Published in final edited form as:

Neurotoxicol Teratol. 2008 ; 30(5): 412–418. doi:10.1016/j.ntt.2008.04.002.

Cocaine Increases Stimulated Dopamine Release more in Periadolescent than Adult Rats

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Abstract

The neural mechanisms responsible for the enhanced adolescent vulnerability for initiating drug abuse are unclear. We investigated whether age differences in dopamine neurotransmission could explain cocaine's enhanced psychomotor effects in the periadolescent rat. Electrical stimulation the medial forebrain bundle of anesthetized post-natal age 28 days (PN28) and PN65 rats elicited dopamine release in caudate nucleus and nucleus accumbens core before and after 15 mg/kg cocaine i.p. Extracellular dopamine concentrations were greater in PN65 than PN28 caudate following 20 and 60Hz stimulations and in the PN65 nucleus accumbens following 60Hz stimulations. Cocaine increased dopamine concentrations elicited by 20 Hz stimulations 3-fold in the adult, but almost 9-fold in periadolescent caudate. Dopamine release rate was lower in the periadolescent caudate although total dopamine clearance was similar to that of adults. The periadolescent caudate achieved adult levels of clearance by compensating for a lower V_{max} with higher uptake affinity. Tighter regulation of extracellular dopamine by the higher uptake/release ratio in periadolescents led to greater increases after cocaine. In nucleus accumbens, dopamine release and V_{max} were lower in periadolescents than adults, but uptake affinity and cocaine effects were similar. Immaturity of dopamine neurotransmission in dorsal striatum may underlie enhanced acute responses to psychostimulants in adolescent rats and suggests a mechanism for the greater vulnerability of adolescent humans to drug addiction.

Keywords

Development; addiction; voltammetry; dopamine neurotransmission; cocaine; adolescence

INTRODUCTION

Adolescence is a critical period for initiation of drug abuse. The onset of substance abuse during adolescence is correlated with a greater severity of addiction involving increased rates of morbidity and mortality [13,62]. Escalation of drug use is more rapid and the risk of dependence is greater in adolescents than adults [14,15,20,55]. Behaviors like risk taking, impulsivity and sensation seeking that are prominent during adolescence are associated with increased risk of drug use [30,42,76,78]. Pharmacologic sensitivity may be an additional key factor in adolescent initiation of substance use.

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Studies directly comparing behavioral responses to addictive drugs in adolescents and adults are few and the results are mixed. Several groups have reported that animals in the periadolescent period, PN 30–40, are in general hyperactive, but have smaller increases in locomotion and stereotypic behaviors than younger or older cohorts after single treatments with amphetamine [8,34,70] or cocaine [8,41,63]. Others have reported that adolescents are more responsive to cocaine [11,12,35], amphetamine [1] or nicotine [4,16]. The reason for the discrepancies is unclear but age differences may be specific to the type of stimulant [71].

Drug exposures during adolescence may have unique effects because of dynamic, ongoing neural development [13,19,24,50,60,62,68]. The involvement of nigrostriatal and mesolimbic dopamine in the mediation of psychostimulant effects on stereotyped and locomotor behavior is supported by a large literature [17,18,32,52,53,59]. The dopaminergic neurons of the substantia nigra and ventral tegmental area play prominent roles in novelty seeking as well as reinforcement [37,61]. Although all of the neurochemical “machinery” for dopaminergic transmission is present soon after birth, a number of indices of dopamine function change markedly during adolescence. Overexpression of dopamine D₁, D₂ and D₄ receptors and subsequent decreases during adolescence have been reported in several studies [3,47,65–67]. Such pruning has also been shown in postmortem studies of adolescent human brain for receptors and the dopamine transporter [44,57]. Knowing how addictive drugs affect dopamine neurotransmission as dopaminergic systems approach final maturation during adolescence is critical to understanding the drug abuse vulnerability of this unique developmental epoch. A dearth of published studies address this issue [2,36]. The present study directly evaluates the impact of the addictive drug cocaine on electrically-stimulated dopamine release in periadolescent animals as a putative mechanism for the stimulant hyperresponsiveness of adolescent rats. We have chosen to evaluate dopamine neurotransmission in nucleus accumbens and dorsal striatum, terminal regions important for mediating behavioral and subjective effects and habit learning [26,27,56].

