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Research report

Repeated cocaine exposure during adolescence alters glutamic acid decarboxylase-65 (GAD_{65}) immunoreactivity in hamster brain: correlation with offensive aggression

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Abstract

Male Syrian hamsters (*Mesocricetus auratus*) treated with low-dose (0.5 mg/kg/day) cocaine throughout adolescence (P27–P56) display highly escalated offensive aggression. The current study examined whether adolescent cocaine exposure influenced the immunohistochemical localization of glutamic acid decarboxylase-65 (GAD_{65}), the rate-limiting enzyme in the synthesis of γ-aminobutyric acid (GABA), a fast-acting neurotransmitter implicated in the modulation of aggression in various species and models of aggression. Hamsters were administered low doses of cocaine throughout adolescence, scored for offensive aggression using the resident–intruder paradigm, and then examined for changes in GAD_{65} immunoreactivity in areas of the brain implicated in aggression control. When compared with saline-treated control animals, aggressive cocaine-treated hamsters showed significant differences in the area covered by GAD_{65} puncta in several notable aggression regions, including the anterior hypothalamus, the medial and central amygdaloid nuclei, and the lateral septum. However, no differences in GAD_{65} puncta were found in other aggression areas, such as the bed nucleus of the stria terminalis, the ventrolateral hypothalamus, and the corticomedial amygdala. Together, these results suggest that altered GABA synthesis and function in specific aggression areas may be involved in adolescent cocaine-facilitated offensive aggression.

Keywords: Adolescence; Cocaine; γ-Aminobutyric acid; GABA; GAD_{65}; Immunohistochemistry; Development; Aggression

1. Introduction

Previously, we have used subadult Syrian hamsters as animal models to examine the link between adolescent cocaine exposure and the behavioral neurobiology of offensive aggression [7,26,29,34,42,43]. Behavioral data from these studies showed that hamsters repeatedly exposed to low doses of cocaine (0.5 mg/kg/day) during adolescent development display highly escalated, adult forms of offensive aggression. This highly escalated behavioral phenotype is characterized by intense bouts of biting and attacking primarily directed towards the flanks, rump, and ventrum of the intruder, as well as high amounts of upright offensive postures and lateral movements (i.e., lateral attacks) toward the intruder. The finding that adolescent cocaine-treated hamsters demonstrated highly escalated adult forms of offensive aggression in the absence of prior social interactions and dominance cues suggested that adolescent exposure to cocaine stimulated aggression directly, perhaps by affecting the development and/or activity of neural circuits that regulate this behavior.

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter system in the central nervous system (CNS). GABA is produced in the brain by the two isoforms of the enzyme glutamic acid decarboxylase, GAD_{65} and GAD_{67}, named for their molecular weights of 65 and 67 kDa, respectively [13]. Although the two isoforms are generally co-expressed in GABAergic neurons, their expression is differentially regulated, and they

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differ in their co-factor-dependent activation and subcellular localization [49]. GAD_{65} has been localized mainly to synaptic terminals and therefore may serve to respond rapidly to demands for GABA with changes in neuronal activity [28]. Both GAD_{65} mRNA expression and the immunohistochemical labeling of GAD_{65} in axon terminals have been localized to various brain regions in rat, including the hypothalamus, lateral septum, bed nucleus of the stria terminalis, and the central and medial amygdaloid nuclei [2,5,14,15,51–53], i.e., areas of the brain implicated in aggressive behavior in hamsters [3,8–10,18,24,30,38,39,48].

Several studies have suggested that GABAergic activity may be involved in the modulation of aggression. For example, in cats reciprocal GABAergic projections between the medial and lateral hypothalamus suppress defensive rage and predatory attack behaviors, respectively [6,25]. Conversely, in mice and hamsters, increased aggressive behavior correlates with significantly higher GABA binding activity and levels and the density of GAD_{65}-containing afferents in the hypothalamus and amygdala [23,27,40], brain areas important for aggression control [3,8–10,18,41]. And, null mutant mice that lack the gene for GAD_{65} and display a marked GABA deficit in the hypothalamus and amygdala show a significant reduction in aggressive behavior [50]. In addition, cocaine appears to influence the activity of GABA neurons. Indeed, in adult rats chronic cocaine administration has been shown to both increase and decrease GABA turnover in a number of brain regions including the nucleus accumbens, globus pallidus, hippocampus, and the somatosensory, cingulated, and pyriform corticies [12,46]. Using brain slice preparations, acute cocaine exposure has been observed to decrease the release of GABA [4]. While these data indicate both stimulatory and inhibitory effects of cocaine on GABAergic tone, it is important to note that none of the aforementioned studies investigated cocaine's effects on GABA function across development and/or in areas of the brain particularly relevant for aggression control, such as the hypothalamus, septum, bed nucleus, or amygdala. This is important since a nearly twofold increase in GABA levels has been observed across the pubertal developmental period, i.e., between the first and second postnatal months of age [50]. It is possible that cocaine exposure during adolescent development escalates aggressive responding by altering GABA activity in brain areas important for aggression control. To date however, it is unknown whether adolescent cocaine exposure has any effects on the development and/or activity of the GABA neural system.

Given this documented association between the GABAergic neural system, aggression, and cocaine, the present study was conducted to establish a link between the expression of GAD_{65}-containing synaptic terminals and adolescent cocaine-induced offensive aggression using the subadult Syrian hamster as an animal model. 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 [35]. 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 aggression [44,56]. To establish whether adolescent cocaine exposure altered GABAergic activity in various brain regions implicated in the control of offensive aggression, we employed immunohistochemistry utilizing an antibody specific against the 65-kDa isoform of glutamate decarboxylase to visualize and quantify GAD_{65}-containing synaptic terminals.

