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Subregion-specific down-regulation of 5-HT_3 immunoreactivity in the nucleus accumbens shell during the induction of cocaine sensitization

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Abstract

Repeated exposure to psychostimulants such as cocaine and amphetamine can result in behavioral sensitization, which is believed to model the onset of drug addiction, as well as neural adaptations that occur after repeated drug abuse that lead to addictive behaviors. Dopamine (DA) in the nucleus accumbens (NAc) has been shown to play an integral role in this phenomenon. However, cocaine also acts on the serotonin (5-HT) system, which has been shown to modulate psychostimulant-induced increases in motor behavior and DA release in the NAc. Recently, it has been demonstrated that the shell portion of the NAc can no longer be considered a homogeneous structure and can be subdivided into at least five separate regions. The present study examines 5-HT_3 receptors in the subdivisions of the NAc in cocaine-sensitized rats. Rats received a sensitization-inducing regimen of cocaine (twice-daily injections of 15 mg/kg ip for five consecutive days). Two or 14 days following the last injection, rats were given a challenge injection of cocaine (15 mg/kg ip) and sacrificed 2 h later. Sections of the NAc were processed for 5-HT_3 immunoreactivity (5-HT_3-IR), and the number of puncta was quantified in each of the subregions of the shell, as well as the core of the accumbens. Repeated cocaine administration resulted in robust sensitization that correlated with a transient decrease in the density of 5-HT_3 immunoreactive puncta in the intermediate zone of the accumbens shell. After a 2-week withdrawal period, sensitized animals no longer showed any differences in any of the areas examined. These data suggest a possible role for 5-HT_3 receptors in the intermediate zone during the induction of cocaine sensitization.

Keywords: Serotonin; 5-HT_3; Dopamine; Immunocytochemistry; Shell; Sensitization

1. Introduction

Repeated intermittent administration of cocaine produces behavioral sensitization in rats (Kalivas et al., 1988; Post and Rose, 1976). Behavioral sensitization or reverse-tolerance to a drug has been defined as an increased behavioral (i.e., locomotor or stereotypic) or neurochemical (e.g., augmented dopamine release) response upon subsequent exposure to a psychostimulant, even after various periods of abstinence (Carlezon and Nestler, 2002; Robinson and Becker, 1986; White and Kalivas, 1998). Recent studies of behavioral sensitization have begun to focus on the two different phases of this phenomenon: induction and expression (for a review, see Pierce and Kalivas, 1997). Briefly, induction is the phase in which behavioral and physiological changes develop with repeated, intermittent exposure to psychostimulants. The expression phase is the resulting long-term behavioral changes that are the result of underlying neuroadaptations. The nucleus accumbens (NAc) is a brain area that is a putative site of action of drug reward (Robinson and Berridge, 1993), as well as a site involved in both the induction and expression phase of behavioral and neurochemical sensitization (Todtenkopf et al., 2002b; Pierce and Kalivas, 1997). Anatomical investigations of the NAc reveal a heterogeneous compartmentalized structure with two main subdivisions, the dorsolateral core (NAc_{CORE}) and the ventromedial shell (NAc_{SHELL}), each of which contain different afferent and efferent projections (Heimer et al., 1997; Zahm, 1999; Zahm and Brog, 1992).

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Increases in motor behavior observed with a challenge dose of cocaine have been shown to be associated with an increase in dopamine (DA) release in NAc_{SHELL} (Barrot et al., 1999; Pierce and Kalivas, 1995).

