ELSEVIER

Behavioural Brain Research 133 (2002) 211–220

BEHAVIOURAL BRAIN RESEARCH

www.elsevier.com/locate/bbr

Research Report

Adolescent cocaine exposure and offensive aggression: involvement of serotonin neural signaling and innervation in male Syrian hamsters

Katrina R. DeLeon [a], Jill M. Grimes [a], Daniel F. Connor [b], Richard H. Melloni, Jr. [a,*]

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

[b] *Department of Psychiatry, The University of Massachusetts Medical Center, 55 Lake Avenue, North, Worcester, MA 01655, USA*

Received 27 August 2001; received in revised form 15 December 2001; accepted 15 December 2001

Abstract

Repeated low-dose cocaine treatment (0.5 mg/kg/day) during adolescence facilitates offensive aggression in male Syrian hamsters (*Mesocricetus auratus*). The current study assessed whether adolescent cocaine-facilitated offensive aggression was inhibited by increased serotonin activity and if cocaine exposure during this developmental period influenced serotonin development in the primary aggression areas of hamster brain. In a first experiment, hamsters were treated with low doses of cocaine throughout adolescence and then scored for offensive aggression following the systemic administration of vehicle or fluoxetine, a selective serotonin reuptake inhibitor. Vehicle-treated hamsters showed high levels of offensive aggression, while treatment with fluoxetine inhibited the cocaine-facilitated aggressive response. Only one out of ten fluoxetine-treated animals both attacked and bit intruders, compared to nine out of ten saline-treated animals. In a second experiment, hamsters were administered low doses of cocaine or saline throughout adolescence, tested for offensive aggression, and then examined for differences in serotonin afferent innervation to regions of the hamster brain implicated in aggressive responding. Aggressive cocaine-treated hamsters showed significant reductions (35–50%) in the number of serotonin immunoreactive varicosities and fibers in several aggression areas, including the anterior hypothalamus, lateral septum, medial amygdala, and bed nucleus of the stria terminalis. Together, these results support a role for serotonin innervation and function in adolescent cocaine-facilitated offensive aggression.

Keywords: Adolescence; Cocaine; Serotonin; Development; Fluoxetine; Aggression

1. Introduction

Studies from the National Institute on Drug Abuse estimate that greater than 22 million Americans have experimented with cocaine, with about 4 million having used the drug during the past year [47]. Of particular interest are reports that cocaine abuse has risen and remains high in the *adolescent* population, with 4.7% of 8th graders reporting use in 1999, up from 4.4% in 1997, 3.5% in 1994 and 2.3% in 1991 [47]. It has been reported that younger youth, beginning at age 12–13 experiment with cocaine [19,33,72]. Increasing trends also exist for high school seniors, with 9.8% reporting use in 1999, as compared with 8.7% in 1997 and 5.9% in 1994 [47]. This early onset of drug use is associated with increased and chronic drug use into adulthood; thus this population of adolescent users may comprise a significant portion of the stable long-term cocaine abusing population [11,33,53].

Clinical studies have demonstrated a positive correlation between the long-term use of cocaine and negative behavioral effects in adolescents [9,17,19,54,61], including increased irritability and aggression. However, whether cocaine exposure can elicit similar behavioral changes in adolescent animal models has only recently been studied. Indeed, behavioral data from our laboratory indicate that Syrian hamsters treated with low doses of cocaine throughout adolescent development display elevated offensive aggression when tested immediately following the treatment period [30]. In these studies hamsters treated with cocaine were greater than

* Corresponding author. Tel.: +1-617-373-3043; fax: +1-617-373-8714

E-mail address: melloni@research.neu.edu (R.H. Melloni, Jr.).

0166-4328/02/$ - see front matter
PII: S0166-4328(02)00004-9

six times more likely and five times faster to attack and bite intruders placed in their home cage. Conversely, these animals show no changes in social communication, sexual motivation or motor activity. The finding that cocaine-treated adolescents displayed heightened offensive aggression on the first behavioral interaction, in the absence of established social interactions and cues, suggested that cocaine exposure during this developmental period may stimulate aggression directly; perhaps by impacting the development and function of specific neural circuits that regulate this behavior.

Serotonin (5-HT) has been implicated in the control of aggression in adolescent and adult human populations [7,14,37,43] and in a number of animal models of aggression [31,38,58,68]. In Syrian hamsters, 5-HT activity in the anterior hypothalamus (AH) and ventrolateral hypothalamus (VLH) has been shown to regulate offensive aggression [16,20,23,25], where 5-HT acts to inhibit aggression. In addition, the activity of neurons located in several other brain sites has been implicated in aggressive responding in hamsters. For instance, offensive aggression is inhibited by electrical stimulation of neurons in the lateral septum (LS) [49] while it is activated by stimulation of cells located in the cortico-medial amygdala (CoMeA) [51]. Similarly, neurons in the CoMeA [51], the medial amygdala (MeA) [15] and the bed nucleus of the stria terminalis (BNST) [15,34] are activated during an aggressive encounter with other hamsters. Perhaps exposure to cocaine during adolescence stimulates aggression in hamsters by disrupting the development of 5-HT afferent fibers into these brain areas. Indeed, the development of the 5-HT neural system continues through adolescence [73] during which it displays remarkable plasticity in response to circulating neurochemical signals [6,39]. In mature animals, acute cocaine treatment increases 5-HT, enhancing 5-HT signaling in brain [2,18,27,29,32,59,62], while chronic cocaine exposure lowers 5-HT levels, disrupting 5-HT signaling [2,18,27,29,32,59,62]. It is possible that chronic cocaine exposure during adolescent development facilitates offensive aggression by a similar mechanism, i.e. by lowering 5-HT activity in areas implicated in aggressive responding, thus reducing 5-HT's inhibitory influence on aggression. To date however, it is unknown whether chronic cocaine exposure during adolescence has any deleterious effects on the development/function of the 5-HT neural system, and/or whether 5-HT signaling plays a significant role in adolescent cocaine-facilitated aggression. This information is important given continued reports that cocaine use during this developmental window is rising [47] and can be associated with an increased incidence of aggression and violence [8,9,17,44,45,65].

