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Repeated Cocaine Exposure: Effects on Catecholamines in the Nucleus Accumbens Septi of Periadolescent Animals

REX MONTGOMERY PHILPOT AND CHERYL L. KIRSTEIN

Departments of Psychology and Pharmacology and Therapeutics, University of South Florida, Tampa, FL 33620

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PHILPOT, R. M. AND C. L. KIRSTEIN. *Repeated cocaine exposure: Effects on catecholamines in the nucleus accumbens septi of periadolescent animals.* PHARMACOL BIOCHEM BEHAV **62**(3) 465–472, 1999.—Substance abuse is a major issue in today's society, and is an issue of critical importance in the adolescent population. Research indicates that substance use is often initiated during the adolescent period, and that brain reward areas are still undergoing changes during this time. Despite this, little research has investigated the effects of repeated drug use on the reward mechanisms of periadolescent animals. For this reason, the present study examined the effects of repeated cocaine administration on the responsiveness of the nucleus accumbens septi (NAcc) to either cocaine or saline challenge. The data indicate that repeated exposure to cocaine produces temporal shifts in the dopaminergic (DAergic) activity of the NAcc, with peak activity occurring earlier. Importantly, following repeated injections of cocaine, saline injections alone elicit increases followed by a subsequent suppression in DA overflow in the NAcc. These results suggest that the context of cocaine administration produces fundamental changes in the way that neurochemical reinforcement mechanisms respond. The expectancy of the drug alone elicits reward-related activity within the NAcc, which may play a critical role in the development of addiction. © 1999 Elsevier Science Inc.

Cocaine Expectancy Conditioning Nucleus accumbens Microdialysis

COCAINE use occurs primarily in individuals from 12–39 years of age (26). A 1995 survey conducted by the National Institute on Drug Abuse indicated that 4.2, 5, and 6% of 8th, 10th, and 12th graders, respectively, were currently using cocaine. Among these individuals there is substantial risk of becoming a cocaine addict, and the morbidity and mortality associated with its use are high (19,34). Because human drug use is frequently initiated during adolescence, animal models should address the effects of cocaine use during this developmental period. To date, most research on the addictive process has been examined in adult animals. Particular attention must be directed toward elucidating the neurobehavioral substrates involved in cocaine use and the development of addiction during the periadolescent period given that, this is the age period of onset of cocaine use, and brain reward systems are still developing. Moreover, drug use during brain development may result in critical changes that subsequently underlie the development of addiction.

Cocaine exerts profound effects on central neurotransmission by inhibiting the reuptake of norepinephrine (NE), serotonin (5-HT), and dopamine (DA). This effect is both rapid and sustained for a prolonged period (20). However, cocaine appears to specifically mediate reinforcement by binding the dopamine transporter protein with high affinity and elevating DA at the synaptic cleft in brain regions involved in reward (13,29). The mesocorticolimbic system, which originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAcc) and prefrontal cortex (7), serves a general role in reinforcement, and consequently, is implicated in cocaine addiction and repeated use of abused substances. In general, this pathway has been shown to be responsible for the reinforcing properties associated with motivated responses such as drinking, eating, and sexual behaviors (8,12,15,22,31,40). Animals trained to lever press for both natural reinforcers (e.g., food/water), or experimental reinforcers (e.g., electrical brain stimulation) rapidly extinguish their responding when

Requests for reprints should be addressed to Cheryl L. Kirstein, Ph.D., Psychology Department-BEH 339, University of South Florida, 4204 E. Fowler Avenue, Tampa, FL 33620.

dopamine receptors are blocked by the antagonist spiroperidol (42). Drugs of abuse (e.g., amphetamine) cause both rapidly induced conditioned place preference and increases in lever pressing for reinforcing brain stimulation (18,49). In addition, studies utilizing self-administration techniques indicate that animals of many species will willingly and rapidly self-administer dopamine agonists such as amphetamine and cocaine (27). Moreover, animals will increase their rate of responding to compensate for reductions in drug concentration or pharmaceutical blockade of the DA receptors (54). These results indicate that the mesocorticolimbic DA pathway plays a critical role in mediating the reinforcing efficacy of a number of natural reinforcers as well as drugs of abuse.

Along with the direct process of mediating reward, the NAcc may serve to provide a link between motivational and motor processes (35). For example, increases in DA metabolism are elicited by food-related stimuli (5,6), and NAcc DA efflux elevates prior to the self-administration of food (25). Ljungberg et al. (33) examined the nature of the information encoded by the mesolimbic DA pathway and found that a novel cue (light) paired with a reinforcer (food) increased firing rate of VTA neurons, prior to and in the initial phases of training. This behavioral change (i.e., learning) correlated with a decrease in VTA response to the primary reinforcer and an increase in response to the previously novel stimulus, indicating neurochemical secondary reinforcing properties. These results suggest that the projections of the mesolimbic DA system may be conditioned with natural reinforcers and serve as a predictor of reward, thus a substrate for expectancy (43).

