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# Metabolic Mapping of the Effects of Cocaine during the Initial Phases of Self-Administration in the Nonhuman Primate

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Because most human studies of the neurobiological substrates of the effects of cocaine have been performed with drug-dependent subjects, little information is available about the effects of cocaine in the initial phases of drug use before neuroadaptations to chronic exposure have developed. The purpose of the present study, therefore, was to define the substrates that mediate the initial effects of cocaine in a nonhuman primate model of cocaine self-administration using the 2-[$^{14}$C]deoxyglucose method. Rhesus monkeys were trained to self-administer 0.03 mg/kg per injection ($N = 4$) or 0.3 mg/kg per injection ($N = 4$) cocaine and compared with monkeys trained to respond under an identical schedule of food reinforcement ($N = 4$). Monkeys received 30 reinforcers per session, and metabolic mapping was conducted at the end of the fifth self-administration session. Cocaine self-administration reduced glucose utilization in the mesolimbic system, including the ventral tegmental area, ventral striatum, and medial prefrontal cortex. In addition, metabolic activity was increased in the dorsolateral and dorsomedial prefrontal cortex, as well as in the mediodorsal nucleus of the thalamus. These latter effects are distinctly different from those seen after the noncontingent administration of cocaine, suggesting that self-administration engages circuits beyond those engaged merely by the pharmacological actions of cocaine. The involvement of cortical areas subserving working memory suggests that strong associations between cocaine and the internal and external environment are formed from the very outset of cocaine self-administration. The assessment of the effects of cocaine at a time not readily evaluated in humans provides a baseline from which the effects of chronic cocaine exposure can be investigated.

*Key words: cocaine; prefrontal cortex; striatum; nucleus accumbens; self-administration; rhesus monkeys*

In recent years neuroimaging studies have provided substantial information about the underlying neural substrates of the effects of cocaine in humans. Studies using such strategies have made central contributions toward the identification of the specific neural circuits and neurotransmitters subserving the distinct functional responses associated with discrete aspects of addiction. The majority of studies investigating the neurobiological basis of the effects of cocaine in humans have been performed with cocaine-dependent subjects, at times in active treatment programs. Because substantial structural and functional changes are thought to accompany chronic cocaine use (for review, see Strickland et al., 1998; Volkow et al., 1999; Kaufman and Levin, 2001), the functional responses of chronic drug abusers used in most human studies are likely to be quite different from those of subjects with minimal drug exposure. Few studies, however, have been performed in human subjects with little or no drug experience. Without an evaluation of the functional response to cocaine in the earliest stages of drug exposure, it is not possible to understand the basis for the transition from casual drug use to addiction.

An alternate approach to studies in humans is the use of animal models in which carefully controlled experiments can be conducted. Studies in our laboratory (Lyons et al., 1996; Porrino and Lyons, 2000) have shown that the acute administration of cocaine to monkeys significantly alters rates of cerebral metabolism in limbic brain regions, including medial and orbitofrontal cortex, medial temporal areas, striatum, and anterior thalamus. Although these studies demonstrated that exposure to cocaine produces a discrete pattern of neural activation in animals that had not previously been exposed to cocaine, cocaine was administered noncontingently.

Self-administration of drugs as compared with their passive or noncontingent administration facilitates the effects of rewarding electrical stimulation (Moolten and Kornetsky, 1990), as well as decreases the stress associated with drug administration (Dworkin et al., 1995; Mutschler and Miczek, 1998). Similarly, dopamine levels are augmented in both the nucleus accumbens (Hemby et al., 1997) and amygdala (Wilson et al., 1994) after cocaine self-administration as compared with yoked controls receiving cocaine passively. Moreover, rats self-administering cocaine displayed a pattern of glucose utilization that is significantly different from animals receiving equivalent amounts of cocaine noncontingently (Graham and Porrino, 1995). These issues have been circumvented in the present study by use of cocaine self-administration.

The purpose of the present study was to characterize the effects of reinforcing doses of cocaine on cerebral metabolism in a nonhuman primate model of cocaine self-administration, using the quantitative 2-[$^{14}$C]deoxyglucose (2-DG) method. In this report we describe the findings from experiments in which mon-

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keys self-administered cocaine for only 5 d, a model of the earliest stages of cocaine exposure, a time when adaptational responses to chronic drug exposure are most likely to be minimal. A second purpose of these studies was to determine the role of the dose of self-administered cocaine in determining the pattern of changes in rates of glucose utilization. Groups of monkeys self-administered either a dose of cocaine (0.03 mg/kg per injection) that maintained peak response rates or a dose (0.3 mg/kg per injection) on the descending limb of the dose–response curve and were compared with control monkeys in which responding was maintained by food presentation under the same schedule of reinforcement.

## MATERIALS AND METHODS

*Subjects.* Twelve experimentally naive adult male rhesus monkeys (*Macaca mulatta*) weighing between 7.6 and 11.5 kg (mean ± SD; 9.5 ± 1.04) at the start of the study served as subjects. Monkeys were individually housed in stainless steel cages with water available *ad libitum*; animals had physical and visual contact with each other. Their body weights were maintained at ~90–95% of free-feeding weights by banana-flavored pellets earned during the experimental sessions and by supplemental feeding of Lab Diet Monkey Chow (PMI Nutrition International, Brentwood, MO), provided no sooner than 30 min after the session. All procedures were performed in accordance with established practices as described in the National Institutes of Health Guide for Care and Use of Laboratory Animals. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of Wake Forest University.