The present studies were conducted to establish a direct link between adolescent cocaine exposure, 5-HT neural signaling and afferent innervation, and offensive aggression using the sub-adult Syrian hamster as an animal model. First, to determine whether 5-HT signaling played a significant role in adolescent cocaine-facilitated aggression we tested whether fluoxetine, a selective serotonin reuptake inhibitor shown previously to elevate 5-HT levels in the hamster brain [20], could inhibit offensive aggression facilitated by adolescent cocaine exposure. Then, to establish whether adolescent cocaine exposure altered the 5-HT afferent innervation to areas of the hamster brain implicated in aggressive responding, we employed immunohistochemistry to visualize and quantify 5-HT varicosities and fibers.

2. Methods

2.1. Animals and experimental treatment

In Syrian hamsters (*Mesocricetus auratus*), the adolescent period of development can be identified as the time between postnatal days 25 and 56 (P25–P56). Weaning generally occurs around P25 with the onset of puberty beginning around P40 [46]. During this developmental time period, hamsters wean from their dams, leave the home nest, establish new solitary nest sites, and learn to defend their territory and participate in social dominance hierarchies [56,71].

In Experiment 1, intact adolescent male hamsters (P25) were obtained from Harlan Sprague–Dawley Labs (Indianapolis, IN), individually housed in Plexiglas cages, and maintained at ambient temperature on a reverse light:dark cycle of (14L:10D; lights on at 19:00). Food and water were provided ad libitum. On P27, animals ($n = 20$ total, i.e. two groups of $n = 10$) received intraperitoneal (IP) injections of cocaine hydrochloride at a concentration of 0.5 mg/kg (Sigma Chemical Co., St. Louis, MO) suspended in isotonic saline, daily for 28 consecutive days (P27–56). This daily dose of cocaine has been shown previously to facilitate aggressive responding in adolescent hamsters [30]. The day following the last injection animals were tested for offensive aggression after one of two different treatments: (1) adolescent cocaine pretreatment in the presence of IP saline administration ($n = 10$) or (2) adolescent cocaine pretreatment in the presence of IP fluoxetine hydrochloride administration ($n = 10$). On the day of testing, cocaine-treated animals were injected with either fluoxetine (20 mg/kg in 0.9% NaCl) or saline vehicle (1 ml/kg) as described in Refs. [16,23]. All injections were performed on unanesthetized animals and took no longer than 10 s. Administration of fluoxetine at this dose has been shown to be very selective for its anti-aggressive properties, with no generalized effects observed on social or sex behavior [23]. After injection, animals were returned to their home cage. One-hour later animals were tested for offensive aggression.

In Experiment 2, P27 hamsters were weighed and randomly distributed into two groups ($n = 6$ animals each). One group of animals received 0.5 mg/kg/day cocaine hydrochloride (Sigma Chemical Co.) in isotonic saline as above, while a second group of hamsters was injected with saline (1 ml/kg) alone. Following the treatment period, animals in both cocaine and saline groups were tested for offensive aggression, sacrificed, and the brains removed and processed for immunohistochemistry as detailed below.

2.2. Aggression testing

Animals were tested for aggressive behavior using the resident/intruder paradigm, a well-characterized and ethologically valid model of offensive aggression in golden hamsters [26,40]. For this measure, a stimulus (intruder) male of similar size and weight was introduced into the home cage of experimental animals and the resident was scored for offensive aggression (i.e. number of attacks, bites, and latency to first attack and bite towards an intruder). In addition, residents were also measured for social interest towards intruders (i.e. total contact time between resident and intruder) to control for nonspecific effects of fluoxetine on animal behavior. Each aggression test lasted for 10 min and was scored by an independent observer uninformed as to the experimental treatment. No stimulus animal was used for more than one behavioral test, and all tests were performed during the first 4 h of the dark phase under dim red illumination and videotaped for behavioral verification of the findings.

2.3. Immunohistochemistry

For immunohistochemical analysis, brains from animals in cocaine- and saline-treatment groups ($n = 6$ per group) were fixed by transcardial perfusion with 4% paraformaldehyde and then incubated in 30% sucrose/phosphate buffered saline (PBS, pH 7.4) overnight at 4 °C for cryoprotection. 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 [23]. Briefly, free floating sections were pretreated with 1% sodium borohydride followed by pre-incubation 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 anti-rabbit (Protos Biotech, Ridgefield, NJ) with 2% NGS and 0.3% Triton X-100 over two nights at 4 °C. After primary incubation, sections were incubated in secondary anti-rabbit followed by tertiary antisera (Vectastain ABC Elite Kit – rabbit, Burlingame, CA) and then labeled with diaminobenzidine (DAB, Vector Labs, Burlingame, CA). 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).