METHODS

Subjects

CD rats were purchased from Charles River Laboratories (Raleigh, NC, U.S.A.) and received at post-natal ages 21 or 55–63, about one week prior to experimentation. Two adults or four periadolescents were housed together in suspended, ventilated plastic cages (Techniplast USA, Exton, PA). Food and water were provided *ad libitum* and the room observed a 12:12 light: dark cycle with lights on at 0530 hr. Animal care was performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 865-23, Bethesda, MD, U.S.A.) and experimental procedures were approved by the Institutional Animal Care and Use Committee.

Electrochemistry

Voltammetry procedures were similar to our previously published methods [72,73]. Fast-scan cyclic voltammetry [46] was conducted with an EI-400 potentiostat (Ensmann Instrumentation, Bloomington, IN, U.S.A.) with hardware modifications as described by Michael et al. [45]. The potential at carbon fiber electrodes was held at –400 mV, ramped to 1V and back to –400 mV at 300 V/sec. Cyclic voltammograms were recorded at 10 Hz. Carbon-fiber microcylinder electrodes, prepared from 7 µm diameter T-300 fibers with about 50 – 100 µm exposed carbon fiber (Amoco, Greenville, SC, U.S.A.), were used in the *in vivo* experiments along with a Ag/AgCl reference wire [9].

Changes in extracellular dopamine were determined by monitoring the current over a 200 mV window at the peak oxidation potential for dopamine. The electroactive substance was

identified as dopamine by comparing background subtracted cyclic voltammograms from the *in vivo* stimulations to those collected at the same electrode *in vitro* after the experiment. Oxidation currents *in vivo* were converted to dopamine concentrations by calibrating the electrodes with dopamine standard solutions in a flow injection system following experimental use.

In Vivo Procedures

Rats were anesthetized with urethane (1.5 g/kg i.p.) and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). Body temperature was maintained at 37.°C with a Deltaphase Isothermal Pad (Braintree Scientific, Braintree, MA). A bipolar stimulating electrode (Plastics One Inc., Roanoke, VA) was positioned in the medial forebrain bundle (MFB) and biphasic stimulation parameters were 300 μ A, 2 ms each phase. The stereotactic coordinates (in mm) anteroposterior (AP) and mediolateral (ML) from bregma and dorsoventral (DV) from dura that follow are based on a brain atlas [51]. The stimulating electrode was placed at: -4.6 AP, +1.4 ML, -7.5 to -9.0 DV. The carbon-fiber microelectrode was directed either at the center of the caudate (+1.2 AP, 2.0 ML, -4.5 to -6.2 DV) or in separate rats at the nucleus accumbens core (+1.4 AP, 1.4 ML, -6.5 to -6.8 DV). The only compensation for the smaller size of the PN28 rats was that the ML placement of the stimulating electrode was +1.35.

The locations of the stimulating and working electrodes were optimized to give maximal dopamine responses. Extracellular dopamine concentrations resulting from sixty pulse stimulation trains at frequencies from 10, 20, 30, 40, 50, and 60 Hz were recorded. Immediately after the final baseline data collection, the rat was administered 15 mg/kg cocaine i.p. The time course of cocaine effects on extracellular dopamine was monitored at 20 Hz because the effect of uptake inhibition is frequency-dependent and most robust at this frequency [75]. Twenty Hz stimulations commenced immediately after cocaine injection (about 1 min) and were repeated at 2.5, 5, 7.5, 10, 15, 30 and 60 min post-cocaine. Cocaine responses to stimulations at the other frequencies were recorded between 20 and 40 min following drug administration.