Multiple studies report conditioned behavioral activation in response to a context previously paired with cocaine administration. These studies show enhanced locomotor activity in context-paired vs. unpaired animals (9,10,17). Additionally, some of these studies report changes in the activity of specific brain regions (e.g., NAcc and striatum) following exposure to a previously cocaine-paired environment (10,17). Specifically, changes in the activity of the NAcc have been observed in a conditioning paradigm by Fontana and Pert (17) and the potential role of DA activity in the "expectancy" (i.e., conditioned neurochemical response) of cocaine has been supported by studies examining cocaine self-administration (20). Others, however, reported no conditioned increases in accumbal DA despite clear changes in locomotor activity (9). Taken together, these data suggest a possible role of NAcc dopamine in the mediation of cocaine reinforcement expectancy and motivation.

A large body of research has shown that repeated cocaine exposure during adulthood results in multiple changes in dopaminergic mechanisms including increased overflow when challenged (11), decreased DA during withdrawal (53), and increased voltage requirements for Intracranial Self Stimulation (ICSS) (16,52). Microdialysis studies of the NAcc also reveal that cessation of cocaine self-administration results in significant reductions in the basal dopamine overflow with peak effects observed 4–6 h after the last lever press (53). In addition, animals that have administered cocaine for multiple days (i.e., 7) exhibit elevated dopamine overflow when challenged with a cocaine injection. This response continues to occur up to a week after the last administration (11). These results indicate that in adults repeated cocaine use results in complex and dynamic changes in the DA system that persist in some form long after use has ended. These changes have been implicated in the addictive process.

In rats, periadolescence, defined in terms of sexual maturation, begins approximately postnatal day 20 (PND 20) for females and PND 30 for males (39). Basal DA levels and turnover rates in the NAcc vary during development. Periadolescent rats exhibit high DA levels in NAcc relative to other DA structures during this developmental period. These DA levels rise to adult levels at about PND 35. Additional changes in DA turnover occur from PND 35–40 (23). Receptor levels also differ in adolescent animals, D_1 and D_2 receptor levels in the NAcc and striatum undergo significant overproduction and pruning during this period (1). Interestingly, Stamford (51) has shown that periadolescent animals exhibit significantly less electrically stimulated DA release in NAcc compared to adults. These young animals also had decreased uptake of DA with the ratio of uptake to release of DA significantly larger than that seen in adults (51). These studies demonstrate dramatic changes in the mesolimbic DA pathway during development. Likewise, measures of the striatum show significant developmental changes. In periadolescent animals, postsynaptic receptor levels and presynaptic DA concentrations gradually increase from levels established at birth to adult levels at the time of puberty (14,36–38). Measures of D_2 receptor mRNA in striatum also increase to levels well above adults at PND 28, and decline to adult levels at puberty (50). Behaviorally, periadolescent animals appear hyperactive relative to adults (47). Pharmacologically, periadolescent pups have been shown to be differentially sensitive to DA agonists across age (30,41), showing reduced sensitivity to DA agonists (e.g., cocaine or amphetamine) and heightened sensitivity to antagonists when compared to adults (45,48). These data suggest a hyposensitivity of the DA system is ongoing during periadolescence that may make these developing animals uniquely susceptible to the effects of repeated exposure to the DA reuptake inhibitor, cocaine. These influences may produce changes in the still developing system not seen following repeated exposure in adulthood.

Clearly, during the periadolescent period the mesocorticolimbic system exhibits individual properties not seen in adults, and as a consequence, may be uniquely affected by repeated use in a fashion equally complex as that seen in adults but specific to the adolescent period. Because drug abuse often begins in this age group, it is critical to examine animals during this time in development. The present study attempts to identify relevant changes in the neurochemical responses to cocaine and cocaine-associated stimuli following repeated cocaine administration during the periadolescent period.

METHOD

Subjects

Fifty-six offspring from Sprague–Dawley breeding pairs (Zivic Miller Laboratories) weighing from 60 to 90 g at the time of testing were used as subjects in these experiments. Pups were sexed and culled to 10 pups per litter on postnatal day 1 (PND 1). Pups remained housed with their respective dams in a temperature-controlled vivarium on a 12L:12D (0700–1900 h) until PND 21. On PND 21 pups were weaned and individually housed.

