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Behavioral Changes and [^{123}I]IBZM Equilibrium SPECT Measurement of Amphetamine-Induced Dopamine Release in Rhesus Monkeys Exposed to Subchronic Amphetamine

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Previously we have shown that twelve weeks of repeated low-dose d-amphetamine (AMPH) exposure in rhesus monkeys induces a long-lasting enhancement of behavioral responses to acute low-dose challenge. The present study was designed to investigate the behavioral and neurochemical consequences of a six-week regimen of low-dose AMPH exposure (0.1–1.0 mg/kg, i.m., b.i.d.) in rhesus monkeys. SPECT imaging of AMPH's (0.4 mg/kg) ability to displace [^{123}I]IBZM bound to D2 dopamine receptors in the striatum of saline control and AMPH-treated animals prior to and following chronic treatment was accomplished using a bolus/constant infusion paradigm. Following chronic AMPH treatment, all monkeys showed an enhanced behavioral response to acute AMPH challenge and a significant decrease in the percent of AMPH-induced displacement of [^{123}I]IBZM in striatum compared to their pretreatment scans. These findings suggest that relatively small changes in presynaptic dopamine function may be reflected in significant alterations in the behavioral response to acute AMPH challenge. ***[Neuropsychopharmacology 22:4–13, 2000]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

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The term sensitization refers to the enhanced behavioral response to psychomotor stimulants, such as d-amphetamine (AMPH) and cocaine, that is shown by subjects who have had prior exposure to these agents (for review see Robinson and Becker 1986; Kalivas and Stewart 1991). Sensitization to psychomotor stimulants has been repeatedly demonstrated using behavioral and biochemical measures in rodents (e.g., Paulson et al. 1991; for reviews also see Robinson and Becker 1986; Kalivas and Stewart 1991; Nestler 1993). Conversely, few studies have documented these same processes in human and nonhuman primates.

Recently, we have shown that repeated intermittent low-dose AMPH exposure in rhesus monkeys produces progressive, persistent, and possibly "permanent" alterations in the behavioral response to an acute low dose (0.4 mg/kg, i.m.) AMPH challenge (Castner and Goldman-Rakic 1999). The long-lasting changes in behavioral response induced in the monkey are consistent

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with the development of behavioral sensitization to a low-dose AMPH challenge and suggest that there may be concomitant changes in the neurochemistry of brain dopamine systems. To date, no attempts have been made to correlate behavioral sensitization to AMPH in nonhuman primates with underlying neurochemical changes in striatum. Uncovering the long-term neurochemical changes induced by repeated low-dose AMPH exposure may provide insights into the etiology of some of the clinical features associated with diseases such as schizophrenia, as well as possible detrimental effects stemming from stimulant abuse in humans.

The development of a nonhuman primate model of repeated low-dose AMPH exposure (sensitization) offers a unique opportunity for the utilization of noninvasive techniques, such as SPECT imaging, to assess the changes underlying behavioral sensitization prior to sacrifice. Recently, there have been significant advances in techniques for imaging neurotransmitter receptors in the brain. One of these techniques, single photon emission computerized tomography (SPECT), can be used to determine Kd, BMax, and, more indirectly, the approximate quantity of neurotransmitter released presynaptically in response to drug administration (e.g., Kung and Kung 1989; Laruelle et al. 1994a, b, 1997). In nonhuman primates, displacement of the bound D2 DA receptor ligand, iodine-123 iodobenzamide ([^{123}I]IBZM), by AMPH provides a reliable index of dopamine (DA) released in the striatum as measured by *in vivo* SPECT imaging using a bolus/constant infusion paradigm (Laruelle et al. 1997). Both SPECT imaging and positron emission tomographic methods have demonstrated increased striatal DA release in response to acute AMPH administration in schizophrenic patients as compared to control subjects (Abi-Dargham et al. 1998; Laruelle et al. 1996; Breier et al. 1997).

In the present study, we employed SPECT imaging using a bolus/constant infusion method to identify possible changes in presynaptic DA release in the striatum resulting from six weeks of repeated low-dose AMPH exposure in nonhuman primates. We also examined the relationship between the behavioral and neurochemical effects of repeated low-dose AMPH exposure.

MATERIALS AND METHODS

Animals

Eight rhesus (*Macaca mulatta*) monkeys, 4 young adult males and 4 young adult females, were used in this study with an age range of 4 to 7 years. Three males and three females underwent six weeks of chronic AMPH treatment. One monkey of each gender served as a paired control and received saline injections during the chronic treatment period (see below). Monkeys were housed individually in standard size cages (male cages: 35″h × 26″d × 49″w; female cages: 30″h × 26″d × 23.5″w) and maintained on a 12:12 light dark cycle with lights on at seven A.M. Two AMPH monkeys were only used for behavioral experiments and did not undergo SPECT imaging.

