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# Effects of Cocaine on Monoamine Uptake as Measured Ex Vivo

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# Effects of cocaine on monoamine uptake as measured *ex vivo*

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## Abstract

The increase in extracellular dopamine (DA) following cocaine administration plays a major role in cocaine abuse. *In vitro*, cocaine binds to DA transporters (DAT) and blocks DA uptake. Moreover, cocaine can increase extracellular DA concentration as measured by *in vivo* neurochemical methods. The present study examined the effects of cocaine and other drugs on DA, NE and 5-HT uptake using an *ex vivo* assay. Rats were injected i.v. with saline or drug and sacrificed at various time points after injections. Brains were dissected for regional monoamine uptake studies *ex vivo*. In most brain regions, cocaine given *in vivo* blocked monoamine uptake as expected. [$^3$H]DA uptake in nucleus accumbens was inhibited with an $ED_{50}$ = 22.3 μmol/kg. Cocaine fully inhibited [$^3$H]NE uptake ($ED_{50}$ = 4.58 μmol/kg) in the occipital cortex and partially inhibited [$^3$H]5-HT uptake (33% at 30 μmol/kg) in the midbrain. However, under the same conditions [$^3$H]DA uptake in the striatum was not inhibited after injections of cocaine up to 56 μmol/kg.. Although the mechanism for this discrepancy is unclear, DA binding and uptake sites may be distinct and/or there may be regional differences in DA transporters.

### Keywords

Cocaine; Monoamine uptake; Ex vivo; Striatum; Nucleus accumbens

Cocaine blocks the uptake of monoamine neurotransmitters *in vitro,* with about equal potency for the three major monoamines, dopamine (DA), norepinephrine (NE), and serotonin (5-HT) [22]. Further, it is widely held that the behavioral effects of cocaine are related to the increase in extraneuronal levels of monoamine neurotransmitters in the CNS [2,18]. A substantial body of data implicates blockade of CNS dopamine (DA) reuptake by dopamine transporters (DAT) as a primary mechanism in the abuse-related effects of cocaine [34,35].

*In vivo* assays also indicate that cocaine can increase the extracellular levels of monoamines. Research with *in vivo* microdialysis has demonstrated an increase in monoamine concentrations in rat striatum and nucleus accumbens following cocaine administration [19], and in monkey striatum as well [29]. Experiments utilizing *in vivo* electrochemistry have reported that systemic administration of cocaine can decrease the clearance of locally-applied dopamine in

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rat striatum and accumbens [4,38], suggesting that blockade of uptake underlies the monoamine elevations.

Although these studies are consistent with the hypothesis that blockade of DA uptake by the DAT is necessary to the behavioral effects of cocaine, other recent data challenge this notion. Self-administration of cocaine is maintained in mice lacking the DAT (DAT knockout mice) [21]. Cocaine-induced place preference was unaffected in mice lacking either the DAT or the serotonin transporter (SERT) [25], but eliminated in mice lacking both DAT and SERT [26]. The present study was designed as an additional test of the hypothesis that cocaine blocks the uptake of monoamine neurotransmitters *in vivo*. Various monoamine uptake blockers were administered i.v. to rats, after which the rats were sacrificed, brains were dissected, and monoamine uptake assays were performed *ex vivo*. The assay has previously been used to study the blockade of DA uptake by cocaine [15] and other drugs [9,31].

All animal use procedures were approved by the University of Mississippi Medical Center's Animal Care and Use Committee and in accordance with National Institutes of Health guidelines.

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing between 250 and 300 g were used. Rats were initially housed in groups of 3 in plastic cages and with a 12:12 light/dark cycle. Food and water were available ad libitum. Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and a femoral catheter was implanted. After surgery, they were housed individually for 72 h then used experimentally.

For *ex vivo* monoamine uptake studies, the method was similar to that published by others [9,15]. Briefly, catheterized rats were injected i.v. with saline or test drugs via the catheter. At designated time points they were sacrificed by decapitation. Striatum, occipital cortex, midbrain (approximately 60 mg tissue/rat) and accumbens (14 mg tissue/rat) were dissected chopped into slices and incubated (37 °C) for 5 min in 1.0 ml of buffer containing [$^3$H]DA (5.0 nM), [$^3$H]NE (5.0 nM), or [$^3$H]5-HT (5.0 nM), respectively. Non-specific uptake was measured under identical conditions but at 4 °C. Other details of the assay have been published [33].