Injections

All animals were given two daily injections of either cocaine (2.0 or 20.0 mg/kg/3 cc, IP), or 0.9% equivalent volume saline for 4 consecutive days starting PND 21 and challenged on PND 25. Doses were chosen based on behavioral data in our laboratory indicating effective doses for olfactory preference conditioning (unpublished observations). Animals in the

acute condition received 0.9% saline injections twice per day on 4 successive days to control for handling and injection stress followed by either saline ($n = 8$) or drug ($n = 8$; low dose; $n = 8$; high dose) during dialysis on PND 25. Animals in the repeated injection condition received 2.0 or 20.0 mg/kg cocaine twice per day from PND 21–24 and cocaine ($n = 16$) at their respective dose on PND 25. In the expectancy condition animals received 20.0 mg/kg cocaine twice per day from PND 21–24 and saline ($n = 8$) during dialysis on PND 25.

Intracranial Microdialysis

Pups were anesthetized on PND 24 using a xylazine/ketamine cocktail (0.15 and 1.0 mg/kg, respectively). An incision was made over the skull and the guide cannula affixed (A:P +3.5 mm M:L +0.5 mm) with cyanoacrylate and cranioplast. On PND 25, microdialysis probes with 1.5 mm membrane tips and 251 μm outer diameter were lowered 5.5 mm, D:V, to accumbens on PND 25. The probe was perfused continuously with artificial cerebrospinal fluid (aCSF; 145 mM NaCl, 2.4 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 0.2 mM Ascorbate, pH = 7.4). Six baseline samples were taken 2 h after initial probe insertion, and the final three samples were used to calculate baseline neurotransmitter levels. Perfusate samples were collected on ice at 10 min intervals in microcentrifuge tubes containing 2.5 μl of 0.25 N perchloric acid ($HClO_4$). Following the collection of six baseline samples animals received an injection of cocaine (2.0 or 20.0 mg/kg IP), or 0.9% saline g/kg IP. After saline or drug challenge, sampling continued at 10 min intervals for an additional 120 min.

Baseline Analysis

To insure neurogenic origin of baseline neurotransmitter levels, calcium dependency ($n = 4$) and tetrodotoxin sensitivity ($n = 4$) were measured. For calcium dependency, following collection of baseline samples the dialysis probe was perfused with a modified aCSF solution with calcium omitted (145.8 mM NaCl, 2.5 mM KCl, 1.0 mM $MgCl_2$, 0.2 mM Ascorbate and 1.0 mM EGTA). Following six Ca^{2+} omitted samples the probe was perfused for a single 10 min sample with an aCSF solution containing elevated potassium levels (87.5 mM NaCl, 60 mM KCl, 1.0 mM $MgCl_2$, 0.2 mM Ascorbate and 1.2 mM $CaCl_2$) (osmolality maintained). Following stimulation with potassium, the modified aCSF was replaced with normal aCSF and samples were collected for an additional 2 h. Results were compared to potassium evoked release without preceding calcium omission to determine calcium dependency of release.

The TTX procedure utilized a modified aCSF solution with 1.0 μM tetrodotoxin added to the aCSF. Following six baseline samples with normal aCSF the dialysis probe was perfused for 1 h with 1.0 μM TTX aCSF. After TTX infusion normal aCSF was perfused and samples collected for an additional 2 h. Postbaseline DA levels were compared to baseline levels to determine non-neurogenic DA levels.

Analytical Procedures

Dialysate samples (12.5 μl) were run immediately or stored at −80°C to prevent breakdown of neurotransmitters until analyzed. Analysis was performed with a reverse-phase high-performance liquid chromatography system coupled with electrochemical detection (HPLC-EC) set to oxidize catecholamines (0.675 V). The mobile phase consisted of 0.15 M chloroacetic acid, 0.05 mM sodium octyl sulfate, 1.0 mM disodium EDTA, and 4% v/v acetonitrile (pH = 2.9). The 12.5-μl samples were injected onto a 5 μm Microsorb-MV C18 column (4.6 × 100 mm), and peaks detected using a BAS LC-4C carbon working electrode, referenced to a Ag/AgCl electrode at 0.675 V. Data were recorded and quantified by a Rainin analytical system and a Macintosh LC III.