Drugs and Chemicals

Cocaine HCl (Sigma-Aldrich, St. Louis, MO) solutions (15 mg/mL) were made fresh in saline and injected intraperitoneally (i.p.) at 1 mL/kg. Urethane and all other chemicals were purchased from Sigma-Aldrich.

Data Analysis

The dynamic changes of extracellular dopamine concentrations resulting from electrical stimulation were modeled as a balance between dopamine release and uptake [43,74,75]. The changes in extracellular dopamine are described by: $d[DA]/dt = f[DA]_p - V_{max} / ((K_m/[DA]) + 1)$ where f is the frequency of stimulation, $[DA]_p$ is the concentration of dopamine released per stimulus pulse, V_{max} and K_m are Michaelis-Menten parameters for the maximal uptake rate and the affinity of the transporter, respectively. Non-linear curve fitting using a simplex algorithm calculated V_{max} , K_m , and $[DA]_p$ using data at each frequency from 20 to 60 Hz simultaneously [77]. All three parameters were typically allowed to float in this analysis although occasionally V_{max} was fixed to the value determined from the slope of the clearance phase of 60 Hz overflow curves [77].

Group averages are expressed as the mean $\pm$ SEM and N is the number of rats. Two-tailed t -tests were used to determine whether the maximum dopamine concentration ($[DA]_{max}$) elicited by 20 and 60 Hz electrical stimulations at baseline were different in PN28 and PN65 rats (Figure 1). Because of age differences in baseline $[DA]_{max}$, cocaine-induced changes in dopamine efflux were expressed relative to the baseline in each rat. Group averages of percent changes

were calculated and displayed in Figure 3. The effects of age (28 vs. 65 day old rats) on brain region (caudate vs. nucleus accumbens core) on percent changes in cocaine-induced dopamine efflux were analyzed by two-way ANOVA with repeated measure on time. Post-hoc analysis used the Newman-Keuls Multiple Comparison Test. Differences were considered to be significant when $p < 0.05$.

RESULTS

Electrically-stimulated dopamine release was recorded at multiple frequencies in the caudate and nucleus accumbens core of PN28 and PN65 male rats. The mean age and range on the day of the experiment was 28 (27–30) and 68 (60–77) for the PN28 and PN65 groups, respectively. Varying the frequency of electrical stimulation evokes steady-state responses at low frequencies (20 Hz) as uptake and release are balanced, and linear increases at high frequencies (60 Hz) as release overwhelms uptake.

Figure 1 shows averaged, baseline overflow curves resulting from 20 and 60 Hz stimulations from all rats tested. Only these two frequencies are depicted to show greater detail in these frequencies characteristic of tonic and phasic firing, respectively. Maximal extracellular dopamine concentrations are greater in the caudate (Figure 1A) of adult rats following 20 Hz ($F(1,20) = 14.4$, $p < 0.002$) and 60 Hz ($p = 0.02$) stimulations. In the nucleus accumbens core (Figure 1B), extracellular dopamine concentrations were significantly greater in the adults following 60 Hz ($F(1,8) = 7.26$, $p = 0.027$) but not 20 Hz stimulations. Figure 1 indicates that electrically-stimulated dopamine release is not functionally mature in the periadolescent in stimulation frequency and brain region specific ways.

Figure 2 shows baseline 20 Hz stimulated dopamine release and the time course of cocaine effects in representative animals. Cocaine (15 mg/kg) rapidly increased evoked extracellular dopamine relative to baseline in both animals but to a greater extent in the periadolescent (Figure 2B). Cocaine effects are rapid, reaching a maximum by 10 or 15 min after injection and waning by 30 and 60 min. The shapes of the overflow curves reveal age-related differences. The recordings from the adult animal (Figure 2A) show that cocaine increased the amplitudes of the steady-state dopamine signals without altering their shapes. In contrast, the steady-state of the baseline is lost in the PN28 as extracellular dopamine concentration continues to rise throughout the 60 pulse stimulation. This age difference in the shape of the overflow curves suggests that the magnitude of the age difference in cocaine effects was determined by the duration of the stimulus train. The results of similar experiments performed in the nucleus accumbens core are shown only as group averages of the maximal cocaine effects in the next figure because there were no significant age differences.

