ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 713–721

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Serotonin modulates offensive attack in adolescent anabolic steroid-treated hamsters

Jill M. Grimes, Richard H. Melloni Jr.*

Behavioral Neuroscience Program, Department of Psychology, 125 Nightingale Hall, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA

Received 21 December 2001; received in revised form 21 April 2002; accepted 17 May 2002

Abstract

Chronic anabolic–androgenic steroid (AAS) treatment during adolescence facilitates offensive aggression in male Syrian hamsters (*Mesocricetus auratus*). The current study assessed whether adolescent AAS-facilitated offensive attack was modulated by serotonin (5-HT) and if AAS exposure during this developmental period influenced 5-HT innervation to areas of hamster brain implicated in aggressive behavior. In a first experiment, hamsters were administered high-dose AAS throughout adolescence, and then scored for offensive attack following the systemic administration of saline or fluoxetine, a selective 5-HT reuptake inhibitor. Saline-treated hamsters showed high levels of offensive attack, while treatment with fluoxetine attenuated the AAS-facilitated aggressive response. In a second experiment, hamsters were administered high-dose AAS or sesame oil throughout adolescence, tested for offensive attack and then examined for differences in 5-HT innervation to areas of the hamster brain important for aggression. Aggressive AAS-treated hamsters showed significant reductions in the number of 5-HT immunoreactive (5-HT-ir) varicosities and fibers in several of these areas, most notably the anterior hypothalamus (AH), ventrolateral hypothalamus (VLH) and medial amygdala (MeA). However, no differences in 5-HT afferent innervation were found in other aggression areas, such as the bed nucleus of the stria terminalis (BNST) and lateral septum (LS). Together, these results support a role for altered 5-HT innervation and function in adolescent AAS-facilitated offensive aggression.

Keywords: Adolescence; Anabolic–androgenic steroids; Serotonin; Development; Fluoxetine; Aggression

1. Introduction

The naturally occurring male hormone testosterone and its synthetic derivatives (collectively termed androgenic–anabolic steroids [AAS]) have been used by professional and amateur athletes and bodybuilders for greater than two decades to enhance athletic performance and overall physical appearance (Yesalis et al., 1988, 1993). Over the past decade, the illicit use of AAS among the adolescent population appears to have reached near epidemic proportions (Yesalis et al., 1997; NIDACapsules, 2001). Indeed, studies from the National Institute on Drug Abuse estimate that more than a half million 8th and 10th grade students are using AAS in the United States each year (NIDACapsules, 2001). Of particular interest are reports that AAS use has risen significantly in this population, with 3.6% of male 10th graders reporting use in 2000, up from 2.8% in 1999, 2.0% in 1997 and 1.8% in 1996 (NIDACapsules, 2001). This pattern of abuse is important since the onset of AAS use during adolescence (15 years of age or less) is correlated with more frequent and heavier use later in life, despite physical or psychological ramifications (Buckley et al., 1988; Yesalis et al., 1988). Further still, the illicit use of AAS during the period of adolescent development portrays a significant health risk since AAS abuse has been associated with several adverse psychiatric and behavioral effects, including increased aggressive behavior.

Using the resident/intruder paradigm, we have shown previously that Syrian hamsters treated with AAS throughout adolescent development direct a significantly high number of offensive attacks and a reduced latency to attack intruders when tested immediately following the treatment period (Melloni et al., 1997; Harrison et al., 2000). The finding that AAS-treated animals attacked more than controls during the first behavioral interaction, in the absence of established social interactions and cues, suggested that AAS

* Corresponding author. Tel.: +1-617-373-3043; fax: +1-617-373-8714.
E-mail address: melloni@research.neu.edu (R.H. Melloni Jr.).

0091-3057/02/$ – see front matter
PII: S0091-3057(02)00880-8

exposure during this developmental period might stimulate aggression directly; perhaps by impacting the activity of specific neural circuits that regulate this behavior. The serotonin (5-HT) neural system has been implicated in the control of aggression in various species and models of aggression (Linnoila et al., 1983; Kruesi et al., 1990; Sijbesma et al., 1990; Higley et al., 1996; Coccaro et al., 1997). In Syrian hamsters, 5-HT activity in the anterior hypothalamus (AH) and ventrolateral hypothalamus (VLH) have been shown to regulate offensive aggression, where 5-HT release acts to suppress aggression (Delville et al., 1996; Ferris, 1996; Ferris and Delville, 1994; Ferris et al., 1997, 1999). Perhaps adolescent AAS exposure facilitates offensive attack in hamsters by disrupting the development of 5-HT afferents into these areas, thus, lowering hypothalamic 5-HT activity and reducing 5-HT's inhibitory influence on aggression. Indeed, the development of the 5-HT neural system in the hypothalamus continues into adolescence during which it displays remarkable plasticity, particularly in response to circulating sex steroids (Ladosky and Gaziri, 1970; Zeisel et al., 1981; Borisova et al., 1996). Sex differences in 5-HT levels and afferent innervation have been reported, with a decrease in 5-HT synthesis, levels and afferent fiber distribution and an increase in 5-HT metabolism observed in male rats (Long et al., 1983; Renner et al., 1985; Wilson et al., 1992; Borisova et al., 1996; Thiblin et al., 1999), especially in the AH brain region (Simerly et al., 1984; Renner et al., 1985). This sexual dimorphism appears to be dependent on androgens, since female rats exposed to testosterone show complete masculinization (i.e., a decrease) of the 5-HT fiber distribution, levels and ratio of 5-HT to its metabolites (Simerly et al., 1985; Gonzalez and Leret, 1992; Sundblad and Eriksson, 1997).

In addition, neurons located in several other brain areas including the amygdala, septum and the preoptic area have been implicated in aggressive behavior in hamsters (Bunnell et al., 1970; Sodetz and Bunnell, 1970; Hammond and Rowe, 1976; Potegal et al., 1981a,b, 1996). For instance, offensive aggression is inhibited by electrical stimulation of neurons in the lateral septum (LS), while it is activated by stimulation of cells located in the cortico-medial amygdala (CoMeA) (Potegal et al., 1981a, 1996). Similarly, neurons in the CoMeA, the medial amygdala (MeA) and the bed nucleus of the stria terminalis (BNST) are activated during an aggressive encounter with other hamsters (Kollack-Walker and Newman, 1995; Potegal et al., 1996; Delville et al., 2000). It is also possible that AAS exposure during adolescent development facilitates aggressive behavior by reducing 5-HT innervation to these brain areas. To date, however, it is unknown whether adolescent AAS exposure has *any* effects on the development of the 5-HT neural system and/or whether 5-HT signaling plays a significant role in adolescent AAS-facilitated offensive aggression. This despite continued reports that AAS use during this developmentally significant window is rising and can be associated with an increased incidence of aggression and violence (Dukarm et al., 1996; Loeber and Hay, 1997; Yesalis et al., 1997; NIDACapsules, 2001).