2.4. 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 [63,64]. The areas analyzed were selected based on data from previous studies implicating these regions in aggressive responding in numerous species and models of aggression, with the notable exception of the caudate putamen ((CPu), i.e. the striatum), a non-aggression area used as a control region. These areas (Fig. 1) included the cingulate cortex (Cg), the intermediate part of the lateral septal nucleus (LS), the medial division of the BNST, medial preoptic nucleus (MPN), the AH, the central amygdaloid nucleus (CeA), the cortico-medial amygdaloid nucleus (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) was utilized to identify the brain region of interest (ROI) at low power (4 ×) using a Nikon E600 microscope. At this magnification, a standard computer-generated parcel was drawn to outline the entire ROI. Each brain region was assigned a separate and distinct ROI parcel, formatted in size specifically for that brain area. Then, under 20 × magnification images were thresholded at a standard RGB-scale level empirically determined by observers unaware of the 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 ROI were quantified. Two to six independent measurements were taken from several consecutive sections of each animal ($n = 5–6$) per treatment group. Then, the number of 5-HT-ir varicosities and fibers was determined for each ROI and standardized per 100 × 100 μm^2 parcel for regional comparison purposes. The number of 5-HT-ir

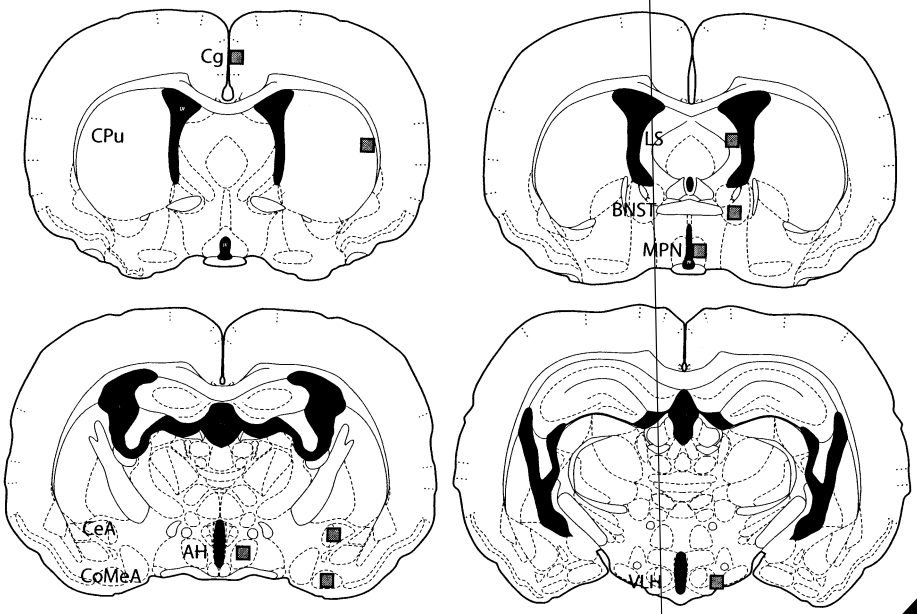


Fig. 1. Diagrams showing the location of the areas selected to quantify 5-HT-ir varicosities and fibers (shaded areas). Di
hamster atlas of Morin and Wood (2001). Abbreviations: AH, anterior hypothalamus; BNST, bed nucleus of the
cortex; CeA, central amygdaloid nucleus; CoMeA, cortico-medial amygdaloid nucleus; CPu, caudate putamen; L
medial preoptic nucleus, VLH, ventrolateral hypothalamus.

varicosities and fibers was then averaged for each brain region per animal and used for statistical analysis.

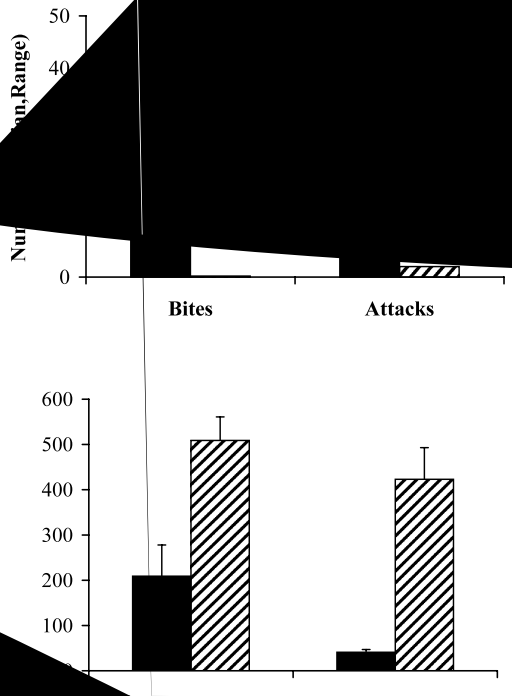
2.5. Statistics

2.5.1. Behavioral studies

The results from the aggression
between fluoxetine- and salin
Experiment 1 and between c
groups in Experimen
number of
Whitney
to first
compare