Recently, further anatomical (Jongen-Rêlo et al., 1993; Wright and Groenewegen, 1995) and functional studies from this laboratory (Todtenkopf et al., 2002a; Todtenkopf and Stellar, 2000) have demonstrated that the NAc_{SHELL} is not a homogenous subdivision of the NAc. Rather, the NAc_{SHELL} can be divided into five separate subdivisions, i.e., the vertex (NAc_{VERT}), the arch (NAc_{ARCH}), the cone (NAc_{CONE}), the intermediate zone (NAc_{INT}), and the ventrolateral zone (NAc_{LAT}), each of which has it's own neurochemical composition as well as afferent and efferent projections (for further details, see Todtenkopf et al., 2002a; Todtenkopf and Stellar, 2000). A previous assessment of tyrosine hydroxylase (TH; the rate limiting enzyme for DA synthesis) immunoreactivity (TH-IR) in these five subregions of the NAc_{SHELL} showed increases of TH-IR varicosities in apposition to perikarya in the NAc_{ARCH} and NAc_{INT} during the induction of cocaine sensitization (Todtenkopf and Stellar, 2000). Additionally, we observed an increase in c-*fos* immunoreactivity (an immediate early gene product) in the NAc_{INT} during the expression of cocaine sensitization (Todtenkopf et al., 2002a).

Serotonin (5-HT) modulates psychostimulant-induced increases in motor behavior and DA release in the NAc (Cunningham et al., 1996; Guan and McBride, 1989; Herges and Taylor, 2000). In addition, both DA and 5-HT reuptake blockade are required to mimic the behavioral and electrophysiological effects of cocaine (White et al., 1993). Indeed, the VTA (where most DA cell bodies projecting to the NAc originate) is rich with 5-HT innervation (Phelix and Broderick, 1995). The distribution of 5-HT and DA is quite similar in the two main subdivisions of the NAc (i.e., the core and shell) in that there is a greater innervation of the shell and the synaptic mechanisms are largely axodendritic (Heimer et al., 1991; Van Bockstaele and Pickel, 1993). However, studies examining the role of 5-HT as a modulator of DA in cocaine-induced behaviors have revealed brain area specific differences. For example, 5-HT has been observed to increase DA release in the NAc when administered into the VTA (Guan and McBride, 1989) while decreases in 5-HT have also been observed to increase DA in the ventral striatum (Nurse et al., 1988). Several 5-HT receptor subtypes have been elucidated in cocaine-induced behaviors. It is likely that the different functional effects of 5-HT in cocaine-related behaviors can be attributed to the differential effects of increased 5-HT at several subtypes of 5-HT receptors in the mesolimbic pathway (Peroutka et al., 1987). For example, stimulation of the $5\text{-}HT_{1B}$ receptors plays a role in the reinforcing (Neumaier et al., 2002; Parsons et al., 1996) and discriminative (Callahan and Cunningham, 1995) properties of cocaine. Blockade of $5\text{-}HT_2$ receptors has also been observed to attenuate cocaine-induced hyperactivity in mice (O'Neill et al., 1999).

Action at the $5\text{-}HT_3$ receptors is also involved in the modulation of cocaine-induced behaviors (Reith, 1990). For example, ondansetron, the $5\text{-}HT_3$ antagonist, has recently been observed to block the behavioral expression of cocaine sensitization in rats (Kankaanpaa et al., 1996; King et al., 2000). Considering that the phenomenon of cocaine sensitization has been largely attributed to the actions of DA in the mesolimbic system, specifically the NAc, and that 5-HT has been shown to effect cocaine-induced behaviors (see above), it is possible that $5\text{-}HT_3$ receptor function may contribute to the initiation or expression of cocaine sensitization. Indeed, $5\text{-}HT_3$ receptors have been observed to modulate DA cell firing in the A9 and A10 nuclei of the VTA (Batsche et al., 1992). Of particular interest is the observation by several pharmacological studies that activity at the $5\text{-}HT_3$ receptors modulates the release of DA in the NAc (Blandina et al., 1989; Chen et al., 1992; Chen et al., 1991; Costall et al., 1987; Jiang et al., 1990; Matell and King, 1997; McNeish et al., 1993).

Because previous examinations of TH-IR, as well as the distribution of c-*fos* in the NAc_{SHELL}, have yielded subregion-specific differences in cocaine-sensitized rats (Todtenkopf et al., 2002a; Todtenkopf and Stellar, 2000), the current study uses immunocytochemistry to examine the distribution of $5\text{-}HT_3$ in each of the five subregions of the NAc_{SHELL} as well as the NAc_{CORE} in cocaine-sensitized animals. Furthermore, we examine the distribution of this receptor in animals that were challenged 2 or 14 days post-repeated treatment in order to identify the role that $5\text{-}HT_3$ receptors might play in the modulation of either the short-term (induction) or the long-term effects (expression) of cocaine sensitization.