*Behavioral apparatus.* Cocaine self-administration and food-reinforced responding occurred in ventilated and sound-attenuated operant chambers (1.5 × 0.74 × 0.76 m; MedAssociates, East Fairfield, VT) designed to accommodate a primate chair (model R001; Primate Products, Redwood City, CA). The chamber contained an intelligence panel (48 × 69 cm), located on the right side and consisted of two retractable levers (5-cm-wide) and three stimulus lights. The levers were positioned within easy reach of the monkey sitting in the primate chair. One gram food pellets were delivered from a feeder located on the top of the chamber. For cocaine self-administering animals, a peristaltic infusion pump (7531–10; Cole-Parmer Co., Chicago, IL) delivered drug injections at a rate of ~1 ml/10 sec. Operation of the chambers and data acquisition were accomplished with a Power Macintosh computer system with an interface (MedAssociates).

*Surgical procedures.* All monkeys, including controls, were surgically prepared, under sterile conditions, with an indwelling intravenous catheter and vascular access port (model GPV; Access Technologies, Skokie, IL). Monkeys were anesthetized with a combination of ketamine (15 mg/kg, i.m.) and butorphanol (0.03 mg/kg, i.m.), and an incision was made near the femoral vein. After blunt dissection and isolation of the vein, the proximal end of the catheter was inserted into the vein for a distance calculated to terminate in the vena cava. The distal end of the catheter was threaded subcutaneously to an incision made slightly off the midline of the back. The vascular access port was placed within a pocket formed by blunt dissection near the incision. Before each experimental session, the back of the animal was cleaned with 95% ethanol and betadine scrub, and a 22 gauge Huber Point Needle (model PG20–125) was inserted into the port leading to the venous catheter, connecting an infusion pump, containing the cocaine solution, to the catheter. Before the start of the session, the pump was operated for ~3 sec, filling the port with the dose of cocaine that was available during the experimental session. At the end of each session, the port was filled with heparinized saline (100 U/ml) to help prevent clotting. In addition at the time of the venous catheterization, each monkey was implanted with a chronic indwelling catheter into the adjacent femoral artery for collection of timed arterial blood samples during the 2-DG procedure. The surgical procedures were identical to those described for the venous catheters.

*Self-administration procedures.* Monkeys were initially trained to respond on one of two levers by reinforcing each response on the correct lever with a 1 gm banana-flavored pellet. Over approximately a 3 week period the interval between availability of food pellets was gradually increased until a 3 min interval was achieved (i.e., fixed interval, 3 min schedule; FI 3 min). Under the final schedule conditions, the first response on the lever after 3 min resulted in the delivery of a food pellet; sessions ended after 30 food presentations. At the end of each session, the response levers were retracted, house lights and stimulus lights were extinguished, and animals remained in the darkened chamber for 30 min before they were returned to their home cages. All monkeys responded under the FI 3 min schedule of food presentation for at least 20 sessions and until stable performance was obtained (±20% of the mean for three consecutive sessions, with no trends in response rates). When food-maintained responding was stable, the feeder was unplugged, and the effects of extinction on responding were examined for 5 consecutive sessions, after which responding was re-established and maintained by food presentation.

After baseline performance had been established, all monkeys were surgically prepared with venous and arterial catheters, as described above, and randomly assigned to one of three groups. One group of monkeys served as controls ($n = 4$) and continued to respond under the FI 3 min schedule of food presentation. The remaining eight monkeys were assigned ($n = 4$/group) to either a low-dose cocaine self-administration group (0.03 mg/kg per injection) or a high-dose cocaine self-administration group (0.3 mg/kg per injection). Food-maintained performance was allowed to stabilize after surgery (~4–6 d) before cocaine self-administration sessions were begun. All sessions ended after animals obtained 30 reinforcers. As during training, animals remained in the darkened experimental chambers with levers retracted for 30 min after the final reinforcer was obtained. Daily experimental sessions, conducted at approximately the same time each day, continued for 5 d. At the end of the session on day 5, the 2-DG procedure was conducted.

*Measurement of local cerebral glucose utilization.* For these experiments, the animals' catheters exited through an opening in the rear of the chamber, allowing all infusions and sampling to be accomplished remotely with minimum disruption to the animal. The 2-DG procedure was initiated at the end of the last session, 2 min into the time out by the infusion of an intravenous pulse of 75 μCi/kg 2-deoxy-D-[$^{14}$C]glucose (DuPont NEN, Boston, MA; specific activity 50–55 mCi/mmol) followed by a flush of heparinized saline. Timed arterial blood samples were drawn thereafter at a schedule sufficient to define the time course of the arterial 2-[$^{14}$C]deoxyglucose and glucose concentrations. Arterial blood samples were centrifuged immediately. Plasma $^{14}$C concentrations were determined by liquid scintillation spectrophotometry (Beckman Instruments, Fullerton, CA), and plasma glucose concentrations were assessed using a glucose analyzer (Beckman Instruments). The animals were killed by an intravenous overdose of sodium pentobarbital (100 mg/kg) ~45 min after tracer injection. Brains were removed rapidly, blocked in three parts, frozen in isopentane (−45°C), and stored at −70°C until they were processed for autoradiography. Coronal sections (20-μm-thick) were cut in a cryostat maintained at −22°C. Four of every 20 sections were thaw-mounted on glass coverslips, dried on a hot plate, and apposed to Kodak (Rochester, NY) MR-1 film for 15–30 d, along with a set of [$^{14}$C]methylmethacrylate standard (Amersham, Arlington Heights, IL) previously calibrated for their equivalent $^{14}$C concentration in 20 μm brain sections. Autoradiograms were developed in Kodak GBX developer, indicator stop bath, and rapid fix at 68°C.