Figure 3 shows the time course of cocaine effects on stimulated dopamine release in groups of periadolescent and adult rats (as in Figure 2). Two-way ANOVA (region $\times$ age) with repeated measures on time indicated that cocaine induced greater dopamine increases overall in the caudate (Figure 3A) and nucleus accumbens (Figure 3B) of 28 day old rats than in adult rats ($F(1,152) = 6.82$, $p = 0.02$). The increases in the caudate (particularly of the periadolescents) tended to be larger but the main effect of brain region did not reach statistical significance ($F(1,152) = 4.44$, $p = 0.052$). The effects of cocaine were clearly time dependent ($p < 0.001$). Time-dependent cocaine effects varied by age ($p < 0.001$) and region ($p < 0.001$). The three-way interaction of age, region and time was also significant ($F(7,152) = 2.61$, $p = 0.016$). Two-way ANOVA for the nucleus accumbens data indicated that age did not affect the cocaine-induced dopamine increases in this region ($F(1,63) = 1.11$, $p = 0.33$). Cocaine effects were highly time-dependent ($p < 0.001$) in nucleus accumbens, but there was no interaction of time and age. In contrast, cocaine-induced increases were greater in the caudate of PN28 relative to PN65 rats ($F(1,77) = 7.17$, $p = 0.025$). The effects of cocaine were also highly time-dependent in dorsal

striatum ($p < 0.001$) and significantly interacted with age ($p < 0.001$). Figure 3 confirms that cocaine increased stimulated dopamine overflow more in the caudate of periadolescent than adult rats.

A similar pattern was found following administration of 10 mg/kg cocaine to other adult and adolescent male rats (not shown). This dose of cocaine maximally increased extracellular dopamine elicited by 20 Hz stimulations to 431 ± 61 percent of baseline (mean $\pm$ SEM) 10 min following cocaine in adolescent caudate and 228 ± 16 percent of baseline 15 min following cocaine in adult caudate. These results mirror those obtained following 15 mg/kg cocaine and show that the age differences in caudate exist for two doses of cocaine. Matching data for the nucleus accumbens were not determined.

Figure 4 shows the frequency dependence of cocaine-induced changes from baseline (within each animal) in the caudate (Figure 4A) and nucleus accumbens (Figure 4B) of periadolescent and adult rats. Cocaine-induced increases in evoked dopamine concentration were frequency dependent ($p < 0.001$) and effects at 20 Hz in both regions were greater than all the others (post-hoc, $p < 0.05$). ANOVA found a main effect of region ($p = 0.018$) as the cocaine effects were greater in the caudate because of the large response in the PN28 rats. Significant interactions of age and frequency ($p < 0.001$) and age, region and frequency ($p = 0.044$) were found. Cocaine increased extracellular dopamine similarly in both ages in the nucleus accumbens and the only significant effect was for frequency ($p < 0.001$). The robust age difference following 20 Hz stimulations in the caudate contributed largely to the significant age $\times$ frequency interaction in this region ($p = 0.002$).

To understand the basis for age differences in baseline electrically-stimulated dopamine release and cocaine effects further, kinetic analysis used overflow curves from individual rats (as in Figure 1 and including additional frequencies) to resolve dopamine release ($[DA]_p$) and the uptake parameters, V_{max} and K_m . Figure 5 shows the values of these parameters at baseline and after cocaine administration for only the rats from Figure 1 that received 15 mg/kg cocaine. The dopamine release term, $[DA]_p$ (Figure 5A), was significantly lower in periadolescent caudate (t-test, $p = 0.003$) and nucleus accumbens ($p = 0.002$) than adults. V_{max} (Figure 5B) was lower in periadolescent caudate ($p < 0.001$) and nucleus accumbens ($p < 0.001$) than adults. K_m (Figure 5C) was lower in periadolescent caudate ($p < 0.001$) indicating greater affinity of the dopamine transporter (DAT) for dopamine than in the adults. No age differences in K_m were found in the nucleus accumbens.