Using the Syrian hamster as an animal model, the present studies were conducted to establish a relationship between adolescent, AAS exposure, 5HT neural signaling and afferent innervation, and offensive aggression. In Syrian hamsters, the adolescent period of development can be identified as the time between postnatal days 27 and 56 (P27–P56). Weaning generally occurs around P25 with the onset of puberty beginning around P40 (Miller et al., 1977). During this developmental time period, hamsters wean from their dams, leave the nest, establish new solitary nest sites, participate in social relationships and learn to defend their territory using offensive attack (Whitsett, 1975; Schoenfeld and Leonard, 1985). Therefore, to determine whether 5-HT signaling played a significant role in adolescent AAS-facilitated offensive attack, we tested whether this aggressive behavioral response could be inhibited by fluoxetine, a selective 5-HT reuptake inhibitor shown to elevate 5-HT levels in the hamster brain (Ferris, 1996). Then, to determine whether AAS exposure altered the 5-HT afferent innervation to areas of the hamster brain implicated in aggressive behavior, we utilized immunohistochemistry to visualize and quantify 5-HT varicosities and fibers in these brain regions.

2. Methods

2.1. Animals

For the experimental treatment paradigm, intact preadolescent male hamsters (P23–P25) were obtained from Harlan Sprague–Dawley Labs (Indianapolis, IN), individually housed in Plexiglas cages, and maintained at ambient room temperature on a reverse light–dark cycle of (14L:10D, lights on at 19:00 h). Food and water were provided ad libitum. For aggression testing, stimulus (intruder) males of equal size and weight to the experimental animals were obtained from Harlan Sprague–Dawley 1 week prior to the behavioral test, group housed at five animals per cage in large Plexiglas cages, and maintained as above to acclimate to the animal facility. All intruders were prescreened for low aggression (i.e., disengage and evade) and submission (i.e., tail-up freeze, flee and fly-away) 1 day prior to the aggression test to control for behavioral differences between stimulus animals, as previously described (Ferris et al., 1997; Melloni et al., 1997). Animals displaying significantly low aggression and/or submissive postures were excluded from use in the behavioral assay.

2.2. Experimental treatment

In the first experiment, P27 hamsters ($n = 36$, i.e., two trials of $n = 18$ animals each) received daily subcutaneous injections (0.1–0.2 ml) of an AAS mixture consisting of

2 mg/kg testosterone cypionate, 2 mg/kg nortestosterone and 1 mg/kg dihydroxytestosterone undecylate (Steraloids, Newport, RI), for 30 consecutive days (P27–P56). This daily treatment of AAS was designed to mimic a chronic 'heavy use' regimen (Pope and Katz, 1988, 1994). The day following the last injection, adolescent AAS-treated animals were tested for offensive attack after an intraperitoneal injection of fluoxetine (20 mg/kg in 0.9% saline) (Sigma, St. Louis, MO) ($n=20$) or vehicle ($n=16$) in a volume of 0.2 ml each. All injections were performed on unanesthetized animals and took no longer than 10 s. Administration of fluoxetine at this dose and manner has been shown previously to be very selective for its antiaggressive properties, with no generalized effects observed on social or sex behaviors (Delville et al., 1996; Ferris and Delville, 1994; Ferris et al., 1997). After injection, animals were returned to their home cage. One hour later, animals were tested for offensive attack.

In a second experiment, P27 hamsters were weighed and randomly distributed into two groups. One group of hamsters ($n=6$) received subcutaneous injections of the AAS mixture as described above, while a second group ($n=5$) was injected with an equal volume of sesame oil vehicle alone. Following the treatment period, animals in both AAS and sesame oil groups were tested for offensive attack, sacrificed and the brains removed and processed for immunohistochemistry as detailed below.

2.3. Aggression testing

Experimental animals were tested for offensive attack using the resident/intruder paradigm, a well-characterized and ethologically valid model of offensive aggression in golden hamsters (Lerwill and Makaings, 1971; Floody and Pfaff, 1977). For this measure, an intruder of similar size and weight was introduced into the home cage of experimental animals and the resident was scored for offensive attack (i.e., number of attacks and latency to attack towards intruders), as previously described (Ferris et al., 1997). Briefly, an attack was scored each time the resident animal would wildly pursue and then either: (1) lunge towards and/or (2) confine the intruder by upright and sideways threat; each generally followed by a direct attempt to bite the intruder's ventrum and/or flank. The latency to attack was defined as the period of time between the beginning of the behavioral test and the first attack of the resident towards an intruder. In the case of no attacks, latency to attack was assigned the maximum latency (i.e., 600 s). Also, in Experiment 1, residents were measured for social interest towards intruders (i.e., contact time between resident and intruder) to control for nonspecific effects of fluoxetine on animal behavior. Contact time was defined as the period of time during which the resident deliberately initiated contact with the intruder either through olfactory investigation (i.e., sniffing) or aggression. Each aggression test lasted for 10 min and was scored by an observer unaware of the hamsters' experimental treatment. No intruder was used for more than one behavioral test and all tests were performed during the first four hours of the dark phase under dim red illumination and videotaped for behavioral verification of the findings.