2. Methods

2.1. Animals

Pre-adolescent male hamsters (P21) were obtained from Charles River Laboratories (Wilmington, MA), individually housed in polycarbonate cages, and maintained at ambient room temperature on a reverse light–dark cycle of (14 L: 10 D; lights off at 07:00) as previously described [7,26]. 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 Charles River Laboratories 1 week prior to the behavioral test (approximately P54–P62 of age), group housed at five animals per cage in large polycarbonate 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 [18,33]. Animals displaying significantly low aggression and/or submissive postures were excluded from use in the behavioral assay. All studies employing live animals were preapproved by the Northeastern University Institutional Animal Care and Use Committee and all methods used were consistent with guidelines provided by the National Institute of Health for the scientific treatment of animals.

2.2. Experimental treatment

P27 Syrian hamsters were weighed and randomly distributed into two groups (n = 10 animals/group). One group of animals received intraperitoneal (IP) injections of 0.5 mg/kg cocaine hydrochloride (Sigma, St. Louis, MO) dissolved in 0.9% saline (1.0 ml/kg total volume injected) for 30 consecutive days during adolescent development (P27–P57), while the second group of hamsters received IP injections of saline (1.0 ml/kg) alone. This dose of cocaine has been shown to induce offensive aggression in

experimental animals in a number of previous studies [7,26,29,34,42,43]. The day following the last injection (P58), animals were tested for offensive aggression using the resident–intruder paradigm, sacrificed 24 h later (i.e., on P59), and the brains removed and processed for immunohistochemistry as previously described [23] and detailed below.

2.3. Aggression testing

Experimental animals were tested for offensive aggression using the resident–intruder paradigm, a well-characterized and ethologically valid model of offensive aggression in Syrian hamsters [20,32]. Briefly, an intruder of similar size and weight was introduced into the home cage of the experimental animal (resident) and the resident was scored for specific and targeted aggressive responses including upright offensive postures, lateral attacks, flank and rump bites, as well as latency to first bite, as previously described [23,42,43]. An attack was scored each time the resident animal would pursue and then either (1) lunge toward and/or (2) confine the intruder by upright and sideways threat; each generally followed by a direct attempt to bite the intruder's dorsal rump and/or flank target area(s). The latency to bite was defined as the period of time between the beginning of the behavioral test and the first bite-containing attack of the resident towards an intruder. In the case of no bites, latencies to bite were assigned the maximum latency (i.e., 600 s). In addition, residents were measured for social interest toward intruders (i.e., contact time between resident and intruder) placed in their home cage. 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 behavioral test was videotaped for 10 min and coded by two observers unaware of the hamsters' experimental treatment. No intruder was used for more than one behavioral test to control for the effects of repeated exposure to conspecifics on the behavior of intruders, and all animals were tested during the first 4 h of the dark cycle under dim-red illumination to control for circadian influences on behavioral responding.

2.4. Immunohistochemistry

For immunohistochemical analysis, hamsters (n = 10 animals/group) were deeply anesthetized with ketamine/xylazine (80 mg/12 mg) and transcardially perfused with a 21 °C saline rinse followed by fixative solution comprised of 4% paraformaldehyde, 0.2% picric acid, and 0.4% glutaraldehyde. Brains were removed, postfixed for 90 min in perfusion fixative, and cryoprotected in 30% sucrose in distilled water at 4 °C overnight. Brains were cut at 35 μm using a freezing microtome in serial, coronal sections and all subsequent immunohistochemical procedures were conducted at 21 °C in one standardized procedure run. Specifically, every third section was washed 3 × 5 min in 0.1 M phosphate-buffered saline (PBS, pH 7.4), then pretreated with 3% H_2O_2 in distilled water for 10 min, and rinsed thoroughly with 0.1 M PBS. Sections were then pretreated with 0.5% sodium borohydride in distilled water for 5 min, 3 × 5 rinsed thoroughly with 0.1 M PBS, and incubated in antibody buffer comprised of 10% normal goat serum and 1% bovine serum albumin (BSA) in PBS for 60 min. Primary antibody (monoclonal GAD_{65} (AB-1), Chemicon, Temecula, CA) was prepared in antibody buffer diluted to final concentration of 0.67 μg/ml and incubation with free-floating brain sections was carried out overnight at 21 °C. Sections were then rinsed 3 × 10 min with PBS, incubated for 60 min in biotinylated secondary goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) in PBS and 1% BSA, rinsed again 3 × 10 min in PBS and incubated for 60 min in avidin–biotin complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) with 1% BSA in PBS. The peroxidase reaction was revealed using 0.5% 3,3′-diaminobenzidine in distilled water as per manufacture's recommendations (DAB Kit. Vectastain; Vector Laboratories, Burlingame, CA). The sections were mounted on gel-coated slides, air-dried, dehydrated through a series of alcohols, cleared with xylene, and coverslipped with Cytoseal (Stephens Scientific, Kalamazoo, MI). Omissions of the primary and secondary antibodies were run as controls during the procedure.

