Abstract—The prevalence of major depressive disorder (MDD) in adult men is roughly half that of women. Clinical evidence supports a protective effect of androgens against depressive disorders in men. The developing brain is subject to androgen exposure but a potential role for this in depression during adulthood has not been considered. In order to explore this question we treated newborn male rat pups with the androgen receptor antagonist flutamide to block endogenous androgen action and then conducted behavioral tests prior to puberty. Depression-like behaviors were assessed with the Forced Swim Test (FST) and the Sucrose Preference Test (SPT), and anxiety-like behaviors were assessed with the Open Field Test (OFT) and the Novelty-Suppressed Feeding Test (NSFT). Compared to the vehicle-treated controls, neonatal-flutamide treatment caused a significant increase in depression-like behaviors in preadolescent male rats but did not cause any significant difference in anxiety-like behaviors. In separate experiments, male pups with and without flutamide treatment were injected with 5-bromo-2$'\text{-}$deoxyuridine-5$'$-monophosphate (BrdU) from postnatal day (PND) 1 to 4 to label newly produced cells or the hippocampi were Golgi-Cox imbedded and pyramidal neurons visualized. Three lines of evidence indicate neonatal flutamide treatment impaired hippocampal neurogenesis and neuronal dendritic spine formation in preadolescent male rats. Compared to vehicle controls, flutamide treatment significantly decreased (1) the number of microtubal associated protein-2$^+$ (MAP-2$^+$) neurons in the CA1 region, (2) the number of MAP-2$^+$ neurons in the dentate gyrus (DG) region of the hippocampus, and (3) the density of dendritic spines of pyramidal neurons in the CA1 region. However, there was no effect of flutamide treatment on the number of gial fibrillary acidic protein (GFAP)$^+$ or GFAP$^+/−$ BrdU$^+$ cells in the hippocampus. This study suggests that the organizational effect of androgen-induced hippocampal neurogenesis is antidepressant. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

EFFECTS OF NEONATAL FLUTAMIDE TREATMENT ON HIPPOCAMPAL NEUROGENESIS AND SYNAPTOGENESIS CORRELATE WITH DEPRESSION-LIKE BEHAVIORS IN PREADOLESCENT MALE RATS

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Key words: depression, androgen, flutamide, hippocampus, neurogenesis.

The prevalence of major depressive disorder (MDD) in adult men is roughly half that of women. Clinical evidence supports a protective effect of androgens against depressive disorders. Lower androgen levels are associated with an increase in the prevalence of depressive disorders in adult males (Seidman et al., 2002, 2003; Shores et al., 2004), and androgen replacement improves depressive symptoms in hypogonadal male patients (Perry et al., 2002; Pope et al., 2003). Interestingly, the prevalence of MDD in males and females is not significantly different during childhood but emerges in the age range of 11–14 years (Angold et al., 1998), paralleling the rise of androgens in adolescent males. This has led to a general assumption that it is the post-pubertal onset of androgen synthesis that underlies the sex difference in the prevalence of depression. However, it is well established in studies of reproductive endpoints that perinatal gonadal steroids act to organize the neural substrate and this changed neuroarchitecture is then activated by gonadal steroids post-puberty in a directed manner. Codified as the classic Organizational/Activational Hypothesis of gonadal steroid action (Phoenix et al., 1959), this tenet has not generally been applied to more complex emotion-based behaviors controlled outside the hypothalamus, such as depression-like behaviors controlled in part by the hippocampus.