Quantitative densitometry of autoradiograms was accomplished with a computer-assisted image-processing system (Imaging Research, St. Catharines, Ontario, Canada). Optical density measurements for each structure were made in a minimum of eight brain sections. Measurements were made bilaterally and averaged across hemispheres. Tissue $^{14}$C concentrations were determined from the optical densities and a calibration curve obtained by densitometric analysis of the autoradiograms of the calibrated standards. Glucose utilization was then calculated using the operational equation of the method (Sokoloff et al., 1977), local-tissue $^{14}$C concentrations, the time course of the plasma 2-[$^{14}$C]deoxyglucose and glucose concentrations, and the appropriate kinetic constants (Kennedy et al., 1978). Because of differences in the baseline levels of glycemia in some animals, the lumped constant was adjusted appropriate to the glucose levels according to procedures based on previous work (Kennedy et al., 1978; Schuier et al., 1990; Suda et al., 1990). Identification of brain structures was accomplished by comparison with adjacent thionin-stained sections.

*Statistical analysis.* Standard statistics software (SPSS for Windows, Chicago, IL) was used for statistical analysis. Response rates maintained by food and cocaine are presented as the mean (± SEM) for all monkeys in a group. Data were analyzed using repeated measures ANOVA. Rates of glucose utilization were measured in 57 discrete brain regions. Global rates of cerebral metabolism were estimated as the mean (weighted by region size) of all measured cerebral regions. Global rates were analyzed

**Table 1. Performance of monkeys responding under a fixed-interval 3 min schedule**

| | Food | Cocaine 0.03 mg/kg per injection | Cocaine 0.3 mg/kg per injection |
|---|---|---|---|
| Pre-extinction food rate (responses/min) | 7.93 ± 1.6 | 6.83 ± 1.7 | 8.03 ± 2.6 |
| Total pre-extinction sessions | 27.0 ± 4.9 | 33.5 ± 8.4 | 25.5 ± 4.0 |
| Food extinction rate (responses/min) | 3.3 ± 3.0 | 0.13 ± .05 | 1.25 ± 0.7 |
| Mean cocaine rate (responses/min) | — | 3.28 ± 1.32 | 0.53 ± 0.13 |
| Post-extinction session length (min) | 143.4 ± 23.7 | 150.0 ± 38.2 | 235.5 ± 19.1* |
| Total cocaine intake (mg/kg) | — | 4.5 ± 0.0 | 45.0 ± 0.0 |

*One-way ANOVA, $F_{(2,9)} = 7.43$; $p < 0.05$. Least significant difference, cocaine 0.03 mg/kg per injection versus 0.3 mg/kg per injection; $p < 0.05$; food control versus 0.3 mg/kg.

by means of a one-way ANOVA. Values of rates of local cerebral glucose utilization obtained for each individual cerebral structure were analyzed by means of a one-way ANOVA followed by least squares difference test for multiple comparisons comparing values of glucose utilization of each group self-administering either 0.03 or 0.3 mg/kg per injection cocaine to values of rates of glucose utilization of food-reinforced controls.

## RESULTS

### Behavior

All monkeys were trained to respond under an FI-3 min schedule of food presentation. There were no differences in baseline response rates among the groups (Table 1). After ~3 weeks of responding under this schedule, the pellet dispenser was unplugged, and responding extinguished over five consecutive sessions, during which time response rates decreased in all monkeys. After extinction, food-maintained responding was re-established, after which time each monkey was surgically prepared with indwelling intravenous and intra-arterial catheters. Cocaine self-administration at either 0.03 mg/kg per injection or 0.3 mg/kg per injection was initiated in two of the groups. This was accomplished by substituting cocaine for food presentation. Self-administration sessions continued for five daily sessions. For the four control monkeys, responding continued to be maintained by food presentation.

After 5 d of cocaine exposure, the mean rate of responding maintained by 0.03 mg/kg per injection was 3.3 responses/min compared with a mean rate of 0.5 responses/min maintained by 0.3 mg/kg per injection (Table 1), whereas the mean rate of responding maintained by food in the control group was 3.1 responses/min. These rates were significantly different from each other ($F_{(2,9)} = 9.16$; $p < 0.05$). Mean session length during the 5 d of cocaine exposure was 143.4 min for food controls and 150.0 min for monkeys self-administering 0.03 mg/kg per injection as compared with 235.5 min for monkeys self-administering 0.3 mg/kg per injection. Session length was significantly longer in this latter group than in the other two groups (Table 1).