2.5.2. 5-H

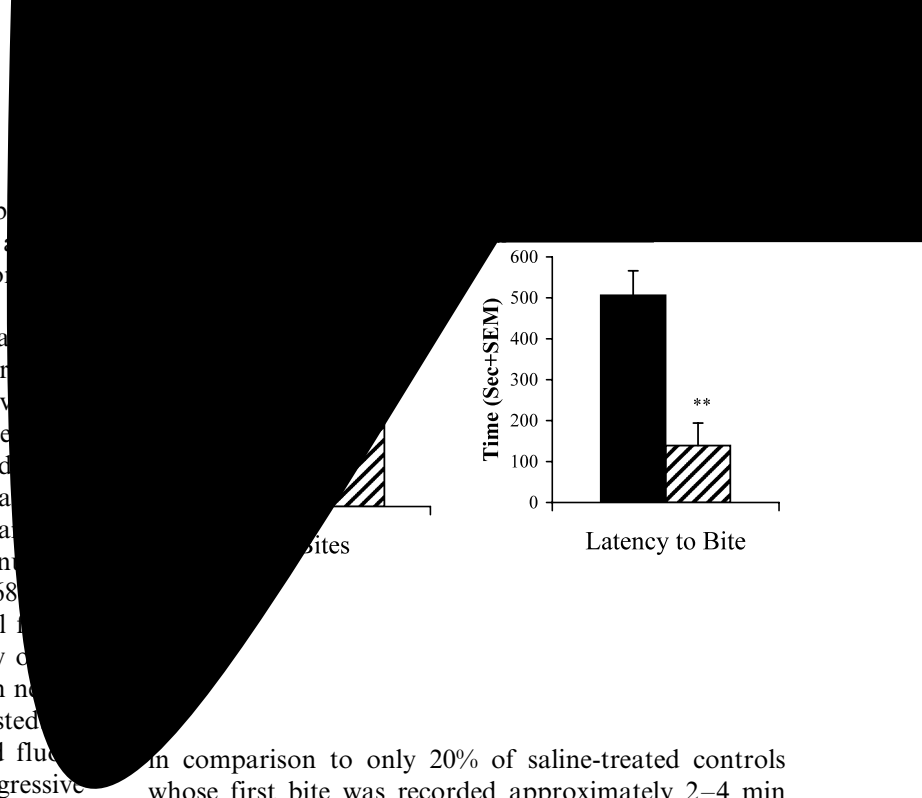
The nu
ed b
area analy



—*serotonin and offensive aggression*

administration of the selective serotonin
nhibitor fluoxetine diminished the aggressive
e of adolescent cocaine-treated animals. As
n in Fig. 2A, hamsters treated with low-dose
caine throughout adolescence showed high levels of

offensive aggression when administered saline p
the aggression test. Indeed, the intensity of the a
sive encounter (i.e. number of attacks and/o
during the test period) was extremely high
attacks, 7 bites) in nearly all (9 out of 10) of the a
tested. Only one animal administered saline prior
aggression test failed to bite intruders. Conv
cocaine-treated animals administered fluoxetine
to the behavioral test period showed a marked red
in the intensity of offensive aggression. Compa
saline-treated controls, fluoxetine-treated ha
showed a statistically significant decrease in the n
of attacks ($Z = 3.25$, $P < 0.01$) and bites ($Z = 2.68$
0.01) during aggression tests. In fact, peripheral
etine treatment completely blocked the intensity o
aggressive encounter (0 attacks and/or 0 bites) in n
three-quarters (7 out of 10) of the animals tested
addition, cocaine-treated hamsters administered flu
etine also displayed a significantly slower aggressive
response towards intruders [latency to attack and bite, $t(18) = -5.17$, $P < 0.0001$ and $t(18) = -3.43$, $P < 0.01$, respectively] than saline-treated animals (Fig. 2B). Indeed, the initiation of the aggressive response was nearly immediate in most all cocaine-treated hamsters administered saline, with nine out of ten of the animals attacking within the first 60 s of the behavioral test. By comparison, only one animal administered fluoxetine prior to the aggression test (10% of the treatment group) attacked within the first minute of testing. The majority of fluoxetine-treated animals (6 out of 10) attacked approximately 6 min later, toward the end of the test period. Finally, although fluoxetine-treated animals showed marked reductions in offensive aggression, the duration of physical contact was similar in fluoxetine- (446.5 ± 37 s) and saline- (447.3 ± 32 s) treated residents ($t(18) = 0.017$, $P > 0.1$).



3.2. Experiment 2—offensive aggression and serotonin afferent innervation

As observed previously [30], animals treated with cocaine during their adolescent development showed significantly heightened measures of offensive aggression (Fig. 3). Specifically, hamsters treated with low-dose cocaine showed a significant increase in the total number of bites ($Z = 2.96$, $P < 0.01$) over saline-treated littermates. The majority of cocaine-treated animals (4 out of 6) scored 10 bites or more during the aggression test. By comparison, the majority of saline-treated hamsters (4 out of 6) failed to score a single bite on opponents. In addition, cocaine-treated hamsters also displayed a significantly quicker aggressive response towards intruders (latency to bite, $t(10) = -4.85$, $P < 0.01$) than saline-treated control animals. Approximately 80% of the cocaine-treated animals (4 out of 6) responded within the first 140 s of the 10 min test period, in comparison to only 20% of saline-treated controls whose first bite was recorded approximately 2–4 min later, toward the middle of the test period.