A large body of literature suggests that sex steroid hormones, including estrogens and androgens, play important roles in hippocampal dimorphism both anatomically and functionally (McEwen, 1983, 1999; Pilgrim and Hutchison, 1994; Cooke et al., 1999). Estrogens are the major focus of these studies (Dawson et al., 1975; Madeira et al., 1991; Madeira and Lieberman, 1995; Stromstedt and Waterman, 1995; Wood et al., 1997; Daniel et al., 1999; Luine et al., 2003; Zhu et al., 2003). Much less research has been done with respect to the effects of androgens on the hippocampus. Testosterone and its 5-alpha reduced metabolite, dihydrotestosterone (DHT), are the major circulating androgenic hormones in the male. In the newborn rat, the testosterone level is five to six times higher in males than in females (Forest, 1979, Angold et al., 1998), paralleling the rise of androgen levels during childhood but emerging in the age range of 11–14 years. The fetal rat brain expresses androgens and estrogens as early as embryonic day (E)12, the
expression peaks on E17–18 and then gradually declines during adulthood (Brannvall et al., 2005). The distribution of AR in the brain is broad and includes the hippocampus, cortex, and lateral septum (Sar and Stumpf, 1973; Lieberburg et al., 1977; Handa et al., 1986; Roselli, 1991). In the hippocampus, AR expression is at a much higher density in CA1 than CA3 or dentate gyrus (DG) (Kerr et al., 1996).

Few studies have examined steroid hormone effects on the developing hippocampus, and even fewer have looked at the potential effect of androgens (McEwen, 1983; Pilgrim and Hutcshon, 1994). Perinatal androgen treatment increases CA3 pyramidal cell layer volume and neuronal soma size, neuronal dendritic length, the number of dendritic branches, and the overall volume of the CA3 region (Isgor and Sengelaub, 1998, 2003; Forgie and Kolb, 2003). These hippocampal structural changes are associated with functional changes. When androgens are eliminated by neonatal gonadectomy, there is a decrease in dendritic spine density in the hippocampus, and poor spatial navigation in adulthood. However, when neonatally gonadectomized rats are treated with testosterone or DHT during pre- or neonatal life, hippocampal dendritic spine density is increased and the spatial navigation performance is significantly improved (Dawson et al., 1975; Isgor and Sengelaub, 1998, 2003).

In the present study we antagonized the androgen receptor in order to examine the neonatal organizational effects of androgens on depression-like behaviors, anxiety-like behaviors, hippocampal neurogenesis, and synaptogenesis during the adolescent period. Our data indicate neonatal androgens play an important role in protecting male rats from depression-like behaviors, and this protection is correlated with hippocampal neurogenesis and an increased dendritic spine density on pyramidal neurons in the hippocampus.

**EXPERIMENTAL PROCEDURES**

**Animals**

Timed-pregnant Sprague–Dawley rats were purchased from Charles River Laboratory (Wilmington, MA, USA). Individually housed pregnant females were checked every morning for the appearance of pups in the nest. The day of birth was defined as postnatal day 0 (PND0). Animals were housed under a 12:12 hour light/dark cycle, with food and water freely available. All animal procedures were approved by the University of Maryland at Baltimore Institutional Animal Care and Use Committee.

**Flutamide and BrdU treatment**

Neonatal treatment with flutamide, an androgen receptor antagonist, was conducted as described previously (Zhang et al., 2008). Briefly, rat pups from multiple dams were randomly distributed into either vehicle or flutamide treatment groups and marked by subcutaneous ink injection in either the front or hind paws for different experimental groups. Pups were removed from dams and placed on a heated pad (37 °C) to maintain temperature during the injection procedure. Flutamide (FLU, 250 μg/0.1 ml, 50 mg/kg) was dissolved in sesame oil. Pups were injected subcutaneously with either flutamide or vehicle on PND 0 and PND 1. The 5-bromo-2′-deoxyuridine-5′-monophosphate (BrdU) was injected peritoneally daily from PND1 to 4 (0.05 ml distilled water containing 50 mg/kg BrdU).

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The flutamide or sesame oil vehicle was injected subcutaneously on PND 0 and PND1, respectively. The BrdU was injected peritoneally daily from PND1 to PND 4 (0.05 ml distilled water containing 50 mg/kg BrdU) to label newly produced cells during neonatal development (Table 1). After each injection, the injection sites were sealed with cyanoacrylate Vetbond Surgical Adhesive (3M Animal Care Product, St. Paul, MN, USA). Pups were randomly distributed to different mother rats after each treatment.