For the monkeys self-administering 0.03 mg/kg per injection cocaine, rates of responding were significantly higher than response rates during extinction (Table 1), indicating that responding was indeed maintained by cocaine presentation. Response rates by monkeys self-administering the higher dose were low, as would be expected for a dose on the descending limb of the cocaine dose–response curve. Over the five consecutive sessions that cocaine was available, each monkey received the maximum number of injections per session (i.e., 30), which totaled, over the course of the study, 4.5 and 45 mg/kg cocaine for the low- and high-dose groups, respectively. A more detailed analysis of these data has been previously reported (Nader et al., 2002).

### Local cerebral glucose utilization

Rates of local cerebral glucose metabolism for the 57 brain regions examined are shown in Table 2. Global rates of metabolism did not differ significantly across groups: food control, 36.6 ± 1.6; cocaine self-administration (0.03 mg/kg per injection), 35.8 ± 1.0; and cocaine self-administration (0.3 mg/kg per injection), 36.1 ± 0.8. Although there were no overall global changes, significant differences in rates of glucose utilization were detected in 12 of 57 discrete brain regions in the low dose self-administration group and 21 of 57 regions analyzed in the higher dose self-administration group, when rates were compared with rates of food-reinforced control monkeys. These differences are described in detail below.

#### *Mesolimbic system and related limbic structures*

Nomenclature for and identifications of structures within the mesolimbic system were according to the atlas of Paxinos et al. (2000), as well as reports of Haber et al. (1995), Haber and McFarland (1999), Martin et al. (1991), and Amaral et al. (1992).

Self-administration of the lower dose of cocaine (0.03 mg/kg per injection) significantly decreased rates of cerebral glucose utilization throughout the ventral striatum and other related limbic areas. These included the rostral portions of the nucleus accumbens (−20.4%), the shell of the nucleus accumbens (−13.5%), as well as the olfactory tubercle (−19.6%). Glucose utilization was also decreased in the bed nucleus of the stria terminalis (−19.8%) and the lateral preoptic nucleus (−14.7%). In contrast, self-administration of the lower dose of cocaine did not significantly alter glucose utilization in any portion of the septum nor amygdala, or in any other portion of the hypothalamus or the ventral tegmental area (Table 2).

Self-administration of the higher dose of cocaine (0.3 mg/kg per injection) significantly decreased rates of cerebral glucose utilization in all portions of the nucleus accumbens (Fig. 1) including, rostral (−21.5%), shell (−27%), and core (−22.6%), as well as in the olfactory tubercle (−25.5%). Self-administration of this dose of cocaine also decreased glucose utilization in the bed nucleus of the stria terminalis (−23.8%) and the ventral tegmental area (−9.8%). Similar to the effects of the self-administration of the lower dose, glucose utilization was not significantly altered in any portion of the septum, nor amygdala, nor hypothalamus (Table 2).

#### *Basal ganglia and related structures*

Nomenclature and identification of structures within the basal ganglia were according to the atlas of Paxinos et al. (2000). Self-administration of the lower dose of cocaine (0.03 mg/kg per injection) significantly decreased rates of cerebral glucose utili-