2. Materials and methods

2.1. Subjects

All procedures were conducted according to NIH guidelines (NIH Publications No. 80–23) and approved by the Institutional Animal Care and Use Committee at Northeastern University. Adult male Sprague–Dawley rats (275–325 g; Taconic Farms; Germantown, NY) were allowed free access to food and water and housed individually in plastic cages on a 12:12-h reversed light/dark cycle (lights on at 1900 h) at an ambient temperature of 22–24 °C with a controlled relative humidity of 55%. Rats were handled before the experiments began and were randomly assigned to the treatment groups.

2.2. Behavioral testing

Two groups of rats were treated as previously described (Todtenkopf et al., 2002a). Briefly, at the beginning of the study, rats were habituated for 1 h to one of four Plexiglas activity monitor chambers (43.2 × 43.2 × 30.5 cm; Med-

Associates, St. Albans, VT) prior to receiving any injections. On the following day, animals received twice-daily injections of either saline (1 ml/kg ip) or cocaine (15 mg/kg ip; Research Biochemicals International, Natick, MA) separated by approximately 6 h (one morning injection, one afternoon injection) for five consecutive days. Half of the rats from each group received a challenge injection of cocaine (15 mg/kg ip) after 2 days of withdrawal, whereas the other half received a challenge injection of cocaine (15 mg/kg ip) after 14 days of withdrawal. Thus, the four treatment conditions are as follows: saline—2 days withdrawal (Sal2D; $n=6$); saline—14 days withdrawal (Sal14D, $n=6$), and cocaine—2 and 14 days withdrawal (Coc2D, $n=10$ and Coc14D, $n=10$, respectively). Behavioral data were collected for the morning injection on the first and last day of the repeated treatment for an initial 20 min of preinjection baseline and an additional 40 min postinjection. After the respective withdrawal periods, all rats were tested with a single challenge injection of cocaine (15 mg/kg ip) as described above. Rats treated with repeated cocaine that did not demonstrate behavioral sensitization were excluded from the immunocytochemical analysis ($n=1$ and $n=2$ for the Coc2D and Coc14D groups, respectively). Sensitization was defined according to the criteria of Pierce et al. (1996a), i.e., an increase in horizontal locomotion of 20% or greater on challenge day when compared to Day 1.

Two hours following administration of the challenge injection, animals were anesthetized with sodium pentobarbital (>65 mg/kg ip) and intracardially perfused with 50 ml ice-cold 0.01 M phosphate buffer (PB; pH 7.4) containing 0.5% procaine and 2.5% sucrose (Sigma, St. Louis, MO), followed by 300 ml of ice-cold 4% paraformaldehyde solution in 0.1 M PB (pH 7.4). The brains were then removed and postfixed in the same solution overnight. Sections (40 μm) were taken through the NAc using a vibratome.