In aggressive, cocaine-treated hamsters, the immunohistochemical staining pattern for 5-HT was altered in several brain regions implicated in the aggressive response in hamsters. For instance, in saline-treated animals, the staining of 5-HT varicosities and fibers 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. 4A). By comparison, aggressive, cocaine-treated animals displayed a less dense pattern of staining for 5-HT-ir varicosities and fibers in the AH brain region (Fig. 4B). Analysis of 5-HT innervation to this brain region showed that cocaine-treated animals had approximately 50% of the 5-HT-ir varicosities and fibers of saline-treated littermates (Fig. 5). This difference was statistically significant ($t(11) = 6.47$, $P < 0.001$). These findings were not restricted to the AH, however, as other areas of the hamster brain implicated in aggression showed similar decreases in 5-HT innervation following adolescent cocaine exposure (Fig. 5). For example, the number of 5-HT-ir varicosities and fibers in the LS of aggressive, cocaine-treated animals was less than 50% that of saline controls, while the BNST and MeA each showed a nearly 35% decrease in the number of 5-HT-ir varicosities and fibers in cocaine-treated animals. In each case the difference in 5-HT varicosity and fiber number between cocaine- and saline-treated animals was statistically significant (LS, $t(28) = 2.79$; BNST, $t(18) = 2.93$; MeA, $t(18) = 3.46$; $P < 0.01$ each comparison).

Not every brain area implicated in the aggressive response showed significant changes in 5-HT afferent innervation following adolescent cocaine exposure. For example, although a slight reduction in 5-HT innervation was observed in the CoMeA of cocaine-treated animals compared to saline-treated controls, this com-Fig.

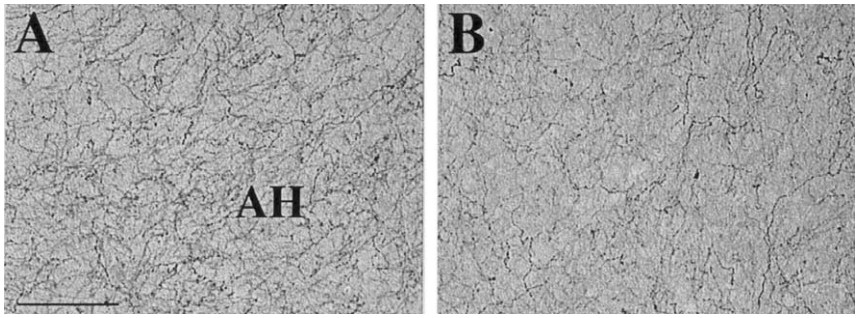


Fig. 4. Brightfield photomicrographs showing immunoreactive labeling of 5-HT varicosities and fibers in the AH of (A) saline- and (B) cocaine-treated hamsters. Bar, 20 μm.

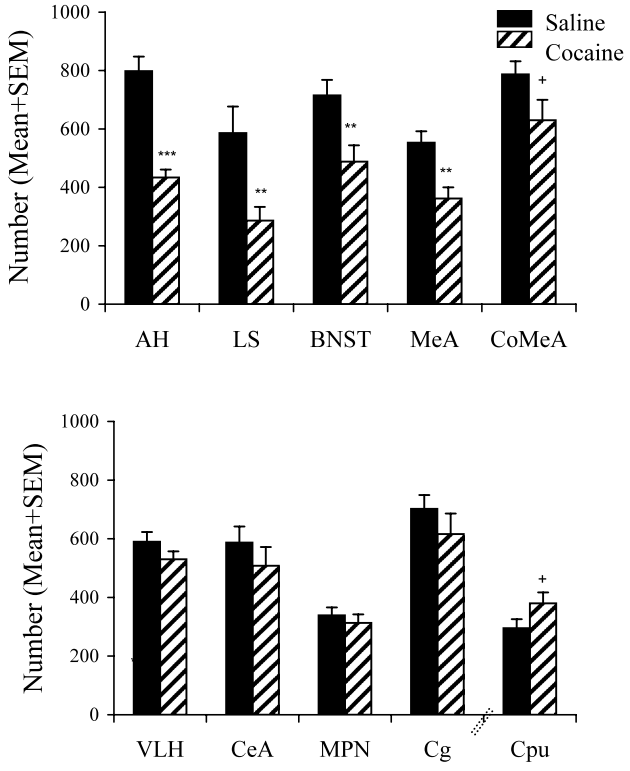


Fig. 5. Comparison of the density of 5-HT-ir varicosities in the aggression areas of saline- vs. cocaine-treated hamsters. Varicosity numbers were normalized to a standard areas (100×100 μm^2) for regional comparisons. $^{***}P < 0.001$, $^{**}P < 0.01$, $+P > 0.05$ and $P < 0.1$; Student's t-test, two-tailed.

parison did not reach statistical significance (CoMeA, $t(20) = 1.88$, $P = 0.074$) (Fig. 5). In addition, similar numbers of 5-HT-ir varicosities were found in the VLH, CeA, MPN, and the Cg of both cocaine-treated and saline control animals (Fig. 5). In each case, no significant difference was observed between treatment groups (VLH, $t(23) = 1.46$; CeA, $t(16) = 0.95$; MPN, $t(27) = 0.65$; Cg, $t(28) = 1.04$; $P > 0.1$ each comparison). Interestingly, a trend towards an increase was observed in the number of 5-HT-ir varicosities and fibers in the striatum (CPu; i.e. a non-aggression area) of cocaine-treated animals compared to saline-treated controls (CPu, $t(22) = 1.86$, $P = 0.077$) (Fig. 5).

