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Speedball induced changes in electrically stimulated dopamine overflow in rat nucleus accumbens

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Abstract

Cocaine/heroin combinations (speedball) induce a synergistic elevation in extracellular dopamine concentrations ($[DA]_e$) in the nucleus accumbens (NAc) that can explain the increased abuse liability of speedball. To further delineate the mechanism of this neurochemical synergism, *in vivo* fast-scan cyclic voltammetry (FSCV) was used to compare NAc DA release and reuptake kinetic parameters following acute administration of cocaine, heroin and speedball in drug-naïve rats. These parameters were extracted from accumbal DA overflow induced by electrical stimulation of the ventral tegmental area. Evoked DA efflux was increased following both cocaine and speedball delivery, whereas heroin did not significantly change evoked DA release from baseline. DA efflux was significantly greater following cocaine compared to speedball. However, DA transporter (DAT) apparent affinity (K_m) values were similarly elevated following cocaine and speedball administration, but unaffected by heroin. Neither drug induced substantial changes in the maximal reuptake rate (V_{max}). These data, combined with published microdialysis and electrophysiological results, indicate that the combination of cocaine-induced competitive inhibition of DAT and the increase in the DA release elicited by heroin is responsible for the synergistic increase in $[DA]_e$ induced by speedball.

Keywords

cocaine; heroin; speedball; dopamine; uptake; release; voltammetry

Introduction

The co-use of cocaine and opiates, termed speedball (Leri et al., 2003a), has been on the rise since the 1970s and represents a growing subpopulation of drug abusers (Craddock et al., 1997; Greberman and Wada, 1994; Kosten et al., 1987). The prevalence of cocaine use among heroin addicts ranges from 30% to 80% (Leri et al., 2003a; Schutz et al., 1994). Negative health and social consequences of such combinations are severe, particularly when cocaine is used intravenously (Schutz et al., 1994). Specifically, the likelihood of positive

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treatment outcomes for opiate addicts decreases severely when high amounts of cocaine are co-abused (Downey et al., 2000). Several hypotheses have been proposed to explain the combined use of cocaine and heroin in humans, including an enhancement of the positive effects of either drug, reduction in the magnitude or duration of undesired side effects, induction of euphoria beyond what either drug alone provides, or independent, non-additive effects even though the drugs are administered concurrently (Foltin and Fischman, 1992; Hemby et al., 1996; Kosten et al., 1987). The hypothesis of enhanced euphorogenic effects in humans is paralleled in preclinical studies, which demonstrate that cocaine and heroin potentiate the reinforcing effects of one another in the self-administration paradigm (Mattox et al., 1997; Rowlett and Woolverton, 1997); however, the specific biological contributors which mediate the amplified reinforcing effects of speedball have not been confirmed.

The potentiated euphorogenic effects in humans and the corresponding enhancement of reinforcing effects in animal models may be based on an augmented neurochemical response in brain pathways underlying reinforcement processes. The mesolimbic dopamine (DA) system is recognized as a critical substrate for the reinforcing effects of drugs of abuse. In general, drugs of abuse activate this pathway and stimulate DA neurotransmission in the nucleus accumbens (NAc) in humans, nonhuman primates and rodents (Lyons et al., 1996; Porrino, 1993; Volkow et al., 1997), which is an effect associated with the abuse liability of such substances. Previous studies have shown that extracellular DA concentrations ($[DA]_e$) in the NAc are significantly elevated (300–400% baseline) during cocaine self-administration (Hemby et al., 1997; Pettit and Justice, 1989; Wise et al., 1995b) and moderately increased (120–150% baseline) by heroin self-administration (Leri et al., 2003b; Wise et al., 1995a) in rats as measured by *in vivo* microdialysis (but see (Hemby et al., 1999; Hemby et al., 1995; Smith et al., 2006) where heroin self-administration did not significantly alter NAc $[DA]_e$). Interestingly, we have demonstrated that intravenous speedball self-administration resulted in synergistic elevations in NAc $[DA]_e$ (~1000% of baseline) compared with cocaine (400%) or heroin alone (no change) (Hemby et al., 1999). Subsequent studies examining self-administration of lower dose combinations, as well as experimenter-administered speedball combinations, have reported similar synergistic elevations (Hemby et al., 1999; Smith et al., 2006; Zernig et al., 1997). While *in vivo* microdialysis studies provide information on the extent to which NAc $[DA]_e$ is increased during speedball self-administration, the mechanisms by which heroin potentiates the effects of cocaine on NAc $[DA]_e$ are not fully understood.