2.4. Immunohistochemistry

One day following the behavioral test for aggression, AAS and sesame oil-treated hamsters in Experiment 2 were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine, and the brains fixed by transcardial perfusion with 4% paraformaldehyde. Brains were then cryoprotected by

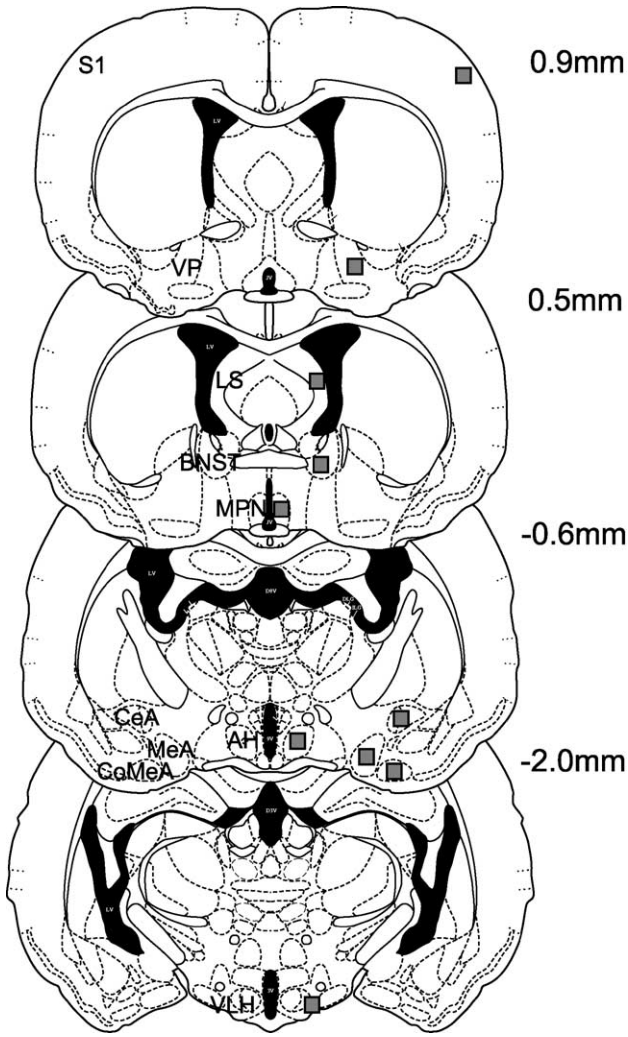

Fig. 1. Diagrams showing the location of the areas selected to quantify 5-HT varicosities and fibers (shaded areas). Plates were modified from hamster atlas of Morin and Wood (2001) and reflect specific positions in the rostral-caudal plane (i.e., distance in millimeters from bregma to the plane of section at the skull surface). Abbreviations: AH, anterior hypothalamus; BNST, medial division of the bed nucleus of the stria terminalis; CeA, central amygdaloid nucleus; CoMeA, cortico-medial amygdaloid nucleus; LS, intermediate part of the lateral septal nucleus; MeA, medial amygdaloid nucleus; MPN, medial preoptic nucleus, S1, S1 neocortex; VLH, ventrolateral hypothalamus; VP, ventral pallidum.

incubating in 30% sucrose in phosphate-buffered saline (PBS; 0.001 M KH_2PO_4, 0.01 M Na_2HPO_4, 0.137 M NaCl, 0.003 M KCl, pH 7.4) overnight at 4 °C. A consecutive series of 35 μm coronal sections were cut on a sliding microtome, collected as free floating sections in 1 × PBS and labeled for 5-HT by single-label immunohistochemistry using a modification of an existing protocol (Ferris and Delville, 1994). Briefly, free floating sections were pretreated with 1% sodium borohydride followed by preincubation in 20% normal goat serum with 1% H_2O_2 and 0.3% Triton X-100. Sections were incubated in primary antiserum (1:1000) for 5-HT antirabbit (Protos Biotech, Ridgefield, NJ) with 2% NGS and 0.3% Triton X-100 for 48 h at 4 °C. After primary incubation, sections were incubated in secondary antirabbit followed by tertiary antisera (Vectastain ABC Elite Kit-rabbit, Vector Labs, Burlingame, CA) for 1 h each at room temperature and then labeled with diaminobenzidine (DAB, Vector Labs). Sections were mounted on gelatin-coated slides, allowed to air dry and dehydrated through a series of ethanol and xylene solutions. Then, slides were coverslipped using Cytoseal-60 mounting medium (VWR Scientific, West Chester, PA).

2.5. Image analysis

The number of 5-HT immunoreactive (5-HT-ir) varicosities and fibers was determined within specific brain areas using the BIOQUANT NOVA 5.0 computer-assisted microscopic image analysis software package as previously described (DeLeon et al., 2002a). The areas analyzed were selected based on previous data implicating these regions as part of the circuit important for aggressive behavior in numerous species and models of aggression, with the notable exception of the S1 neocortex (S1) and ventral pallidum (VP), i.e., nonaggression areas used as a control regions. These areas (see Fig. 1) included the intermediate part of the lateral septal nucleus (LS), the medial division of the BNST, the medial preoptic nucleus (MPN), the MeA, the AH, the central amygdaloid nucleus (CeA), the CoMeA and the VLH, which included the medial aspects of the medial tuberal nucleus and the ventrolateral part of the ventromedial hypothalamic nucleus. Slides from each animal were coded by an experimenter unaware of the experimental conditions and BIOQUANT NOVA 5.0 image analysis software running on a Pentium III CSI Open PC computer (R&M Biometrics, Nashville, TN, USA) was utilized to identify the brain region of interest at low power (4 ×) using a Nikon E600 microscope. At this magnification, a standard computer-generated box was drawn to fit within the particular region of interest. Then, under 20 × magnification, images were thresholded at a standard RGB-scale level empirically determined by observers blinded to treatment conditions, such as to allow detection of stained 5-HT-ir elements with moderate to high intensity, while suppressing lightly stained elements. This threshold value was then applied across subjects to control for changes in background staining and differences in foreground staining intensity between animals. The illumination was kept constant for all measurements. 5-HT-ir varicosities and fibers were identified in each field using a mouse driven cursor, and then 5-HT-ir counts were performed automatically by the BIOQUANT software. Measurements at 20 × continued until 5-HT-ir elements throughout the entire region of interest were quantified. Two to three independent measurements of 5-HT-ir elements were taken from several consecutive sections ($n > 3$) of each animal per treatment group depending upon: (1) identification of the exact position of the nucleus within the region of interest and (2) the size of the nucleus in the rostral-caudal plane. Then, the number of 5-HT-ir varicosities and fibers was determined for each region of interest, standardized per 100 × 100 μm parcel for regional comparison purposes, and then used for statistical analysis.