To verify the effects of cocaine in a different tissue preparation, *ex vivo* uptake experiments were conducted using whole homogenized tissue. Rats were sacrificed five min after cocaine injections. Rather than chopping, brain tissue was homogenized using 10 strokes with a Teflon pestle homogenizer (Glas-Col, Terre Hante, IN) at 1000 rpm.

To verify the presence of cocaine in tissues, we also studied ex vivo [$^3$H]cocaine binding and, in other rats, measured the concentration of [3H]cocaine in striatum. Catheterized rats were injected i.v. with [$^3$H]cocaine (20 μCi/rat) and sacrificed by decapitation at various time points up to 10 min after injection. Striatum and cerebellum were dissected, put into separate 10 ml glass vials and 10 μl/mg tissue Solvable® was added. After 24 hours, 1 μl/mg tissue glacial acetic acid was added to neutralize Solvable. Radioactivity was counted using a scintillation counter (Top Count®, Packard Instruments, Downers Grove, IL). The concentration of cocaine achieved in striatum was estimated by multiplying total injected cocaine (30 μmol/kg) by the proportion of [$^3$H]cocaine bound in striatum relative to the total injected.

All uptake data from drug-pretreated rats were converted to percentages of control, with data from rats pretreated with saline on the same experimental day serving as control values. $ED_{50}$ values were calculated using nonlinear regression assuming sigmoidal dose-responses with variable slopes (Prism 4.0, Graphpad, San Diego, CA). For [$^3$H]cocaine binding, striatum/cerebellum ratios of binding were calculated. One-way ANOVA with Bonferroni's multiple comparison was used with $p < 0.05$ considered statistically significant.

There was a dose-related inhibition of uptake of [$^3$H]DA in accumbens (Figure 1, circles; ED50 =1.0 μmol/kg) and striatum (Figure 1, squares; $ED_{50}$ = 5.45 μmol/kg) of rats given GBR 12909. Similarly, dose-dependent and complete inhibitions of [$^3$H]5-HT uptake in the midbrain and [$^3$H]NE uptake in the occipital cortex were seen in rats given citalopram and DMI, respectively (Figure 1, triangles: $ED_{50}$ citalopram = 3.26 μmol/kg; inverted triangles: $ED_{50}$ DMI = 0.50 μmol/kg). DMI (10 μmol/kg) failed to block [$^3$H]NE and [$^3$H]DA uptake (105 ± 4 % and 98.9 ± 18 % of control respectively) and citalopram (30 μmol/kg) failed to block [$^3$H]DA uptake (120 ± 17 % of control) in accumbens.

As seen with GBR 12909, there was a dose-related inhibition of [$^3$H]DA uptake in accumbens (Figure 2, circles; $ED_{50}$ = 22.3 μmol/kg) of rats given cocaine (3–30 μmol/kg, 1–10 mg/kg, i.v.). However, cocaine had no effect on [$^3$H]DA uptake in the striatum over this same dose range. A higher dose (56 μmol/kg, 19 mg/kg) was lethal in all rats tested (n = 3). Rats pretreated with diazepam (2.0 mg/kg, i.v.) survived an injection of 56 μmol/kg cocaine, but with no evidence of blockade of [$^3$H]DA uptake in the striatum (Figure 2, squares). Blockade of striatal [$^3$H]DA uptake was also not evident when the time between cocaine (30 μmol/kg) injection and sacrifice was varied (Table). Indeed, [$^3$H]DA uptake increased in the striatum when rats were sacrificed 15 min after the injection [$F$ (9,20) = 4.8; $P < 0.001$]. Cocaine completely blocked uptake of [$^3$H]NE in the occipital cortex (Figure 2, inverted triangles; $ED_{50}$ = 4.58 μmol/kg). Uptake of [$^3$H]5HT in the midbrain was decreased to 67 % of control at 30 μmol/kg cocaine [Figure 2, triangles; $F$ (3,8) = 9.9; $P < 0.005$].

In experiments using homogenized tissue, [$^3$H]DA uptake in striatum was increased to 121 ± 3 % of saline control after injections of cocaine (30 μmol/kg, $P < 0.005$). [$^3$H]DA uptake in accumbens was reduced to 74 ± 2.5 % of saline control, [$^3$H]NE uptake in occipital cortex was reduced to 8 ± 4 % of saline control, and [$^3$H]5-HT uptake in mid-brain was reduced to 42 ± 5 % of saline control (all comparisons $P < 0.001$).

