. fluoxetine), may have behavioral effects as a result of increasing neurosteroidogenesis of 3α,5α-THP. Both plasma and brain progesterone and 3α,5α-THP levels have been shown to be increased following olanzapine administration; however, as discussed previously, it is not clear the extent to which these effects are independent of the peripheral glands and/or due to de novo production of 3α,5α-THP in the brain by glial cells. There is evidence that altering the production of 3α,5α-THP in the brain can mediate anxiety. Administration of a neurosteroidogenic agent, FGIN 1-27, increases anxiolysis of male rats in the elevated plus-maze and shock-probe burying tasks (Bitran et al., 2000). Neurosteroidogenesis of 3α,5α-THP can also be increased by specific serotonin reuptake inhibitors, such as fluoxetine, which enhance the efficiency of enzymatic metabolism from dihydroprogesterone (Griffin and Mellon, 1999). Fluoxetine can induce 3α,5α-THP elevations in adrenalectomized and gonadectomized male rats (Uzonov et al., 1996). In addition, fluoxetine reverses the 3α,5α-THP deficits and reduces agonistic behavior of mice reared in social isolation (Guidotti et al., 2001). Elevations of 3α,5α-THP in the cerebral spinal fluid of depressed patients occur subsequent to fluoxetine administration and are correlated with improved depressive symptomology (Uzunova et al., 1998). Based on these findings, it is likely that endogenously altering 3α,5α-THP levels can enhance behavioral functioning, which may explain, in part, the clinical efficacy of olanzapine's effects to enhance social interactions. The extent to which olanzapine's effects are due to neurosteroidogenesis is an important question for future investigation. Using this animal model, it will be possible to determine whether olanzapine's effects on performance in the open field, elevated plus maze, social interaction, and defensive freezing tasks are associated with increases in 3α,5α-THP in specific brain regions important for these behaviors (such as the hippocampus and the amygdala).

Acknowledgements

This research was supported by The National Science Foundation (98-96263). We appreciate the assistance of Alicia Walf in the implementation of the studies and in preparation of the manuscript. Olanzapine was generously donated by Eli Lilly and Company.

References

Akwa, Y., Purdy, R.H., Koob, G.F., Britton, K.T., 1999. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. Behav. Brain Res. 106, 119–125.

Bakshi, V.P., Swerdlow, N.R., Braff, D.L., Geyer, M.A., 1998. Reversal of isolation rearing-induced deficits in prepulse inhibition by seroquel and olanzapine. Biol. Psychiatry 15, 436–445.

Bardo, M.T., Valone, M.J., 1994. Morphine-conditioned analgesia using a taste cue dissociation of taste aversion and analgesia. Psychopharmacology 114, 269–274.

Belanger, A., Cusan, L., Caron, S., Barden, N., Dupont, A., 1981. Ovarian progestins,androgens and estrogen throughout the day estrous cycle in the rat. Biol. Reprod 24, 591–596.

Bitran, D., Foley, M., Audette, D., Leslie, N., Frye, C.A., 2000. Activation of peripheral mitochondrial benzodiazepine receptors in the hippocampus stimulates allopregnanolone synthesis and produces anxiolytic-like effects in the rat. Psychopharmacology 151, 64–71.

Bitran, D., Hilvers, R.J., Kellogg, C.K., 1991. Anxiolytic effects ofhydroxy-5([(]-pregnane-20-one: endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res. 561, 157–161.

Bitran, D., Shiekh, M., McLeod, M., 1995. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain $GABA_A$ receptors. J. Neuroendocrinol. 7, 171–177.

Brudzynski, S.M., Krol, S., 1997. Analysis of locomotor activity in the rat: parallelism index — a new measure of locomotor exploratory pattern. Physiol. Behav. 62, 635–642.

Butcher, R.L., Collins, W.E., Fugo, N.W., 1974. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94, 1704–1708.

Cheeta, S., Irvine, E.E., Kenny, P.J., File, S.E., 2001. The dorsal raphe nucleus is a crucial structure mediating nicotine's anxiolytic effects and the development of tolerance and withdrawal responses. Psychopharmacology 155, 78–85.