In vivo voltammetry is an electrochemical technique that is commonly used in conjunction with *in vivo* microdialysis to further characterize DA dynamics (Mateo et al., 2004; Ng et al., 1991; Oleson et al., 2009a; Oleson et al., 2009b; Stamford et al., 1989; Wu et al., 2001a). Voltammetry facilitates analysis of DA uptake kinetics aside from DA release or metabolism contributions (Wu et al., 2001b; Zimmerman and Wightman, 1991). Importantly also, the higher temporal resolution of this technique allows the time course of DA uptake inhibition to be determined more precisely (Budygin and Jones, 2008; Budygin et al., 2000). This is principally beneficial when drugs are administered intravenously and therefore have a fast onset neurochemical response (Mateo et al., 2004). In the present study, we utilized *in vivo* fast-scan cyclic voltammetry (FSCV) to assess electrically evoked DA release and DAT kinetic parameters in rat NAc in pursuit of unveiling the mechanisms of neurochemical synergy of speedball administration.

2. Methods

2.1 Animals

Male Fisher F-344 rats (120–150 days; 270–320 g; Charles River, Wilmington, MA) were housed in acrylic cages in a temperature-controlled vivarium on a 12 h reversed light/dark

cycle (lights on at 6:00 PM). Rats were group-housed before surgery and singly housed after catheterization. Food was restricted to maintain starting body weight and water was available *ad libitum*, except during experimental sessions which were conducted during the dark phase. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) revised in 1996.

2.2 Intravenous catheterization

Drug-naïve rats ($n = 15$) were anesthetized with 1.5 g/kg urethane (i.p.) and implanted with chronic indwelling venous catheters as described previously (Hemby et al., 1999; Hemby et al., 1997; Hemby et al., 1996). Catheters were inserted into the right jugular vein, terminating just outside the right atrium and anchored to muscle near the point of entry into the vein. The distal end of the catheter was attached to a syringe containing heparinized saline. Immediately following catheterization, while still under urethane anesthesia, *in vivo* fast-scan cyclic voltammetry experiments were performed.

2.3 In vivo fast-scan cyclic voltammetry

While under urethane anesthesia, rats were secured in a stereotaxic frame in a flat skull position. Holes were drilled into the skull above the NAc and ventral tegmental area (VTA) (AP: +1.3, L: +1.3 and AP: -5.2, L: +1.0 in mm relative to bregma, respectively). An additional hole was drilled into the skull of the contralateral hemisphere into which an Ag/AgCl reference electrode was implanted just below the surface of the skull and connected to a voltammetric amplifier. A carbon fiber microelectrode (approximately 80 to 200 μm in length beyond the glass capillary in which it was contained) was secured to the stereotaxic frame arm and also connected to the voltage amplifier. The carbon fiber electrode was placed into the hole above the NAc, and lowered approximately 5 mm from the surface into the striatum. A bipolar stimulating electrode was connected to a voltage output box and lowered into the hole above the VTA approximately 7.5 mm. Voltammetric recordings were made at the recording electrode every 100 ms for a 15 s duration by applying a triangular waveform (-0.4 to +1.2 V, 400 V/s). The biphasic stimulation applied by the stimulating electrode consisted of 60 rectangular pulses at 60 Hz, 300 μA and was activated at 5 s into each recording. Recorded signals showed an oxidation peak at +0.6 V and a reduction peak at -0.2 V (vs. Ag/AgCl reference), ensuring that the released chemical was indeed DA. The depths of the carbon fiber microelectrode and bipolar stimulating electrode were adjusted from -6.2 to -7.2 and -7.5 to 8.3 mm, respectively, in order to optimize electrically evoked DA release in the NAc core.

Once evoked DA recordings were optimized, stable readings were collected every 5 min for at least 50 min. When baseline recordings were within 10% of each other for at least 5 measurements, drug (1 mg/kg cocaine, 0.03 mg/kg heroin or 1 mg/kg cocaine/0.03 mg/kg heroin combination; $n = 5$ per drug) was injected i.v. over a 6 sec period, immediately followed by 0.2 ml of heparinized saline i.v. over another 6 sec. Importantly, selected doses of the drugs resulted in reliable synergistic elevations in extracellular DA levels measured by microdialysis in rat NAc during speedball self-administration (Hemby et al., 1999). Stimulated recordings were then made at 1, 5 and 10 min and thereafter every 10 min up to 120 min following drug injection.