**Behavioral tests**

Behavioral tests were conducted in a sequence from the least to the most stressful procedures: the Sucrose Preference Test, the Novelty Suppressed Feeding Test, and the Forced Swim Test. The Open Field Test was conducted in a separate batch of rats.

**The Sucrose Preference Test (SPT)**. The SPT was performed as described by Banasr and colleagues (Banasr and Duman, 2008). Briefly, on PND 22, rats were habituated with 1% sucrose for 48 h without any food or water. Then, after 24 h of deprivation, each rat was provided two identical bottles containing either water or 1% sucrose solution in an individual cage for 1 h. After 1 h, the amount of water and 1% sucrose intake were recorded. Data were expressed as percentage of sucrose intake from total intake (sucrose/sucrose + water).

**The Novelty-Suppressed Feeding Test (NSFT)**. The NSF was conducted as described (Britton and Britton, 1981; Bodnoff et al., 1988; Santarelli et al., 2003) with modifications. Briefly, the test was conducted in an open field box measured 76×76×46 cm^3^ All food was removed from the home cages 24 h before the test. Two grams of food pellets were placed on a white round paper (d=6.25 cm) in the center of the open field box. During the test, the rat was put at the corner of the testing box for 5 min to measure the latency to bite the food pellets. The rat was then put back in its cage with food. The amount of food the rat ate during the next 5 min period was measured.

**The Forced Swim Test (FST)**. The test was conducted accounting to previously established protocols (Porsolt et al., 1977; Detke and Lucki, 1996; Siuciak et al., 1997; Shirayama et al., 2002; Pechnick et al., 2008; Reed et al., 2008). Briefly, on the first day (PND 27), rats swam for 15 min in a 22.5 cm diameter glass cylinder filled with 25 °C water up to 30 cm high. On the second day (PND 28), rats swim for 5 min and were video-taped. Three behaviors were scored automatically with the Forced SwimScan system (Cleversys Inc. Reston, VA, USA, Detke et al., 1995; Tonelli et al., 2008): (1) climbing, rat made an active attempt to escape from the container; (2) swimming, rat stayed afloat, pedaling, and making circular movements around the tank; and (3) immobility, rat did not make any active movements.

**The Open Field Test (OFT)**. Testing followed the standard procedure described by Lacroix et al. (1998). The test room was dimly illuminated (two 60 W lights, indirectly), and rats placed in a square arena (76.5×76.5×49 cm^3^), divided into two areas: a peripheral area and a square center (40×40 cm^3^), and allowed to explore for 5 min. The measurement parameters included the number of square-crossings and the time spent in the peripheral and central areas of the open field.
**Immunohistochemistry**

**Brain tissue treatment.** On PND 28, rats were deeply anesthetized with ketamine (10 mg intraperitoneal) and placed in a stereotaxic instrument. The skulls were removed and the dorsal hippocampus was exposed. The tissue was immersed in 4% paraformaldehyde solution for 24 h. After dehydration in a graded series of ethanols, the tissue was embedded in paraffin. Sections (6 μm thick) were cut and mounted onto slides. The slides were deparaffinized in xylene, hydrated through a graded series of ethanols, and then rehydrated in distilled water. The slides were then incubated in 3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. The slides were then rinsed in distilled water and incubated in a blocking solution (10% normal goat serum in 0.1% Triton X-100 in PBS) for 1 h at room temperature. The slides were then incubated with primary antibodies overnight at 4°C. The primary antibodies used were rabbit polyclonal antibodies against MAP-2 (1:10,000 in PBS-T) and BrdU (1:1000 in PBS-T) followed by biotin-labeled secondary antibodies. The slides were then incubated with 2N HCl for 1 h at 37°C to denature DNA. After the reaction, the slides were rinsed and mounted onto gelatin-subbed slides, dehydrated, and coverslipped. The number of MAP-2 positive and BrdU/MAP double positive neurons were counted in the CA1 and DG regions of the rostral hippocampus. For each region, the number of neurons was determined in a 50 μm segment which was at least 50 μm counting frame with a 40× objective. Ten to15 sections were collected throughout the rostral hippocampus from each animal. The number of MAP-2 positive and BrdU/MAP double positive neurons were counted bilaterally only in whole sections (free of any tears or other physical defects) that matched anatomically across all animals, resulting in four sections per animal. Numbers were averaged to give one final value per animal. The treatment conditions of the animals from which the sections were generated were unknown to the investigator doing the analysis.