**Table 2. Effects of cocaine self-administration on rates of local cerebral glucose utilization in rhesus monkeys[a]**

| | Food | Cocaine 0.03 mg/kg/inj | Cocaine 0.3 mg/kg/inj |
|---|---|---|---|
| Prefrontal cortex | | | |
| Orbital (Areas 11, 12, 13) | 44.3 ± 2 | 44.3 ± 3 | 47.4 ± 3 |
| Medial (Areas 14, 24, 25, 32) | 36.4 ± 2 | 29.3 ± 1* | 30.3 ± 1* |
| Dorsolateral (Areas 45, 46) | 44.6 ± 4 | 51.5 ± 4* | 55.8 ± 2* |
| Anterior insular (Iai, Iam, Ial, G) | 34.2 ± 3 | 25.2 ± 2* | 23.9 ± 3* |
| Dorsomedial (Area 9) | 42.5 ± 3 | 54.1 ± 3* | 59.0 ± 2* |
| Other cortex | | | |
| Area 6 | 48.7 ± 3 | 48.8 ± 5 | 45.8 ± 1 |
| Area 4 | 49.1 ± 1 | 46.1 ± 5 | 50.1 ± 3 |
| Area 5 | 50.4 ± 5 | 54.6 ± 5 | 57.2 ± 4 |
| Area 7 | 46.8 ± 3 | 44.4 ± 4 | 47.0 ± 5 |
| Temporal pole | 40.3 ± 2 | 40.6 ± 2 | 39.0 ± 3 |
| Entorhinal cortex | 36.2 ± 4 | 34.8 ± 3 | 31.8 ± 2 |
| Precommissural striatum | | | |
| Rostral caudate | 59.8 ± 4 | 46.5 ± 4* | 48.3 ± 2* |
| Rostral putamen | 60.0 ± 5 | 48.3 ± 5 | 50.0 ± 2 |
| Caudal caudate | 60.5 ± 3 | 51.0 ± 5 | 49.2 ± 2* |
| Caudal putamen | 61.8 ± 4 | 50.5 ± 5 | 47.7 ± 2 |
| Rostral accumbens | 48.0 ± 5 | 38.2 ± 3* | 37.7 ± 2* |
| Nucleus accumbens-shell | 44.5 ± 3 | 33.3 ± 4* | 32.5 ± 2* |
| Nucleus accumbens-core | 46.5 ± 3 | 40.2 ± 7 | 36.0 ± 2* |
| Olfactory tubercle | 47.0 ± 3 | 37.8 ± 3* | 35.0 ± 3* |
| Basal ganglia | | | |
| Ventral tegmental area | 31.0 ± 1 | 27.8 ± 2 | 27.2 ± 1* |
| Parabrachialis pigmentosus | 31.3 ± 1 | 30.2 ± 2 | 30.0 ± 2 |
| Substantia nigra pars compacta | 46.8 ± 1 | 50.9 ± 4 | 45.4 ± 2 |
| Substantia nigra pars reticulata | 39.9 ± 1 | 37.4 ± 3 | 43.9 ± 2* |
| Globus pallidus-external | 29.8 ± 3 | 31.0 ± 3 | 36.5 ± 3* |
| Globus pallidus-internal | 30.8 ± 4 | 32.0 ± 4 | 40.5 ± 2* |
| Ventral pallidum | 36.2 ± 2 | 27.8 ± 3 | 30.3 ± 3 |
| Subthalamic nucleus | 56.5 ± 8 | 64.5 ± 9 | 68.3 ± 5* |
| Red nucleus | 42.5 ± 4 | 50.5 ± 4 | 56.3 ± 6* |
| Thalamus | | | |
| Ventral anterior | 38.5 ± 5 | 39.3 ± 3 | 41.8 ± 1 |
| Anterior ventral | 49.0 ± 5 | 55.5 ± 2 | 62.2 ± 3* |
| Paraventricular | 34.6 ± 2 | 34.7 ± 2 | 36.8 ± 3 |
| Mediodorsal-magnocellular | 45.5 ± 4 | 55.3 ± 4* | 61.8 ± 4* |
| Mediodorsal-parvicellular | 49.2 ± 4 | 61.5 ± 4 | 70.5 ± 5* |
| Centromedian | 40.0 ± 5 | 46.0 ± 2 | 50.3 ± 7 |
| Parafascicularis | 42.5 ± 3 | 51.6 ± 3* | 53.3 ± 3* |
| Ventralposterior | 38.1 ± 5 | 44.2 ± 2 | 48.0 ± 5 |
| Ventroposterolateral | 33.1 ± 3 | 38.8 ± 4 | 40.8 ± 3 |
| Limbic Regions | | | |
| Lateral amygdala | 36.1 ± 2 | 35.5 ± 2 | 39.5 ± 2 |
| Basal-medial amygdala | 41.4 ± 1 | 39.8 ± 4 | 44.0 ± 3 |
| Basal-lateral amygdala | 36.1 ± 1 | 33.4 ± 3 | 37.5 ± 3 |
| Central amygdala | 24.8 ± 1 | 22.8 ± 3 | 25.2 ± 1 |
| Medial amygdala | 27.5 ± 1 | 25.5 ± 3 | 28.8 ± 1 |
| Medial septum | 31.6 ± 3 | 31.0 ± 3 | 28.8 ± 2 |
| Lateral septum | 28.8 ± 3 | 27.7 ± 3 | 23.8 ± 2 |
| Bed nucleus of stria terminalis | 28.2 ± 2 | 22.6 ± 4* | 21.5 ± 1* |
| Extended amygdala | 32.4 ± 2 | 24.8 ± 3 | 26.6 ± 2 |
| Hippocampus (CA1) | 40.2 ± 2 | 41.5 ± 3 | 40.0 ± 3 |
| Hippocampus (CA3) | 34.3 ± 1 | 36.0 ± 2 | 34.5 ± 3 |
| Hippocampus (Dentate gyrus) | 35.8 ± 1 | 38.5 ± 3 | 35.3 ± 2 |
| Subiculum | 42.5 ± 2 | 43.5 ± 3 | 43.0 ± 2 |
| Hypothalamus | | | |
| Medial preoptic area | 34.1 ± 3 | 28.4 ± 1 | 29.3 ± 4 |
| Lateral preoptic area | 30.0 ± 2 | 25.6 ± 1* | 27.6 ± 1 |
| Lateral | 28.3 ± 1 | 24.1 ± 2 | 30.1 ± 3 |
| Dorsomedial | 26.2 ± 2 | 24.6 ± 2 | 26.0 ± 2 |
| Ventromedial | 29.0 ± 1 | 24.3 ± 1 | 26.7 ± 2 |
| Paraventricular | 26.3 ± 2 | 23.8 ± 2 | 25.3 ± 2 |
| Posterior | 38.3 ± 6 | 28.8 ± 5 | 32.1 ± 6 |

[a]Data represent rates of glucose utilization (μmol/100 gm/min) expressed as mean ± SEM.

*$p < 0.05$ different from food control, one-way ANOVA followed by a *post hoc* least squares difference test comparing self-administration groups to food controls.

zation throughout the caudate nucleus as measured in both the rostral (−22.2%) and caudal (−15.7%) portions of the precommissural striatum, levels at which the nucleus accumbens was represented. Self-administration of this dose of cocaine did not alter rates of glucose utilization in any other portion of the basal ganglia (Table 2).

Self-administration of the higher dose of cocaine (0.3 mg/kg per injection) significantly decreased glucose utilization in the precommissural caudate nucleus (rostral, −19.2%; caudal, −18.7%), as did self-administration of the lower dose (Fig. 1), but in contrast to the lower dose also significantly elevated rates of glucose utilization in both the internal (+31.5%) and external (+22.5%) globus pallidus. Elevated rates were also observed in the substantia nigra pars reticulata (+10.0%) and the red nucleus (+32.5%). No other significant alterations in glucose utilization were noted throughout other portions of the basal ganglia.