Carbon fiber microelectrodes were post-calibrated *in vitro* with known concentrations of DA (2–5 μM). Calibrations were performed in triplicate and the average value for the current at the peak oxidation potential was used to normalize recorded *in vivo* current signals to DA concentration. The kinetic parameters of DAT (DAT apparent affinity or the Michaelis-Menten constant, K_m , and maximal velocity of uptake rate, V_{max}) were calculated using

LVIT software (UNC, Chapel Hill, NC). DA reuptake by DAT was assumed to follow Michaelis-Menten kinetics and the change in DA concentration during and after stimulated release was fit using the equation:

$$d[DA]/dt = (f)[DA]_p - (V_{max}/((K_m/[DA]) + 1)),$$

where f is the stimulation frequency (Hz), and $[DA]_p$ is the concentration of DA released per stimulus pulse. V_{max} is the Michaelis-Menten parameter for maximal uptake rate of a first-order enzymatic reaction, such as DA reuptake by DAT. The Michaelis-Menten constant, or DAT reuptake apparent affinity, K_m , is the $[DA]_e$ required for DAT-mediated reuptake rate to reach one half of V_{max} . Baseline K_m value was previously determined for DAT in rat brain synaptosomes to be 0.16 μ M (Near et al., 1988). The integral form of this equation was used to model DA response for individual rat at all time points before and after drug injections (Wu et al., 2001b). Under current condition, there are two substrates competing for DAT binding, which are cocaine and endogenous DA. Therefore, K_m is actually apparent K_m under this circumstance, but for convenience will be referred to as just K_m .

To perform an alternative analysis of DA uptake changes induced by cocaine and speedball administration, we fit a decaying exponential to the descending part of the electrically evoked DA efflux. The numbers reported in this study were obtained by fitting an exponential function of the form

$$y = y_0 + Ae^{-(x-x_0)/t},$$

to the DA concentration versus time before and 1 min after cocaine and heroin, when the maximal effects of drugs on DA efflux were observed. Here y represents the DA concentration, x represents the time, and y_0 , x_0 , A , and t are fitting parameters. The quantity y_0 is the background level of the DA concentration in the absence of any electrical stimulation, x_0 is the time at which the DA concentration begins to decline after an electrical stimulation, A is the amplitude (peak) of the DA concentration at time x_0 , and t represents the decay time for the decrease of DA concentration due to the reuptake mechanism. The portion of the curves that are fit are the points corresponding to about 70% of the peak to where the concentration is less than 5% above the background concentration value. The fitting was carried out in OriginLab's OriginPro8 data analysis and fitting program.

2.4 Data analysis

Statistical analyses were performed using SigmaStat 3.1 software. One-way repeated-measures analyses of variance (ANOVA) were used to compare effects after acute i.v. drug administration to predrug baseline values for evoked peak DA, DAT apparent affinity (K_m), and maximal uptake rate (V_{max}) values within each drug group. These data were also analyzed using two-way ANOVA with drug group (cocaine, heroin or speedball) and time as the factors. Evoked DA values were analyzed as a percent of baseline values (baseline defined as the average of five measurements taken at 25, 20, 15, 10 and 5 min before drug injection). All *post hoc* analyses were performed using Bonferroni t-tests to compare postdrug injection values to baseline (for one-way repeated-measures ANOVAs) and for pairwise comparisons between drugs.

3. Results

3.1 Electrically evoked peak DA

The amplitude of current as detected by FSCV in rat NAc markedly increased after cocaine (1.0 mg/kg, i.v.) and speedball (1.0 mg/kg cocaine + 0.03 mg/kg heroin, i.v.), but not after heroin (0.03 mg/kg, i.v.) injection (Fig. 1). As determined by one-way repeated-measures ANOVA, cocaine significantly increased electrically evoked peak DA values from baseline ($p < 0.001$) at 1, 5, 10, 20, 30, 40 and 50 min after injection ($p < 0.01$), and speedball also significantly increased baseline electrically evoked DA levels ($p < 0.001$) at 1, 5, 10, 20, and 30 min ($p < 0.005$) after drug injection, as determined by Bonferroni *post hoc* analyses. Acute i.v. heroin did not significantly change evoked DA from baseline in drug-naïve animals ($p = 0.529$) at any time after injection. Two-way ANOVA revealed significant differences in evoked peak DA values between drug groups ($F_{(2, 204)} = 64.24$; $p < 0.001$). Cocaine elicited an overall greater increase ($p < 0.001$) in electrically evoked DA levels than speedball in drug-naïve animals, significantly at 1 min after drug injections ($p < 0.05$; Fig. 2). Both speedball and cocaine provoked significant increases in evoked peak DA compared to heroin from the time of injection until 30 and 40 min after drug injections, respectively, as determined by *post hoc* analyses. A significant group $\times$ time interaction was also observed ($F_{(16, 204)} = 16.92$; $p < 0.001$).