When [$^3$H]cocaine was injected, the striatum/cerebellum ratio of binding was 1.6 within one min after injection. This ratio ranged between 1.6 and 1.8 for at least 10 min after the injection [$F$ (17,64) = 15.7; $P < 0.0001$). Five minutes after injection, a dose of 30 μmol/kg of cocaine was estimated to achieve 1200 nM of cocaine in the ex vivo uptake incubation buffer.

Together with previously published results [9,31], the present results confirm the utility of this *ex vivo* assay for the study of *in vivo* monoamine uptake blockade. When administered i.v., GBR 12909 caused a dose-related blockade of the uptake of [$^3$H]DA in striatum and accumbens. This result is consistent with results of both *in vitro* and *in vivo* studies with GBR 12909 [12,3]. Similarly, our results with DMI and citalopram are consistent with existing *in vitro* [11,24] and *in vivo* [19,17] results.

By-and-large, the effects of i.v. cocaine were as expected. Cocaine caused a dose-related blockade of the uptake of [$^3$H]DA in accumbens. This result is consistent with both *in vitro* and *in vivo* studies with cocaine and DA in the accumbens [22,38]. The failure of DMI or citalopram to block [$^3$H]NE or [$^3$H]DA uptake in the accumbens argues that blockade of [$^3$H]DA uptake involved a DAT action of cocaine. The effective dose was 3-30 μmol/kg (approximately 1–10 mg/kg), appropriately lower than the i.p. doses of 20 and 30 mg/kg reported to increase extracellular DA *in vivo* [38], though somewhat higher than the 0.25–1.25 mg/kg i.v. dose range reported to increase extracellular DA [18]. The present results also support, at least qualitatively, the conclusion that blockade of DA uptake in the accumbens is involved in the behavioral effects of i.v. cocaine. Quantitatively, however, the range of effective doses in the present study was higher than the range of 0.25–1.25 mg/kg/injection of cocaine reported to maintain i.v. self-administration and increase extracelluar DA in accumbens [18]. However, animals can accumulate injections in a typical self-administration study. Moreover,

for *in vivo* microdialysis neurotransmitter concentrations are measured continuously over several minutes rather than precisely at five min post-injection. Therefore, the present data do not seriously challenge the hypothesis that blockade of DA uptake in the accumbens plays a central role in the reinforcing effect of cocaine.

Potency relationships between cocaine and GBR 12909 with regard to DA uptake were consistent with the literature. *In vitro*, GBR 12909 has approximately 10-fold higher DAT binding affinity than cocaine [30]. As noted, cocaine doses of 20–30 mg/kg, i.p. increase extracellular DA *in vivo* while the effective dose of GBR 12909 for *in vivo* microdialysis was approximately 10-fold lower [2.5–5 mg/kg, i.p.; 27]. Similarly, GBR 12909 was 10-fold more potent than cocaine in the present study. These comparisons strengthen the validity of the present assay. Interestingly, doses of GBR 12909 that are self-administered by rats [0.5–1.5 mg/kg/inj; 28] are not notably different from cocaine doses that are self-administered by rats. The reason(s) for this discrepancy is not clear, though it also may relate to the availability of multiple doses and longer duration of action of GBR 12909 relative to cocaine.

As with DMI, [$^3$H]NE uptake was decreased in occipital cortex following i.v. cocaine. This is consistent with studies using *in vivo* microdialysis [19] and supports an *in vivo* mechanism of blockade of NE uptake by cocaine. It has also well established that cocaine can block the uptake of 5-HT *in vitro* [22]. An increase in extracellular 5-HT following cocaine has also been demonstrated *in vivo* [16]. Unexpectedly, cocaine only partially blocked [$^3$H]5-HT uptake in the present study, up to lethal doses. Several investigators have proposed an involvement of 5-HT in the behavioral effects of cocaine [32]. Taken at face value, the present results argue that the effects of cocaine that involve increased 5-HT neurotransmission are a consequence of only partial blockade of 5-HT uptake.