Monkeys had unrestricted access to food and water (30 biscuits of standard monkey chow, fruit, and peanuts each day). The quantity of food eaten each day was recorded and the monkeys' diets were supplemented as necessary. Animals were also provided with standard enrichment devices: logs, dog toys, plastic chains, and mirrors. Animals were maintained in accordance with Yale Animal Care and Use Committee guidelines for nonhuman primates.

Baseline Behavioral Observations

Monkeys were observed in their home cage during the late morning/early afternoon to establish a baseline behavioral profile for each monkey prior to treatment. Observations were made using a focal time-sampling procedure (2.5 min per monkey) on a Macintosh laptop computer using MonkeyWatcher, a program specially developed for our purposes by Jonathon Traupman. Each monkey was observed for no less than 10 min. The MonkeyWatcher program records the total duration, frequency, number of occurrences, and average duration of a given behavior. Twenty-three keys on the keyboard were assigned specific behaviors, e.g., pacing, circling, eating, drinking, watching, scanning, presenting, lipsmacking, responding-to-stimuli, and so forth (see Castner and Goldman-Rakic 1999 for details). All behaviors exhibited by the monkeys that were not covered by the other 23 keys were recorded on the 24th key. These miscellaneous ("etcetera") behaviors most often included either individual specific responses or behaviors that were augmented by repeated AMPH exposure, e.g., responses "independent of stimuli," static posturing, parasitotic-like grooming, gnawing on toys/cage bars, twirling, swinging on bars of cage.

For the present manuscript, both the behaviors assigned to the 23 keys, as well as "etcetera" behaviors, have been reduced to three categories. Category I: Locomotor Stereotypies; Category II: Responses "Independent of Stimuli;" and Category III: Other AMPH-induced behaviors (see Table 1 of Castner and Goldman-Rakic, in preparation, for a description of each behavioral category).

Briefly, locomotor stereotypies include repetitive gross locomotor behaviors such as pacing, circling, etc. Responses "independent of stimuli" refer to behavioral responses to nonapparent stimuli, e.g., checking, tracking nothing or batting at air. Other AMPH-induced behaviors include vocalizations, grooming, fine-motor stereotypies, static posturing, submissive behaviors, and oral stereotypies or dyskinesias.

Drugs

S(+)-amphetamine sulfate was obtained from RBI (Natick, MA). Amphetamine was dissolved in sterile saline solution and filtered for i.v. injections. All injections received in the home cage were given i.m. During SPECT scans, AMPH was administered i.v.

Chronic AMPH Treatment

Monkeys received twice daily injections of AMPH or saline five days per week with weekends off for six weeks. The dose of AMPH was increased by increments of 0.1 mg/kg every three days (starting dose 0.1 mg/kg; ending dose 1.0 mg/kg). The AMPH doses used in the present experiment have been shown to produce a robust behavioral enhancement to subsequent acute challenge when administered over a 12-week period (Castner and Goldman-Rakic 1999). Paired controls received twice-daily injections of saline to control for the stress of injection and handling. During chronic treatment, the monkeys' behavioral responses to each AMPH dose were recorded by the investigator on computer and on videotape.

Pre- and Post-treatment Acute AMPH Challenges

Behavioral Assessment of the Effects of Repeated Low-Dose AMPH Exposure. Acute injections of 0.4 mg/kg AMPH were administered before and after chronic treatment and used to establish behavioral enhancement in the treated monkeys [challenge times: pretreatment and post-treatment (5, 11, and 21 days, and 6.5–8 months)]. Saline-treated control monkeys received acute AMPH challenges for behavioral assessment at only two time points: once prior to treatment and again at 21 days post-treatment.

Behavioral responses to acute challenges were recorded on computer and on videotape. After each challenge, behavioral observations were made at regular intervals for up to 96 hours post-injection to allow for behavioral "baseline" (defined as prechallenge behavior) to be reestablished.

SPECT Measurement of the Effects of Repeated Low-Dose AMPH Exposure. RADIOTRACER PREPARATION FOR SPECT EXPERIMENTS. [^{123}I]IBZM was prepared by electrophilic radioiodination of BZM [(*S*)-(–)-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide] as previously described (Kung and Kung 1989, peracetic acid as a superior oxidant).