2.3. 5-HT_3 immunocytochemistry

Tissue samples from the saline treatment group, as well as samples from cocaine-sensitized animals, were processed for 5-HT_3 immunoreactivity (5-HT_3-IR) using a protocol developed in our laboratory prior to the beginning of the study. Sections were washed 3 times for 10 min (3×10) in phosphate-buffered saline (PBS) containing 0.6% triton-X 100 (PBSTx). Sections were then pretreated with 5% H_2O_2 in dH_2O for 8 min, rinsed thoroughly with PBSTx, and incubated in 1% sodium borohydride in dH_2O for 5 min. After being rinsed thoroughly in PBSTx, the tissue was incubated in PBSTx containing 10% normal goat serum (NGS) and 8% bovine serum albumin (BSA) for 90 min. The tissue was then incubated with a polyclonal rabbit anti-5-HT_3 receptor antibody (Ab-1; Oncogene Research, Cambridge, MA) diluted 1:1000 overnight at 21 °C on a slowly heating rotator in 10% NGS, 8% BSA in PBSTx. The sections were then washed 3×10 min in PBSTx followed by a 90-min incubation in biotin-conjugated goat anti-rabbit secondary antiserum (1:200; Vector Laboratories, Burlingham, CA) in PBSTx containing 0.3% BSA. The sections were rinsed (3×10) in PBSTx and incubated for 90 min in avidin–biotin complex (Vectastain ABC kit; Vector Laboratories) with 0.3% BSA in PBSTx. The peroxidase reaction was revealed using 3,3′ -diaminobenzidine (DAB Kit, Vectastain; Vector Laboratories) at one half the recommended concentration in distilled water. The sections were mounted on gel-coated slides and dehydrated through a graded series of ethanol and xylenes and coverslipped using Cytoseal 60 mounting medium (VWR Scientific, West Chester, PA).

Immunocytochemical controls consisted of either omission of the primary antisera during the overnight incubation or a preabsorption method. Briefly, the preabsorption control consisted of incubating the primary antibody solution with the 5-HT_3 receptor-peptide antigen (Anaspec) in 10, 100, and 1000× molar excess of the primary antibody at 4 °C for 90 min prior to incubation with control tissue sections.

2.4. Microscopic and statistical analysis

Slides were codified and analyzed under blinded conditions using a computer-assisted microscopic image analysis software package (Todtenkopf and Benes, 1998; Todtenkopf et al., 2000; Todtenkopf and Stellar, 2000). Each subregion was identified at low power (×2) based on the 5-HT_3-IR staining pattern (which was similar as previously described for TH (Todtenkopf and Stellar, 2000, see Fig. 1A) on a Nikon Eclipse E600 microscope (Microscope Video Instruments, Avon, MA, USA) equipped with an Optronics DEI-750 CE video camera (Optronics, Goleta, CA, USA) interfaced with a BioQuant NOVA 98 software package running on a Pentium PC computer (R&M Biometrics, Nashville, TN, USA). Subsequently, a ×100 oil immersion lens was used to quantify 5-HT_3-IR puncta within a computer-generated "box" (2705.87 μm^2 for the NAc_{CORE}, NAc_{VERT}, NAc_{CONE}, NAc_{INT}, NAc_{LAT}, and 609.66 μm^2 for the NAc_{ARCH}) placed in three separate locations within each subregion as a counting field. Using a touch count subroutine (Todtenkopf et al., 2000), the number of 5-HT_3-IR puncta was counted in each of the three areas. This procedure was repeated for all of the subdivisions of the NAc.

The number of puncta was expressed as a numerical density; that is, the number of puncta was divided by the field area for each box, and then these numbers were averaged within subregion and again across animals in each treatment group to generate a "mean-of-means." Two-way analyses of variance (ANOVAs) were performed, with subregion as the within-subjects factor and treatment group (Coc2D, Sal2D, Coc14D, and Sal14D) as the between-subjects factor. Fisher's protected least significant difference