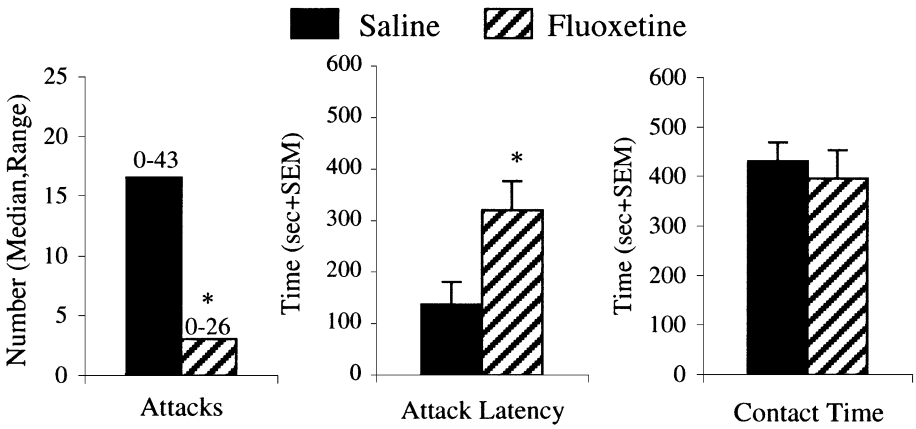

Fig. 2. Fluoxetine treatment decreases offensive attack behavior in adolescent AAS-treated hamsters. Number of attacks, attack latency and total contact time in saline- and fluoxetine-pretreated residents. * $P < .05$, Mann–Whitney, two-tailed (attacks), Student's t tests, two-tailed (attack latency and contact time). Bars denote S.E.M.

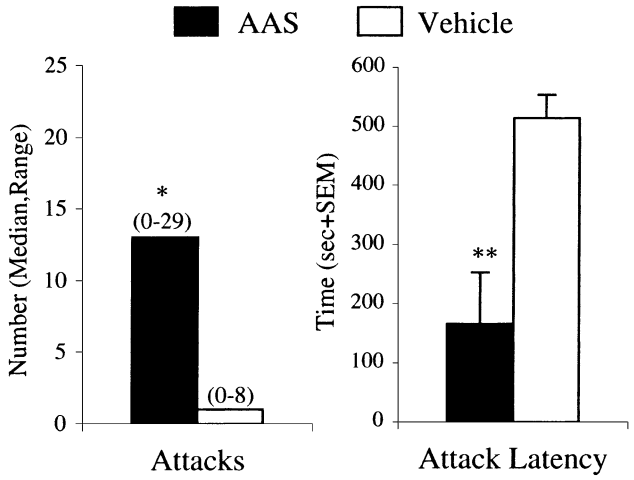

Fig. 3. Adolescent AAS treatment increases offensive attack. Number of attacks and attack latency in AAS- and vehicle-treated residents. Bars denote S.E.M. $^{**}P<.01$, $^{*}P<.05$, Mann–Whitney, two-tailed (number of attacks), Student's t test, two-tailed (attack latency).

2.6. Statistics

2.6.1. Behavioral studies

For Experiment 1, the results from the aggression tests were compared between fluoxetine- and saline-treatment groups. For Experiment 2, results from the aggression tests were compared between AAS- and sesame oil-treatment groups. In both experiments, nonparametric data (number of attacks) were compared by Mann–Whitney U tests (two-tailed), while parametric data (attack latency and contact time) were compared by Student's t test (two-tailed).

2.6.2. 5-HT afferent innervation

The number of 5-HT-ir varicosities and fibers was compared between treatment groups by Student's t test (two-tailed) for each area analyzed.

3. Results

3.1. Experiment 1—5-HT and offensive attack

Peripheral administration of the selective 5-HT reuptake inhibitor fluoxetine diminished the aggressive response of adolescent AAS-treated animals. As shown in Fig. 2, hamsters treated with high-dose AAS throughout adolescence showed high levels of offensive attack when administered saline prior to the aggression test. Indeed, half of the animals tested (8 out of 16) scored greater than 15 attacks on intruders, with only 25% (4 out of 16) scoring less than 10 attacks during the test period. The remainder of the

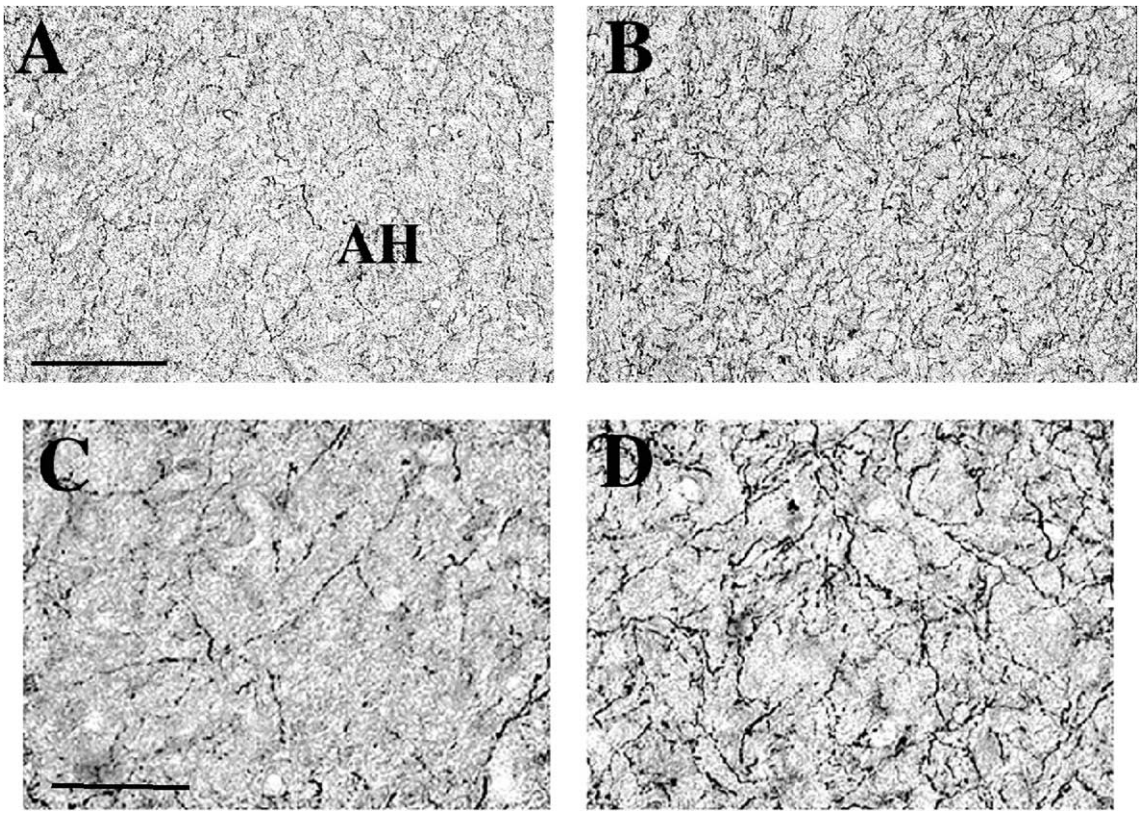

Fig. 4. Brightfield photomicrographs showing 5-HT-ir varicosities and fibers in the AH of (A, C) AAS- and (B, D) oil-treated hamsters. Bar, 20 μm (A, B) and 2 μm (C, D).