2.5. Image analysis

The area covered by GAD_{65} immunoreactive (GAD_{65}-ir) puncta was determined within specific brain areas using the BIOQUANT NOVA 5.0 computer-assisted microscopic image analysis software package as previously described [7,22,23,29,42]. The areas analyzed were selected based on previous data implicating these regions as part of the neural circuit important for aggressive behavior in numerous species and models of aggression, with the notable exception of the S1 neocortex (S1) (i.e., a non-aggression area used as a control region) [3,8–10,18,24,30,38,39,48]. These areas (see Fig. 1) included the intermediate part of the lateral septal nucleus (LS), the medial division of the bed nucleus of the stria terminalis (BNST), the anterior hypothalamus (AH), the corticomedial amygdaloid nucleus (CoMeA), the medial amygdaloid nucleus (MeA), the central amygdaloid nucleus (CeA), and the ventrolateral hypothalamus (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,

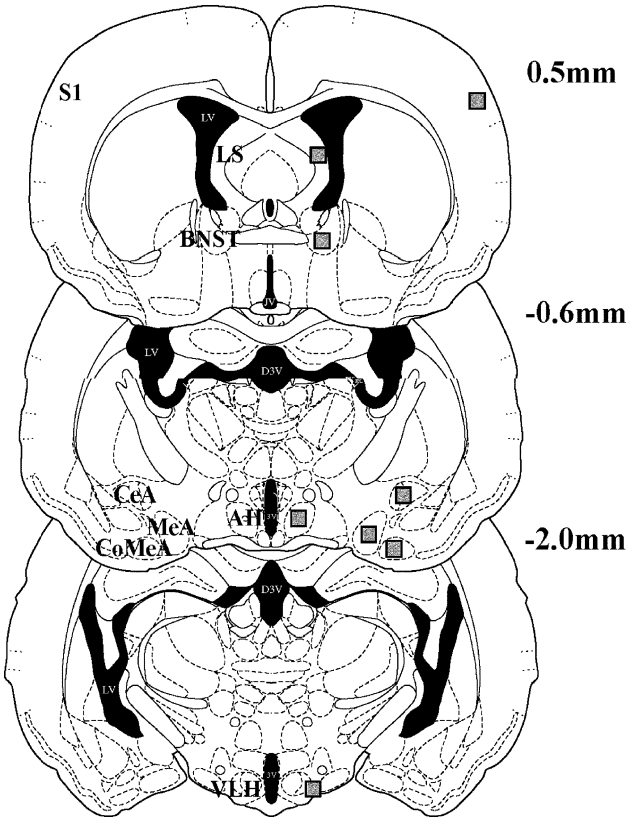

Fig. 1. Diagram showing the location of the areas selected to quantify GAD_{65}-ir puncta (shaded areas). Plates were modified from hamster atlas of Morin and Wood [37] and reflect specific positions in the rostral–caudal plane (i.e., distance in mm from bregma to the plane of section at the skull surface). Abbreviations: AH, anterior hypothalamus; BNST, medial division of the bed nucleus of the stria terminalis; CeA, central amygdala; CoMeA, corticomedial amygdala; LS, intermediate part of the lateral septal nucleus; MeA, medial amygdala; S1, S1 neocortex; VLH, ventrolateral hypothalamus.

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 to allow detection of stained GAD_{65}-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. GAD_{65}-ir puncta were identified in each field using a mouse-driven cursor and then GAD_{65}-ir counts were performed automatically by the BIOQUANT software. Measurements at 20× continued until GAD_{65}-ir elements throughout the entire region of interest were quantified. Two to three independent measurements of GAD_{65}-ir elements were taken from several consecutive sections ($n > 2$) of each animal per treatment group depending upon the following: (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 area covered by GAD_{65}-ir puncta was determined for each region of interest, standardized per 100 × 100-μm parcel for regional comparison purposes, and then used for statistical analysis.

2.6. Statistics

Results from the aggression tests were compared between saline and cocaine treatment groups. All behaviors were compared using one-way ANOVA followed by Fischer's PLSD post hoc (two tailed) when applicable. Immunohistochemical data were compared between cocaine- and saline-treated controls in brain areas associated with aggression and relative densities were analyzed using ANOVA followed by pairwise planned comparisons. The α level for all experiments was set at 0.05.

3. Results

3.1. Aggressive behavior

As previously observed in a number of studies, low-dose cocaine exposure throughout the developmental period of adolescence increased offensive aggression in hamsters [7,26,42,43] specifically offensive attack [$F(3,36) = 7.64$, $P < 0.001$] and bite behaviors [$F(3,36) = 8.05$, $P < 0.001$]. Animals treated with cocaine showed significantly heightened levels of threat and attack patterning toward intruders when compared to their saline-treated littermates (Fig. 2). Specifically, cocaine-treated animals showed increased upright offensive postures compared to saline-treated littermates [$t(9) = 4.3$, $P < 0.01$]. Indeed, cocaine-treated animals displayed anywhere from 5 to 30 postures during the 10-min test period compared to saline-treated animals that displayed between 0 and 5 such postures. Cocaine-treated hamsters also showed significant increases in the number of lateral attacks animals compared to saline controls [$t(9) = 2.6$, $P < 0.05$], with cocaine-treated residents displaying as many as 47 lateral attacks towards intruders in one test period. The number of specifically targeted bites also differed between cocaine- and saline-treated animals with cocaine-treated hamsters directing a significantly greater number of bites to the flank [$t(9) = 3.6$, $P < 0.01$] and rump region [$t(9) = 2.5$, $P < 0.05$] of intruders. In fact, all but one animal in the cocaine condition executed bites to the flank and only two animals in the saline condition exhibited any flank bites during the test period. In addition, hamsters administered cocaine were quicker to bite intruders [$t(9) = 3.5$, $P < 0.01$] than vehicle-treated control animals with cocaine animals being more than twice as fast at initiating bite behavior than saline-treated littermates (i.e., 231.5 vs. 510 s, respectively). In contrast, there was no difference between saline- and cocaine-treated animals with respect to the total amount of time the resident and intruder were in contact [$t(9) = 0.49$, $P = 0.78$].