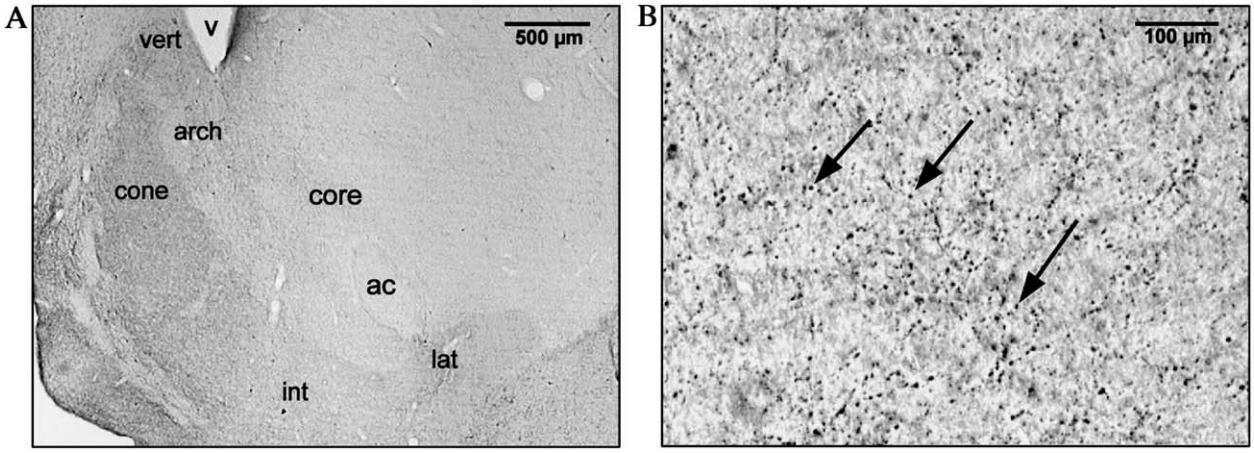

Fig. 1. (A) Low power (× 2) photomicrograph depicting the distribution of 5-HT_3-IR in the different subregions of the NAc. (B) High power (× 100) photomicrograph of the immunoreactive puncta quantified in the present study. Arrows indicate representative puncta quantified. ac = anterior commissure, v= lateral ventricle, vert= NAc_{VERT}, arch = NAc_{ARCH}, cone = NAc_{CONE}, int = NAc_{INT}, lat = NAc_{LAT}.

(PLSD) post hoc analyses were done where appropriate. Horizontal locomotor activity was analyzed with one-way repeated measures ANOVAs, with time as the repeated measure. Between-group comparisons were also analyzed with one-way ANOVAs using a Fisher's PLSD post hoc analysis where indicated.

3. Results

Repeated treatment with cocaine resulted in a progressive increase in locomotion that was still elevated upon a cocaine challenge dose. This continued elevation was present in both groups (Coc2D and Coc14D), whereas repeated saline injections had no appreciable effect on locomotor activity in either group (Sal2D and Sal14D) (Table 1). For each treatment group, the total distance traveled (cm) on the first day was compared with that of the last day of the repeated treatment and the challenge days. In addition, between-group comparisons were made on each of the days in which behavior was recorded. Cocaine-treated animals demonstrated a significant increase in horizontal locomotor behavior by the end of the treatment period [$F(1,15) = 7.53$, $P < .01$], while saline-treated animals did not [$F(1,9) = 0.49$, *ns*]. In addition, distances traveled were significantly greater in the cocaine treatment groups than the distance of the animals that were challenged with cocaine but had been treated with repeated saline both after 2 and 14 days of withdrawal [$F(1,13) = 15.86$, $P < .01$ and $F(1,11) = 11.96$, $P < .01$, respectively].

Upon gross examination (× 2) of the tissue after immunocytochemical processing, there were no noticeable staining pattern differences between saline- and cocaine-treated animals. In all of the sections, the immunoperoxidase staining revealed a staining pattern throughout the NAc (Fig. 1A) that is similar to patterns previously observed with several neuropeptides and neurotransmitters (Todtenkopf and Stellar, 2000). That is, the subregions of the NAc can be easily identified at low magnification based on the 5-HT immunoreactivity. At high magnification (× 100) (Fig. 1B), many immunoreactive puncta were seen throughout the neuropil. Tissue from one rat (from the Sal14D group) was not usable due to poor staining during processing and was excluded from microscopic analysis. Control sections, which did not include primary antisera or that had been used in the peptide blocking condition, did not yield any specific immunoreaction staining (data not shown).

