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Research Report

One hour, but not six hours, of daily access to self-administered cocaine results in elevated levels of the dopamine transporter

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ABSTRACT

We have previously shown that brief (1 h) and extended (6 h) daily access to IV cocaine self-administration produce different behavioral and neural consequences following 2 weeks of drug withdrawal. Brief daily access produced stable consumption of the drug and, after withdrawal, a sensitized locomotor response and an enhanced c-Fos labeling to a single cocaine challenge. In contrast, extended daily cocaine self-administration produced escalation of drug consumption over trials but no enhanced behavioral or neurochemical response after withdrawal. Cocaine affects dopaminergic (DA) function by binding to the presynaptic transporter and thereby preventing reuptake of the neurotransmitter—an action thought to be responsible for the drug's reinforcing properties. In an extension of our previous work, the current study, using receptor autoradiography, compared binding (by [3H]WIN35428) of the dopamine transporter (DAT) in animals having experienced either brief or extended daily access to cocaine over 8 days, followed by 14 days of withdrawal. DAT densities were found to increase in the nucleus accumbens core (N.Acc Core) and the dorsal striatum (but not in the N.Acc shell, medial prefrontal cortex (mPFC), or ventral tegmental area (VTA)) of the 1-h, but not 6-h, subjects. In other words, elevations in DAT density were not associated with the 6-h access group, the group that models patterns of drug-use in human addicts, and therefore are likely to be independent of the neuroadaptations that occur in the "addictive" process. Such conclusions are also consistent with brain-imaging studies of human cocaine addicts. Additional research will be needed to identify the specific neural changes relevant to addiction.

1. Introduction

Every year, about three million Americans use cocaine at least once, many of them teenagers. Of these, roughly 6% will become addicted in the span of 2 years (Banken, 2004; Chen and Kandel, 2002; O'Brien and Anthony, 2005; Sloboda, 2002). While our knowledge regarding the neuronal substrates that support cocaine self-administration and the neuroadaptations that accompany limited use of cocaine has advanced tremendously in the last few decades, the changes that mediate the transition from limited, controlled cocaine administration to escalated, uncontrolled cocaine administration are still largely unknown. In order to study these changes, we adopted a model in which rats are given either brief daily access to cocaine and show stable pattern of cocaine administration, or extended daily access to cocaine

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and show an escalated pattern of cocaine self-administration (Ahmed and Koob, 1998; Ben-Shahar et al., 2004). We reasoned that looking at the differences between these two groups, and comparing them to control animals that receive access to saline alone, will advance our knowledge about the neuroadaptations that mediate the transition from drug naïve (our saline control animals) to recreational drug use (the brief 1 h/day access group), and from recreational drug use to addiction (the extended 6 h/day access condition).

We previously found that after 14 days of withdrawal, rats from the brief access group showed a sensitized locomotor response and elevated c-Fos reactivity to self-administered cocaine challenge. In contrast, extended daily access to cocaine did not produce such sensitized responses, but rather induced locomotor and immunoreactive c-Fos responses to the self-administered cocaine challenge that were not different from those of saline control animals (Ben-Shahar et al., 2004). These results support the notion that the brief and extended access conditions result in qualitatively different neuroadaptations and that the transition to addiction likely involves changes in brain function that either counteract, or are simply different from, those associated with recreational drug use. The current project sought to continue exploring the differences and changes in brain function that are associated with these two conditions of drug access.

Cocaine binds to catecholamine transporters and to muscarinic and sigma receptors in the central nervous system. However, it was shown that cocaine's ability to bind to the dopaminergic transporter (DAT) is critical for its reinforcing effects (Ritz et al., 1987, 1988). Similarly, in human cocaine addicts, it was found that cocaine induced-euphoria was correlated with levels of DAT occupancy by cocaine (Volkow et al., 1996a,b, 1997). We therefore chose to monitor changes in the function of the DAT as reflected by changes in binding, in order to further examine the neuroadaptations that mediate the transition from recreational to escalated compulsive drug use. More specifically, in the current project, we monitored levels of the DAT in saline control (Sal group), brief 1-h access (Coc1h group), and extended 6-h access animals (Coc6h group) after 14 days of withdrawal.