animals (4 out of 16) scored moderate-to-high levels of offensive attack, directing between 10 and 15 attacks towards intruders. Conversely, AAS-treated animals administered fluoxetine prior to the behavioral test period showed a statistically significant decrease in the number of attacks ($Z = 2.48$, $P < .05$) during aggression tests. In fact, peripheral fluoxetine treatment completely eliminated offensive attack in nearly half (9 out of 20) of the animals tested. Of the remaining animals (11 out of 20), only one animal showed high levels of offensive attack (i.e., greater than 15 attacks during the test). In addition, AAS-treated hamsters administered fluoxetine also displayed a significantly increased latency to attack intruders [$t(35) = -2.42$, $P < .05$] compared to saline-treated animals. In most all AAS-treated hamsters administered saline, attack latencies were decreased with 12 out of 16 (75%) of the animals attacking within the first 130 s of the behavioral test. By comparison, the majority (60%) of fluoxetine-treated animals (12 out of 20) attacked intruders after the first 130 s of the test period. Finally, although fluoxetine-treated hamsters showed marked reductions in offensive attack, no significant difference was measured in contact time between fluoxetine- and saline-treated animals ($P > .1$).

3.2. Experiment 2—offensive aggression and 5-HT afferent innervation

As characterized extensively in our previous studies (Melloni and Ferris, 1996; Melloni et al., 1997; Harrison et al., 2000), animals treated with AAS during adolescent development showed significantly heightened measures of offensive attack (Fig. 3). Specifically, hamsters treated with high-dose AAS showed a significant increase in the number of attacks ($Z = 2.09$, $P < .05$) over vehicle-treated littermates. Indeed, half of the AAS-treated animals (3 out of 6) scored greater than 15 attacks during the aggression test. By comparison, the majority of saline-treated hamsters (4 out of 5) scored a single attack or less on opponents. In addition, AAS-treated hamsters also displayed a significantly decreased attack latency towards intruders [latency to attack, $t(10) = -3.35$, $P < .01$] than vehicle-treated control animals. Nearly 84% of AAS-treated animals (five out of six) responded within the first 130 s of the 10-min test, in comparison to all saline-treated control animals (five out of five) whose first recorded attack occurred approximately 4–5 min later, toward the end of the test period.

In aggressive AAS-treated hamsters, the immunohistochemical staining pattern for 5-HT was altered in several areas of hamster brain important for aggressive behavior, including those in the hypothalamus. For example, in sesame oil-treated controls, the staining of 5-HT varicosities in the AH displayed a dense pattern of 5-HT-ir indicative of the normal distribution of synaptic input onto neurons in this brain region (Fig. 4B and D). By comparison, animals treated with AAS during adolescence display a less dense pattern of staining for 5-HT-ir varicosities and fibers in the AH (Fig. 4A and C). Quantitative analysis of 5-HT innervation to the AH showed that AAS-treated animals had approximately 40% of the 5-HT-ir varicosities and fibers of oil-treated littermates (Fig. 5). This difference was statistically significant [$t(12) = -8.29$, $P < .001$]. Similar results were found in the VLH where AAS-treated animals had about 25% of the 5-HT-ir varicosities and fibers of oil-treated littermates (Fig. 5). This difference was statistically significant [VLH, $t(8) = -4.93$, $P < .001$]. These findings were not restricted to the hypothalamus, however, as other hamster circuits implicated in aggression showed similar decreases in 5-HT innervation following adolescent AAS exposure (Fig. 5). For instance, the number of 5-HT-ir varicosities and fibers in the MeA and CeA of AAS-treated animals was less than 50% that of sesame oil controls. In each case, the difference in 5-HT varicosity number between AAS- and oil-treated animals was significantly significant [MeA, $t(8) = -3.88$; CeA, $t(9) = -4.26$, $P < .01$ each].

Not every brain region that has been implicated in aggressive behavior showed significant changes in 5-HT afferent innervation following adolescent AAS exposure. For instance, similar numbers of 5-HT-ir varicosities and fibers were found in the medial division of the BNST, the

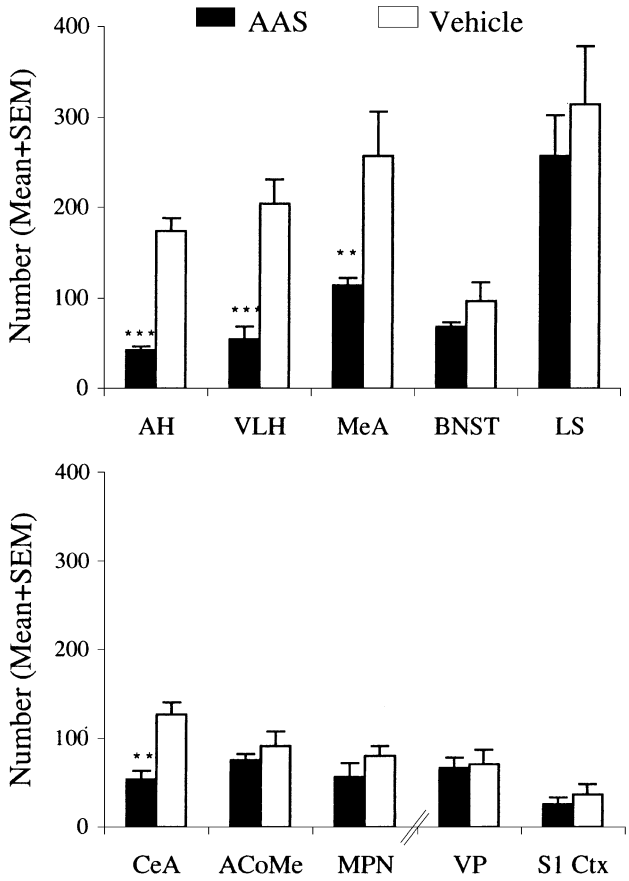

Fig. 5. Density of 5-HT-ir varicosities and fibers in brains of AAS- vs. vehicle-treated hamsters. Numbers were normalized to a standard area (100×100 μm) for regional comparisons. $***P < .001$, $**P < .01$, Student's t test, two-tailed.