The two-way ANOVA yielded a significant Treatment × Subregion interaction effect [$F(15,120) = 2.12$, $P = .01$], yet no main effect [$F(3,24) = 1.01$, *ns*]. Further analysis comparing cocaine- and saline-treated rats after 2 days of withdrawal again demonstrated a similar significant

Table 1
Cocaine-induced locomotion

Treatment	Day 1	Day 5	2-Day challenge	14-Day challenge
Cocaine	12234.08 ± 1471.40[#]	18576.46 ± 2104.06*,[#]	26200.01 ± 2900.00*,[#]	24856.88 ± 2343.38*,[#]
Saline	5073.20 ± 664.33	5288.75 ± 706.15	11463.53 ± 1969.13	11661.30 ± 2205.52

Data are presented as the mean ± S.E.M. total distance traveled (cm) recorded on Days 1, 5, and the respective challenge day. Day 1 indicates the first day of the repeated treatment for all the animals, while Day 5 indicates the first injection of the final day of the repeated treatment. Each group was then subdivided and challenged either 2 days or 14 days following the last injection of the treatment period with cocaine (15 mg/kg ip).

* = $P < .05$ compared to Day 1.

[#] = $P < .05$ compared to saline-treated controls.

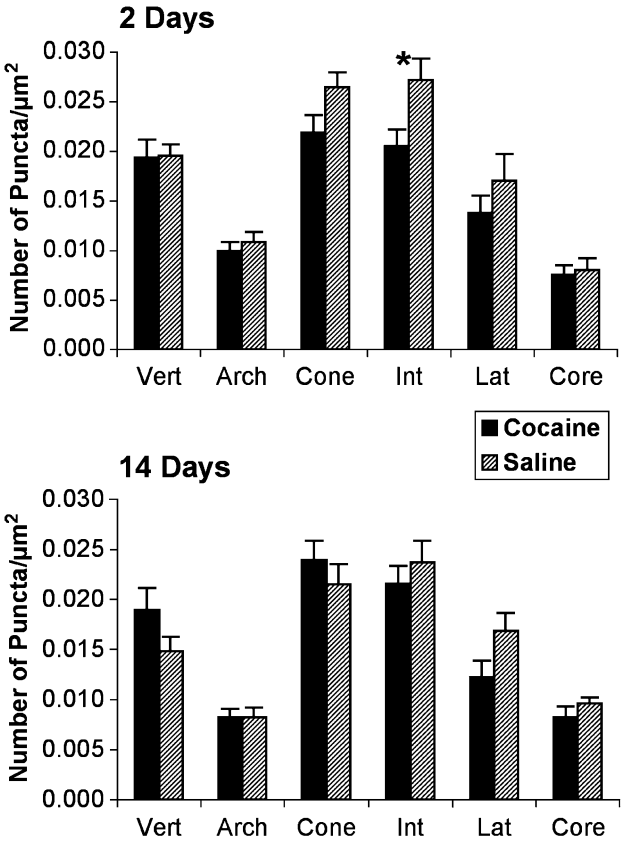

Fig. 2. Density of 5-HT_{3R}-IR puncta in six subregions of the NAc in control and sensitized animals challenged 2 days (top panel) or 14 days (bottom panel) after the last day of the repeated treatment. * $P < .05$.

interaction effect [$F(5, 65) = 2.40$, $P < .05$]. Post hoc Fisher's PLSD tests indicated that there was significantly less 5-HT_3-IR only in the NAc_{INT} [$F(1,13) = 5.94$, $P = .03$] of rats challenged after 2 days of withdrawal (see Fig. 2, top panel). When comparing cocaine- and saline-treated rats challenged after 14 days of withdrawal, there was no significant difference between the groups [$F(1,11) = 0.02$, *ns*] (see Fig. 2, bottom panel).

4. Discussion

5-HT_3-IR was decreased in the NAc_{SHELL} of cocaine-sensitized animals, specifically in the intermediate zone after 2 days of withdrawal. This decrease was no longer observed on Day 14 of withdrawal in animals that still expressed sensitization to a challenge dose of cocaine. There were no other changes in the density of 5-HT_3 immunoreactive puncta in any of the other subregions of the NAc_{SHELL} or in the NAc_{CORE} after 2 or 14 days of withdrawal. These observations suggest that 5-HT_3 receptors may play a critical region-specific role during the early stages of cocaine sensitization. Since this change appears to be transient, 5-HT_3 receptors may serve a different function during the expression phase of cocaine sensitization.