4. Discussion

In previous studies, we have shown that repeated low-dose cocaine treatment (0.5 mg/kg/day) throughout adolescent development significantly increases offensive aggression in male Syrian hamsters [30]. One mechanism by which chronic cocaine may facilitate offensive aggression is by altering the activity of neurotransmitters implicated in this behavioral response. For example, there is abundant evidence that 5-HT inhibits aggressive behavior in humans [7,14,37,43] and in many animal models and species [31,38,58,68], including hamsters [16,20,23,25]. Perhaps exposure to cocaine during adolescence suppresses 5-HT activity, thereby stimulating offensive aggression. To address this question, we performed experimental manipulations elevating extracellular 5-HT in the brains of adolescent cocaine-treated hamsters to determine whether enhancing 5-HT activity would attenuate cocaine-stimulated offensive aggression. The behavioral data presented here support our hypothesis that 5-HT plays an important role in adolescent cocaine-facilitated aggression. For instance, during the present experiments, the IP administration of saline to cocaine-treated hamsters had no effect on aggressive responding. These animals showed levels of offensive aggression analogous to that observed in our previous studies [30] and presented here (Fig. 3). Nearly all saline-treated animals (9 out of 10) showed a very high intensity of aggression (defined by the total number bites and/or attacks per test period) and a quick onset (initiation) of the aggressive response (defined by the latency to the first attack and/or bite). Conversely, peripheral injections of fluoxetine resulted in a nearly complete blockade of adolescent cocaine-facilitated aggression. Fluoxetine-treated animals were remarkably less aggressive and slower to initiate the aggressive response than saline-treated counterparts. Fluoxetine-

treated animals showed a >95% decrease each in the number of bites and attacks as well as a >50% decrease in the latency to first bite and attack 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 data are important and novel in that they directly show that 5-HT activity in brain plays a critical role inhibiting the aggressive phenotype that arises in response to adolescent cocaine exposure, and that enhanced 5-HT activity does not block cocaine-facilitated offensive aggression through a nonspecific behavioral inhibition.

Since experimental manipulations that enhance 5-HT activity in brain block adolescent cocaine-stimulated offensive aggression, perhaps cocaine exposure during this developmental period predisposes animals to respond aggressively by depressing the normal developmental profile of the 5-HT neural system regulating aggression. In Syrian hamsters, the activity of 5-HT neural system in two particular brain regions, i.e. the VLH and AH, has been shown previously to regulate offensive aggression [16,20,23,25], where 5-HT acts to suppress aggression. Adolescent cocaine exposure may depress 5-HT neural development in these brain regions by decreasing the extent to which 5-HT neurons innervate synaptic partners, functionally dis-inhibiting the neural circuits stimulating offensive aggression. To determine this, we quantified the 5-HT innervation to the VLH and AH of cocaine- and saline-treated hamsters. In the VLH, no ostensible differences were observed in the number of 5-HT-ir varicosities and fibers between treatment groups, suggesting that adolescent cocaine-facilitated offensive aggression may not be regulated by the development of 5-HT afferents to this brain region. Conversely, animals exposed to cocaine during adolescence had nearly 2-fold fewer 5-HT-ir varicosities and fibers within the AH, suggesting a decreased afferent innervation or depletion of AH-5-HT in response to adolescent cocaine. From a functional standpoint, this decrease in serotonergic tone may activate the AH neural circuit implicated in the aggressive response. In hamsters, activity of the arginine vasopressin (AVP) neural system in the AH facilitates offensive aggression [21,24,50]. Experimental manipulations that increase 5-HT in this brain region effectively block AVP-facilitated aggression [20,22,23,25]. It is plausible that AVP cells in the AH receive a diminished inhibitory 5-HT input as a result of adolescent cocaine exposure, culminating in the stimulation of AH AVP and offensive aggression in these animals. There is support for this notion from experiments examining the effects of acute and chronic cocaine on the function of the hypothalamic-pituitary-adrenal (HPA) axis in adult rats. Acute cocaine treatment increases hypothalamic 5-HT correlated with the activation of the HPA [41]. 5-HT stimulates HPA by activating hypothalamic AVP/CRH (corticotropin-releasing hormone) neurons that influence pituitary function [48,66], stimulating the release of AVP from hypothalamic neurons [55]. Depletion of 5-HT or destruction of 5-HT neurons prevents the cocaine-mediated surge in HPA function [41]. Interestingly, chronic cocaine treatment dampens HPA function by reducing hypothalamic 5-HT signaling [3,32,42,67]. Thus, the chronic effects of cocaine on the HPA are distinct from the acute effects and reflect a decrease in 5-HT signaling. While there exists contrasting data regarding the effects of acute and chronic cocaine on the 5-HT neural system, data support the notion that cocaine-mediated disturbances in 5-HT signaling occur in brain areas that contain AVP neurons i.e. the AH. Experiments examining whether chronic cocaine exposure during adolescence stimulates AH AVP activity due to a reduction in AH 5-HT signaling are currently underway in the laboratory.