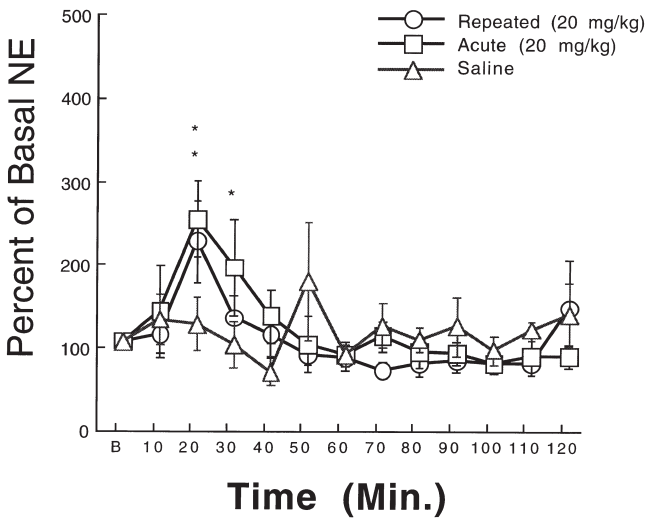

FIG. 1. Percent of basal NE following either saline (triangles; $n = 8$), acute (squares; $n = 8$), or repeated (circles; $n = 8$) administration of 20.0 mg/kg IP cocaine. *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

Histology

All microdialysis drop sites were verified histologically after completion of the microdialysis experiment. Animals were sacrificed via decapitation, the brain removed, and quickly frozen in 2-Methylbutane at −40°C. Probe placements were

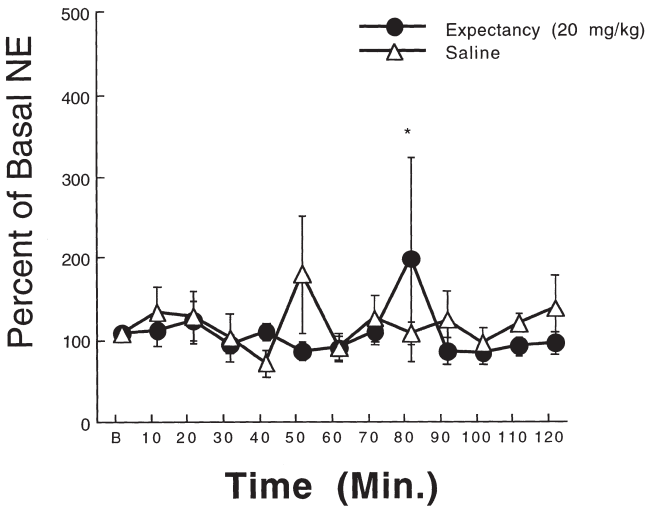

FIG. 2. Norepinephrine levels following repeated saline or 20.0 mg/kg IP cocaine with subsequent saline challenge (saline—triangles; $n = 8$, expectancy—closed circles; $n = 8$). *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

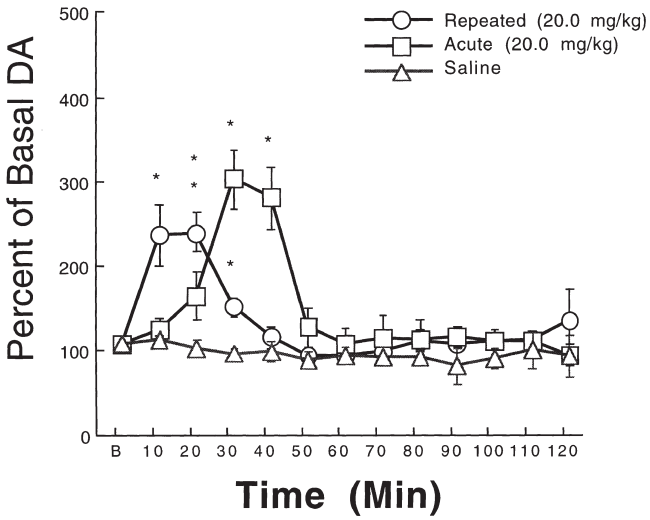

FIG. 3. Percent of basal DA following either saline (triangles; $n = 8$), acute (squares; $n = 8$), or repeated (circles; $n = 8$) administration of 20.0 mg/kg IP cocaine. *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

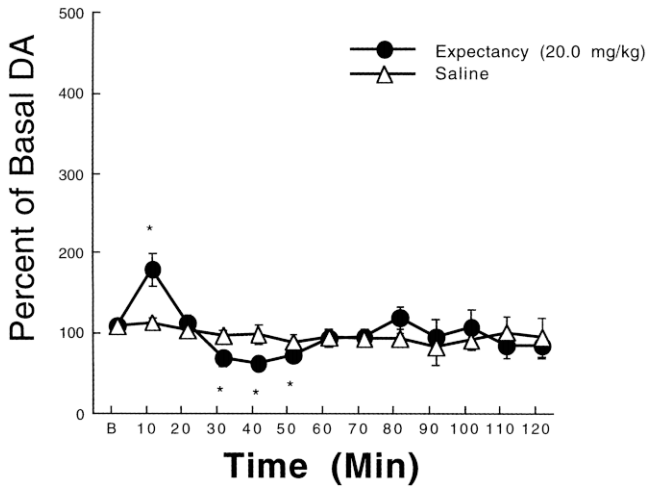

FIG. 5. Dopamine levels following repeated saline or 20.0 mg/kg IP cocaine with subsequent saline challenge (saline—triangles $n = 8$, expectancy—closed circles; $n = 8$). *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

histologically verified using 40-μm sections stained in cresyl violet.