ANIMAL PREPARATION FOR SPECT EXPERIMENTS. Animals were immobilized with ketamine (150 mg, i.m.) and atropine and subsequently anesthetized for the duration of the experiment with 2–2.5% isoflurane via an endotracheal tube. Glycopyrrolate (10 μg/kg, i.m.), a long-acting peripheral anticholinergic drug that does not cross the blood brain barrier (Proakis and Harris 1978) was administered at the beginning of the study to decrease respiratory secretions. Vital signs (pulse, respiratory rate, and body temperature) were monitored every 15 min. Body temperature was maintained and stabilized with a heated recirculating water-filled blanket.

Two intravenous indwelling catheters were used, one for AMPH administration and blood collection and the other for radiotracer infusion. During a given experiment, animals received 6.00 ± 0.94 mCi of the radiotracer IBZM, which was divided into a bolus injection (1.5 ± 0.25 mCi) administered at the beginning of the experiment and a constant infusion at a rate of (0.5 ± 0.06 mCi/h) for the remainder of each scan. Scans lasted for approximately 7 to 8 hours.

SPECT IMAGING. The animal's head was immobilized using a vacuum-pack bean-bag device, which molds to the head and hardens upon evacuation (Olympic Medical, Seattle, WA). They were positioned for image collection in planes parallel to the cantho-meatal (CM) line. Images were acquired using a CERASPECT camera (Digital Scintigraphics, Cambridge, MA). The CERASPECT camera is a multislice brain dedicated device consisting of a stationary single annular crystal surrounding three parallel hole collameters (Holman et al. 1990).

The resolution of a point source in a scattering medium is 10–12 mm FWHM (full-width at half maximum) in all three planes. Scans were acquired in step and shoot mode for 10 to 15 min throughout the experiment which lasted 480 min on average.

Images were reconstructed from photopeak events with a 20% energy window set at 143–177 keV. Tomographic images were reconstructed with a fixed filter defined as the default filter level of the acquisition recording the highest level of counts with a cutoff of 1 cm^{-1}. Images were displayed on a 64 × 64 × 32 matrix (voxel dimensions: 3.3 × 3.3 × 3.3 cm^3). Images were corrected for attenuation within an ellipse drawn around the skull, assuming uniform attenuation (μ = 0.15 cm^{-1}) equal to that of water (Zubal et al., 1990). All data were decay-corrected to time of injection.

Detailed kinetic analyses were performed by outlining regions of interest (ROIs): right and left striata, occipital lobe, and cerebellum. The same ROI template was then used for all acquisitions from subsequent studies. Data were expressed in CPM's (i.e., counts per min per pixel) derived from the ROIs and reflected the average density of regional activity (i.e., total activity divided by the area of the ROI). For each study, the raw data was corrected for infusion rate by dividing the raw counts for each ROI (e.g., striatum, cerebellum) for each 10 min acquisition period by the infusion rate of the isotope for that study.

Two periods, during each scan, were chosen for comparison to determine the ability of an acute AMPH injection to displace [^{123}I]IBZM: 1) a baseline that included six 10-min acquisitions immediately prior to AMPH chal-