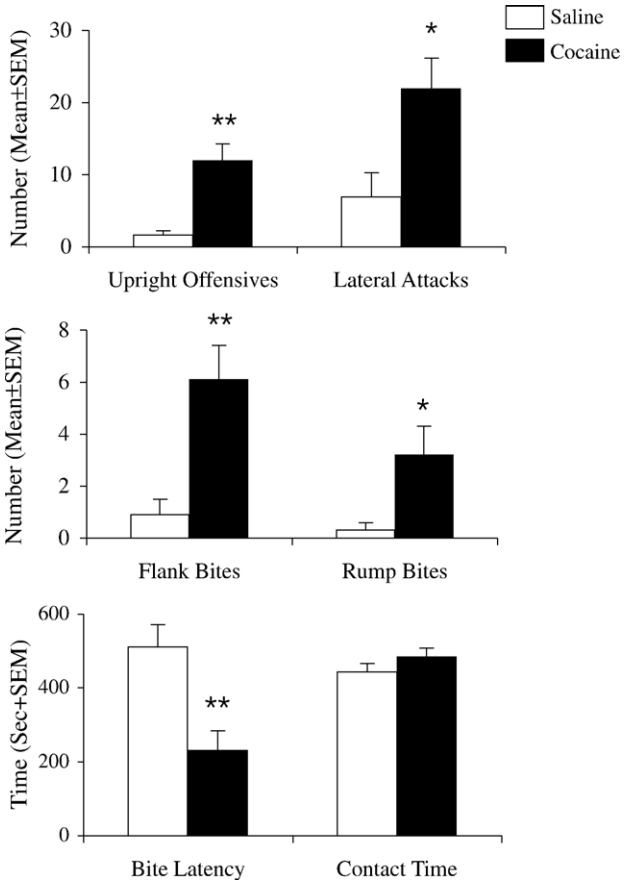

Fig. 2. Adolescent cocaine treatment increases offensive aggression. Number of upright offensive and lateral attacks; flank and rump bites; as well as latency to first attack and bite; and total contact time in cocaine- and saline-treated residents. Bars denote SEM. $^{**}P < 0.01$; $^{*}P < 0.05$; Student's t test, two tailed.

3.2. GAD_{65} immunohistochemistry

Gross analysis of overall combined brain areas implicated in aggression revealed no differences in GAD_{65} immunoreactivity between cocaine- and saline-treated animals [$F(1,12) = 1.54$, $P = 0.22$]. However, significant brain–treatment interactions (area × treatment) were found

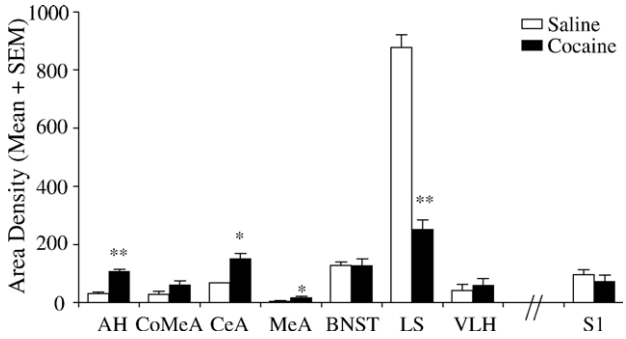

Fig. 4. Density of glutamic acid decarboxylase (GAD_{65}) immunoreactive puncta in brains of cocaine- vs. saline-treated hamsters. Numbers were normalized to a standard area (100 × 100 μm) for regional comparisons. $^{***}P < 0.001$, $^{**}P < 0.01$; $^{*}P < 0.05$; Student's t test, two tailed.

[$F(1,6) = 28.9$, $P < 0.001$]. GAD_{65}-ir was altered in several areas of hamster brain important for aggressive behavior. For example, cocaine-treated hamsters exhibited a dense staining pattern of GAD_{65}-ir puncta in the AH (Fig. 3A) when compared to saline-treated controls (Fig. 3B). Quantitative analysis of GAD_{65}-ir puncta showed that cocaine-treated animals had over a threefold increase in the density of GAD_{65}-ir puncta in the AH when compared to saline-treated littermates (Fig. 4). This difference was statistically significant [$t(8) = 7.07$, $P < 0.0001$]. Similar results were observed in the CeA and the MeA where cocaine-treated animals had greater than 2–3 times the density of GAD_{65}-ir puncta of saline-treated littermates, respectively (Fig. 4). These differences were statistically significant ([CeA]$t(8) = 3.4$, $P < 0.01$, and [MeA] $t(8) = 2.66$, $P < 0.05$). Changes in GAD_{65}-ir between cocaine- and saline-treated animals were not limited to areas of the amygdala and hypothalamus. For instance, the density of GAD_{65}-ir puncta in the LS was more than three times greater in saline-treated animals than cocaine-treated animals [$t(8) = 11.4$, $P < 0.0001$] (Fig. 4).