**Golgi-Cox method.** The Golgi-Cox stain was conducted as described with modifications (Glascher and de Loos, 1981; Mong et al., 1999; Mong and McCarthy, 1999; Shors et al., 2001; Amateau and McCarthy, 2004). Briefly, rats were deeply anesthetized with ketamine and fresh control tissue was collected for impregnation in potassium dichromate solution and incubated in a dark box for 2 weeks. Impregnated brains were then sectioned (100 μm) and mounted on a cryostat for observation.

**Double-label immunohistochemistry.** MAP-2+ neurons. Newly-produced mature neurons were detected with BrdU/MAP-2 double labeling immunochemical techniques (Yang et al., 2009). Briefly, tissue sections were treated with 1% sodium borohydride in phosphate buffered saline (PBS) for 20 min, rinsed, and incubated with 0.04% phenylhydrazine in PBS for 20 min. Tissue sections were then incubated with monoclonal antibody against MAP-2 in PBS with 0.4% Triton X-100 (PBS-T) overnight. After being thoroughly rinsed, tissue sections were incubated with biotinylated secondary antibody, followed by rinses and addition of Vectastain Elite ABC reagents (Vector). MAP-2 positive neurons were detected by the dianaminobenzidine (DAB) method. Then, tissue sections were further incubated with 2N HCl for 1 h at 37 °C to denature DNA. After a thorough rinse, tissue sections were incubated with 5% goat serum in PBS-T for 60 min. Monoclonal antibody against BrdU (Caltag Lab, 1:10000 in PBS-T) was applied in PBS-T for 1 h at room temperature and then for 48 h at 4 °C. The next day, tissue sections were incubated with biotinylated secondary antibody against mouse IgG, followed by rinses and addition of Vectastain Elite ABC reagents (Vector). BrdU positive cells were detected with Nickel-diaminobenzidine in sodium acetate, giving a deep blue color to BrdU-positive nuclei (Fig. 2B). After the reaction, tissue sections were rinsed and mounted onto gelatin-subbed slides, dehydrated, and coverslipped. The number of MAP-2 positive and BrdU/MAP double positive neurons were counted in the CA1 and DG regions of the rostral hippocampus. For each region, the number of neurons was determined in a 5×10 μm counting frame with a 40× objective. Ten to15 sections were collected throughout the rostral hippocampus from each animal. The number of MAP-2 positive and BrdU/MAP double positive neurons were counted bilaterally only in whole sections (free of any tears or other physical defects) that matched anatomically across all animals, resulting in four sections per animal. Numbers were averaged to give one final value per animal. The treatment conditions of the animals from which the sections were generated were unknown to the investigator doing the analysis.

**GFAP+ cells.** Newly-produced glial cells were detected with glial fibrillary acidic protein (GFAP)/BrdU double labeling immunochemical technique as described (Zhang et al., 2008). Briefly, the tissue sections were treated as described above. Sections were incubated with antibody (goat anti-mouse monoclonal antibody) against BrdU and sequentially with biotin-labeled secondary antibody (rabbit anti-goat). BrdU positive cells were detected with Nickel-diaminobenzidine (DAB) in sodium acetate, giving a deep blue color to BrdU-positive nuclei (Fig. 4B). After being thoroughly rinsed overnight, the tissue sections were sequentially incubated with antibodies against GFAP (Sigma, 1:10,000), and incubated with biotin-labeled secondary antibodies. The GFAP positive cells were detected with DAB. The number of GFAP+ cells was counted in the CA1 and DG regions of the rostral hippocampus, using the Neurolucida program package (Microbrightfield version 2.01) and a counting frame (80×80 μm²) with a 60× objective. The final value of each animal was averaged from four sections and expressed as mean±SEM.