Analysis

Data were analyzed in four separate 2 (acute vs. repeated) × 3 (dose) × 13 (sample) factorial analysis of variance (ANOVA) with planned comparisons (Newman–Keuls comparison to a mean) to isolate effects across sample time.

RESULTS

Analysis of calcium dependency and tetrodotoxin sensitivity revealed that neurotransmitter release was enhanced by potassium evoked stimulation for all substances analyzed. However, these effects were blocked by omission of calcium [NE, $F(14, 44) = 57.01$, $p < 0.05$; DA, $F(14, 44) = 73.37$, $p < 0.05$; DOPAC, $F(14, 44) = 20.96$, $p < 0.05$; HVA, $F(14, 44) = 27.94$, $p < 0.05$] or administration of TTX [NE, $F(14, 44) = 65.62$, $p < 0.05$; DA, $F(14, 44) = 92.31$, $p < 0.05$; DOPAC, $F(14, 44) = 94.11$, $p < 0.05$; HVA, $F(14, 44) = 143.61$, $p < 0.05$], indicating that in the case of each eluant, measured changes were both calcium dependent and action potential dependent.

Acute administration of 20.0 mg/kg cocaine elevated NE over basal and saline levels at 20 and 30 min while repeated cocaine (20 mg/kg) produced elevations over baseline at 20 min only following drug challenge (Fig. 1). Low-dose cocaine (2.0 mg/kg) did not elevate NE over basal values in either

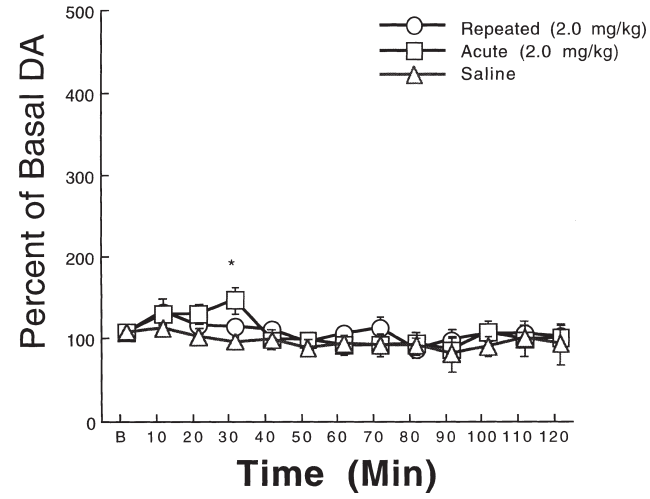

FIG. 4. Percent of basal DA following either saline (triangles; $n = 8$), acute (squares; $n = 8$), or repeated (circles; $n = 8$) administration of 2.0 mg/kg IP cocaine. *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

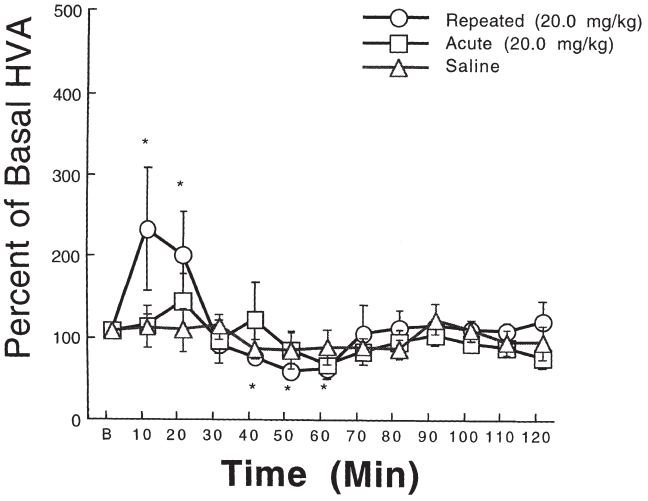

FIG. 6. Percent of basal HVA following either saline (triangles; $n = 8$), acute (squares; $n = 8$), or repeated (circles; $n = 8$) administration of 20.0 mg/kg IP cocaine. *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

acute or repeated conditions. Expectancy-induced changes in NE were observed only at 80 min postinjection (Fig. 2). Analysis of basal NE values revealed no significant differences across dose, $F(3, 66) = 0.620$, $p > 0.05$; sex, $F(1, 66) = 1.502$, $p > 0.05$; or treatment, $F(2, 66) = 0.458$, $p > 0.05$. Overall analysis revealed a significant sample by dose interaction, $F(12, 408) = 2.906$, $p < 0.05$, as well as a significant sample by treatment interaction, $F(12, 408) = 1.594$, $p < 0.05$.