However, not every brain region implicated in aggressive behavior in hamsters showed significant changes in the density of GAD_{65}-ir puncta following adolescent cocaine exposure. For instance, there were no discernable differ-

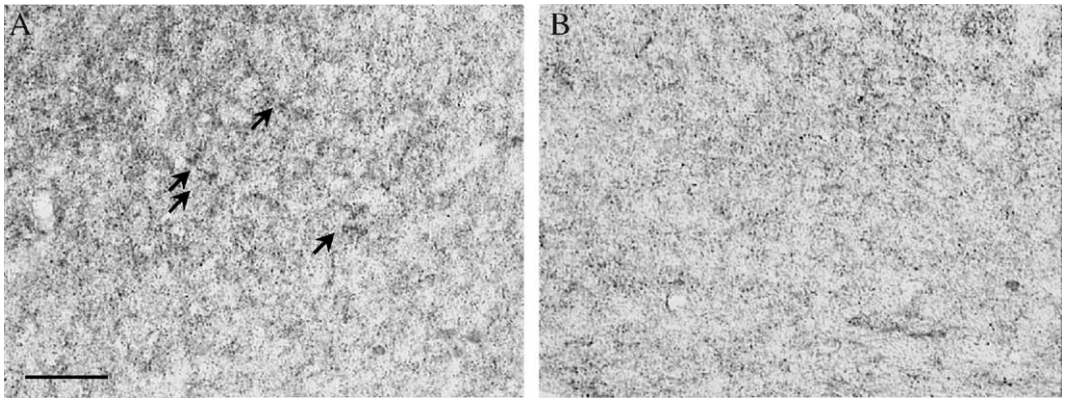

Fig. 3. Bright-field photomicrographs of a coronal section through the Syrian hamster hypothalamus. Shown are glutamic acid decarboxylase (GAD_{65}) immunoreactive puncta (arrows) within the anterior hypothalamus (AH) of (A) cocaine-treated and (B) saline-treated hamsters. Scale bar, 20 μm.

ences in the density of GAD_{65}-ir puncta found in the VLH, the medial division of the BNST, or the CoMeA between cocaine- and saline-treated animals (Fig. 4). Similarly, no differences between treatment groups were found in the density of GAD_{65}-ir puncta in the S1 cortex ($P > 0.2$) (Fig. 4), a brain area not involved in aggressive behavior in the hamster.

4. Discussion

Our previous research has shown that low-dose cocaine exposure throughout the developmental period of adolescence facilitates offensive aggression in male Syrian hamsters [7,26,42,43]. From a mechanistic standpoint one possible explanation for the augmented aggressive phenotype observed in adolescent cocaine-treated animals is that cocaine alters the activity of neurotransmitter systems underlying this behavioral response. In various species, including hamsters, increases in GAD_{65} enzymatic activity, GABA levels, and $GABA_A$ receptor activity have been shown to facilitate aggressive behavior. For instance, highly aggressive hamsters and mice display elevated GABA levels [27,40], and $GABA_A$ receptor agonism facilitates attack behavior toward a conspecific in a neutral arena in rats [11]. Similarly, compounds acting to inhibit GABA from binding its receptor have also been shown to dose-dependently decrease offensive aggression in rats and squirrel monkeys [55]. Together, these findings suggest an activational role for GABA in aggressive behavior. Moreover, the GAD_{65} enzyme, its mRNA transcript, and $GABA_A$ receptors have been localized to the hypothalamus, septum, amygdala, and the bed nucleus of the stria terminalis [2,5,14,15,51–53], i.e., brain regions implicated in the facilitation (and inhibition) of aggression in hamsters [3,8–10,18,24,30,38,39,48], further supporting a role for GABA activity in offensive aggression. The current hypothesis was that low-dose cocaine exposure throughout the adolescent developmental period would facilitate offensive aggression in hamsters by altering GABAergic function in areas of the brain implicated in aggression control. To test our hypothesis the brains of aggressive, adolescent cocaine-treated animals, and non-aggressive saline-treated littermates were examined for GAD_{65} immunoreactive staining as a measure of GABAergic tone.

As previously reported in a number of studies, adolescent cocaine-treated animals showed highly elevated levels of offensive aggression when compared to saline-treated controls [7,26,42,43]. The brains of these aggressive, cocaine-treated animals showed changes in GAD_{65}-ir in several areas involved in aggression control including the AH, LS, and both the CeA and MeA. Specifically, compared to saline-treated controls, cocaine-treated residents showed significant increases in the density of GAD_{65}-ir puncta in the AH, CeA, and MeA, suggesting either a localized increase in the expression of GAD_{65} protein and/or the density of GABAergic terminals in these brain areas. One potential mechanism by which cocaine-induced increases in GAD_{65}-ir in these brain sites may facilitate the development of the aggressive phenotype is by augmenting GABA inhibition of neuronal populations and/or neural afferent fiber systems suppressing aggression (e.g., serotonin [5] afferent fibers). Support for the latter proposed mechanism comes from studies in hamsters showing that 5-HT release and activity in the AH inhibit offensive aggression [9,16–19] and those in rats indicating a functional relationship between 5-HT and GABA in the modulation of aggression [47]. While the site(s) and mechanism of action of 5-HT/GABA interactions are not completely understood, studies have localized GABA receptors to 5-HT afferent terminals [21], and in turn, 5-HT receptors have been localized to GABA cells [36]. Thus, it is possible that changes in signaling between 5-HT and GABA terminals in the AH, CeA, and MeA may facilitate adolescent cocaine-induced offensive aggression. Further support for this hypothesis comes from work from our laboratory showing dramatic reductions in 5-HT afferent innervation to several of these brain regions (i.e., the AH and MeA) in aggressive, adolescent cocaine-treated hamsters compared with saline-treated controls [7]. Mechanistically, the increased density of GAD_{65}-ir puncta in the AH and MeA observed in the present study may represent a neural plastic response to the loss of GABA receptor-containing 5-HT afferent terminals in these regions of the hamster brain important for offensive aggression. Lending credibility to this hypothesized mechanism of action are similar 5-HT/GABA correlations observed in aggressive, adolescent anabolic steroid-treated hamsters [22,23]. Together, these data support the notion that signaling between 5-HT and GABA inhibitory control systems are linked in certain brain areas and suggest a mechanism whereby enhanced GABA activity could cause the disinhibition of a behavioral state (i.e., offensive aggression) that is regulated by 5-HT. This hypothesis is currently under investigation in the laboratory.