CoMeA, the intermediate part of the LS and the MPN of both AAS-treated and control animals. These counts were not significantly different between treatment groups ($P>.1$ each comparison). Similarly, no significant differences were found in several brain areas not involved in aggressive behavior in the hamster. For example, the number of 5-HT-ir varicosities and fibers were not significantly different between treatment groups in the VP and S1 cortex ($P>.1$ each comparison).

4. Discussion

In previous studies, we have shown that repeated high-dose AAS treatment throughout adolescence specifically increased offensive aggression in male Syrian hamsters (Melloni and Ferris, 1996; Melloni et al., 1997; Harrison et al., 2000). To determine whether adolescent AAS-facilitated aggression was modulated by 5-HT activity, we examined the behavioral effects of increasing extracellular 5-HT in aggressive, adolescent AAS-treated hamsters. For these studies, aggressive AAS-treated animals were administered saline or fluoxetine, a selective 5-HT reuptake inhibitor, prior to the aggression test. The administration of saline to AAS-treated hamsters had no effect on aggression, as animals showed high levels of offensive attack analogous to that observed in our previous studies (Melloni et al., 1997; Harrison et al., 2000) and presented here (see Fig. 3). Conversely, fluoxetine treatment resulted in a marked reduction of adolescent AAS-facilitated attack. These animals showed a nearly 80% reduction in the number of attacks as well as a greater than two-fold increase in attack latency during the test period compared to saline-treated controls. Conversely, there was no difference in contact time between saline- and fluoxetine-treated animals, indicating that animals in both groups were equally interested in intruders. Together, these behavioral data are important and novel in that they indicate that 5-HT activity in brain plays a role inhibiting the aggressive phenotype that arises in response to adolescent AAS exposure and that enhanced 5-HT activity does not block AAS-facilitated offensive attack through a nonspecific behavioral inhibition.

In Syrian hamsters, 5-HT activity in the VLH and AH has been shown previously to inhibit offensive aggression (Delville et al., 1996; Ferris, 1996; Ferris and Delville, 1994; Ferris et al., 1997, 1999). Since experimental manipulations that enhance 5-HT activity reduce adolescent AAS-facilitated attack, perhaps AAS exposure during this time period predisposes animals to respond aggressively by decreasing the extent to which 5-HT neurons innervate synaptic partners in these brain areas, functionally disinhibiting the neural circuits activating offensive attack. To determine whether adolescent AAS exposure depressed 5-HT neural development in these brain regions, we quantified 5-HT afferent innervation in AAS- and oil-treated hamsters. Animals exposed to AAS during adolescence had two-fold less 5-HT-ir varicosities and fibers within both the VLH and AH, suggesting a decreased afferent innervation and/or depletion of VLH−5-HT and AH−5-HT in response to adolescent AAS. From a functional standpoint, this decrease in serotonergic tone may activate the VLH and AH neural circuit implicated in the aggressive response. In hamsters, activity of the arginine vasopressin (AVP) neural system in the VLH and AH facilitates offensive aggression (Ferris and Delville, 1994; Ferris and Potegal, 1988; Ferris et al., 1989, 1997; Potegal and Ferris, 1989). Experimental manipulations that increase 5-HT in the VLH and AH effectively block AVP-facilitated aggression implicating a functional relationship between these neurochemical signals in the regulation of aggression (Delville et al., 1996; Ferris, 1996; Ferris et al., 1997, 1999). It is plausible that AVP-ergic cells in the VLH and AH receive a diminished inhibitory 5-HT input as a result of adolescent AAS exposure, resulting in the activation of AVP neural circuits in these areas and the intensification of the aggressive response in these animals. There is support for this notion from our previous studies showing that aggressive, AAS-treated hamsters possess increased AVP innervation and levels in the AH (Harrison et al., 2000) and AVP $V1_A$ receptor binding in the VLH (DeLeon et al., 2002b). Thus, adolescent AAS exposure appears to disrupt the signaling equilibrium between hypothalamic 5-HT and AVP, likely contributing to the development of the aggressive phenotype in these animals.

In hamsters, activity of neurons in the MeA and CeA has also been implicated in the aggressive response. Our results show that animals exposed to high-dose AAS during adolescence display marked decreases in 5-HT-ir varicosities and fibers in the MeA and CeA. Neurons in the MeA regulate aggressive response patterns in rats, mice, prairie voles and hamsters (Bunnell et al., 1970; Shibata et al., 1982; Vochteloo and Koolhaas, 1987; Koolhaas et al., 1990, 1991; Albert et al., 1992; Kollack-Walker and Newman, 1995; Wang et al., 1997; Delville et al., 2000; Gammie and Nelson, 2001), and increased neuronal activation has been observed in this brain region in aggressive, experienced fighter hamsters (Delville et al., 2000). It is possible that the decrease in 5-HT signaling in this brain area following adolescent AAS exposure functionally disinhibits neurons in the MeA, facilitating aggression. The CeA has been implicated in defensive aggression in cats (Zagrodzka et al., 1998), physical aggression in rats (Bedard and Persinger, 1995) and scent marking in hamsters (Bamshad et al., 1997). Although previous reports have not shown the CeA to be directly involved in offensive aggression in hamsters, this brain region is known to be a component of the neural circuit controlling flank marking in Syrian hamsters, a motor behavior that is part of the response pattern of offensive aggression that is influenced by testosterone (Bamshad et al., 1997). It is possible that 5-HT signaling here functions to inhibit the activation of cells critical to this response. Reduced 5-HT innervation to the CeA resulting from adolescent AAS exposure may cause

the functional activation of neurons in this region, increasing flank marking. Indeed, our recent studies showing that hamsters exposed to AAS during adolescence display increased flank marking behavior support this hypothesis (DeLeon et al., 2002b). Taken together, these data above are novel and significant in that they show that exposure to high-dose AAS during adolescence can alter the developmental patterns of innervation of 5-HT afferents to areas of the brain which have been implicated in aggression and social communication in hamsters. From a neuroanatomical standpoint, these data implicate reduced 5-HT neural signaling in these key areas as potential neural substrates for adolescent AAS-facilitated aggression.

To determine whether exposure to high-dose AAS during adolescence altered 5-HT anatomical development in non-aggression regions of the brain, we examined the 5-HT afferent innervation to the VP and S1 neocortex. Our data show no differences in 5-HT innervation between AAS- and oil-treated hamsters in either of these brain regions. Together, these data suggest that there is a nonuniform effect of adolescent AAS exposure on 5-HT development across the neuraxis.