3.2 DAT apparent affinity

Cocaine induced a significant increase in K_m from baseline ($p < 0.001$) with *post hoc* analyses showing a significant effect ($p < 0.05$) at time points 1, 5, 10, 20, 30, 40, 50, 60 and 70 min following injection. Speedball resulted in a similar increase in K_m values ($p < 0.001$) after drug injection, with significant increases from baseline at time points from 1 to 90 min after drug injection ($p < 0.05$). Heroin had no effect on baseline K_m values ($p = 0.841$). Two-way ANOVA showed no difference in K_m between cocaine and speedball following drug injection ($p = 1.000$) (Fig. 3). Both speedball and cocaine significantly increased K_m compared to heroin ($F_{(2, 204)} = 277.0$; $p < 0.001$). A significant group $\times$ time interaction was also observed ($F_{(16, 204)} = 64.88$; $p < 0.001$).

3.3 DAT-mediated reuptake rate

No significant changes in V_{max} were seen from baseline values after cocaine ($p = 0.880$). Heroin also had no effect on baseline V_{max} values ($p = 1.000$). Speedball injection did produce a modest (~ 10%) but significant increase ($p < 0.05$) in V_{max} (Fig. 4). *Post hoc* analyses revealed that the increase in V_{max} from baseline (1816.4 ± 478.6 nM/s) was significant at time points from 1 to 100 min (2023.6 ± 732.7 nM/s) ($p < 0.005$). However, two-way ANOVA comparing average V_{max} values between drugs showed no statistical difference between the average V_{max} following cocaine (1817.4 ± 662.4 nM/s) and speedball or heroin ($F_{(2, 204)} = 3.883$; $p > 0.05$). To avoid any confusion regarding drug-induced uptake changes, we performed alternative analysis fitting a single exponential decay function to the descending phase of electrically evoked DA signal. No significant difference ($t = 1.749$, $df = 8$; $p = 0.1184$, unpaired t test) was revealed between effects of cocaine and speedball on the decay time (t) for the decrease of DA concentration due to reuptake (1.28 ± 0.28 vs 1.85 ± 0.18 s). The same analysis demonstrated a significant increase in the t after cocaine (0.95 ± 0.13 s for baseline value vs 1.85 ± 0.18 s after cocaine, $t = 5.621$, $df = 4$; $p < 0.005$; paired t test) and speedball (0.68 ± 0.10 s for baseline value vs 1.28 ± 0.28 s after speedball, $t = 3.263$, $df = 4$; $p < 0.05$; paired t test). Taken together, these results indicate the marked decrease in the accumbal DA reuptake following acute cocaine and speedball administration.

4. Discussion

The proposed mechanism of speedball's actions on the mesolimbic DA system is a result of combinatory effects of cocaine and heroin on NAc [DA]_e. Cocaine blocks DAT on dopaminergic terminals as well as cell bodies to increase extracellular DA concentrations (Koob and Bloom, 1988; Pettit and Justice, 1989; Ritz et al., 1987; Volkow et al., 1997). The DA uptake inhibition leads to several different pharmacological consequences. The inhibition of soma firing rate due to the activation of DA autoreceptors is the one of well described outcomes that should diminish basal DA release in the NAc. However, the DA release induced by electrical stimulation and measured by FSCV is increased, since cocaine inhibits DA reuptake between multiple electrical pulses (Mateo et al., 2004; Oleson et al., 2009a; Oleson et al., 2009b). Heroin binds μ -opioid receptors to inhibit VTA GABAergic interneurons and remove a tonic inhibition of DA neurons, thus upregulating cell firing rates and consequently DA release at NAc terminals (Johnson and North, 1992; Leone et al., 1991; Self et al., 1995). We evaluated the changes in kinetic parameters of DA release and uptake in order to better understand the neurobiological substrates involved in the potentiating effects of speedball on NAc [DA]_e.