These data notwithstanding, one finding in the current study is inconsistent with the aforementioned hypothesis. Our previous study showed a significantly reduced 5-HTergic afferent innervation to the BNST in aggressive, cocaine-treated hamsters compared to saline-treated controls. Thus, under our hypothesis, an increase in the density of GAD_{65}-ir in the BNST of aggressive, cocaine-treated animals should have been observed when compared to controls. However, no differences were observed in GAD_{65}-ir in the BNST between treatment groups. While it is true that the BNST has previously been reported to be active following bouts of agonistic behavior in hamsters [30] and voles [54], this brain region has not been actively characterized as regulating offensive aggression. In fact, the heightened activation seen in the BNST following an aggressive encounter was only observed in voles with social/sexual experience [54]. Similarly, in hamsters the

BNST has been shown to play a role in mating and scent marking [1,30,31]. Therefore, the heightened activational state of this brain region following an agonistic interaction may be dependent upon and/or part of a more complex set of social/sexual behaviors. So, no change in GABA activity in this brain region in the present studies may indicate less of an affect (if any) of this area on purely offensive responding, remaining consistent with our central hypothesis regarding the site specific nature of 5-HT/GABA interactions and the development of the offensive aggressive phenotype. Interestingly, aggressive, cocaine-treated hamsters showed a significant reduction in GAD_{65}-ir density in the LS compared with controls. Decreased GABA levels have been observed in the septum of isolation-induced aggressive mice but these effects appear to be dependent upon the housing condition rather than the aggressive phenotype [45]. Since offensive aggression has been shown to be inhibited by electrical stimulation of the LS [38,39], adolescent cocaine-induced decreases in GAD_{65}-ir in this area could potentially cause a disinhibition of an inhibitory neural system (e.g., 5-HT), subsequently increasing an inhibitory influence on LS neurons and facilitating offensive aggression. Taken together, these data above are novel and significant in that they show that exposure to low-dose cocaine during adolescent development can alter patterns of GAD_{65}-containing afferent terminals to areas of the brain which have been implicated in offensive aggression in hamsters. From a neurobiological standpoint, these data implicate increased GABA neural signaling in several key areas as potential neural substrates for adolescent cocaine-induced offensive aggression. Further, the different patterns of GAD_{65}-ir staining in several brain regions suggest that there is a non-uniform effect of adolescent cocaine-treatment on GABA neurons across the neuraxis.

In summary, the role of GABAergic mechanisms in the elicitation of cocaine-induced offensive aggression appears to be complicated and likely interactive with other neurotransmitter systems, as indicated by our previous work showing that aggressive, adolescent cocaine-treated animals have region-specific compromised 5-HT neural systems. That notwithstanding, the studies presented in this paper provide data regarding the neurobiological effects of chronic low-dose cocaine exposure during adolescent development and propose basic neurobiological mechanisms by which these agents may exert their aggression-stimulating effects. These findings indicate that low-dose cocaine exposure during adolescent development produces both increases and decreases in GAD_{65}-ir in several areas of hamster brain important for aggressive behavior. These, together with previous findings from our laboratory, provide evidence that multiple neural systems including GABA are important in linking adolescent cocaine exposure and offensive aggression in hamsters. Further studies are needed to elucidate whether the aggression-stimulating effects of cocaine exposure occur as a direct result of cocaine's influence on the GABA system or whether the changes in GAD_{65}-ir observed here were representative of neural plastic alterations in the GABA system that occur as an indirect result of changes in other neurochemical systems in areas of the brain implicated in offensive aggression.