**Data analysis**

All data were expressed as mean±SEM. Two-tailed Student’s t-test was used to compare the mean between groups, with P<0.05 required for statistical significance.

**RESULTS**

**Neonatal flutamide-treatment and brain and body weights in PND 28 rats**

Neonatal flutamide treatment did not affect either brain (t=0.84, df=23, P=0.41) or body weights (t=1.69, df=24, P=0.10) in PND 28 rats as indicated in Table 2.

**Neonatal flutamide-treatment increased the expression of depression-like behaviors in PND 25–28 rats**

The level of depression-like behaviors was significantly increased in both the FST and the SPT after male pups were treated with flutamide neonatally. In the first day of FST, there was no significant difference in the immobility between the vehicle- and flutamide-treated male rats. During the first 5 min, the duration of immobility was 21.6±4.4 (n=5) in vehicle-treated and 19.7±6.9 s (n=6) in flutamide-treated males (t=0.237, df=8, P=0.82). However, on the second day of the test, during a 5 min recording session the duration of immobility was 15.9±5.5 (n=16) in

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Male pups were collected from four dams, and distributed randomly to different groups. The pups were treated with flutamide (50 mg/kg) or sesame oil vehicle on PND 0 and PND 1 as indicated. All rats were euthanized on PND 28. Body and brain weights were obtained, and expressed as mean±SEM.
Though flutamide-treated rats took a longer time to find and eat the food, the difference between these two groups was not statistically significant ($P=0.38$). In the OFT, the number of crossings of outer squares was $98.7\pm9.3$ ($n=17$) in vehicle-treated and $77.8\pm11.4$ ($n=9$) in flutamide-treated male rats ($t=1.20$, $df=19$, $P=0.17$); the number of inner square crossings was $9.6\pm1.8$ ($n=8$) in vehicle-treated and $8.0\pm2.1$ ($n=9$) in flutamide-treated male rats ($t=0.58$, $df=18$, $P=0.56$). The percentage of time in inner squares was $5.3\pm1.1\%$ ($n=17$) in vehicle-treated and $6.8\pm1.9\%$ ($n=9$) in flutamide-treated male rats ($t=0.70$, $df=13$, $P=0.50$). There were no significant differences between treatment groups on any measured parameters (data not shown).

Neonatal flutamide-treatment decreased the number of MAP2+ neurons in the hippocampus

Representative photomicrographs of neurons of MAP-2+/BrdU-neurons are shown in Fig. 2A and neurons double labeled for MAP2 and BrdU are shown in Fig. 2B. Compared to the vehicle-treated controls, the number of MAP-2+ neurons was significantly decreased in both CA1 and DG regions of the hippocampus of flutamide-treated male rats. In the CA1 region, the mean number of MAP-2+ neurons was significantly higher in vehicle-treated ($n=8$) than in flutamide-treated ($n=6$) male rats ($t=2.69$, $df=7$, $P=0.03$); likewise in the DG region of the same animals there were significantly more MAP2+ neurons in vehicle-treated versus flutamide-treated male rats ($t=4.71$, $df=9$, $P=0.001$, Fig. 2C). A significant difference in the number of BrdU+/MAP-2+ neurons (Fig. 2A) was observed in the DG region with vehicle treated males having more double labeled neurons compared to flutamide-treated male rats ($t=2.75$, $df=12$, $P=0.02$). There was no effect of treatment on double labeled neurons in the CA1 region ($t=0.57$, $df=10$, $P=0.27$, Fig. 2D).

Neonatal flutamide-treatment decreased the density of dendritic spines on pyramidal neurons in CA1 hippocampus

Representative photomicrographs of Golgi-Cox impregnated pyramidal neurons are shown in Fig. 3A, C, D. The density of dendritic spines was significantly decreased in neonatally flutamide-treated males compared to vehicle-treated controls. The data were expressed as the number of spines per $\mu$m ($t=2.58$, $df=8$, $P=0.03$, $n=7–8$; Fig. 3B).