Analysis of DA revealed an elevation over basal values at 20–40 min for acute administration of 20.0 mg/kg cocaine, while repeated 20 mg/kg injection produced an elevation that was shifted to the left compared to acute animals, with significant elevations occurring at 10–30 min (Fig. 3). Acute cocaine at the low dose (2.0 mg/kg) produced a significant elevation over baseline at 30 min (Fig. 4), while repeated exposure resulted in no significant changes in dopamine levels. Previous administration of 20 mg/kg cocaine followed by saline challenge (expectancy) produced an elevation over basal values at 10 min followed by a decrease in DA levels at 30–50 min (Fig. 5). Analysis of basal DA levels indicated no significant differences in DA across dose, $F(3, 66) = 1.061$, $p > 0.05$; sex, $F(1, 66) = 0.00009$, $p > 0.05$; or treatment, $F(2, 66) = 0.466$, $p > 0.05$. Overall analysis revealed a significant effect of dose, $F(1, 34) = 29.217$, $p < 0.05$; treatment, $F(2, 34) = 15.242$, $p < 0.05$; sample, $F(12, 408) = 5.490$, $p < 0.05$; sample by drug, $F(12, 408) = 5.880$, $p < 0.05$; sample by treatment, $F(24, 408) = 8.081$, $p < 0.05$; and sample by drug by treatment, $F(12, 408) = 6.552$, $p < 0.05$.

Acute cocaine challenge at 20 mg/kg produced an elevation in HVA 20 min after administration that was followed by a depression below basal levels at 50–70 min and at 120 min postinjection. Repeated cocaine (20 mg/kg) produced elevations over basal HVA at 10–20 min postinjection and suppression of HVA levels at 40–60 min (Fig. 6). Cocaine (2 mg/kg) produced no changes in HVA levels. Expectancy resulted in a decrease in HVA levels at 20–70 min (Fig. 7). Basal HVA levels were not significantly different across conditions. Overall analysis of HVA revealed a significant interaction of sample by dose, $F(12, 408) = 2.482$, $p < 0.05$; and sample by treatment, $F(24, 408) = 2.057$, $p < 0.05$. Basal DOPAC levels were not significantly different across conditions. Overall analysis revealed no significant changes in DOPAC levels (Fig. 8).

DISCUSSION

The present findings demonstrate that cocaine administration produces multifaceted changes in the neurochemical activity of the NAcc in periadolescent animals. The most dramatic changes were observed in DA overflow in the present study. This is not surprising, given that expectancy-induced changes in neurochemistry have been associated with DA rather than the other measured neurochemicals. The low dose used in the present study increased DA levels in the acute group only. This dose was not sufficient to effect NE or metabolite levels, and repeated administration failed to continue to elicit DA overflow similar to that observed in the acute group. Acute administration of the high dose of cocaine produced dramatic elevations in extracellular DA, with maximal elevations occurring at 30 min. Acute administration of the low dose produced significant increases that were smaller than the high dose but showed the same temporal pattern. This is in contrast to repeated cocaine administration, which resulted in elevations that appeared somewhat blunted in comparison to acute administration and produced peak effects at an earlier time point than cocaine administered acutely. Low doses of repeated cocaine produced no significant changes in DA levels. At the high dose, the time course of DA release in acute vs. repeated cocaine indicates that repeated administration produces a temporal shift in DA responsiveness. There was a more rapid increase in the onset of DA levels following repeated administration, exhibiting substantial elevations 10 min following cocaine administration in comparison to 20 min in the acute animals. Although the increase occurs more rapidly in the repeated condition, the DA response also decreases more rapidly, thus shifting the entire profile earlier than responses observed in the acute condition.