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References

[1] M. Bamshad, H.E. Albers, Neural circuitry controlling vasopressin-stimulated scent marking in Syrian hamsters (*Mesocricetus auratus*), J. Comp. Neurol. 369 (1996) 252–263.
[2] G. Bowers, W.E. Cullinan, J.P. Herman, Region-specific regulation of glutamic acid decarboxylase (GAD) mRNA expression in central stress circuits, J. Neurosci. 18 (1998) 5938–5947.
[3] B.N. Bunnell, F.J. Sodetz Jr., D.I. Shalloway, Amygdaloid lesions and social behavior in the golden hamster, Physiol. Behav. 5 (1970) 153–161.
[4] D.L. Cameron, J.T. Williams, Cocaine inhibits GABA release in the VTA through endogenous 5-HT, J. Neurosci. 14 (1994) 6763–6767.
[5] F. Chen, A. Rezvani, B. Jarrott, A.J. Lawrence, Distribution of GABAA receptors in the limbic system of alcohol-preferring and non-preferring rats: in situ hybridisation histochemistry and receptor autoradiography, Neurochem. Int. 32 (1998) 143–151.
[6] J.M. Cheu, A. Siegel, GABA receptor mediated suppression of defensive rage behavior elicited from the medial hypothalamus of the cat: role of the lateral hypothalamus, Brain Res. 783 (1998) 293–304.
[7] K.R. DeLeon, J.M. Grimes, D.F. Connor, R.H. Melloni Jr., Adolescent cocaine exposure and offensive aggression: involvement of serotonin neural signaling and innervation in male Syrian hamsters, Behav. Brain Res. 133 (2002) 211–220.
[8] Y. Delville, K.M. Mansour, C.F. Ferris, Serotonin blocks vasopressin-facilitated offensive aggression: interactions within the ventrolateral hypothalamus of golden hamsters, Physiol. Behav. 59 (1996) 813–816.
[9] Y. Delville, K.M. Mansour, C.F. Ferris, Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus, Physiol. Behav. 60 (1996) 25–29.
[10] Y. Delville, G.J. De Vries, C.F. Ferris, Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters, Brain Behav. Evol. 55 (2000) 53–76.
[11] A. Depaulis, M. Vergnes, Elicitation of conspecific attack or defense in the male rat by intraventricular injection of a GABA agonist or antagonist, Physiol. Behav. 35 (1985) 447–453.
[12] S.I. Dworkin, C. Co, J.E. Smith, Rat brain neurotransmitter turnover rates altered during withdrawal from chronic cocaine administration, Brain Res. 682 (1995) 116–126.
[13] M.G. Erlander, A.J. Tobin, The structural and functional heterogeneity of glutamic acid decarboxylase: a review, Neurochem. Res. 16 (1991) 215–226.