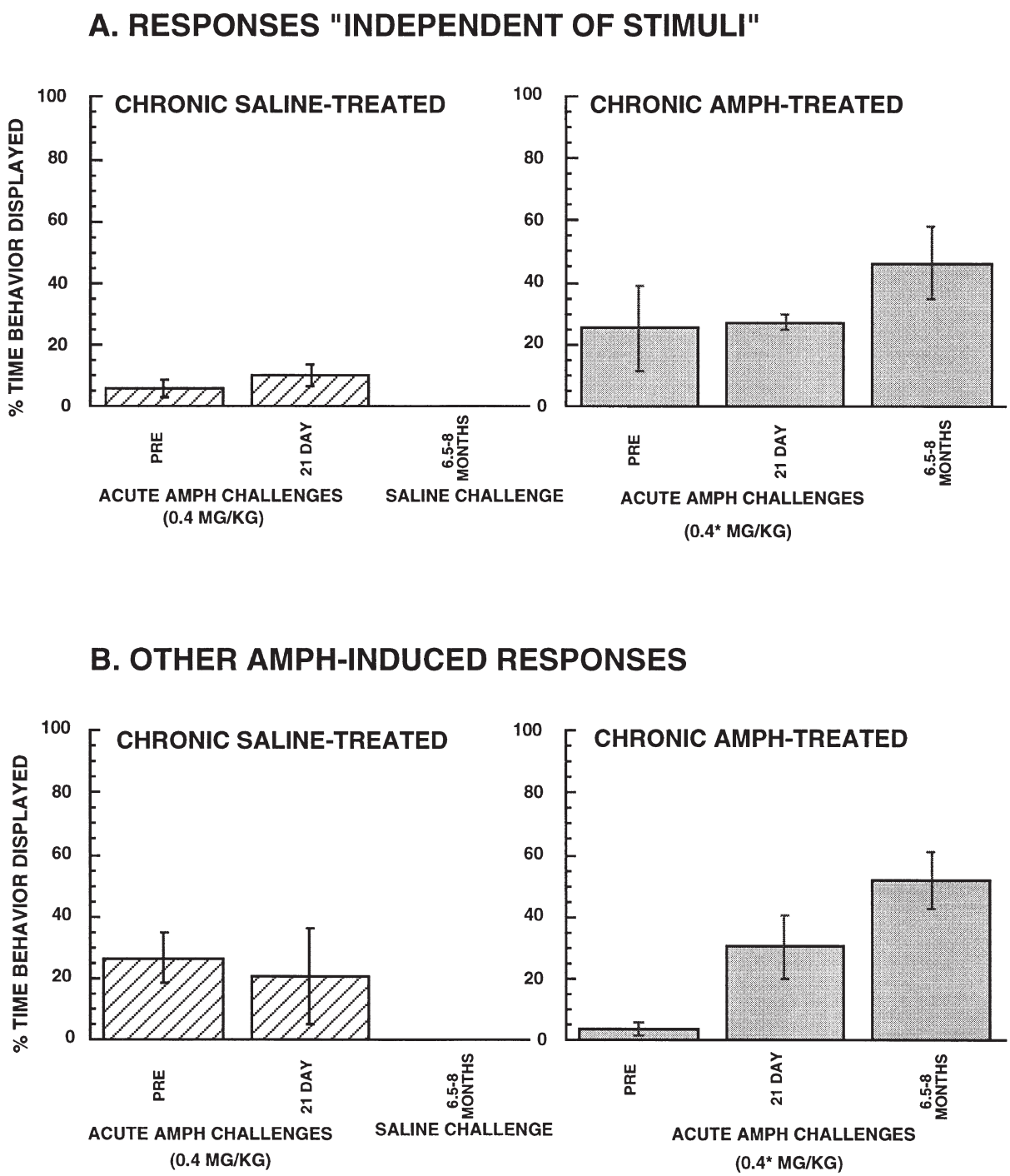

Figure 1. Panels **(A)** and **(B)** show responses "independent of stimuli" and other AMPH-induced responses for the saline-treated and AMPH-treated monkeys in response to pre- and post-treatment AMPH challenges. As can be seen on the left side of panels A and B, the chronic saline-treated monkeys did not show signs of enhanced behavioral responses to acute AMPH challenge at 21 days post-treatment. By contrast, the monkeys that received six weeks of repeated low dose AMPH exposure showed a trend for increased expression of responses "independent of stimuli" and a significant increase (see Results) in other AMPH-induced behaviors in response to acute AMPH challenge post-treatment.

lenge; and 2) another six 10-min acquisitions taken usually during the second hour post-AMPH, after the peak dopamine release has subsided. The percent change in displacement of each ROI in each study was calculated using the following formula: {[average count (baseline) − average count (post-AMPH)]/ average count (baseline)}* 100. The ratio of percent change in the striatal/cerebellar ratio was calculated by dividing the percent

Table 1. Percent Bound [^{123}I]IBZM Displaced in Response to Acute AMPH Challenge Prior to and Following Repeated Low-Dose AMPH Exposure

Monkey Name	Chronic Treatment	Scan/AMPH Challenge Time	Striatum/Cerebellum
BRIS (M)	AMPH	pretreatment	12.70*
"	"	post-treatment (30 d)	9.50*
WEST (M)	AMPH	pretreatment	8.34*
"	"	post-treatment (30 d)	−0.50
PHYL (F)	AMPH	pretreatment	13.00*
"	"	post-treatment (30 d)	−2.50
MARG (F)	AMPH	pretreatment	9.91*
"	"	post-treatment (30 d)	5.22*
BRON (M)	SALINE	pretreatment	5.68*
"	"	post-treatment (30 d)	8.64*
MAE (F)	SALINE	pretreatment	1.61
"	"	post-treatment (30 d)	2.77