Neonatal flutamide treatment did not affect hippocampal gliogenesis in PND 28 male rats

Representative photomicrographs for GFAP+ cells (Fig. 4A) and GFAP+/BrdU+ cells (Fig. 4B) are shown. In the CA1 region, the number of GFAP+ cells was $22.5\pm10.7$ ($n=9$) in vehicle-treated and $20.5\pm2.2$ ($n=6$) in flutamide-treated male rats ($t=0.86$, $df=6$, $P=0.42$), whereas the number of BrdU+/GFAP+ cells was $13.5\pm1.0$ ($n=9$) in vehicle-treated and $13.6\pm2.5$ ($n=6$) in flutamide-treated male rats ($t=0.03$, $df=7$, $P=0.98$). In the DG region, the number of GFAP+ cells was $24.1\pm1.1$ ($n=9$) in vehicle-treated and $23.2\pm2.4$ ($n=6$) in flutamide-treated male rats ($t=0.36$, $df=7$, $P=0.73$); whereas the number of BrdU+/GFAP+ cells was $13.9\pm0.8$ ($n=9$) in vehicle-treated and
DISCUSSION

The biological basis of gender bias in the prevalence of major depressive disorder remains unknown. We present evidence here that male rats exhibited more depression-like but not anxiety-like behaviors during preadolescence when they were treated with flutamide, an androgen receptor antagonist, neonatally. This increase in depression-like behaviors was associated with a decrease in the number of neurons and a decrease in the density of dendritic spines on the pyramidal neurons, but not with gliogenesis, in the hippocampus. To our knowledge, this is the first exploration of a potential contributing effect of neonatal androgen exposure on the adult onset of depressive-like symptoms.

There are organizational effects of androgens on depression-like behaviors

Androgens begin to affect hippocampal development prenatally (Bayer, 1980; Bingaman et al., 1994; Isgor and Sengelaub, 1998; Brannvall et al., 2005). Circulating androgens are synthesized in the fetal testis beginning around E16, peaking at E18 and rising again 4 days later at birth (Weisz and Ward, 1980). During this critical time androgens not only affect fetal brain development but can also determine how the brain functions during adulthood. In this study, we observed that flutamide administration in neonatal male rats resulted in an increase in depression-like behaviors during preadolescence in both the FST and the SPT, suggesting that perinatal androgens are protective against a later onset of depression. Behavioral tests were conducted prior to PND 28 to avoid the surge of androgen production at puberty. The FST has been successfully conducted using perinatal rats in antidepressant studies (Pechnick et al., 2008; Reed et al., 2008). Our results are therefore consistent with an organizational effect of androgens on depression-like behaviors. Organizational effects of androgens have been reported for the hypothalamic-pituitary-adrenal axis (McCormick et al., 1998), spatial memory (Isgor and Sengelaub, 1998), and adult social behaviors (Schulz et al., 2009). Flutamide is a specific androgen receptor antagonist and widely used to block androgen’s effect both in vivo and in vitro (Isgor and Sengelaub, 1998;
There are no organizational effects of androgens on anxiety-like behaviors

Acute anti-anxiety effects of androgens are reported in both human (Kessler, 2003; Heyns et al., 2003, Howell and Shalet, 2001; Kaminetsky, 2005; Palomba et al., 2008) and animal studies (Bitran et al., 1993; Bing et al., 1998; Osborne et al., 2009). Our goal was to explore the organizational actions of androgens. We used the open field test (OFT) and the Novelty Food Suppressed test (NFST) to assess anxiety-like behaviors, but did not observe any effects of neonatal flutamide treatment. This may be a result of the behavioral tests we employed. Zuloaga and colleagues (2008), using mice with the testicular feminization mutation (tfm) which renders androgen receptors non-functional throughout life, observed an increase in anxiety-like behaviors as detected in the novel object test (NOT) and the Light/Dark (LD) Box, but not in the OFT or the elevated plus maze (EPM) test. In contrast, Stewart et al. (1975) found that injections of high doses of testosterone to neonatal females decreased anxiety-like behavior in the