In hamsters, activity of neurons located in the LS, MeA, and BNST have also been implicated in offensive aggression. It is possible that the normal development of the 5-HT neural system in these primary aggression areas is affected by adolescent cocaine exposure, functionally dis-inhibiting neurons active in the aggressive response. Indeed, our results show that animals exposed to low-dose cocaine during adolescence display marked decreases in 5-HT innervation to each of these aggression areas, identifying these brain regions as potential sites where 5-HT activity may modulate adolescent cocaine-facilitated aggression. For example, the activation of neurons in the BNST and MeA correlate with aggressive response patterns in rats [1,35,36,57,69], mice [28], prairie voles [70], and hamsters [10,15,34]. It is possible that 5-HT signaling in these brain regions functions [49] to inhibit the activation of cells critical to the aggressive response. Reduced 5-HT innervation in these regions resulting from adolescent cocaine exposure may cause the functional activation of neurons in these regions, facilitating a heightened aggressive response. However, if 5-HT activity in the LS is involved in adolescent cocaine-facilitated aggression, then our finding that cocaine decreases 5-HT innervation to this region suggests a reciprocal behavioral response (i.e. the attenuation of aggression following cocaine exposure) since stimulation of the LS inhibits offensive aggression. Nevertheless, the development of the aggressive phenotype following adolescent cocaine exposure may be explained by the subtype(s) of 5-HT receptors expressed in the LS. Recently, we have localized the expression of the 5-HT3 subtype receptor (i.e. an excitatory 5-HT receptor [4,5,13]) within the LS [52]. A reduction in 5-HT3 signaling in the LS due to the loss of 5-HT afferents following adolescent cocaine exposure would effectively inhibit LS neurons, facilitating the establish-

ment of the aggressive phenotype. Taken together, the data above are novel and significant in that they show that exposure to low-dose cocaine during adolescence can alter the developmental patterns of innervation of 5-HT afferents to areas of the hamster brain implicated in aggressive responding. No studies to date have shown

extracellular concentrations of serotonin and dopamine. Pharmacol Biochem Behav 1994;49:107–13.
[19] Estroff TW, Schwartz RH, Hoffmann NG. Adolescent cocaine abuse. Addictive potential, behavioral and psychiatric effects. Clin Pediatr (Phila) 1989;28:550–5.
[20] Ferris CF. Serotonin diminishes aggression by suppressing the activity of the vasopressin system. Ann NY Acad Sci 1996;794:98–103.
[21] 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.
[22] Ferris CF, Delville Y. Vasopressin and serotonin interactions in the control of agonistic behavior. Psychoneuroendocrinology 1994;19:593–601.
[23] Ferris CF, Melloni RH, Jr., 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.
[24] Ferris CF, Potegal M. Vasopressin receptor blockade in the anterior hypothalamus suppresses aggression in hamsters. Physiol Behav 1988;44:235–9.
[25] Ferris CF, Stolberg T, Delville Y. Serotonin regulation of aggressive behavior in male golden hamsters (*Mesocricetus auratus*). Behav Neurosci 1999;113:804–15.
[26] 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.
[27] Galloway MP. Regulation of dopamine and serotonin synthesis by acute administration of cocaine. Synapse 1990;6:63–72.
[28] Gammie SC, Nelson RJ. cFOS and pCREB activation and maternal aggression in mice. Brain Res 2001;898:232–41.
[29] Hadfield MG. Cocaine. Selective regional effects on central monoamines. Mol Neurobiol 1995;11:47–53.
[30] Harrison RJ, Connor DF, Nowak C, Melloni RH, Jr.. Chronic low-dose cocaine treatment during adolescence facilitates aggression in hamsters. Physiol Behav 2000;69:555–62.
[31] Higley JD, Mehlman PT, Taub DM, Higley SB, Suomi SJ, Vickers JH, et al. Cerebrospinal fluid monoamine and adrenal correlates of aggression in free-ranging rhesus monkeys. Arch Gen Psychiat 1992;49:436–41.
[32] Johnson RG, Fiorella D, Rabin RA. Effects of chronic cocaine administration on the serotonergic system in the rat brain. Pharmacol Biochem Behav 1993;46:289–93.
[33] Kandel D, Yamaguchi K. From beer to crack: developmental patterns of drug involvement. Am J Public Health 1993;83:851–5.
[34] 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.
[35] 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, 1991:213–9.
[36] Koolhaas JM, Van den Brink THC, Roozendal B, Boorsma F. Medial amygdala and aggressive behavior: interaction between testosterone and vasopressin. Aggres Behav 1990;16:223–9.
[37] Kruesi MJ, Rapoport JL, Hamburger S, Hibbs E, Potter WZ, Lenane M, et al. Cerebrospinal fluid monoamine metabolites, aggression, and impulsivity in disruptive behavior disorders of children and adolescents. Arch Gen Psychiat 1990;47:419–26.
[38] Kyes RC, Botchin MB, Kaplan JR, Manuck SB, Mann JJ. Aggression and brain serotonergic responsivity: response to slides in male macaques. Physiol Behav 1995;57:205–8.
[39] Ladosky W, Gaziri LC. Brain serotonin and sexual differentiation of the nervous system. Neuroendocrinology 1970;6:168–74.
[40] Lerwill CJ, Makaings P. The agonistic behavior of the golden hamster. Anim Behav 1971;19:714–21.
[41] Levy AD, Li QA, Kerr JE, Rittenhouse PA, Milonas G, Cabrera TM, et al. Cocaine-induced elevation of plasma adrenocorticotropin hormone and corticosterone is mediated by serotonergic neurons. J Pharmacol Exp Ther 1991;259:495–500.
[42] Levy AD, Rittenhouse PA, Bonadonna AM, Alvarez Sanz MC, Bethea CL, Van de Kar LD. Repeated exposure to cocaine produces long-lasting deficits in the serotonergic stimulation of prolactin and renin, but not adrenocorticotropin secretion. Eur J Pharmacol 1993;241:275–8.
[43] 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.
[44] 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.
[45] Loiselle JM, Baker MD, Templeton JM, Schwartz G, Drott H. Substance abuse in adolescent trauma. Ann Emerg Med 1993;22:1530–4.
[46] 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.
[47] NIDACapsules, 2001. Available from: http//www.nida.nih.gov/NIDACapsules/NCIndex.html.
[48] Pergola PE, Sved AF, Voogt JL, Alper RH. Effect of serotonin on vasopressin release: a comparison to corticosterone, prolactin and renin. Neuroendocrinology 1993;57:550–8.
[49] Potegal M, Blau A, Glusman M. Effects of anteroventral septal lesions on intraspecific aggression in male hamsters. Physiol Behav 1981;26:407–12.
[50] Potegal M, Ferris C. Intraspecific aggression in male hamsters in inhibited by intrahypothalamic vasopressin receptor antagonists. Aggres Behav 1989;15:311–20.
[51] 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.
[52] Potter L, Melloni RH Jr. Sex differences in the distribution of serotonin type-3 receptor in Syrian hamster brain. J Comp Neurol, submitted for publication.
[53] Raskin-White H. Longitudinal patterns of cocaine use among adolescents. Am J Drug Alc Abuse 1988;14:1–15.
[54] Salloum IM, Daley DC, Cornelius JR, Kirisci L, Thase ME. Disproportionate lethality in psychiatric patients with concurrent alcohol and cocaine abuse. Am J Psychiat 1996;153:953–5.
[55] Sarnyai Z, Vecsernyes M, Laczi F, Biro E, Szabo G, Kovacs GL. Effects of cocaine on the contents of neurohypophyseal hormones in the plasma and in different brain structures in rats. Neuropeptides 1992;23:27–31.
[56] Schoenfeld TA, Leonard CM. Behavioral Development in the Syrian Golden Hamster. In: Siegel HI, editor. The hamster: reproduction and behavior. New York: Plenum Press, 1985.
[57] 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.
[58] 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.
[59] Simms D, Gallagher JP. Modification of serotonin responses in rat dorsolateral septal nucleus neurons by acute and chronic cocaine. J Pharmacol Exp Ther 1996;276:1292–303.
[60] Snyder-Keller AM, Keller RW, Jr.. Prenatal cocaine increases striatal serotonin innervation without altering the patch/matrix