2. Results

2.1. Self-administration

As expected, self-administration rates of the saline control animals (n = 6) were very low (5 lever-presses/infusions per session on average). Coc1h (n = 6) animals exhibited stable self-administration patterns and showed no change in self-administration rates between the first and last day of the 8-day period (consuming on average 3.7 ± 0.2 mg on the first day and 4.1 ± 0.4 mg on the last day; see Fig. 1, panel A or B). Coc6h (n = 5) animals showed increased rates of self-administration (i.e., escalation) from the first to the last day of the 8-day period (consuming on average 3.4 ± 0.2 mg and 21 ± 2 mg on the first hour or the whole session, respectively, of the first day, and 7.4 ± 0.8 mg and 31 ± 2 mg on the first hour or the whole session, respectively, of the last day). Thus, a two-way ANOVA analyzing rates of self-administration during the first hour of the session (see Fig. 1, panel A) yielded a significant main effect for Day ($F(1,9) = 63.551$, $P < 0.0001$), a significant main effect for Group ($F(1,9) = 7.002$, $P < 0.027$), and a significant Day X Group interaction ($F(1,9) = 40.104$, $P < 0.0001$). One-way ANOVA revealed no difference between day 1 and day 8 for the coc1h group, but a significant difference for the coc6h group ($F(1,4) = 49.231$, $P < 0.002$). Increases in self-administration responding during the whole session were also seen in the coc6h animals (see Fig. 1, panel B). Thus another two way ANOVA revealed a significant main effect for Day ($F(1,9) = 45.701$, $P < 0.0001$), a significant main effect for Group ($F(1,9) = 156.578$, $P < 0.0001$), and significant interaction for Day X Group ($F(1,9) = 37.970$, $P < 0.0001$). For the coc1h group, the first hour comprised the whole session, therefore there was no need to repeat the simple effect analysis for this group. However, a one-way ANOVA for the coc6h group revealed a significant effect for day ($F(1,4) = 34.725$, $P < 0.004$).

2.2. DAT density

One-way ANOVAs followed by Tukey post hoc comparisons were utilized to compare DAT densities in the VTA, N.Acc core

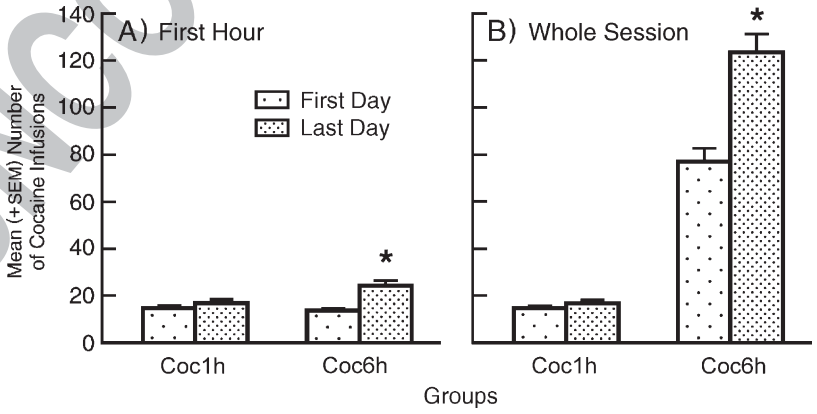

Fig. 1 – Self-administration patterns—this figure illustrates mean number of self-administered cocaine infusions during the first hour of each trial (panel A) and throughout the session (panel B) on the first and last day of the 8 days of self-administration post-training. *Signifies significant difference between first and last day ($P < 0.004$).

and shell, dorsal striatum, and mPFC of animals from the Sal, Coc1h, and Coc6h groups. Fig. 2 shows a sample section from the N.Acc and striatum that was stained for the DAT.

The Coc1h condition resulted in higher densities of the DAT in the N.Acc Core relative to both the Sal and the Coc6h groups (see Fig. 3). This was evident from a significant one-way ANOVA ($F(2,16) = 8.09$, $P < 0.005$) and significant post hoc comparisons between the coc1h group and the Sal group ($P < 0.005$) or the Coc6h group ($P < 0.024$). DAT densities in the dorsal striatum were also higher in the Coc1h group as compared to the two other groups (see Fig. 4). Thus, a one-way ANOVA revealed a significant effect for Group ($F(2,16) = 4.845$, $P < 0.025$) and the post hoc tests confirmed a significant difference between the Sal and the Coc1h groups ($P < 0.039$) and a significant difference between the Coc1h and the Coc6h groups ($P < 0.052$). DAT densities in the N.Acc Shell, the mPFC, or the VTA, were not significantly different for the three experimental groups and are described in Table 1.