[14] S. Feldblum, M.G. Erlander, A.J. Tobin, Different distributions of GAD65 and GAD67 mRNAs suggest that the two glutamate decarboxylases play distinctive functional roles, J. Neurosci. Res. 34 (1993) 689–706.
[15] V.S. Fenelon, W. Sieghart, A.E. Herbison, Cellular localization and differential distribution of GABAA receptor subunit proteins and messenger RNAs within hypothalamic magnocellular neurons, Neuroscience 64 (1995) 1129–1143.
[16] C.F. Ferris, Serotonin diminishes aggression by suppressing the activity of the vasopressin system, Ann. N. Y. Acad. Sci. 794 (1996) 98–103.
[17] C.F. Ferris, Y. Delville, Vasopressin and serotonin interactions in the control of agonistic behavior, Psychoneuroendocrinology 19 (1994) 593–601.
[18] C.F. Ferris, R.H. Melloni Jr., G. Koppel, K.W. Perry, R. Fuller, Y. Delville, Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters, J. Neurosci. 17 (1997) 4331–4340.
[19] C.F. Ferris, T. Stolberg, Y. Delville, Serotonin regulation of aggressive behavior in male golden hamsters (*Mesocricetus auratus*), Behav. Neurosci. 113 (1999) 804–815.
[20] O.R. Floody, D.W. Pfaff, Aggressive behavior in female hamsters: the hormonal basis for fluctuations in female aggressiveness correlated with estrous state, J. Comp. Physiol. Psychol. 91 (1977) 443–464.
[21] K. Gale, Evidence for GABA receptors on serotonergic afferent terminals in the substantia nigra and on nigral efferent projections to the caudal mesencephalon, Brain Res. 231 (1982) 209–215.
[22] J.M. Grimes, R.H. Melloni Jr., Serotonin modulates offensive attack in adolescent anabolic steroid-treated hamsters, Pharmacol. Biochem. Behav. 73 (2002) 713–721.
[23] J.M. Grimes, L.A. Ricci, R.H. Melloni Jr., Glutamic acid decarboxylase (GAD65) immunoreactivity in brains of aggressive, adolescent anabolic steroid-treated hamsters, Horm. Behav. 44 (2003) 271–280.
[24] M.A. Hammond, F.A. Rowe, Medial preoptic and anterior hypothalamic lesions: influences on aggressive behavior in female hamsters, Physiol. Behav. 17 (1976) 507–513.
[25] Y. Han, M.B. Shaikh, A. Siegel, Medial amygdaloid suppression of predatory attack behavior in the cat: II. Role of a GABAergic pathway from the medial to the lateral hypothalamus, Brain Res. 716 (1996) 72–83.
[26] R.J. Harrison, D.F. Connor, C. Nowak, R.H. Melloni Jr., Chronic low-dose cocaine treatment during adolescence facilitates aggression in hamsters, Physiol. Behav. 69 (2000) 555–562.
[27] M. Haug, S. Simler, L. Ciesielski, P. Mandel, R. Moutier, Influence of castration and brain GABA levels in three strains of mice on aggression towards lactating intruders, Physiol. Behav. 32 (1984) 767–770.
[28] D.L. Kaufman, C.R. Houser, A.J. Tobin, Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions, J. Neurochem. 56 (1991) 720–723.
[29] I. Knyshevski, D.F. Connor, R.J. Harrison, L.A. Ricci, R.H. Melloni Jr., Persistent activation of select forebrain regions in aggressive, adolescent cocaine-treated hamsters, Behav. Brain Res. (in press).
[30] S. Kollack-Walker, S.W. Newman, Mating and agonistic behavior produce different patterns of fos immunolabeling in the male Syrian hamster brain, Neuroscience 66 (1995) 721–736.
[31] S. Kollack-Walker, S.W. Newman, Mating-induced expression of c-*fos* in the male Syrian hamster brain: role of experience, pheromones, and ejaculations, J. Neurobiol. 32 (1997) 481–501.
[32] C.J. Lerwill, P. Makaings, The agonistic behavior of the golden hamster, Anim. Behav. 19 (1971) 714–721.
[33] R.H. Melloni Jr., D.F. Connor, P.T. Hang, R.J. Harrison, C.F. Ferris, Anabolic–androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters, Physiol. Behav. 61 (1997) 359–364.
[34] R.H. Melloni Jr., D.F. Connor, M.S. Todtenkopf, K.R. DeLeon, P. Sanyal, R.J. Harrison, Repeated cocaine treatment activates flank marking in adolescent female hamsters, Physiol. Behav. 73 (2001) 561–570.
[35] L.L. Miller, J.M. Whitsett, J.G. Vandenbergh, D.R. Colby, Physical and behavioral aspects of sexual maturation in male golden hamsters, J. Comp. Physiol. Psychol. 91 (1977) 245–259.
[36] M. Morales, F.E. Bloom, The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon, J. Neurosci. 17 (1997) 3157–3167.
[37] L.P. Morin, R.I. Wood, A Stereotaxic Atlas of the Golden Hamster Brain, Academic Press, 2001.
[38] M. Potegal, A. Blau, M. Glusman, Effects of anteroventral septal lesions on intraspecific aggression in male hamsters, Physiol. Behav. 26 (1981) 407–412.
[39] M. Potegal, A. Blau, M. Glusman, Inhibition of intraspecific aggression in male hamsters by septal stimulation, Physiol. Psychol. 9 (1981) 213–218.
[40] M. Potegal, A.S. Perumal, A.I. Barkai, G.E. Cannova, A.D. Blau, GABA binding in the brains of aggressive and non-aggressive female hamsters, Brain Res. 247 (1982) 315–324.
[41] M. Potegal, M. Hebert, M. DeCoster, J.L. Meyerhoff, Brief, high-frequency stimulation of the corticomedial amygdala induces a delayed and prolonged increase of aggressiveness in male Syrian golden hamsters, Behav. Neurosci. 110 (1996) 401–412.
[42] L.A. Ricci, J.M. Grimes, R.H. Melloni Jr., Serotonin type-3 receptors modulate the aggression-stimulating effects of adolescent cocaine exposure, Behav. Neurosci. 118 (2004) 1097–1110.
[43] L.A. Ricci, I. Knyshevski, R.H. Melloni Jr., Serotonin type-3 receptors stimulate offensive aggression in Syrian hamsters, Behav. Brain Res. 156 (2005) 19–29.
[44] T.A. Schoenfeld, C.M. Leonard, Behavioral development in the Syrian golden hamster, in: H.I. Siegel (Ed.), The Hamster: Reproduction and Behavior, Plenum Press, New York, 1985.
[45] S. Simler, S. Puglisi-Allegra, P. Mandel, Gamma-aminobutyric acid in brain areas of isolated aggressive or non-aggressive inbred strains of mice, Pharmacol. Biochem. Behav. 16 (1982) 57–61.
[46] J.E. Smith, T.R. Koves, C. Co, Brain neurotransmitter turnover rates during rat intravenous cocaine self-administration, Neuroscience 117 (2003) 461–475.
[47] B. Soderpalm, A.I. Svensson, Naloxone reverses disinhibitory/aggressive behavior in 5,7 DHT-lesioned rats; involvement of GABA (A) receptor blockade? Neuropharmacology 38 (1999) 1851–1859.
[48] F.J. Sodetz, B.N. Bunnell, Septal ablation and the social behavior of the golden hamster, Physiol. Behav. 5 (1970) 79–88.
[49] J.J. Soghomonian, D.L. Martin, Two isoforms of glutamate decarboxylase: why? Trends Pharmacol. Sci. 19 (1998) 500–505.
[50] O. Stork, F.Y. Ji, K. Kaneko, S. Stork, Y. Yoshinobu, T. Moriya, S. Shibata, K. Obata, Postnatal development of a GABA deficit and disturbance of neural functions in mice lacking GAD65, Brain Res. 865 (2000) 45–58.
[51] C. Sur, L. Fresu, O. Howell, R.M. McKernan, J.R. Atack, Autoradiographic localization of alpha5 subunit-containing GABAA receptors in rat brain, Brain Res. 822 (1999) 265–270.
[52] M.L. Tappaz, M.J. Brownstein, Origin of glutamate–decarboxylase (GAD)-containing cells in discrete hypothalamic nuclei, Brain Res. 132 (1977) 95–106.
[53] M.L. Tappaz, M.J. Brownstein, I.J. Kopin, Glutamate decarboxylase (GAD) and gamma-aminobutyric acid (GABA) in discrete nuclei of hypothalamus and substantia nigra, Brain Res. 125 (1977) 109–121.
[54] Z. Wang, T.J. Hulihan, T.R. Insel, Sexual and social experience is associated with different patterns of behavior and neural activation in male prairie voles, Brain Res. 767 (1997) 321–332.
[55] E.M. Weerts, W. Tornatzky, K.A. Miczek, Prevention of the pro-aggressive effects of alcohol in rats and squirrel monkeys by benzodiazepine receptor antagonists, Psychopharmacology (Berl) 111 (1993) 144–152.
[56] J.M. Whitsett, The development of aggressive and marking behavior in intact and castrated male hamsters, Horm. Behav. 6 (1975) 47–57.