organization of intrinsic cell types. Brain Res Dev Brain Res 1993;74:261–7.
[61] Sosin DM, Koepsell TD, Rivara FP, Mercy JA. Fighting as a marker for multiple problem behaviors in adolescents. J Adolesc Health 1995;16:209–15.
[62] Teneud LM, Baptista T, Murzi E, Hoebel BG, Hernandez L. Systemic and local cocaine increase extracellular serotonin in the nucleus accumbens. Pharmacol Biochem Behav 1996;53:747–52.
[63] Todtenkopf MS, Benes FM. Distribution of glutamate decarboxylase65 immunoreactive puncta on pyramidal and nonpyramidal neurons in hippocampus of schizophrenic brain. Synapse 1998;29:323–32.
[64] Todtenkopf MS, Stellar JR. Assessment of tyrosine hydroxylase immunoreactive innervation in five subregions of the nucleus accumbens shell in rats treated with repeated cocaine. Synapse 2000;38:261–70.
[65] Valois RF, McKeown RE, Garrison CZ, Vincent ML. Correlates of aggressive and violent behaviors among public high school adolescents. J Adolesc Health 1995;16:26–34.
[66] Van de Kar LD. Neuroendocrine pharmacology of serotonergic (5-HT) neurons. Annu Rev Pharmacol Toxicol 1991;31:289–320.
[67] Van de Kar LD, Bonadonna AM, Rittenhouse PA, Kerr JE, Levy AD, Iyer L, et al. Prior chronic exposure to cocaine inhibits the serotonergic stimulation of ACTH and secretion of corticosterone. Neuropharmacology 1992;31:169–75.
[68] Vergnes M, Depaulis A, Boehrer A, Kempf E. Selective increase of offensive behavior in the rat following intrahypothalamic 5,7-DHT-induced serotonin depletion. Behav Brain Res 1988;29:85–91.
[69] Vochteloo JD, Koolhaas JM. Medial amygdala lesions in male rats reduce aggressive behavior: interference with experience. Physiol Behav 1987;41:99–102.
[70] 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.
[71] Whitsett JM. The development of aggressive and marking behavior in intact and castrated male hamsters. Horm Behav 1975;6:47–57.
[72] Windle M, Miller-Tutzauer C. Antecedents and correlates of alcohol, cocaine, and alcohol-cocaine abuse in early adulthood. J Drug Educ 1991;21:133–48.
[73] 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.