Shown are the times of AMPH challenge for the AMPH-treated (Bris, West, Phyl, and Marg) and saline-treated monkeys (Bron and Mae). Pretreatment refers to scans conducted prior to chronic exposure (AMPH or saline) and post-treatment indicates scans performed at approximately 30 days after chronic treatment. F and M indicate which monkeys are females and males, respectively. The right column shows percent change in striatal/cerebellar ratios (corrected for infusion rates; see formula in Methods section) from pre- to post-AMPH injection for pre- and post-treatment scans for each monkey. As can be seen, all AMPH-treated monkeys showed a significant decrease in the percent displacement of bound [^{123}I]IBZM in the striatum in response to acute AMPH at the 30 day post-treatment challenge compared to their pretreatment challenge. Conversely, both control animals showed increased displacement after chronic saline treatment.

* indicate significance at an alpha level of .05 in a paired *t*-test.

change in striatum by the percent change in cerebellum.

CONTROL SPECT SCANS. Three animals were scanned to determine an adequate bolus to infusion ratio of the radiotracer in rhesus monkeys that can presumably generate a state of equilibrium in the brain and plasma over a period of time during which an AMPH challenge can be administered (Laruelle et al. 1994a, b). After bolus injection of [^{123}I]IBZM as a (1.3 ± 0.4 mCi), the animals were scanned for up to 8 hrs with a constant infusion of radiotracer (0.4 ± 0.1 mCi/hr).

AMPH SPECT SCANS. To assess the effects of chronic AMPH exposure, monkeys received two scans: 1) pretreatment AMPH scan; and 2) post-treatment AMPH scan. All monkeys received at least one post-treatment AMPH scan done at approximately 30–35 days post-withdrawal. The data were expressed as a percent ratio using a graphics program (KaleidaGraph™ version 2.1, Synergy Software, Redding, PA). Statistical calculations were made using a paired (two-tailed) *t*-test.

PLASMA CONCENTRATIONS OF PARENT COMPOUND. The plasma concentration of parent tracer was measured by extraction and chromatography as described for [^{123}I]IBF (Baldwin et al. 1994).

RESULTS

Pre- and Post-treatment Acute AMPH Challenges

Behavioral Assessment of the Effects of Repeated Low-Dose AMPH Exposure. In contrast to the saline-treated monkeys, the chronic AMPH-treated monkeys showed a trend for an increase in the time spent engaged in responses "independent of stimuli" across challenges as indicated by paired two-tailed *t*-tests (see Figure 1A; saline (left panel): paired *t*-value = −0.43, p = .7422; AMPH: (right panel) 21-day vs. pre-paired *t*-value = −1.00, p = .3626; 6.5–8 mos vs. pre-paired *t*-value = −1.82, p = .1285); and a significant (significance defined as $p < .05$) enhancement in other AMPH-induced behaviors (e.g., fine-motor stereotypies, oral stereotypies, buccolingual dyskinesias, parasitotic-like grooming, static posturing) from pre- to post-treatment challenges (Figure 1B; saline (left panel): paired *t*-value = 0.48, p = .716; AMPH: (right panel) 21-day vs. pre-paired *t*-value = −2.96, p = .03; and 6.5–8 mos vs. pre-paired *t*-value = −7.02, p = .0009).

As reported previously, not all monkeys displayed each AMPH-induced behavioral response, as responses augmented by repeated low-dose AMPH exposure were specific to each individual monkey. Moreover, six weeks of intermittent AMPH exposure did not produce an enhancement in gross locomotor stereotypies (Category I) in response to acute challenge post-treatment (saline : paired *t*-value = −0.90, p = .5318; AMPH: 21-day vs. pre-paired *t*-value = −2.09, p = .0912; and 6.5–8 mos vs. pre-paired *t*-value = −1.26, p = .2617; data not shown).

CONTROL SPECT SCANS. Time activity curves generated from the SPECT images showed the expected concentration of activity in striatum, a brain region that has a high density of D2 dopamine receptors. Other regions, e.g., occipital cortex and cerebellum, showed less activity, consistent with a lower density of D2 receptors in these re-