In summary, the studies presented in this paper provide data examining the neurobehavioral effects of chronic high-dose AAS exposure during adolescent development on aggression and the basic neurobiological mechanisms by which these psychopharmacological agents may exert their aggression-stimulating effects. These findings indicate that increases in offensive attack resulting from adolescent AAS treatment are modulated, at least in part, by 5-HT and that AAS exposure during this developmental period produces marked reductions in 5-HT afferent innervation to several areas of the hamster brain implicated in aggressive behavior. These findings provide a link between adolescent AAS, 5-HT and the stimulation of aggression, indicating a role of reduced 5-HT innervation and function in AAS-facilitated aggression in adolescent hamsters.

Acknowledgments

The authors would like to acknowledge Dr. Mark S. Todtenkopf for assistance with image analyses and for critical reading of the manuscript. R.H.M. would like to extend special thanks to Dr. K.A. Melloni for support and encouragement. This publication was made possible by grant number 10547 from NIDA to R.H.M. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIDA.

References

Albert DJ, Jonik RH, Walsh ML. Hormone-dependent aggression in male and female rats: experiential, hormonal, and neural foundations. Neurosci Biobehav Rev 1992;16:177–92.

Bamshad M, Karom M, Pallier P, Albers HE. Role of the central amygdala in social communication in Syrian hamsters (*Mesocricetus auratus*). Brain Res 1997;744:15–22.

Bedard AW, Persinger MA. Prednisolone blocks extreme intermale social aggression in seizure-induced, brain-damaged rats: implications for the amygdaloid central nucleus, corticotrophin-releasing factor, and electrical seizures. Psychol Rep 1995;77:3–9.

Borisova NA, Proshlyakova EV, Sapronova AY, Ugrumov MV. Androgen-dependent sex differences in the hypothalamic serotoninergic system. Eur J Endocrinol 1996;134:232–5.

Buckley WE, Yesalis CE, Friedl KE, Anderson WA, Streit AL, Wright JE. Estimated prevalence of anabolic steroid use among male high school seniors. JAMA 1988;260:3441–5.

Bunnell BN, Sodetz FJ, Shalloway DI. Amygdaloid lesions and social behavior in the golden hamster. Physiol Behav 1970;5:153–61.

Coccaro EF, Kavoussi RJ, Hauger RL. Serotonin function and antiaggressive response to fluoxetine: a pilot study. Biol Psychiatry 1997;42:546–52.

DeLeon KR, Grimes JM, Melloni RH. Adolescent cocaine exposure and offensive aggression: involvement of serotonin signaling and innervation in male Syrian hamsters. Behav Brain Res 2002a;133:211–20.

DeLeon KR, Grimes JM, Melloni RH. Repeated anabolic–androgenic steroid treatment during adolescence increases vasopressin v1a receptor binding in Syrian hamsters: correlation with offensive aggression. Horm Behav 2002b (in press).

Delville Y, Mansour KM, Ferris CF. Serotonin blocks vasopressin-facilitated offensive aggression: interactions within the ventrolateral hypothalamus of golden hamsters. Physiol Behav 1996;59:813–6.

Delville Y, De Vries GJ, Ferris CF. Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. Brain Behav Evol 2000;55:53–76.

Dukarm CP, Byrd RS, Auinger P, Weitzman M. Illicit substance use, gender, and the risk of violent behavior among adolescents. Arch Pediatr Adolesc Med 1996;150:797–801.

Ferris CF. Serotonin diminishes aggression by suppressing the activity of the vasopressin system. Ann NY Acad Sci 1996;794:98–103.

Ferris CF, Delville Y. Vasopressin and serotonin interactions in the control of agonistic behavior. Psychoneuroendocrinology 1994;19:593–601.

Ferris CF, Potegal M. Vasopressin receptor blockade in the anterior hypothalamus suppresses aggression in hamsters. Physiol Behav 1988;44:235–9.

Ferris CF, Axelson JF, Martin AM, Roberge LF. Vasopressin immunoreactivity in the anterior hypothalamus is altered during the establishment of dominant/subordinate relationships between hamsters. Neuroscience 1989;29:675–83.

Ferris CF, Melloni RH, Koppel G, Perry KW, Fuller RW, Delville Y. Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. J Neurosci 1997;17:4331–40.

Ferris CF, Stolberg T, Delville Y. Serotonin regulation of aggressive behavior in male golden hamsters (*Mesocricetus auratus*). Behav Neurosci 1999;113:804–15.

Floody OR, Pfaff DW. Aggressive behavior in female hamsters: the hormonal basis for fluctuations in female aggressiveness correlated with estrous state. J Comp Physiol Psychol 1977;91:443–64.

Gammie SC, Nelson RJ. cFOS and pCREB activation and maternal aggression in mice. Brain Res 2001;898:232–41.

Gonzalez MI, Leret ML. Extrahypothalamic serotonergic modification after masculinization induced by neonatal gonadal hormones. Pharmacol, Biochem Behav 1992;41:329–32.

Hammond MA, Rowe FA. Medial preoptic and anterior hypothalamic lesions: influences on aggressive behavior in female hamsters. Physiol Behav 1976;17:507–13.

Harrison RJ, Connor DF, Nowak C, Nash K, Melloni RH. Chronic anabolic–androgenic steroid treatment during adolescence increases anterior hypothalamic vasopressin and aggression in intact hamsters. Psychoneuroendocrinology 2000;25:317–38.

Higley JD, Mehlman PT, Poland RE, Taub DM, Vickers J, Suomi SJ, Linnoila M. CSF testosterone and 5-HIAA correlate with different types of aggressive behaviors. Biol Psychiatry 1996;40:1067–82.