Total dopamine clearance rate is determined by the Michaelis-Menton parameters, V_{max} and K_m . The net clearance rate for baseline stimulations can be visualized in the plot of the decay portions of the averaged overflow curves (Figure 1). The close overlap and nearly simultaneous return to baseline of the decay curves from both ages indicate that despite the age differences in V_{max} and K_m , net dopamine clearance is similar in the periadolescent and adult caudate. In the periadolescent caudate, V_{max} contributes less and K_m contributes more to total clearance rate than in the adult.

Figure 5 also shows the results of the kinetic analysis of the cocaine data. As expected, the competitive uptake inhibitor, cocaine increased K_m overall ($p < 0.001$) without changing V_{max} . ANOVA also indicated that cocaine induced a significant increase in $[DA]_p$ ($p = 0.022$) considering both ages and both regions together. There were no significant post-hoc differences in the effects of cocaine on $[DA]_p$.

DISCUSSION

The present study showed that cocaine elicited greater increases in electrically-stimulated extracellular dopamine in adolescent than in adult rats. By using cyclic voltammetry, we were

able to show that this difference exists because adolescents and adults use a different balance of mechanisms to attain comparable rates of dopamine clearance. Relative to adults, adolescents had a higher ratio of uptake/release. In addition, the component mechanisms of uptake were different in the periadolescent than the adult caudate: higher affinity uptake in the PN28 rats compensated for a lower $V_{\max}$. This resulted in an unusual finding of lower electrically-stimulated dopamine release even at relatively “physiologic” stimulation frequencies. This uptake dominated situation likely leads to lower extracellular dopamine levels and greater relative disruption by dopamine uptake inhibitors in adolescents than adults. This developmentally specific form of regulation could influence both environment- and drug-induced activation of the dopamine system.

The present results show that DAT inhibition by cocaine produced greater effects on extracellular dopamine concentration attained after electrical stimulation. A moderate dose of cocaine increased dopamine efflux about 3-fold in adult striatum but nearly 9-fold in the caudate of PN28 rats. Similar results have been reported by Stamford [64] who showed that nomifensine (10 mg/kg, i.p.) increased electrically-stimulated dopamine release more in the striatum of young (30 day) rats relative to adults. Our study used a lower stimulation frequency than that of Stamford and this likely contributed to a greater relative age difference because the effects of competitive dopamine uptake inhibitors are greatest at 10–20 Hz [75]. Stamford attributed the age difference in nomifensine effects to age-related differences in baseline dopamine regulation as both uptake and release were greater in adults than young rats but their relative ratio (uptake/release) was greater in the striatum of young rats. The present results demonstrate that despite the lower $V_{\max}$ in periadolescent caudate, total dopamine clearance was very similar to that of adults because of the lower K_m . By using the kinetic modeling that cyclic voltammetry allows, we also were able to show that dopamine release is lower in the periadolescent. Thus, we also find uptake/release is greater in periadolescent than adult caudate. This “uptake-dominated” situation favors greater relative disruption by competitive uptake inhibitors.

This striking difference was region-specific: age differences were only observed in the striatum and not in the core of the nucleus accumbens. A similar phenomenon has been reported for nomifensine, which was found to increase stimulated dopamine efflux more in caudate nucleus than nucleus accumbens core in brain slices of adult rats [28]. These authors speculated that the regional difference in potency (K_i) could be due to a greater degree of DAT glycosylation in the nucleus accumbens [39]. This regional difference could have important implications for age differences in behavioral response to addictive drugs. The seminal work of Kelly and others [31] suggest that the balance of locomotor stimulation and stereotypy after psychostimulants should vary in adolescents and adults. The present findings would predict a greater tendency toward stereotypy in response to high doses of psychostimulants. Indeed, this is exactly what we have reported [10]. The relevance for addiction is far from clear, but recent findings which support a more important role for the dorsal striatum in habit-learning related to self-administration of addictive drugs suggest that adolescents might be at greater risk for this transition [21,54,69]. The rewarding effects of cocaine do not appear to be enhanced in adolescent rats because the majority of studies do not show greater i.v. self administration [5, 22,29,33,38]. We did not find differences in cocaine effects in nucleus accumbens, the region thought to mediate the rewarding effects of cocaine.