### *Thalamus*

Nomenclature for and identification of thalamic nuclei was according to Olzewski (1952). Self-administration of the lower dose of cocaine (0.03 mg/kg per injection) produced robust increases in cerebral metabolism in the mediodorsal nucleus of the thalamus. Elevated rates were observed in both the magnocellular (+21.5%) and parvicellular (+25%) divisions. In addition, glucose utilization was increased in the parafascicular nucleus (+21.4%). Similar to the lower dose, self-administration of the higher dose of cocaine also produced increased rates of cerebral metabolism within the magnocellular (+35.8%) and parvicellular (+43.3%) divisions of the mediodorsal thalamus (Fig. 2, Table 2). Significant increases were also noted in the anterior ventral thalamus (+26.9%), parafascicular nucleus (+25.4%), and the ventral posterior nucleus (+26.0%).

### *Cerebral cortex*

The prefrontal cortex was parcellated according to Carmichael and Price (1994). Other cortical areas were subdivided according to the atlas of Paxinos et al.(2000). Changes in rates of glucose utilization were restricted to the territories of the prefrontal cortex (Table 2). Within limbic prefrontal cortices, self-administration of the lower dose of cocaine decreased rates of glucose utilization in the prefrontal cortex along the medial wall (−19.5%), including areas 14, 24, 25, and 32, as well as within the anterior insula (−26.3%), including areas Iai, Iam, Ial, and G). In contrast, significant increases in rates of glucose utilization were

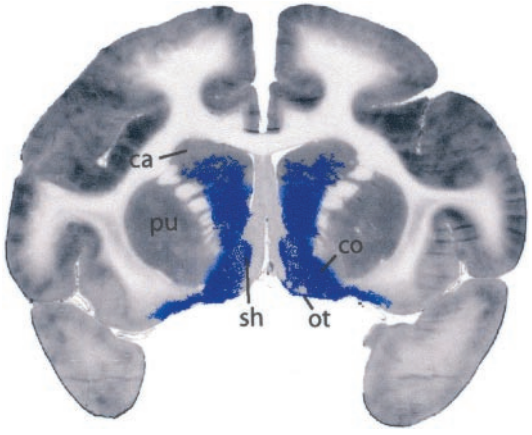


*Figure 1.* Areas of cerebral metabolic response produced by self-administered cocaine in the striatum of rhesus monkey. Shown are representative autoradiograms of 2-[$^{14}$C]deoxyglucose uptake in coronal sections at a caudal level of the precommissural striatum, +2.5 from bregma (Paxinos et al., 2000). Areas shown in *blue* depict those areas in which significant decreases in rates of glucose utilization were measured. *sh,* Nucleus accumbens-shell; *co*, nucleus accumbens-core; *ot,* olfactory tubercle; *cc,* caudal caudate; *cp*, caudal putamen.

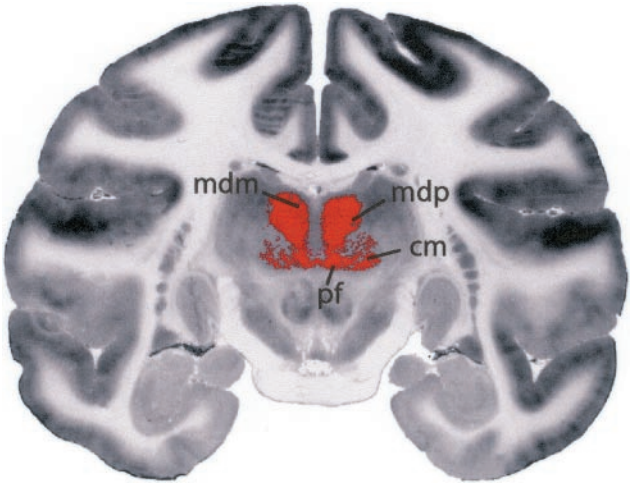


*Figure 2.* Areas of cerebral metabolic response produced by self-administered cocaine in the thalamus of rhesus monkey. Shown are representative autoradiograms of 2-[$^{14}$C]deoxyglucose uptake in coronal sections at the level of the mediodorsal nucleus, −14 from bregma (Paxinos et al., 2000). Areas shown in *red* indicate significant increases in rates of glucose utilization. *mdm*, Mediodorsal nucleus-magnocellular division; *mdp*, mediodorsal nucleus-parvicellular division; *pf,* parafascicular nucleus; *cm*, centromedian nucleus.

found in the dorsomedial prefrontal cortex (area 9; +27.3%) and in the regions of the lateral prefrontal cortex (areas 45 and 46; +15.5%). Cocaine self-administration at this dose did not alter cerebral metabolism in any other portion of cortex analyzed, including areas 5, 6, 7, 8 and the temporal pole (Table 2).

Self-administration of the higher dose of cocaine produced similar cortical changes in glucose utilization as reported for the lower dose group. These changes included decreases in medial (−16.8%) and anterior insula cortex (−30.1%), and increases in dorsomedial (+38.8%) and dorsolateral (+25.1%) prefrontal cortex (Fig. 3, Table 2). Again, no other changes were observed in other cortical regions after self-administration of this dose of cocaine.