A significant increase in the magnitude of evoked DA release was observed following both cocaine and speedball injections. The maximal effect of intravenously administered drugs on evoked DA release in the NAc was found to occur one minute after administration, which is consistent with previous findings in our lab (Mateo et al., 2004; Oleson et al., 2009b) and correlates with the time it takes for cocaine to reach peak brain levels (Fowler et al., 1998). Kinetic analyses of the electrically evoked DA signals recorded during cocaine and speedball time courses indicated that both effects are associated with an increase in the apparent K_m for DA uptake. Importantly, it was previously indicated that the cocaine-induced increase in the electrically evoked DA release measured by FSCV *in vivo* is preferentially due to the decrease in DA reuptake (increase in the apparent K_m) (Mateo et al., 2004; Oleson et al., 2009a; Oleson et al., 2009b). Since the K_m changes were indistinguishable between cocaine and speedball groups, the possibility that heroin makes cocaine more efficient in inhibiting DA uptake when these two drugs are combined can be ruled out. Because K_m was unaffected by heroin, the elevation in K_m due to speedball is likely attributable to the cocaine component alone. Therefore, changes in the affinity of the DAT cannot be involved in the potentiating effect of speedball on extracellular DA concentrations.

The magnitude of the increase in evoked DA concentrations was significantly greater following cocaine in comparison with speedball administration. At first glance, these voltammetric results may appear contradictory to previously published microdialysis data (Hemby et al., 1999; Smith et al., 2006; Zernig et al., 1997), but the findings are in fact compatible. Microdialysis provides an assessment of the steady state extracellular levels of DA in the region of interest, but with relatively poor temporal resolution (minutes) (Jones et al., 1999). In contrast, FSCV does not allow assessment of basal DA concentrations, but detects evoked DA concentrations with subsecond resolution, allowing DA dynamics to be measured in real time (Budygin and Jones, 2008). Therefore, prompt adaptive changes in DA release and uptake can be detected with this approach. Taking this into account, the attenuated amplification in electrically evoked DA effluxes observed following speedball administration compared to cocaine alone is likely due to feedback induced by greater accumulation of DA in NAc extracellular space after the speedball administration.

Several mechanisms can be considered in this regard. Post-synaptic D₂DA receptors in the NAc provide indirect feedback to DA neurons via long pathways (Hommel and Bunney, 1980), while pre-synaptic D₂DA receptors on dopaminergic terminals and somatodendrites

function as autoreceptors (Bunney et al., 1991; Starke et al., 1989; Wolf and Roth, 1990). Pre-synaptic D₂ autoreceptors maintain dopaminergic homeostasis via feedback mechanisms, which include regulation of DA release (Cardozo and Bean, 1995; Herdon et al., 1987; Uchimura et al., 1986; Wu et al., 2002). For example, microdialysis assessment of DA in wild type and D₂ receptor knockout mice (D₂R^{-/-}) following acute injections of cocaine or morphine showed that D₂R^{-/-} mice exhibited a significantly greater increase in [DA]_e in the striatum than wild type mice (Rouge-Pont et al., 2002). *In vitro* voltammetric assessment found an increase in electrically evoked DA release when amphetamine was applied in the presence of the D₂ antagonist sulpiride compared to amphetamine alone. Moreover, a greater peak DA amplitude from amphetamine was observed in striatal slices from D₂R^{-/-} mice in comparison with wild type mice (Schmitz et al., 2002). Even though amphetamine is known to elicit DA overflow in the NAc (Fischer and Cho, 1979; Jones et al., 1998; Raiteri et al., 1979; Sulzer et al., 1995; Sulzer et al., 1993), stimulation-dependent DA release is decreased by amphetamine (Jones et al., 1998), mostly due to release-regulating D₂ autoreceptor activation which inhibits electrically evoked DA release (Schmitz et al., 2002). Similarly, markedly increased extracellular DA induced by cocaine (~400 %) and speedball (~1000%) likely activates release-regulating autoreceptors, decreasing electrically evoked DA release. However, post-synaptic D₂ receptors which provide release inhibition via long, indirect feedback loops can be also involved in this effect. Since the magnitude of NAc [DA]_e is greater following speedball compared to cocaine administration, D₂ feedback initiated by DA overflow from cocaine would be of lesser magnitude compared to speedball. Therefore, electrically evoked DA release detected by voltammetry is reduced following speedball administration compared to cocaine alone.