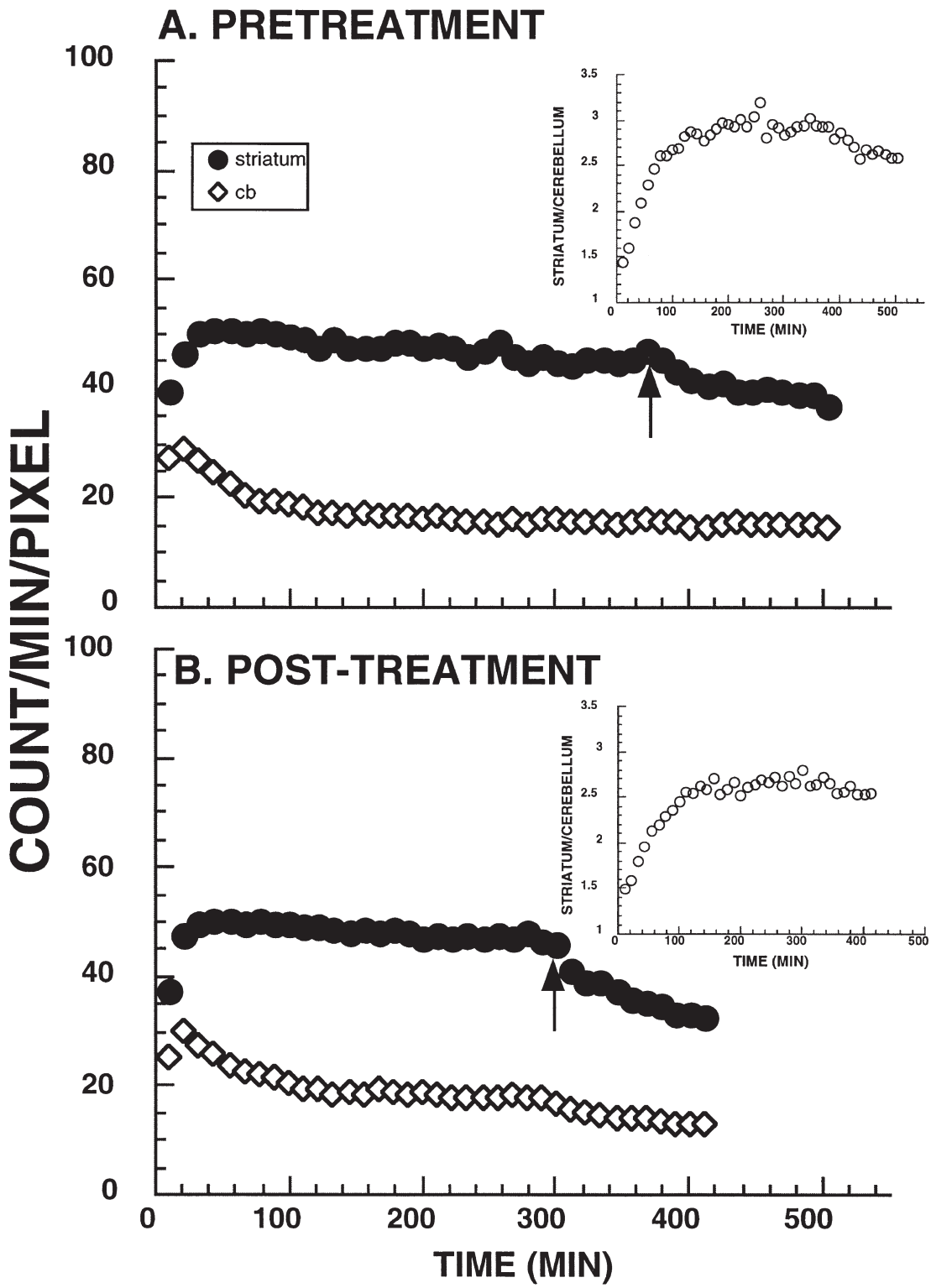

Figure 2. Shown are pre- **(A)** and post-treatment **(B)** AMPH scans for an AMPH-treated female monkey. Activity in the striatum and cerebellum is shown in panels A and B in counts/min/pixel. The insets in each panel reflect the striatal/cerebellar ratio for each experiment. The time of AMPH injection is indicated by an arrow. As can be seen in the inset to panel B, repeated AMPH exposure induced a significant decrease in the percentage of bound [^{123}I]IBZM displaced in the striatum in response to an acute AMPH challenge.

gions. The period of stability prior to AMPH injection varied between scans depending on the bolus/infusion ratio.

SPECT Measurement of the Effects of Repeated Low-Dose AMPH Exposure. For almost all AMPH scans, there was a significant change in the percent displacement as measured by the striatal/cerebellar ratio in response to an acute AMPH injection (0.4 mg/kg, i.v.) which provided an indirect measure of dopamine-induced displacement of bound [^{123}I]IBZM in the striatum (Table 1; * indicate paired *t*-tests for which the *p* value obtained was less than .05).