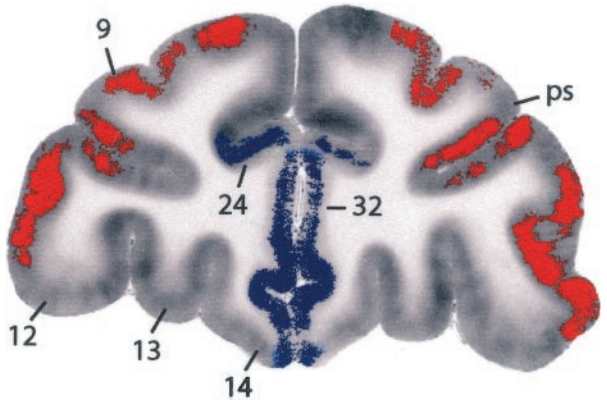


*Figure 3.* Areas of cerebral metabolic response produced by self-administered cocaine in the prefrontal cortex of rhesus monkey. Shown are representative autoradiograms of 2-[$^{14}$C]deoxyglucose uptake in coronal sections midway through the prefrontal cortex, +9.5 from bregma (Paxinos et al., 2000). Areas shown in *red* indicate significant increases in rates of glucose utilization. Areas shown in *blue* depict those areas in which significant decreases in rates of glucose utilization were measured. *12*, Area 12; *13*, area 13; *14*, area 14; *32*, area 32; *24*, area 24; *9*, area 9; *ps*, principal sulcus.

## DISCUSSION

The present findings demonstrate that self-administration of cocaine produces alterations in functional activity, as reflected by rates of local cerebral glucose utilization, within a broadly distributed, yet highly interconnected, constellation of brain regions. These changes were focused in limbic-related brain areas and included portions of brain reward circuitry, i.e., the nucleus accumbens, extended amygdala, ventral tegmental area, and medial and ventral prefrontal cortices. But in addition to these regions, cocaine self-administration activated the dorsolateral and dorsomedial prefrontal cortex and the thalamic nuclei with which they are reciprocally connected (Kievit and Kuypers, 1977; Goldman-Rakic and Porrino, 1985). This pattern of activation represents the effects of cocaine during the initial phases of drug self-administration, just after the acquisition of this behavior but before the likely advent of any significant biological and behavioral adaptations. Although these animals were exposed to cocaine for only 5 d, their response rates on the fifth day of self-administration indicate that the drug functioned as a reinforcer. This early time point was chosen to model initial drug experimentation in humans, when cocaine use is still considered casual or recreational before a transition to an addictive state. To date, investigations of the substrates of cocaine exposure in humans have used subjects that have had long and variable histories of cocaine use, frequently combined with extensive experience with a variety of legal and illegal substances. The effects of cocaine administration in human studies encompass a broad expanse of sensorimotor, association, and limbic cortex (London et al., 1990; Stapleton et al., 1995; Breiter et al., 1997; Gollub et al., 1998). This is in sharp contrast to the effects observed here in the earliest phases of cocaine exposure where only prefrontal cortex was involved. This suggests that the adaptations to chronic cocaine use may be marked by a widening influence of cocaine throughout the brain. The initial experience with the subjective sensations induced by cocaine, particularly the positive reinforcing effects, is the starting point from which these adaptations develop. Therefore, the present findings in a nonhuman primate model provide a baseline from which the time course and pro-

gression of the adaptations that accompany chronic cocaine use can be compared.

### Comparisons with effects of noncontingent cocaine presentation

The effects of cocaine self-administration reported here differ from previous work in which cocaine was administered noncontingently by the experimenter to drug-naive animals (Lyons et al., 1996). Cocaine self-administration resulted in a more restricted distribution of changes in functional activity the striatum and medial and orbital prefrontal cortex. In other regions, such as the bed nucleus of the stria terminalis, alterations in functional activity were present only in self-administering animals. These differences are consistent with previous reports in which self-administration and passive cocaine administration produced different behavioral (Dworkin et al., 1995; Mutschler and Miczek, 1998), neurophysiological (Carelli et al., 1993), and neurochemical (Wilson et al., 1994; Hemby et al., 1997; Broadbear et al., 1999) consequences, and emphasize the importance of the behavioral context of drug administration as a critical determinant of the functional response to cocaine.

The most striking difference between the distribution of functional changes associated with self-administration and passive administration, however, was the elevation in cerebral metabolic rates within the mediodorsal thalamus (Fig. 2) and the dorsolateral and dorsomedial prefrontal cortex (Fig. 3) of the self-administering animals. These, like other elevations in rates of cerebral metabolism, result from increases in synaptic activity as well as increased afferent input to these brain regions. The dorsolateral and dorsomedial cortices are among those areas activated during the performance of a broad range of cognitive tasks that require the monitoring of complex information in working memory or the planning of behavior for future goals (for discussion, see Baddeley, 1986 or Fuster, 1997). A common feature among these tasks is the ability to keep or maintain information online over time. In the course of performing cognitive tasks, neurons in this area can display sustained activity during the delays between stimulus presentation and a required response. The cortical activation seen in the present study may reflect a continued representation of the environmental context associated with cocaine self-administration that persists after the end of the session. This continued activation at a time when access to cocaine has ceased may constitute the basis for the formation of memories for the multifactored cocaine cues that can elicit strong cravings, even after long periods of abstinence. In fact, elevations in rates of glucose utilization within these areas are similar to the effect observed in investigations of cue-elicited craving in human cocaine abusers (Grant et al., 1996; Garavan et al., 2000). The effects of cocaine, therefore, are not restricted solely to limbic networks, but involve those brains areas that mediate complex cognitive processes, even at the outset of experience with self-administration.