**Fig. 3.** The effect of neonatal flutamide treatment on the density of dendritic spines in the CA1 region of the hippocampus. (A) Golgi-Cox impregnated pyramidal neurons. *: Pyramidal neuron, arrow: basal spines, scale bar: 50 μm. (B) High modification of a dendritic spines from vehicle-treated and (C) flutamide-treated male rats. Scale bar: 10 μm; (D) the density of dendritic spines in the pyramidal neurons in the CA1 hippocampus was significantly decreased (P<0.016) in neonatally flutamide-treated male rats compared to their counterparts. * P<0.05.

**Fig. 4.** GFAP+ cells in the hippocampus of PND 28 male rats. (A) GFAP+ Glial cells; (B) BrdU+/GFAP+ cell. GFAP, glial fibrillary acidic protein. Scale bar: 25 μm.
OFT. Other investigators have reported that neonatal castration decreases anxiety behaviors in the EPM in adult male rats (Lucion et al., 1996). These differences in animal models, behavioral tests, and treatment paradigms preclude making definitive conclusions regarding the organizational effects of androgens on adult anxiety-like behavior. The lack of effect may be also related with the neural substrates involved in the expression of emotional behaviors revealed by these tests. While the FST is sensitive to manipulations affecting hippocampal structure and function, the OFT is not. This provide additional evidence of a relationship between the effects of Flu in the hippocampus and the expression of depressive-like behavior.

There are organizational effects of androgens on hippocampal neurogenesis

We have previously reported that neonatal testosterone administration increases the number of hippocampal neurons in female rats (Zhang et al., 2008). In the current study, the number of mature (MAP-2+/H11001) neurons was significantly decreased in flutamide-treated male rats compared to controls in the DG region. Based on co-labeling with the cell division marker, BrdU, some of these neurons were born during the first four postnatal days and persisted until PND 28. Androgens may promote hippocampal neurogenesis by at least three different routes: the proliferation of new cells, the survival of new cells, and the differentiation of new cells into neurons. Androgens increase new cells via both AR as well as estrogen receptor (ER) mediated mechanisms since both DHT and estradiol increase the number of BrdU+ cells in the neonatal hippocampus (Zhang et al., 2008) and estradiol can be synthesized from testosterone in vivo. Androgens may promote neurogenesis via indirect mechanisms. Both testosterone and estradiol up-regulate the expression of brain derived neurotrophic factor (BDNF) in the brain (Rasika and Alvarez-Buylla, 1999) including in the hippocampus (Solum and Handa, 2002). BDNF promotes neuronal survival and differentiation (Alderson et al., 1990; Ghosh et al., 1994; Jones et al., 1994). Therefore, it is possible that androgens may program undifferentiated neuronal progenitor cells to respond to local cues such as BDNF to become mature neurons in their later development, an organizational effect of androgens. It is also possible that androgens may cause an increase in neurons by preventing neuronal apoptosis or death (Hammond et al., 2001; Hsu et al., 2001).

In the current study the number of mature neurons (MAP-2+ neurons) in the CA1 region was significantly decreased in flutamide-treated male rats compared to controls. However, in contrast to our findings in the DG region, neonatal flutamide treatment did not affect the number of BrdU+/MAP-2+ neurons. This may be a simple artifact of the smaller number of neurons being born in CA1 at this time, versus the large number proliferating in the DG. Regardless, our observation that there were fewer overall MAP-2+ neurons in the CA1 of flutamide treated males is consistent with an overall decrease in neurons in this region when androgen action is antagonized.

There are no organizational effects of androgens on gliogenesis

In postmortem studies of patients with MDD, a loss of glial cells is detected in fronto–limbic regions (Ongur et al., 1998; Rajkowska et al., 1999; Cotter et al., 2001, 2002), but not in the hippocampus (Stockmeier et al., 2004). Our data indicate that neonatal flutamide administration does not affect the number of glial cells in both the CA1 and DG regions of the hippocampus, suggesting that androgens may not be the major contributor to gliogenesis during neonatal hippocampal development. It also suggests that gliogenesis may not be the major mechanism for the antidepressant effects of androgens observed in this study.