Considering these findings, 5-HT_3 receptor down-regulation may affect the induction of cocaine-induced sensitization via a change in the modulation of other monoamines. This hypothesis is consistent with pharmacological evidence provided in studies investigating the role of these receptors in other stimulant-induced behaviors (Costall et al., 1987; Kankaanpaa et al., 1996; McNeish et al., 1993; Reith, 1990). Indeed, previous assessment of TH-IR in the five subregions of the NAc_{SHELL} demonstrated an increase of TH-IR in the intermediate zone after 2 days of withdrawal, but not after 14 days (Todtenkopf and Stellar, 2000). Microstructural research indicates that serotonergic cells typically contact GABAergic cells in the NAc, with only few 5-HT cells contacting TH-IR-containing cells (Bayer and Pickel, 1991). It is possible that GABA cells in the intermediate zone of the NAc_{SHELL} express 5-HT_3 receptors that are down-regulated during the induction of sensitization and that their inhibitory role on DA signals is transiently decreased, thereby producing an observable increase in TH-IR. Hence, it is possible that the function of the GABAergic neurons in the NAc_{INT} is suppressed during the induction of cocaine sensitization and restored (or increased) during expression. It is also possible that this mechanism is confined to the NAc_{SHELL} since there has not been an observable change in the terminals of the GABA neurons located in the ventral pallidum (De Leon et al., 2000), the main projection site of the NAc (Churchill and Kalivas, 1994; Zahm and Heimer, 1990). Further evidence suggests that the NAc_{INT} may be a critical component to the response to drugs of abuse. For example, microinjections of cocaine or other indirect DA agonists (nomifensine and phencyclidine) directly into the NAc_{INT} established reliable lever pressing while microinjections in neighboring NAc regions did not (Carlezon et al., 1995; Carlezon and Wise, 1996). Together, these data indicate that the NAc_{INT} may be critically involved in mediating sensitivity to stimulant drugs.

The present data suggest that there could be a dynamic interaction among at least three neurotransmitter systems (DA, 5-HT, and GABA) in the NAc_{INT}. There is some evidence to support this hypothesis: mRNA labeling in specific brain regions suggests that 5-HT_3 receptors indirectly modulate DA via GABAergic contacts with DA cell bodies (Tecott et al., 1993). However, since there was no change in 5-HT_3 receptor density in the NAc_{ARCH}, this hypothesis does not explain the previously reported up-regulation of TH-IR in the NAc_{ARCH} after 2 days of withdrawal (Todtenkopf and Stellar, 2000). Furthermore, it has been shown that the NAc_{INT} contains relatively low levels of neurotensin (Zahm et al., 1998, p. 380), a neuropeptide that is mutually regulatory with DA (Nemeroff et al., 1983). Upon psychostimulant exposure, neurotensin levels were elevated throughout the NAc_{SHELL} except in the intermediate zone (Zahm et al., 1998). Together, these data suggest that there maybe be a deficiency in regulated DA effects over GABA, and that this is due, in part, to a reduction in 5-HT_3 receptors in the NAc_{INT}. It is

possible the change in 5-HT_3-IR was restricted to the intermediate zone of the shell as a consequence of the differing densities of 5-HT in the two main subregions of the NAc (i.e., core and shell). Indeed, the density of the 5-HT innervation in the caudomedial NAc_{SHELL} is greater than that in other subterritories (Brown and Molliver, 2000). An examination of 5-HT innervation of the more specific subregions of the NAc_{SHELL} may yield similar differences in 5-HT density, which in turn may help to elucidate the implications of the current findings.