Cheng, Y.J., Karavolas, H.J., 1973. Conversion of progesterone to 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnan-20-one by rat medical basal hypothalami and the effects of estradiol and stage of estrous cycle on the conversion. Endocrinology 93, 1157–1162.

Conley, R.R., Mahmoud, R., 2001. A randomized double-blind study of risperidone and olanzapine in the treatment of schizophrenia or schizoaffective disorder. Am. J. Psychiatry 158, 765–774.

Corbett, R., Camacho, F., Woods, A.T., Kerman, L.L., Fishkin, R.J., Brooks, K., Dunn, R.W., 1995. Antipsychotic agents antagonize non-competitive N-methyl-D-asparate antagonist-induced behaviors. Psychopharmacology 120, 67–74.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Davis, J.M., Chen, N., 2001. The effects of olanzapine on the 5 dimensions of schizophrenia derived by factor analysis: combined results of the North American and international trials. J. Clin. Psychiatry 62, 757–771.

Dunn, R., Copeland, P., Reed, T., Frye, C.A., 1998. Anti-anxiety effects of nitric oxide inhibitors. Neuropharmacology 37, 899–904.

Fernandez-Guasti, A., Picazo, O., 1999. Sexual differentiation modifies the allopregnanolone anxiolytic actions in rats. Psychoneuroendocrinology 24, 251–267.

File, S.E., Pellow, S., Braestrup, C., 1985. Effects of the beta-carboline, FG2, in the social interaction test of anxiety and the holeboard: correlations between behaviour and plasma concentrations. Pharmacol. Biochem. Behav. 22, 941–944.

Finn, D.A., Gee, K.W., 1994. The estrous cycle, sensitivity to convulsants and the anticonvulsant effect of a neuroactive steroid. J. Pharmacol. Exp. Ther. 271, 164–170.

Frye, C.A., 2001. The role of neurosteroids and non-genomic effects of progestins in the ventral tegmental area in mediating sexual receptivity of rodents. Horm. Behav. 40, 226–233.

Frye, C.A., Bayon, L.E., 1998. Seizure activity is increased in endocrine states characterized by decline in endogenous levels of the neurosteroid 3α,5α-THP. Neuroendocrinology 68, 272–280.

Frye, C.A., Bayon, L.E., 1999. Mating stimuli influence endogenous variations in the neurosteroids 3α,5α-THP and 3α-Diol. J. Neuroendocrinol. 11, 839–847.

Frye, C.A., Bayon, L.E., Pursnani, N.K., Purdy, R.H., 1998. The neurosteroids, progesterone and 3α5α-THP, enhance sexual motivation, receptivity, and proceptivity in female rats. Brain Res. 808, 72–83.

Frye, C.A., Duncan, J.E., 1994. Progesterone metabolites, effective at the $GABA_A$ receptor complex, attenuate pain sensitivity in rats. Brain Res. 643, 194–203.

Frye, C.A., Duncan, J.E., 1995. Estradiol benzoate potentiates neuroactive steroids' effects on pain sensitivity. Pharmacol. Biochem. Behav 53, 27–32.

Frye, C.A., Lacey, E.H., 2000. Progestins influence performance on cognitive tasks independent of changes in affective behavior. Psychobiology 28, 550–563.

Frye, C.A., McCormick, C.M., Coopersmith, C., Erskine, M.S., 1996. Effects of paced and non-paced mating stimulation on plasma progesterone, 3α-Diol and corticosterone. Psychoneuroendocrinology 21, 431–439.

Frye, C.A., Petralia, S.M., Rhodes, M.E., 2000. Estrous cycle and sex differences in performance on anxiety tasks coincide with increases in hippocampal progesterone and 3α,5α-THP. Pharmacol. Biochem. Behav. 67, 587–596.

Frye, C.A., Seliga, A.M., 2001. Testosterone increases analgesia, anxiolysis, and cognitive performance of male rats. Cogn. Affect. Behav. Neurosci. 1, 371–381.

Frye, C.A., Seliga, A.M., 2002. Olanzapine and progesterone have dose-dependent and additive effects to enhance lordosis and progestin concentrations of female rats. Physiol. Behav. (in press).