3. Discussion

Extended 6-h daily access to self-administered cocaine resulted in escalated drug use, whereas brief 1-h access yielded stable consumption, as shown before (Ahmed and Koob, 1998; Ben-Shahar et al., 2004, 2005). After 14 days of withdrawal, DAT levels in the N.Acc core and dorsal striatum were higher in the brief access condition relative to both 6-h access and saline control animals, where DAT levels were similar. These data parallel our previous results, in that the 1-h access condition that resulted in a heightened (i.e., sensitized) locomotor response and elevated c-Fos labeling (in the N.Acc core) upon cocaine challenge after 14 days of withdrawal (Ben-Shahar et al., 2004), resulted also in increased DAT density, compared to saline controls. Similarly, the 6-h access rats that exhibited neither a sensitized locomotor response nor an elevated c-Fos reactivity to cocaine, also exhibited no change in DAT density. These results are also consistent with the data of Alburges et al., (1993; using [3H] BTCP) and of Claye et al., (1995; using [3H]GBR-12935) who showed increased levels of DAT in the nucleus accumbens or striatum of rats receiving a cocaine treatment regimen previously shown to resulting behavioral sensitization. Finally, the current results are consistent with the data of Letchworth et al., (2001; [3H]WIN35,428) showing increased DAT density in the N.Acc of rhesus monkeys self-administering cocaine in a pattern resembling recreational drug use in humans. The current findings, therefore, strengthen our argument that these two access conditions result in distinctive patterns of neuronal adaptations.

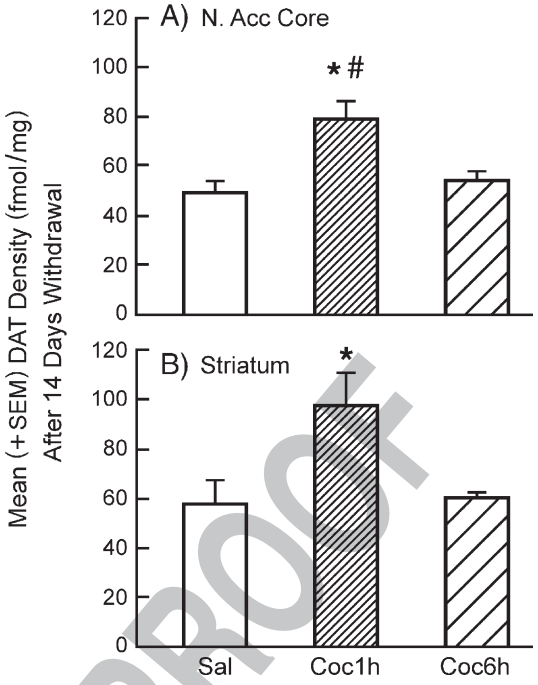

Fig. 3 – DAT Density—Mean density of the DAT in the N.Acc core (panel A) and the dorsal striatum (panel B) of the Sal, Coc1h, and Coc6h subjects. *Signifies a significant difference between the Coc1h and Sal condition ($P < 0.05$); #Signifies a significant difference between the Coc1h and Coc6h conditions ($P < 0.04$).

The literature regarding changes of DAT levels in human addicts consists of conflicting results. Specifically, DAT levels were found to increase, decrease, or stay unchanged in post mortem examinations of the brains of cocaine addicts by different researchers (Little et al., 1998b, 1999; Malison et al., 1998; Mash et al., 2002; Hitri et al., 1994; Hurd and Herkenham, 1993; Wilson et al., 1996). Some of this variability can be accounted for by the different procedures