There are organizational effects of androgens on synaptic formation

The density of dendritic spines on pyramidal neurons in the CA1 region of the hippocampus was significantly decreased in flutamide-treated preadolescent rats, suggesting neonatal androgen exposure is important for dendritic spine formation in the developing hippocampus. Multiple studies demonstrate that androgens promote and enhance dendritic spine formation in the adult hippocampus (Leranth et al., 2003; MacLusky et al., 2006; Parducz et al., 2006). However, our data now indicate that neonatal effects of androgens on dendritic spines can be extended into preadolescence. There are two possible explanations for our observation. The first one is that neonatal androgens promote dendritic spine formation and flutamide blocked this effect. Under this hypothesis, the decrease in dendritic spines occurred during the neonatal stage when flutamide was administrated and blocked the action of endogenous androgen. The flutamide-induced spine deficiency sustained into preadolescence, most likely due to lack of endogenous androgens before adolescence. The second possibility is that neonatal androgens prepare hippocampal neurons to respond to environmental cues for more spine formation in later stages of development. Under this hypothesis, neonatal flutamide administration blocked the organizational effect of androgens on dendritic spine formation in the hippocampus. This possibility needs to be further explored.

As the primary loci of excitatory synaptic transmission in the CNS, a change in the density of dendritic spines is associated with many functional changes in the CNS (Colonnier, 1968; Jones et al., 1997; Sorra and Harris, 2000). The density of dendritic spines can be regulated by many factors. For example, the spine density of neurons can be increased by learning and training (Jones et al., 1997; Moster et al., 1997; O’Malley et al., 2000), by estrogens in female rats (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992, 1993), and by androgens in male rats (Leranth et al., 2003; MacLusky et al., 2006; Parducz et al., 2006). Accumulated evidence suggests that the plasticity of dendritic spine may associate with depressive disorders and their treatment. For example, chronic administration of fluoxetine, an antidepressant, induces a signif-
icant increase in the density of dendritic spines in the rat hippocampus (Hajszan et al., 2005).

**Hippocampal neurogenesis and depression**

Major Depressive Disorder is a complicated brain disorder. Structural changes have been detected in depressive patients using MRI (Sheline et al., 1996; Bremmer et al., 2000) and postmortem studies (Rajkowska, 2000, 2002), including orbital and medial prefrontal cortex, amygdala, ventral striatum, inferior anterior cingulate, and the hippocampus (Sheline et al., 1998; Bremmer et al., 2000; Drevets, 2001; Beyer and Krishnan, 2002). Hippocampal atrophy is consistently detected in patients with recurrent depression (Sheline et al., 1996) as well as in patients with first episode of depression (Fordl et al., 2002), suggesting that reduced hippocampal volume may be a risk factor for depression. In the adult, hippocampal neurogenesis is inhibited by stress or glucocorticoids, and reduced neurogenesis is associated with depressive behaviors both in animals and humans (Brown et al., 1999; McEwen et al., 1999; McEwen, 2000; Sapolsky, 2000). In animal studies, effective antidepressants, such as desipramine, fluoxetine, and electroconvulsive therapy, enhance hippocampal neurogenesis (Malberg et al., 2000; Scott et al., 2000). Interestingly, by blocking hippocampal neurogenesis using radiation, Santarelli et al. (2003) indicated that the antidepressant effect of fluoxetine is dependent on hippocampal neurogenesis. Developmentally, prenatal stress significantly inhibits hippocampal neurogenesis, and this effect persists until adolescence (Coe et al., 2003) with the affected offspring exhibiting higher rates of depression-like behaviors (Sapolsky, 2001; Schmitz et al., 2002). Interestingly, the increase in depression-like behaviors following prenatal stress is seen only in females (Zhu et al., 2004), suggesting androgens may protect the hippocampus either by promoting hippocampal neurogenesis or preventing its atrophy.

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