For the AMPH-treated monkeys, there was a significant decrease in the percent displacement of bound [^{123}I]IBZM in the striatum by acute AMPH following chronic treatment (Figure 2; Table 1). Conversely, both of the saline-treated monkeys showed increased displacement following chronic saline treatment (see Table 1).

Plasma Concentrations of Parent Compound. Plasma concentrations of the parent tracer remained relatively constant throughout the duration of both pre- and post-treatment scans. For scans in which plasma samples were taken, fluctuations in plasma concentrations of the parent compound, i.e., altered hepatic metabolism of IBZM, did not account for changes in the binding of IBZM to striatal D2 dopamine receptors.

DISCUSSION

The present study is the first to use the bolus/constant infusion method to examine AMPH's ability to displace [^{123}I]IBZM bound to striatal D2 dopamine receptors in rhesus monkeys. We provide evidence that six weeks of repeated intermittent low-dose AMPH exposure induces a long-lasting (i.e., $>$ 6.5 mos) enhancement of behavioral responses to subsequent acute low dose AMPH challenge, and is consistent with our previous findings for a 12-week AMPH regimen (Castner and Goldman-Rakic 1999). Second, this first demonstration of AMPH-induced displacement of bound [^{123}I]IBZM in the striatum of rhesus monkeys indicates that repeated low-dose AMPH exposure produces a significant decrease in the percent displacement of bound [^{123}I]IBZM in the striatum by acute AMPH challenge.

The most likely explanation for the long-lasting behavioral enhancement observed in the AMPH-treated monkeys is the development of behavioral sensitization, a phenomenon which has been well-documented in rodents (for review see Robinson and Becker 1986; Kalivas and Stewart 1991; also see Paulson et al. 1991). In monkeys, 12 weeks of repeated low-dose AMPH exposure induces a long-lasting behavioral enhancement to acute AMPH challenge that is persistent through more than three and one-half years post-withdrawal (Castner SA, Goldman-Rakic PS (unpublished observations, in preparation) Selective prefrontal cortical lesions in rhesus monkeys entrance behavioral sensitization to the locomotor-stimulating effects of amphetamine).

While the present behavioral data supports the phenomenon of behavioral sensitization to AMPH in monkeys, this conclusion is tentative since the possibility of repeated low-dose AMPH exposure inducing either "permanent" or reversible neural toxicity of brain dopamine systems can not be entirely ruled out until the animals are sacrificed and their brains carefully examined (e.g., Ridley et al. 1983; Ellison and Ratan 1982; Ellison et al. 1978; Melega et al. 1997; Villemagne et al. 1998). However, since the decrease in AMPH's ability to displace bound [^{123}I]IBZM in striatum was relatively small, and, preliminary histological evidence supports the integrity of dopaminergic markers in the striatum of an AMPH-treated monkey (Castner and Goldman-Rakic 1999), extensive neural toxicity of this system is unlikely.

This study has demonstrated that repeated low-dose AMPH exposure produces a decrease in the percent displacement of bound [^{123}I]IBZM to striatal D2-like dopamine receptors by acute AMPH challenge. While the absolute percent change in displacement between pre- and post-treatment scans observed here was relatively small, the pattern was consistent across all AMPH-treated monkeys. The most obvious question to confront is why the effects of repeated low-dose AMPH exposure appear to be much greater for behavioral as compared to neurochemical measures. On the one hand, it is possible that small changes in presynaptic dopamine function can be reflected in significant changes in behavioral responsivity to AMPH. Alternatively, there are a number of potential/methodological as well as theoretical explanations for the discrepancy. One possibility is that 30-day post-withdrawal is too short to observe neurochemical evidence of sensitization in the primate. However, enhancement of AMPH-stimulated striatal dopamine release has been observed as early as 21 days post-withdrawal in rodents and behavioral enhancement to repeated AMPH exposure as early as 5 days post-withdrawal in monkeys (Paulson et al. 1991; Castner and Goldman-Rakic 1999).

It is possible that significant differences exist between rodents and nonhuman primates in the neurochemical basis underlying sensitization to AMPH, i.e., alterations in dopaminergic neural transmission in the striatum may be less affected by repeated AMPH exposure in the primate in light of the expansion of the dopamine system in primates (Williams and Goldman-Rakic 1998). However, coincident *in vivo* microdialysis experiments, as well as MRI co-registration, might help to identify more specific ROIs. Also, our imaging results with the bolus/constant infusion method suggest that there are significant differences in the metabolism of IBZM in rhesus as compared to both baboons and humans. Thus, in future studies it might be useful to

conduct more control (non-AMPH) scans in each animal in order to determine an optimal bolus/infusion ratio specific to each animal. In addition, it might be possible to minimize metabolic variance between animals by using only ovariectomized females.