Adolescents exhibited an unusual frequency-dependent pattern of dopamine release: release was lower than adults even after lower (20Hz) stimulation frequencies. Typically, differences in maximal release capacity are “buffered” at 20 Hz stimulation frequency so that extracellular dopamine concentrations are uniform at different combinations of release and uptake rates [6]. For example, Garriss et al. [23] determined electrically-stimulated dopamine release in the caudate of rats with partial 6-hydroxydopamine lesions of the substantia nigra. They found that

60 Hz stimulations were lower in parallel with decreased tissue dopamine content, but 20 Hz stimulations were not attenuated by the lesion. Likewise, greater release and uptake rates in female caudate were accompanied by greater overflow following 60 Hz but not 20 Hz stimulations in the females [73]. The unusually low overflow following 20 Hz stimulations indicates that extracellular dopamine regulation is substantially different in periadolescent caudate.

The robust age difference in extracellular dopamine evoked by 20 Hz stimulations is best explained by a lower K_m in adolescents, because the steady-state concentration of evoked dopamine is linearly related to K_m [77]. Developmental differences in DAT glycosylation represent one potential explanation for the lower K_m for dopamine uptake observed in adolescents. Patel et al. [49] found that DAT from the striatum of adult rats had a higher molecular weight than DAT from young rats at 0, 4, and 14 days of age. Chemical removal of N-linked sugars reduced the age-related differences in DAT size, indicating that adult DAT is more glycosylated than DAT in immature rats. Human DAT mutants with glycosylation sites removed exhibit lower molecular weights and when expressed in cells, their altered functional dopamine uptake is relevant to the current results [40]. Non-glycosylated mutant DAT tended to be less represented at the cell surface and endocytosis of mutants was enhanced. The V_{max} for dopamine uptake into cells expressing non-glycosylated mutants was significantly lower than those expressing wild-type DAT and most importantly, the K_m for dopamine uptake was also lower in mutants [40]. Furthermore, cocaine was more potent for inhibition of dopamine uptake into cells expressing the least glycosylated mutant. The functional *in vivo* kinetics in periadolescent caudate (lower V_{max} and K_m) exhibit the same pattern seen in non-glycosylated mutants in the Li et al. [40] study and suggest that DAT in PN28 caudate is less glycosylated than adult caudate DAT. If such a developmental difference exists in human, then these results would have broader implications for pharmacotherapy with DAT inhibitors in adolescence.

The dopamine uptake/release mismatch at baseline might be expected to result in a lower basal (non-stimulated) level of extracellular DA during adolescence. In fact, basal extracellular dopamine concentrations measured by microdialysis are reported to be lower than adult values in 33–43 day old rats [2] and in 35–36 day olds [36]. In contrast to lower extracellular dopamine in adolescents, striatal D_1 and D_2 receptor levels are higher in adolescent than adult humans [47,48,58] and rats [65,66]. The combination of more dopamine being released due to DAT inhibition onto more, or more sensitive, receptors could synergistically contribute to the greater behavioral response to cocaine that we reported [11].

A limited number of studies in humans suggest that a similar balance of uptake and release might influence drug responses in adolescent humans. Transporter density reaches maximal expression during adolescence while stores/synthetic capacity achieve maximal levels later [25]. The mechanism for how a hypofunctional dopaminergic system would produce greater vulnerability to addiction is not fully understood. One possibility is that adolescent animals have lower baseline dopamine activity and an enhanced threshold for rewarding stimuli [7]. If so, a greater relative dopamine response induced by psychostimulant administration renders adolescents biologically vulnerable to addiction. Our results showing greater dopamine stimulation in dorsal striatum relative to nucleus accumbens are consistent with the observations that adolescent rats transition into stereotypy at lower doses of cocaine than adults [11] but do not self administer more cocaine and that enhanced habit learning in the dorsal striatum could underlie the adolescent vulnerability to addiction.