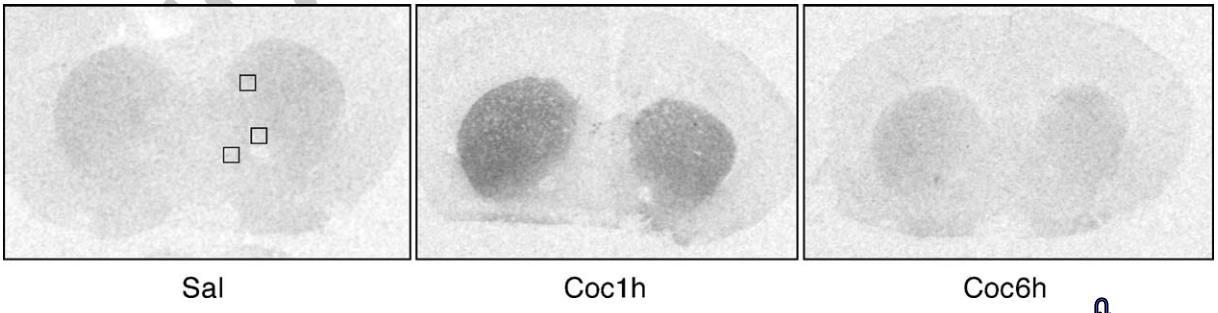

Fig. 2 – Sample DAT autoradiographs—schematics of all the brain areas sampled and their coordinates are illustrated in panel A. Panel B includes sample autoradiographs of sections including the nucleus accumbens and striatum of representative animals from the Saline, Coc1h, and Coc6h groups. Squares designate the approximate area from which measurements were taken (lower medial square—N.Acc Shell; lower lateral square—N.Acc Core; upper square—Dorsal Striatum).

Table 1 – DAT Density — Mean density of the DAT in the N.Acc Shell, VTA, and mPFC where the Sal, Coc1h, and Coc6h groups did not differ significantly from each other

Brain area	Saline	Coc1h	Coc6h
N.Acc Shell	28 ± 2.7	36 ± 5.3	30 ± 5
VTA	*	*	*
mPFC	9.5 ± 3.2	12.6 ± 1.8	16.9 ± 3

* Levels detected were not significantly different than zero.

and ligands used by the different researchers. Thus, for example, Wilson et al. (1994) reported that in rats, immediately at the end of unlimited access to IV cocaine [3H]GBR-12935 binding was increased in the substantia nigra and VTA while [3H]WIN35,428 binding was increased in the N.Acc and striatum. After 3 weeks of withdrawal [3H]GBR-12935 was reduced in the substantia nigra and VTA while [3H]WIN35,428 was reduced in the N.Acc. Hitri et al. (1996) found that in rats, continuous infusion of cocaine resulted in no change in [3H]GBR-12935 binding, but in increased binding of [3H]WIN35,428 in the striatum. In contrast, intermittent administration of cocaine resulted in decreased [3H]GBR-12935 binding in PFC.

Another very important source of variability is the different samples of cocaine addicts examined. For example, Mash et al., (2002) observed directionally opposite changes in two different populations of cocaine addicts. Moreover, all of the studies cited above were conducted post mortem, where the length of withdrawal from cocaine, if any, is unknown, as is the cause of death. This is particularly important considering the data of Malison et al., (1994) showing an almost significant increase of DAT in cocaine addicts when scanned a few hours after detoxification, but no change in levels of DAT between matched controls and addicts at 2–4 weeks of withdrawal. Malison and colleagues' data highlight the importance of in vivo analysis, in which one has a higher level of control over, and knowledge of, the independent variables (i.e., cause of death, severity of intoxication, etc.). Furthermore, Malison and colleagues' data present the possibility that the increases in DAT levels found in cocaine addicts were specifically tied to a short period of detoxification (i.e., a few hours); the loss of these increases after 2 weeks of detoxification is consistent with the current results. In another in vivo analysis, Volkow et al., (1996b) found a decrease in cocaine uptake and no change in DAT availability after either 2–4 weeks, or 3 months, of detoxification. Our observation of no change in DAT levels in the 6-h group after 2 weeks of withdrawal corresponds perfectly with the latter two studies and further reinforces our claim that this condition is the one most relevant to the study of the neuroadaptations that mediate addiction.