When interpreting the current findings, one must also consider the complications that arise when evaluating the role of 5-HT_3 receptors in cocaine-induced sensitization because cocaine may have its own direct and indirect effects on the function of 5-HT receptors. Cocaine increases synaptic 5-HT levels by blocking the 5-HT transporter (Koe, 1976; Ross and Renyi, 1969), thereby augmenting the effects of 5-HT at 5-HT_3 receptors in cocaine-treated animals. In addition, cocaine itself has an affinity for 5-HT_3 receptors (Kilpatrick et al., 1987) and has been observed to act at the receptor as an antagonist (Fozard et al., 1979). The direct effects of cocaine at 5-HT_3 receptors, while less likely to occur than those of endogenous 5-HT, are not inconsequential. Several potent and selective 5-HT_3 antagonists are cocaine derivatives (Fozard, 1984; Richardson et al., 1985). Considering the cocaine activity at this receptor, it is possible that the receptors may respond to overstimulation induced by repeated cocaine administration, as indicated by the observed down-regulation. That the observed down-regulation realized in the rats challenged and sacrificed after 2 days of withdrawal is limited to only one area of the NAc_{SHELL} does not explain the absence of a change in the other subregions. Detailed examinations of the innervation of 5-HT to the five subregions of the NAc may provide insight into the specific nature of the current findings, as well as studies examining the effects of localized microinjections of 5HT receptor antagonists into the NAc_{SHELL}.

Recently, it has been observed that ondansetron (a 5-HT_3 antagonist) can eliminate tolerance to continuous cocaine administration, which is hypothesized to occur via a proposed functional down-regulation of 5-HT_3 receptors (King et al., 1999). Ondansetron has also been observed to block the expression of both short-term (King et al., 2000) and long-term cocaine sensitization (King et al., 2002). The work of King et al. raises the possibility that there would be a functional down-regulation of 5-HT_3 receptors in animals that have developed tolerance to cocaine and a correspondingly opposite effect (i.e., an increase in 5-HT_3 receptors) in cocaine-sensitized animals. Remarkably, the blockade of sensitization by ondansetron lasted as long as 28 days (King et al., 2002), while the current changes in 5-HT_3 were not present after 14 days of withdrawal. The current observations are not consistent with the hypothesized up-regulation of 5-HT_3 receptors. However, a recent study demonstrated that pretreatment with 5-HT_3 antagonists attenuated the locomotor response to cocaine during the induction of sensitization while not blocking the long-term locomotor response in sensitized rats (Szumlinski et al., 2003). Therefore, during the repeated treatment period, the effects (i.e., attenuated locomotor response to cocaine) of these receptors may due to a transient down-regulation, while the long-term role of 5-HT_3 receptors may be one that is not necessarily related to the quantity of these receptors, but rather the alterations in the affinity or sensitivity of these receptors that occur during repeated exposure to cocaine. Interestingly, after 14 days of withdrawal, the NAc_{INT} shows an increase in cell activation, as indicated by c-*fos* immunoreactivity (Todtenkopf et al., 2002a), which was not present after 2 days of withdrawal. Furthermore, it has been shown that 5-HT_3 receptors are required for amphetamine-induced striatal c-*fos* expression (Genova and Hyman, 1998). These data may be indicative of the role of 5-HT_3 receptors in the response of NAc_{INT} neurons. Together with our previous report of an increase of TH-IR after 2 days of withdrawal (Todtenkopf and Stellar, 2000), there appears to be a functional down-regulation of NAc_{INT} activity during the treatment period, which may lead to a long-term hyperactivity of these neurons in this subregion (Todtenkopf et al., 2002a).

The present study demonstrates that 5-HT systems do play a role during the induction phase of behavioral sensitization to cocaine. However, this role may be limited to the intermediate zone of the NAc. Interestingly, several reports from this laboratory have yielded results implicating the NAc_{SHELL} in the induction of sensitization (Todtenkopf et al., 2002b; Todtenkopf and Stellar, 2000; Todtenkopf et al., 2002c), whereas other reports have emphasized the NAc_{SHELL} during the expression of sensitization (Cador et al., 1995; Kalivas, 1995; Perugini and Vezina, 1994; Pierce et al., 1996b). Taken together, the NAc_{SHELL} may be a critical brain region with respect to behavioral sensitization, both during the induction and expression phases. However, the role of the NAc (and/or subregion) may differ depending on the time point in which the animals are assessed.

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