The results obtained here may have been limited by the resolution of the camera used to acquire the images, e.g., if changes occurred in the nucleus accumbens they would not have been resolved. Such changes have been shown to be critical for expression of behavioral sensitization in rodents (e.g., Nestler 1993). However, preliminary studies in our laboratory do indicate that dopamine turnover (HVA/DA ratio as assessed by HPLC) is increased in the dorsal striatum following repeated low dose AMPH exposure (Castner et al. unpublished observations). Finally, as IBZM is not specific to the D2 receptor but also has an affinity for D3 receptors, changes in D2 receptor densities might not be easy to detect.

In addition to methodological explanations, there are also structural and/or functional changes which could help to explain the divergence between behavioral and neurochemical effects of repeated AMPH treatment in monkeys. It is possible that repeated low-dose AMPH exposure in the monkey induces postsynaptic changes in receptor function that are not able to be detected by the present methodology. For instance, while the evidence for AMPH-induced changes in absolute receptor densities is confusing and linked to the time of examination relative to withdrawal, there is convincing evidence for changes in the functional, i.e., second messenger systems, state of post-synaptic dopamine receptors in brain regions linked to sensitization to drugs of abuse (e.g., Miserendino and Nestler 1995; Nestler and Aghajanian 1997; Nestler 1993; Terwilliger et al. 1991; Duman et al. 1988).

In conjunction with these types of functional changes, it is also possible that repeated exposure to AMPH induces a reduction in the affinity of postsynaptic striatal dopamine receptors for endogenous dopamine. In this scenario, increases in AMPH-stimulated dopamine release following chronic exposure to the drug might not be detectable by simply examining AMPH's ability to displace [^{123}I]IBZM bound to striatal D2-like dopamine receptors. Such alterations in postsynaptic efficacy could help to account for both the decreased ability of AMPH to displace [^{123}I]IBZM bound to striatal D2 dopamine receptors as well as behavioral enhancement to acute AMPH challenge post-treatment.

Another possible explanation for the relatively small neurochemical effects of repeated AMPH exposure might reside pre-synaptically. For instance, it may be the case that increases in the presynaptic dopamine response to AMPH are not positively correlated with increased behavioral responses to the same stimulus. In fact, there are a number of studies in the rodent that indicate that AMPH's ability to augment dopamine release and its ability to augment behavioral responses following either acute or chronic treatment are dissociable (e.g., Kuczenski et al. 1991; Kuczenski and Segal 1989, 1990). Moreover, some recent microdialysis studies on behavioral sensitization to AMPH in rodents suggest that increases in the release of other striatal neurotransmitters, e.g., acetylcholine, more closely parallel the time course of the emergence of behavioral sensitization (Bickerdike and Abercrombie 1997). In nonhuman primates, it is certainly conceivable that changes in other neuronal systems, such as the nucleus accumbens and prefrontal cortex, might be more important for the expression of behavioral sensitization to AMPH than changes in the striatum (Castner and Goldman-Rakic, in preparation).

Finally, unlike the imaging results, the behavioral measures were obtained in the animals home cages without the potential confounds of other drugs in the system. While it is unlikely that ketamine administered six hours prior to AMPH interfered with it's ability to release DA, isolflurane's potential effects on brain dopamine systems are not well understood. Therefore, DA concentrations measured in the anesthetized state may not mimic DA's response to AMPH in the awake, behaving state. Moreover, this interpretation could help to explain the differences between the findings in the present experiment for sensitized monkeys and those previously reported for schizophrenics (Abi-Dargham et al. 1998; Laruelle et al. 1996; Breier et al. 1997).

In summary, the findings of the present study are consistent with findings from imaging studies in humans. For example, imaging studies in former cocaine addicts have also shown decreased dopamine responsivity in the striatum (e.g., Volkow et al. 1997). Moreover, even in the human SPECT studies (Abi-Dargham et al. 1998; Laruelle et al. 1996) done with IBZM only approximately one third of the schizophrenics showed increased dopamine release in the striatum in response to acute AMPH challenge as compared to control subjects. Therefore, the present findings do not invalidate the value of behavioral sensitization to AMPH as a useful animal model and suggest that relatively small changes in striatal dopamine function can have significant effects on behavioral output.

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