In agreement with this speculation, the dose of heroin used in this study did not significantly affect electrically evoked DA release. As mentioned previously, heroin administration increases dopaminergic cell firing rates via activation of mu opiate receptors on VTA GABAergic neurons causing hyperpolarization and removal of tonic inhibition on dopaminergic cells (Johnson and North, 1992; Leone et al., 1991; Self et al., 1995). *In vivo* extracellular electrophysiological recordings have shown that acute heroin decreases VTA GABAergic cell firing rates (Steffensen et al., 2006) and microdialysis studies have shown modest increases in NAc [DA]_e following heroin administration (Leri et al., 2003b; Wise et al., 1995a; Zernig et al., 1997). Perhaps a higher dose of heroin would better facilitate detection of heroin-induced increases in extracellular DA levels, which would likely be observable as a decrease in electrically evoked DA release. In agreement with such a suggestion, we observed decreased electrically evoked DA release following high doses of ethanol (Budygin et al., 2001; Jones et al., 2006), which reliably increased basal DA levels measured by microdialysis (Di Chiara and Imperato, 1988; Weiss et al., 1993; Weiss et al., 1996; Yoshimoto et al., 1992).

DA D₂ receptor activation not only inhibits release but can also upregulate uptake through DAT (Cass and Gerhardt, 1994; Hoffman and Donovan, 1994; Wu et al., 2002). The attenuated increase in electrically evoked DA efflux observed after speedball administration could be a consequence of other feedback mechanisms resulting from D₂ autoreceptor induced upregulation of DAT. However, no evidence was obtained in our experiments to support this hypothesis. Indeed, the maximal uptake rate was not notably increased following cocaine and its combination with heroin.

Although these data revealed significant changes in electrically evoked DA overflow in response to the enhanced NAc [DA]_e elicited by speedball, mechanistically they cannot explain the potentiation of this drug combination, since the ultimate outcome of D₂ DA autoreceptor-mediated feedback is a decrease in extracellular DA concentration. At the same time, we have clearly demonstrated that alterations in DAT affinity cannot contribute to the

potentiation of $[DA]_e$ observed with speedball. Therefore, our voltammetric results, when combined with published microdialysis and electrophysiological data, indicate that the combination of cocaine-induced competitive inhibition of the DAT (apparent K_m increase, but not V_{max} changes) and the modest increase in the DA release elicited by heroin via the enhancement of firing rate, explains the synergistic increase in extracellular DA concentrations induced by speedball in the NAc.

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Fig. 1.

Representative traces of electrically evoked DA signals detected by FSCV in rat NAc before (solid lines) and 1 min after (dashed lines) cocaine (1.0 mg/kg, i.v.), heroin (0.03 mg/kg, i.v.) and speedball (1.0 mg/kg cocaine + 0.03 mg/kg heroin, i.v.) injection in drug-naïve animals (upper panel). These signals had an oxidation peak at +0.6 V and a reduction peak at -0.2 V vs. Ag/AgCl reference, identifying the released species as DA. Representative color plots topographically depict the voltammetric data before drug administration, with time on the x-axis, applied scan potential on the y-axis and background-subtracted faradaic current shown on the z-axis in pseudo-color (lower panel).

Fig. 2.

Electrically evoked DA efflux in the NAc of anesthetized drug-naïve rats following a single i.v. injection of cocaine (1.0 mg/kg), heroin (0.03 mg/kg) and speedball (1.0 mg/kg cocaine + 0.03 mg/kg heroin). Injections were given at time = 0 min (↑). Evoked DA efflux values are represented as a percent of the pre-drug baseline DA concentration. All data are means $\pm$ SEM of five rats per group. * $p < 0.05$ for cocaine versus speedball (two-way ANOVA followed by Bonferroni tests). Values significantly different from baseline readings and from the heroin group are not indicated on plots (see 3.1).

Fig. 3. DAT apparent affinity (K_m , or Michaelis-Menten rate constant) in the NAc of anesthetized drug-naïve rats following a single i.v. injection of cocaine (1.0 mg/kg), heroin (0.03 mg/kg) and speedball (1.0 mg/kg cocaine + 0.03 mg/kg heroin). Injections were given at time = 0 min ($\uparrow$). All data are means $\pm$ SEM of five rats per group. Values significantly different from baseline readings and from the heroin group are not indicated on plots (see 3.2).

Fig. 4.

DAT-mediated maximal reuptake rate (V_{max}) in the NAc of anesthetized drug-naïve rats following a single i.v. injection of cocaine (1.0 mg/kg), heroin (0.03 mg/kg) and speedball (1.0 mg/kg cocaine + 0.03 mg/kg heroin). Injections were given at time = 0 min ($\uparrow$). All data are means $\pm$ SEM of five rats per group. Values significantly different from baseline readings and from the heroin group are not indicated on plots (see 3.3).