Fu, Y., Zhu, Z.T., Chen, L.J., Yu, L.P., Jin, G.Z., 2000. Behavioral characteristics of olanzapine: an atypical neuroleptic. Acta Pharmacol. Sin. 21, 329–334.

Gallo, M.A., Smith, S.S., 1993. Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: a possible rat model of PMS anxiety. Pharmacol. Biochem. Behav. 46, 897–904.

Ge, J., Barnes, N.M., Costall, B., Naylor, R.J., 1997. Effect of aversive stimulation on 5-hydroxytryptamine and dopamine metabolism in the rat brain. Pharmacol. Biochem. Behav. 58, 775–783.

Griffin, L.D., Mellon, S.H., 1999. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. Proc. Natl. Acad. Sci. USA 96, 13512–13517.

Guidotti, A., Dong, E., Matsumoto, K., Pinna, G., Rasmusson, A.M., Costa, E., 2001. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5α-dihydroprogesterone in psychiatric disorders. Brain Res. Brain Res. Rev. 37, 110–115.

Heidbreder, C.A., Foxton, R., Cilia, J., Hughes, Z.A., Shah, A.J., Atkins, A., Hunter, A.J., Hagan, J.J., Jones, D.N., 2001. Increased responsiveness of dopamine to atypical, but not typical antipsychotics in the medial prefrontal cortex of rats reared in isolation. Psychopharmacology 156, 338–351.

Inoue, T., Tsuchiya, K., Koyama, T., 1996. Effects of typical and atypical antipsychotic drugs on freezing behavior induced by conditioned fear. Pharmacol. Biochem. Behav. 55, 195–201.

Johnston, A.L., File, S.E., 1991. Sex differences in animal tests of anxiety. Physiol. Behav. 49, 245–250.

Kapur, S., Remington, G., Wilson, A.A., 1998. 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. Am. J. Psychiatry 155, 921–928.

Kelly, D.L., Conley, R.R., Tamminga, C.A., 1999. Differential olanzapine plasma concentrations by sex in a fixed-dose study. Schizophr Res. 40 (2), 101–104.

Laconi, M.R., Casteller, G., Garguilo, P.A., Bregonzio, C., Cabrera, R.J., 2001. The anxiolytic effect of allopregnanolone is associated with gonadal hormonal status in female rats. Eur. J. Pharmacol. 417, 111–116.

Marx, C.E., Duncan, G.E., Gilmore, J.H., Lieberman, J.A., Morrow, A.L., 2000. Olanzapine increases allopregnanolone in the rat cerebral cortex. Biol. Psychiatry 47, 1000–1004.

McCarthy, M.M., Felzenberg, E., Robbins, A., Pfaff, D.W., Schwartz-Giblin, S., 1995. Infusions of diazepam and allopregnanolone into the midbrain central gray facilitate open-field behavior and sexual receptivity in female rats. Horm. Behav. 29, 279–295.

Meehan, K., Zhang, F., David, S., Tohen, M., Janicak, P., Small, J., Koch, M., Rizk, R., Walker, D., Tran, P., Breier, A., 2001. A double-blind, randomized comparison of the efficacy and safety of intramuscular injections of olanzapine, lorazepam, or placebo in treating acutely agitated patients diagnosed with bipolar mania. J. Clin. Psychopharmacol. 21, 389–397.

Mintzer, J., Faison, W., Street, J.S., Sutton, V.K., Breier, A., 2001. Olanzapine in the treatment of anxiety symptoms due to Alzheimer's disease: a post hoc analysis. Int. J. Geriatr. Psychiatry 16, S71–S77.