DAT trafficking and membrane expression is regulated by its substrates, two of which are dopamine and cocaine (which binds to the DAT and thus block the re-uptake of dopamine). While the exact mechanism by which cocaine and dopamine affect DAT membrane expression is still unknown, two critical variables for this process are influx of Na+ into the cell and activation of protein kinase C (Kahlig and Galli, 2003; Jayanthi and Ramamoorthy, 2005; Little et al., 2002). The 6-h animals consume significantly more cocaine and for a much longer time compared to the 1-h animals. In addition, the 6-h subjects maintain significantly higher levels of dopamine in the nucleus accumbens (Ahmed et al., 2003) as compared to 1-h subjects. Densities of DAT, as measured by receptor autoradiography, reflect both changes in the affinity for of the ligand used and membrane expression of the DAT. It is therefore reasonable to assume that the difference in DAT densities between the brief and extended cocaine access conditions reflects a difference in membrane expression that resulted from higher exposure of DAT to two of its substrates, cocaine and dopamine, in the 6-h condition. The 6-h animals first experienced 1-h sessions with cocaine. It is possible that DAT first increased in these animals (while they were still given only 1 h of daily access to cocaine), and then decreased, as a result of the transfer to longer daily access. This later decrease in the DAT could have happened either while subjects experienced extended access to cocaine, or during withdrawal. This hypothesis is consistent with the results of both Letchworth et al., (2001) showing a decrease followed by an increase in DAT density in animals having 1-h access to cocaine, and Wilson et al., (1994) showing a decrease in DAT after 24 h of access to cocaine.

In summary, the current data confirm and extend our previous results showing that daily access of 1-h vs. 6-h to cocaine self-administration results in different neuronal adaptations. Given the escalating nature of cocaine self-administration observed only in the 6-h group, it would seem that in order to understand the neurobiological mechanisms at the root of addiction, one needs to employ extended daily access conditions to model the development of addiction. The lack of change in membrane expression of DAT, combined with the results of Ahmed et al. (2003) showing the same DA release in 1-h and 6-h animals in response to one IV administration of cocaine, is consistent with the notion that the relevant adaptations in the dopaminergic function of these animals are post- and not pre-synaptic. Clearly, more research is needed to illuminate these mechanisms.

4. Experimental procedures

4.1. *Subjects*

The subjects ($n = 24$, 17 of which finished the experiment) were male albino Sprague–Dawley rats weighing 300–350 g at the beginning of the experiment obtained from Charles River Laboratories (Hollister, CA). The animals were housed individually in wire-hanging cages located within a temperature-controlled (22 °C), 12/12 h light/dark cycle (lights on at 0700) vivarium located in the Psychology Department at UCSB. Subjects had ad libitum access to food and water, except during operant training for food reinforcement (see Food Training below). All procedures were conducted in strict adherence to the NIH *Guide for the Care and Use of Laboratory Animals* and were reviewed and approved by the UCSB Institutional Animal Care and Use Committee.

4.2. *Surgery*

Rats were implanted with chronic intravenous silastic catheters in the right jugular vein under Isoflurane gas anesthesia (Abbott Laboratories, North Chicago, IL; 4% for induction; 2.0–2.5% for maintenance). A single dose of atropine (0.04 mg/kg IM) was administered to minimize respiratory congestion during anesthesia. Banamine (2 mg/kg SC), a non-opiate analgesic, was provided to treat post-surgical pain. Catheters were 13 cm long (0.3 mm inner diameter, 0.64 mm outer diameter; Dow Corning Corporation, Midland, MI), and cemented to a 22 gauge guide cannula (Plastics One, Roanoke, VA) that was in turn secured with Bard Mesh (C.R. Bard Inc., Cranston, RI) to the animals' back. The other end of the catheter was passed subcutaneously around the shoulder to the neck where it was inserted into the jugular vein and secured in place by suture. Animals were allowed 10 days for recovery. Catheter patency was maintained by flushing the IV system with a solution of 30 units heparin in 0.1 ml sterile saline, each day. Catheter patency was confirmed in all animals with the fast acting anesthetic sodium methohexital (1 mg/0.1 ml saline), once a week and at the end of the last session of cocaine self-administration.