Kollack-Walker S, Newman SW. Mating and agonistic behavior produce

different patterns of Fos immunolabeling in the male Syrian hamster brain. Neuroscience 1995;66:721–36.
Koolhaas JM, Van den Brink THC, Roozendal B, Boorsma F. Medial amygdala and aggressive behavior: Interaction between testosterone and vasopressin. Aggress Behav 1990;16:223–9.
Koolhaas JM, Moor E, Hiemstra Y, Bohus B. The testosterone-dependent vasopressinergic neurons in the medial amygdala and lateral septum: involvement in social behavior of male rats. In: Jard S, Jamison R, editors. Vasopressin. Paris: John Libbey Eurotext, 1991. p. 213–9.
Kruesi MJ, Rapoport JL, Hamburger S, Hibbs E, Potter WZ, Lenane M, Brown GL. Cerebrospinal fluid monoamine metabolites, aggression, and impulsivity in disruptive behavior disorders of children and adolescents. Arch Gen Psychiatry 1990;47:419–26.
Ladosky W, Gaziri LC. Brain serotonin and sexual differentiation of the nervous system. Neuroendocrinology 1970;6:168–74.
Lerwill CJ, Makaings P. The agonistic behavior of the golden hamster. Anim Behav 1971;19:714–21.
Linnoila M, Virkkunen M, Scheinin M, Nuutila A, Rimon R, Goodwin FK. Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. Life Sci 1983;33:2609–14.
Loeber R, Hay D. Key issues in the development of aggression and violence from childhood to early adulthood. Annu Rev Psychol 1997;48:371–410.
Long JB, Youngblood WW, Kizer JS. Effects of castration and adrenalectomy on in vitro rates of tryptophan hydroxylation and levels of serotonin in microdissected brain nuclei of adult male rats. Brain Res 1983;277:289–97.
Melloni RH, Ferris CF. Adolescent anabolic steroid use and aggressive behavior in golden hamsters. Ann NY Acad Sci 1996;794:372–5.
Melloni RH, Connor DF, Hang PT, Harrison RJ, Ferris CF. Anabolic–androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters. Physiol Behav 1997;61:359–64.
Miller LL, Whitsett JM, Vandenbergh JG, Colby DR. Physical and behavioral aspects of sexual maturation in male golden hamsters. J Comp Physiol Psychol 1977;91:245–59.
Morin LP, Wood RI. A stereotaxic atlas of the golden hamster brain. San Diego: Academic Press, 2001.
NIDACapsules, Available at: http//www.nida.nih.gov/NIDACapsules/NCIndex.html, 2001.
Pope HG, Katz DL. Affective and psychotic symptoms associated with anabolic steroid use. Am J Psychiatry 1988;145:487–90.
Pope HG, Katz DL. Psychiatric and medical effects of anabolic–androgenic steroid use. A controlled study of 160 athletes. Arch Gen Psychiatry 1994;51:375–82.
Potegal M, Ferris C. Intraspecific aggression in male hamsters in inhibited by intrahypothalamic vasopressin receptor antagonists. Aggress Behav 1989;15:311–20.
Potegal M, Blau A, Glusman M. Effects of anteroventral septal lesions on intraspecific aggression in male hamsters. Physiol Behav 1981a;26:407–12.
Potegal M, Blau A, Glusman M. Inhibition of intraspecific aggression in male hamsters by septal stimulation. Physiol Psychol 1981b;9:213–8.
Potegal M, Ferris CF, Hebert M, Meyerhoff J, Skaredoff L. Attack priming in female Syrian golden hamsters is associated with a c-*fos*-coupled process within the corticomedial amygdala. Neuroscience 1996;75:869–80.
Renner KJ, Biegon A, Luine VN. Sex differences in long-term gonadectomized rats: monoamine levels and [^{3}H]nitroimipramine binding in brain nuclei. Exp Brain Res 1985;58:198–201.
Schoenfeld TA, Leonard CM. Behavioral development in the Syrian golden hamster. In: Siegel HI, editor. The hamster: reproduction and behavior. New York: Plenum, 1985. p. 289–318.
Shibata S, Yamamoto TY, Ueki S. Differential effects of medial, central and basolateral amygdaloid lesions on four models of experimentally-induced aggression in rats. Physiol Behav 1982;28:289–94.
Sijbesma H, Schipper J, De Kloet ER. The anti-aggressive drug eltoprazine preferentially binds to 5-HT1A and 5-HT1B receptor subtypes in rat brain: sensitivity to guanine nucleotides. Eur J Pharmacol 1990;187:209–23.
Simerly RB, Swanson LW, Gorski RA. Demonstration of a sexual dimorphism in the distribution of serotonin-immunoreactive fibers in the medial preoptic nucleus of the rat. J Comp Neurol 1984;225:151–66.
Simerly RB, Swanson LW, Gorski RA. Reversal of the sexually dimorphic distribution of serotonin-immunoreactive fibers in the medial preoptic nucleus by treatment with perinatal androgen. Brain Res 1985;340:91–8.
Sodetz FJ, Bunnell BN. Septal ablation and the social behavior of the golden hamster. Physiol Behav 1970;5:79–88.
Sundblad C, Eriksson E. Reduced extracellular levels of serotonin in the amygdala of androgenized female rats. Eur Neuropsychopharmacol 1997;7:253–9.
Thiblin I, Finn A, Ross SB, Stenfors C. Increased dopaminergic and 5-hydroxytryptaminergic activities in male rat brain following long-term treatment with anabolic androgenic steroids. Br J Pharmacol 1999;126:1301–6.
Vochteloo JD, Koolhaas JM. Medial amygdala lesions in male rats reduce aggressive behavior: interference with experience. Physiol Behav 1987;41:99–102.
Wang Z, Hulihan TJ, Insel TR. Sexual and social experience is associated with different patterns of behavior and neural activation in male prairie voles. Brain Res 1997;767:321–32.
Whitsett JM. The development of aggressive and marking behavior in intact and castrated male hamsters. Horm Behav 1975;6:47–57.
Wilson CA, Gonzalez I, Farabollini F. Behavioural effects in adulthood of neonatal manipulation of brain serotonin levels in normal and androgenized females. Pharmacol, Biochem Behav 1992;41:91–8.
Yesalis C, Herrick R, Buckley W, Friedl KE, Brannon D, Wright JE. Estimated incidence of anabolic steroids use among elite powerlifters. Phys Sportsmed 1988;16:91–108.
Yesalis CE, Kennedy NJ, Kopstein AN, Bahrke MS. Anabolic–androgenic steroid use in the United States. JAMA 1993;270:1217–21.
Yesalis CE, Barsukiewicz CK, Kopstein AN, Bahrke MS. Trends in anabolic–androgenic steroid use among adolescents. Arch Pediatr Adolesc Med 1997;151:1197–206.
Zagrodzka J, Hedberg CE, Mann GL, Morrison AR. Contrasting expressions of aggressive behavior released by lesions of the central nucleus of the amygdala during wakefulness and rapid eye movement sleep without atonia in cats. Behav Neurosci 1998;112:589–602.
Zeisel SH, Mauron C, Watkins CJ, Wurtman RJ. Developmental changes in brain indoles, serum tryptophan and other serum neutral amino acids in the rat. Brain Res 1981;227:551–64.