Moore, N.A., Tye, N.C., Axton, M.S., Risius, F.C., 1992. The behavioral pharmacology of olanzapine, a novel "atypical" antipsychotic agent. J. Pharmacol. Exp. Ther. 262, 545–551.
Nowakowska, E., Chodera, A., Kus, K., 1999. Influence of olanzapine on cognitive functions and catalepsy in rats after single and chronic administration. Pol. J. Pharmacol. 51, 295–300.
Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.
Pellow, S., File, S.E., 1986. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24, 525–529.
Perry, P.J., Lund, B.C., Sanger, T., Beasley, C., 2001. Olanzapine plasma concentrations and clinical response: acute phase results of the North American Olanzapine Trial. J. Clin. Psychopharmacol. 21, 14–20.
Petty, F., Brannan, S., Casada, J., Davis, L.L., Gajewski, V., Kramer, G.L., Stone, R.C., Teten, A.L., Worchel, J., Young, K.A., 2001. Olanzapine treatment for post-traumatic stress disorder: an open-label study. Int. Clin. Psychopharmacol. 16, 331–337.
Purdy, R.H., Moore, P.H. Jr, Rao, P.N., Hagino, N., Yamaguchi, T., Schmidt, P., Rubinow, D.R., Morrow, A.L., Paul, S.M., 1990. Radioimmunoassay of α-hydroxy-5α-pregnan-20-one in rat and human plasma. Steroids 55, 290–296.
Purdy, R.H., Morrow, A.L., Blinn, J.R., Paul, S.M., 1990b. Synthesis, metabolism, and pharmacological activity of 3α-hydroxy steroids which potentiate GABA-receptor-mediated chloride ion uptake in rat cerebral cortical synaptoneurosomes. J. Med. Chem. 33, 1572–1581.
Rodbard, D., Hutt, D.M., 1974. Statistical analysis of radioimmunoassay and immunoradiometric assays: a generalized, weighted iterative, least squares method for logistic curve fitting. In: International Atomic Energy Agency (Ed.), Symposium on Radioimmunoassay and Related Procedures in Medicine. Uniput, New York, pp. 209–233.
Sams-Dodd, F., 1997. Effect of novel antipsychotic drugs on phencyclidine-induced stereotyped behaviour and social isolation in the rat social interaction task. Behav. Pharmacol. 8, 196–215.
Santosh, P.J., Baird, G., 2001. Pharmacotherapy of target symptoms in autistic spectrum disorders. Indian J. Pediatr. 68, 427–431.
Smythe, J.W., McCormick, C.M., Rochford, J., Meaney, M.J., 1994. The interaction between prenatal stress and neonatal handling on nociceptive response latencies in male and female rats. Physiol. Behav. 55, 971–974.
Squitieri, F., Cannella, M., Piorcellini, A., Brusa, L., Simonelli, M., Ruggieri, S., 2001. Short-term effects of olanzapine in Huntington's disease. Neuropsychiatry, Neuropsychol. Behav. Neurol. 14, 69–72.
Stanford, J.A., Fowler, S.C., 1997. Similarities and differences between the subchronic and withdrawal effects of clozapine and olanzapine on forelimb force steadiness. Psychopharmacology 132, 408–414.
Steinpreis, R.E., Anders, K.A., Branda, E.M., Kruschel, C.K., 1999. The effects of atypical antipsychotics and phencyclidine (PCP) on rotorod performance. Pharmacol. Biochem. Behav. 63, 387–394.
Treit, D., 1985. The inhibitory effect of diazepam on defensive burying: anxiolytic vs. analgesic effects. Pharmacol. Biochem. Behav. 22, 47–52.
Treit, D., Pinel, J.P., Fibiger, H.C., 1981. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. Pharmacol. Biochem. Behav. 15, 619–626.
Trevitt, J.T., Carlson, B.B., Salamone, J.D., 1999. Behavioral assessment of atypical antipsychotics in rats: studies of the effects of olanzapine (Zyprexa). Psychopharmacology 145, 309–316.
Uzonov, D.P., Cooper, T.B., Costa, E., Guidotti, A., 1996. Fluoxetine-elicited changes in brain neurosteroid content as measured by negative ion mass fragmentography. Proc. Natl. Acad. Sci. USA 93, 12599–12604.
Uzunova, V., Sheline, Y., Davis, J., Rasmusson, A., Uzunov, D., Costa, E., Guidotti, A., 1998. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc. Natl. Acad. Sci. USA 95, 3239–3244.
Zanarini, M.C., Frankenburg, F.R., 2001. Olanzapine treatment of female borderline personality disorder patients: a double-blind, placebo-controlled pilot study. J. Clin. Psychiatry 62, 849–854.
Zangrossi, H., File, S.E., 1992. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain Res. Bull. 29, 381–388.