4.3. *Apparatus*

Six standard (29 cm wide × 25 cm long × 30 cm high) operant chambers were used for all behavioral training and testing. Each chamber was equipped with a non-retractable (fixed) lever and a retractable lever, each positioned 7.0 cm above the grid floor on either side of a food pellet trough that was situated 2 cm above the grid floor. Food dispensers were located outside the chambers. A center house light (2.8 W) was situated 28 cm above the grid floor in the center of the back panel. Two cue lights (2.8 W) were located 6–7 cm above each lever. In the current study, only the right cue light was used. All behavioral testing equipment and data acquisition were controlled by a desktop personal computer running Med Associates software (MED-PC for Windows, Version 1.17). A custom-made liquid swivel was located above the center of each operant chamber permitting the animals to freely move about the chamber without strain on the PE tubing. The inlet of the liquid swivel was connected with polyethylene tubing (Plastics One; outer diameter 0.127 cm, inner diameter 0.058 cm) to a 10-ml syringe containing the self-administration solutions and seated in a syringe pump (Med Associates Inc., St. Albans, VT). An additional length of PE tubing passed through a cannula connector (C313CT Plastic One) from the swivel overhead to the animal where it was connected to the external cannula on the animal's back. Intravenous infusions were administered by activation of the syringe pump.

4.4. *Drugs*

Cocaine hydrochloride (provided by the National Institute on Drug Abuse) was dissolved in 0.9% physiological saline. The concentration used for intravenous (IV) administration was 0.25 mg/0.1 ml that was infused at a volume of 0.1 ml over a 4-s period.

4.5. *Procedure*

The procedure was the same as described previously (Ben-Shahar et al., 2004). Briefly, to facilitate acquisition of operant responding for cocaine, rats were initially trained to lever press for food (45 mg Noyes pellets) prior to catheter implantation. Rats were trained on an FR-1 schedule followed by a time-out (TO) period. The TO period lasted 1 s initially and then was lengthened to 10 s, and finally to 20 s. Surgical implantation of catheters was performed one to 2 days after a rat completed the food-training regimen. Ten days after surgery, cocaine self-administration training began. Training consisted of 1-h daily sessions on an FR-1 TO 20 schedule. The reinforcer was either 0.1 ml physiological saline or 0.25 mg cocaine in 0.1 ml physiological saline. Once a rat exhibited a stable response rate for cocaine (i.e., no more than 15% variability over 3 consecutive days) and had experienced at least seven self-administration sessions, it was assigned to either the Coc1h group or the Coc6h group for the next 8 days. Saline animals (Sal group) continued to have access to IV saline for 1 h each day.

At the end of this 8-day period, rats were given 14 days of withdrawal during which they had no access to cocaine (or saline) and were never placed in the operant boxes. On the 14th day of withdrawal, all subjects were given the fast-acting anesthetic sodium methohexital (2 mg/kg IV) via their catheters and were decapitated immediately, their brains removed, rapidly frozen in isopentane on dry ice, and then transferred to dry ice. Brains were then stored at –80 °C until processing. Coronal sections of brain tissue (16 μm) were cut on a cryostat and immediately mounted on 1.5% gelatin-coated slides. Using the Paxinos and Watson atlas (1986) as a guide, the mPFC, N.Acc Core, N.Acc Shell, dorsal striatum, and VTA were sampled.

4.6. *Quantitative receptor autoradiography*

Brain sections were pre-incubated at room temperature for 20 min in 50 mM Sodium Phosphate buffer (pH 7.4) containing 50 mM NaCl. Total binding was measured from sections that had been incubated for 120 min in the same buffer with 10 nM [3H]WIN35428. We chose [3H]WIN35428 since it is one of the best characterized dopamine transporter ligand, and it is more specific to the mesolimbic dopaminergic system (Wilson et al., 1994). In addition, [3H]WIN35428 show cross tolerance to cocaine (Katz et al., 1993), which suggests that it binds to similar sites on the DAT and is highly sensitive to cocaine-induced changes in DAT function. Non-specific binding was determined from adjacent sections by adding 30 μM cocaine to the binding buffer. Sections were subsequently washed in ice-cold buffer (3 × 5 min), rinsed in ice-cold distilled water, and left to dry overnight. Slides were then exposed to film for 48 days. Autoradiograms were analyzed with a computerized image-analysis system (ImageJ, National Institute of Health, USA).

5. ~~Uncited reference~~

~~Little et al., 1998a~~

Acknowledgments

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