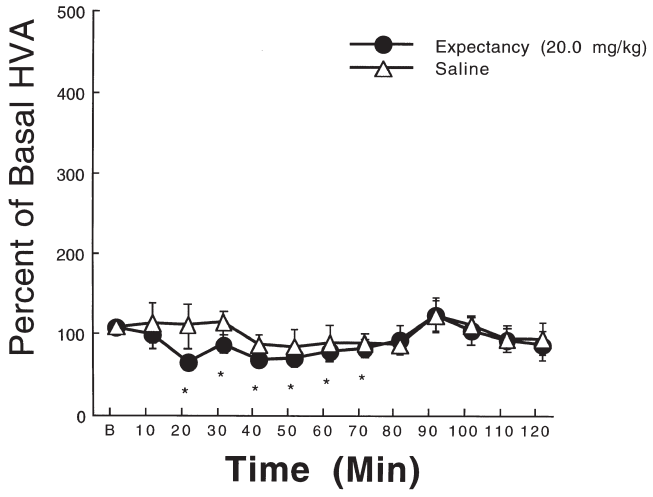

FIG. 7. Homovanillic Acid levels following repeated saline or 20.0 mg/kg IP cocaine with subsequent saline challenge (saline—triangles; $n = 8$, expectancy—closed circles; $n = 8$). *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

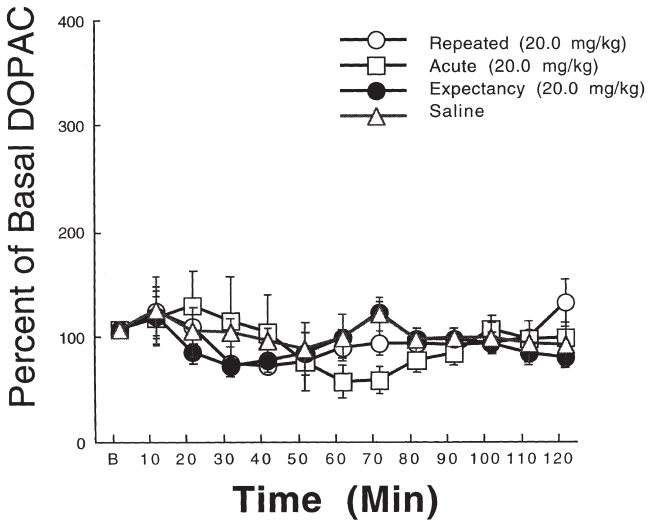

FIG. 8. DOPAC levels following either saline (triangles; $n = 8$), acute (squares; $n = 8$), or repeated (circles; $n = 8$) administration of 20.0 mg/kg IP cocaine or repeated 20.0 mg/kg IP cocaine with subsequent saline challenge (expectancy—closed circles; $n = 8$). *Indicates significant differences from basal values ($p < 0.05$). Error bars denote SEM.

The temporal shift seen in the DA response to cocaine is best addressed in conjunction with the DA expectancy response. The association of an environment or aspect of the environment, with peripheral or central nervous system effects, confers on the environment alone the ability to initiate behavioral or neurochemical changes (10,17,20). In the present study, immediately following saline injections, animals in the expectancy condition exhibited elevations in DA approximately 200% greater than baseline values. The more rapid onset of DA overflow seen in the repeated cocaine condition is most likely a combination of the effect of cocaine and the expectancy-induced overflow. Thus, the initial elevation at 10 min may be primarily expectancy driven, and this elevation is potentially maintained by the pharmacological action of cocaine. An interesting aspect of the cocaine expectancy condition is the subsequent decrease in DA, which corresponds temporally with elevations seen in animals in the acute condition. The dopaminergic depression observed may be due to a withdrawal response to the repeated cocaine condition, given that some researchers have found reductions in stimulated output following a period of withdrawal greater than 24 h (21). However, following a 24 h period of abstinence, Kalivas and Duffy (24) found that repeated cocaine (30 mg/kg $\times$ 5 days) followed by saline challenge produced no changes in locomotor activity or striatal DA levels. Animals in our studies experienced no greater than 12 h of withdrawal from the drug before drug administration during dialysis; consequently, withdrawal seems an unlikely explanation. It has been suggested that DA in the accumbens is involved in prediction and attention to relevant stimuli, given that ventral tegmental area activity is reduced when expected rewards do not occur (43). It is possible that the initial elevation seen in the expectancy situation is a predictive response, and the subsequent decrease in DA is a suppression of response when prediction and physiological reality do not coincide. Alternately, this suppression of DA could represent the action of an opponent process or counteradaptation mechanism that serves to maintain homeostasis (28,46).