Acknowledgments

This work was supported by grant #DA09079

Abbreviations

PN, post-natal (age); [DA]_p, concentration of dopamine released per stimulus pulse; [DA]_{max}, maximal evoked dopamine concentration.

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Figure 1.

Electrically-stimulated extracellular dopamine concentrations in the caudate (A) and nucleus accumbens core (B) of anesthetized adolescent and adult male rats were determined at carbon-fiber microelectrodes using fast cyclic voltammetry. The circles represent average extracellular dopamine concentrations recorded at 10 Hz (N= 12 adolescents and 10 adults in the caudate and 5 for both ages in the nucleus accumbens). Standard error bars are shown for every third point for clarity. Background subtracted cyclic voltammograms (not shown) matched those collected at the same electrode perfused with a standard dopamine solution *in vitro* after the experiment, thereby identifying the electroactive substance *in vivo* as dopamine. Solid lines under the recordings indicate the duration of 20 and 60 Hz electrical stimulations (60 pulses at 300 μA). Vertical scale bars are specific to each frequency and indicate dopamine concentration in both regions. The inset box on the right shows the clearance phases of the 60 Hz caudate curves, time-shifted so that equimolar dopamine concentrations start at the same point.

Figure 2.

Voltammetric recordings in caudate of individual PN28 (A) and PN65 (B) rats following 20 Hz 60 pulse electrical stimulations of the medial forebrain bundle. Baseline recordings for each age prior to drug administration are shown on the far left. Evoked dopamine concentrations were determined at the times indicated following 15 mg/kg cocaine i.p. The scale bars for each animal are identical. The bar under each recording indicates when the electrical stimulation was applied.

Figure 3.

Age differences in the time course of cocaine effects on dopamine overflow in caudate (A) and nucleus accumbens core (B) of anesthetized rats. Cocaine effects are expressed as a percent of the change in DA_{max} (cocaine / pre-drug baseline). For the caudate, [DA]_{max} was averaged across groups of PN28 (N=7) and PN65 (N=5) rats and for the nucleus accumbens, PN28 (N=4) and PN65 (N=5). The average [DA]_{max} before administration of cocaine was significantly greater in adults than periadolescents only in the caudate (Figure 1). The effect of cocaine was first determined at 1 min after injection, then at 2.5, 5, 7.5 10, 30 and 60 min. The dashed lines serve as a visual reference for the baseline values. *significantly greater than the baseline value of the same age (P < 0.05).

Figure 4.

Frequency dependence of cocaine effects on stimulated dopamine overflow in caudate (A) and nucleus accumbens core (B) of anesthetized rats. The groups of rats described in Figure 3 were also tested at these additional frequencies from 20 to 40 min after 15 mg/kg cocaine i.p. The maximal responses to 20 Hz stimulations in Figure 3 are also shown in this figure. Cocaine effects were maximal at 20 Hz in both regions and age differences were significant only in the caudate. *significantly greater than PN65 at the same frequency ($P < 0.05$).

Figure 5.

In vivo dopamine release and uptake kinetics at baseline and following 15 mg/kg cocaine i.p. in caudate and nucleus accumbens core. Overflow curves recorded following 20 – 60 Hz stimulations (as in Figure 1) were analyzed simultaneously to resolve release (A) and uptake parameters, V_{max} (B) and K_m (C) (see Methods). Baseline values shown here were restricted to the subset of rats from Figure 1 that were subsequently given 15 mg/kg cocaine to compare cocaine-induced changes from baseline. The N for the caudate groups were PN28 (N=7) and PN65 (N=5); and for the nucleus accumbens, PN28 (N=4) and PN65 (N=5). *significantly different than baseline (P < 0.05). # significantly different than PN28 (P < 0.05).