### Topography of cerebral metabolic alterations

In a number of brain regions the effects of self-administered cocaine on rates of glucose utilization were primarily dose-dependent. This was most evident within regions of the basal ganglia where dose-dependent increases were seen in the substantia nigra reticulata, the internal and external segments of the globus pallidus, subthalamic nucleus, and red nucleus. This pattern in cerebral metabolic change within the basal ganglia reflects the motor activating effects of cocaine. Monkeys displayed increasing levels of behavioral agitation with higher doses, and in some cases there was some evidence of stereotypies when animals were removed from their experimental chambers after self-administration sessions. The presence of a clear dose–response relationship in these regions indicates that the direct pharmacological actions of cocaine self-administration are responsible for these changes.

There was an absence of a dose–response relationship, however, within the mesolimbic system (Fig. 1), including the ventral tegmental area and the nucleus accumbens. These metabolic alterations were essentially of equivalent magnitude, despite the 10-fold difference in total intake between groups. Although the absence of a dose–response relationship may reflect a full saturation of dopamine transporters and/or receptors by dopamine in these areas, it is also possible that during acquisition of cocaine reinforcement, the environmental context and the behavioral repertoire associated with self-administration may have made a greater contribution to these changes than the actual dose of cocaine itself.

The measurement of rates of glucose utilization took place during a scheduled timeout at the conclusion of the final session, thus eliminating the confounds of differing response rates of individual animals during the experimental sessions. This timing also eliminated the presence of any anticipatory or expectancy effects that would have been present if the 2-DG procedure had taken place during or before self-administration sessions. This may help to explain the absence of significant alterations of functional activity in the amygdala. Nuclei within the amygdala are central to the acquisition and expression of stimulus–reward relationships (for review, see Everitt et al., 1999) and have been shown to be involved in conditioned aspects of cocaine self-administration as well as cue- and drug-related craving (Grant et al., 1996; Childress et al., 1999; Kilts et al., 2001). Because these states were not a factor in the current design, the failure of cocaine self-administration to alter functional activity within the amygdala is consistent with its role in conditioned reinforcement.

### Basis of the changes in cerebral metabolism

The decreases in functional activity prominent in dopamine-rich areas, particularly the striatum, are in keeping with what is known about the actions of cocaine on cellular activity. In acute electrophysiological studies, the predominant response of striatal neurons to dopamine agonists is a reduction in cell excitability (Hu and White, 1996; O'Donnell and Grace, 1996; Zhang et al., 1998; Nicola and Deadwyler, 2000). Perhaps more pertinent for the present findings are the results of studies in which *in vivo* electrophysiological recordings of nucleus accumbens neurons were performed in rats self-administering cocaine (Peoples et al., 1998). There was a significant decrease in mean firing rates within the ventral striatum over the course of self-administration sessions when compared with firing rates during pre-drug and post-drug periods. This overall tonic inhibition seen *in vivo*, as well as reduced cell excitability seen in acute preparations, is likely to translate into reduced rates of glucose utilization.

The direction of the changes in striatal glucose utilization accompanying cocaine exposure is opposite to the increased neural activation of some previous reports (Breiter et al., 1997; Howell et al., 2002). These reports are based on hemodynamic measurements accomplished with magnetic resonance imaging or positron emission tomography. Blood flow measures are generally short in duration with the signal measured over seconds or a few minutes, whereas metabolic measures used in the present study

are on a much longer time scale, requiring up to 60 min for signal acquisition. The changes in the activity of ventral striatal neurons that accompany cocaine self-administration occur on multiple time scales as well. Depressed neuronal firing rates seen across the session contrast with increased firing rates in the seconds just before and after a reinforced lever press (Peoples et al., 1998, 1999; Nicola and Deadwyler, 2000). Decreased metabolic rates are likely to reflect the tonic decreases in excitability across the session, whereas the increased signal observed in studies using hemodynamic measures may reflect the increased activation that occurs in anticipation of and just after cocaine administration. The inconsistencies then, may be a function of the underlying substrates of the measurements. Furthermore, subtle differences in the nature of the drug experience measured by these different imaging methods, especially the presence of expectancy in human studies, may exist as well.

In summary, cocaine self-administration in its earliest stages resulted in changes in functional activity in a widely distributed network of brain regions that included not only mesocorticolimbic pathways, but corticothalamic circuits involving the dorsolateral and dorsomedial prefrontal cortex as well. The involvement of cortical areas subserving working memory suggests that strong associations between cocaine and the internal and external environment are formed from the very outset of experience with cocaine. Although intravenous self-administration in nonhuman primates is only a model of drug use in humans and may not completely represent all of the elements of human cocaine seeking, the assessment of the effects of cocaine self-administration at a time point not readily evaluated in humans establishes a baseline from which shifts in the effects of cocaine that accompany chronic exposure can be investigated in future studies. Understanding this progression may provide insights into the neurobiological basis of the transition to addiction.

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