The absence of change in basal DOPAC levels regardless of cocaine manipulation is most likely due to the process of DOPAC metabolism. DOPAC is a product of intracellular DA metabolism (4). Because cocaine acts as a reuptake inhibitor, elevations in extracellular DA are not mirrored intracellularly; therefore, DOPAC levels should not be elevated, given that DOPAC is produced intracellularly following DA reuptake. However HVA, which is produced from DA metabolized by catechol-*O*-methyl transferase (COMT) and monamine oxidase (MAO) in the extracellular space, does exhibit changes in response to cocaine administration. Acute administration of cocaine produces an initial elevation at 20 min and a subsequent decrease at 50–70 min. This increase represents an increase in metabolism of extracellular DA. However, under normal conditions, HVA is not the primary metabolite of DA in rats (4), and the lack of prolonged elevation may represent an inability to sufficiently metabolize such substantially elevated levels in the acute case. This idea is supported by the repeated HVA response, in which there is a significant increase in HVA at 10–20 min. Not only is the duration of elevated metabolite levels extended, but the levels of metabolite are higher and correspond to repeated DA levels implying an adaptation in the system to compensate for the inability to rapidly turnover cocaine-induced elevations in DA. The lack of HVA elevation seen in the expectancy condition is possibly due to the short duration of increases in DA in addition to the absence of reuptake inhibition, which allows for DOPAC production. Specifically, the decrease in HVA seen in the expectancy condition could be the result of a general decrease in DA efflux during expectancy (Fig. 5) and the combined ability to metabolize the brief, small elevation of DA into both DOPAC and HVA.

The present study examined the effects of acute or repeated cocaine as well as drug expectancy on NE levels in part as a control to separate cocaine's effects on reuptake mechanisms vs. observed expectancy-induced effects specific to DA activity. Drug expectancy did not increase NE in the dramatic fashion observed with DA; however, there was a significant increase in NE at 80 min following drug administration, an effect that appears to be due to an aberrant data point. Acute administration of the high dose of cocaine produced a predicted elevation in NE levels given the established effects of cocaine on the NE transporter (32). The duration of this effect was reduced following repeated administration of cocaine (20 mg/kg), indicating a decrease in responsiveness of the system due to repeated exposure. This shift in duration does not appear to be due to changes in basal release because basal NE values, as well as that of all of the neurochemicals measured, did not differ across acute and repeated conditions. This effect may reflect an upregulation of NE autoreceptors or an increase in autoreceptor sensitivity that results in a more rapid inhibition of neuron activity (2). Alternatively, this effect could be due to a change in number or efficacy of the NE transporters (3). An increase in transporter number or decrease in efficacy of cocaine would result in a diminished duration of NE elevation. However, it would be predicted that in addition to decreases in duration, maximal elevations would be reduced. Because this is not the case, it seems more likely that there is a change in autoreceptor density or overall responsiveness.

Taken together, the present data demonstrate that cocaine not only exerts an influence on DA mechanisms of periadolescent animals but also that repeated use results in a temporal shift in neurochemical response, and most importantly, a neurochemical expectancy for cocaine. These results indicate that the NAcc of PND 25 animals is functionally responsive to drugs of abuse in a dynamic fashion. Repeated cocaine clearly altered the neurochemical responsiveness to the drug. Repeated use resulted in a temporal shift in the DA response in the NAcc. After multiple administrations of cocaine, elevations in DA were produced earlier in time (i.e., 10 min) than those observed after a single administration (i.e., 30 min). It has been shown previously that activity of the mesolimbic DA system can mimic priming cues in cocaine-seeking behavior. For example, administration of D_2-like specific agonists can reinstate nonreinforced lever pressing and enhance cocaine-seeking behavior (44). Additionally, transient increases in NAcc DA have also been reported in humans to correspond with a recurrent desire to use cocaine (20). The present results could represent a neurochemical expectancy response from previously associated cues (injection). Administration of saline challenge following repeated cocaine validates this hypothesis. Following multiple cocaine injections, an IP injection of saline elicited an elevation in DA that is similar in time course to that seen in repeated cocaine-challenged animals, although the magnitude of the increase is not as substantial. This initial increase is followed by a shift below basal values at the time that increases are observed in acute cocaine animals. This indicates that the association with the injection itself comes to elicit an anticipatory neurochemical response within the mesolimbic DA system that may cue addictive (e.g., cocaine-seeking) behaviors, which are later depressed when the

expected neurochemical activity does not correspond to actual stimulation.

In conclusion, the results demonstrate that the developing mesocorticolimbic DA system is responsive to the effects of cocaine, and that this system is changed by either repeated or expected drug administration. Given the vast hormonal (39) and neurochemical (41) changes that are ongoing during periadolescence in both rats and humans as well as the fact that this is the age period in which drug use is most often initiated, it is critical to examine this system throughout ontogeny to elucidate the mechanisms that underlie the development of addiction.

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