The most surprising result of the present study was that under conditions that were effective for inhibiting [$^3$H]DA uptake in the accumbens, cocaine failed to block [$^3$H]DA uptake in striatum, even when the dose was increased to the lethal range. No such difference has been seen *in vitro* [1,7]. This finding is even more surprising given the blockade of [$^3$H]DA uptake in the striatum after GBR 12909. Differences in the effects of cocaine on extracellular DA in accumbens and striatum have been reported previously *in vivo* [4,13], though these have been potency differences as opposed to the efficacy difference reported herein. The present results demonstrating increased potency of GBR 12909 in the accumbens relative to the striatum extend those observations. While the affinity of the transporter for DA in the two areas appears to be the same, the DAT density in the accumbens and the amount of DA are lower than in the striatum. Cass et al. [4] proposed that the increased sensitivity of the accumbens derives from the fact that there are fewer DATs to inhibit. Others have argued that a lower density of uptake sites should reduce the effect of an uptake inhibitor on neurotransmitter levels [23]. Wu et al. [37] have proposed that lower rates of DA release and uptake in the accumbens underlie regional differences in the effects of cocaine. If DA is released at higher rates resulting in higher concentrations DA at the DAT in the striatum as compared to the accumbens, then it would seem possible that cocaine binding *in vivo* would be displaced more effectively in the striatum than accumbens. Such an effect would contribute to the diminished potency of GBR 12909 in the striatum relative to accumbens observed in the present study.

The possibility that pharmacokinetics may contribute to the present results should also be considered. The estimated concentration of cocaine achieved in the striatum was clearly adequate to block DA uptake. Perhaps cocaine's penetration to the DAT is slower in the striatum than in accumbens. This seems untenable considering data demonstrating competitive *in vivo* DAT binding by cocaine in striatum 2–10 min after i.v. injection [37]. The *in vivo* [$^3$H]cocaine binding results in the present paper also showed [$^3$H]cocaine binding in striatum at 1–10 min after i.v. injection. Others have reported striatal binding of [$^3$H]cocaine over 1–

10 min after i.v. injection [8]. It is also conceivable that some or all of the [$^3$H]cocaine binding in those studies was to inactive transporter sites.

Cocaine was not, however, without effect in striatum. Striatal DA uptake was actually increased at 15 min after injection. Using a similar *ex vivo* assay, Daws et al. also found that 30 min after an i.p. injection of 30 mg/kg cocaine, [$^3$H]DA uptake into synaptosomes was increased in accumbens [6]. Although we did not see increased uptake in accumbens, we did not vary the time of sacrifice in our experiments with accumbens. Additionally, we used slice and whole homogenized tissue while Daws et al. [6] used P2 synaptosomes. Daws et al. also reported that cocaine can upregulate the surface expression of DAT in HEK 293 cells incubated for 10 min with 10 μM cocaine [6], and that this could account for increased uptake. In their voltammetry study [6], a low dose of cocaine increased DA clearance in rat striatum. David et al. found similar result in the shell of rat nucleus accumbens (5). In another study [10], DA uptake by human DAT-transfected mouse neuroblastoma cells was increased after 24 of exposure to 1 μM of cocaine. A similar mechanism could be operative in the present experiment. Together, these results support that suggestion that cocaine has effects *in vivo* that are not predicted in vitro.

In summary, the present study demonstrates several effects of cocaine in an *ex vivo* assay that are consistent with existing data regarding mechanism of action of cocaine. However, although cocaine is generally found *in vitro* to be equipotent as an uptake blocker across the monoamines [22], the present results suggest that this may not be the case *in vivo*. Moreover, cocaine failed to block DA uptake in the striatum. Because the assay behaved as expected in all other respects, including evident blockade of DA uptake in the accumbens, the mechanism for this discrepancy remains unclear. It is conceivable that the DAT that is expressed in the striatum has different properties from the DAT in the accumbens. For example, the DAT in striatum and accumbens have different apparent molecular weights [14]. It is also interesting that the predominant cellular location of DAT in the striatum is on DA terminals originating in the nigra, whereas the predominant cellular location of DAT in the accumbens is on terminals originating in the ventral tegmental area [20]. Hence, the possibility exists that these different cell groups may process the DAT protein differently resulting in distinct pharmacodynamic profiles. Also the effects of cocaine in *ex vivo* DAT binding and *ex vivo* DA uptake are apparently disassociated in striatum, suggesting that DA binding sites and uptake sites may differ. It is also possible that cocaine's binding to the striatal DAT does not necessarily result in DA uptake inhibition.

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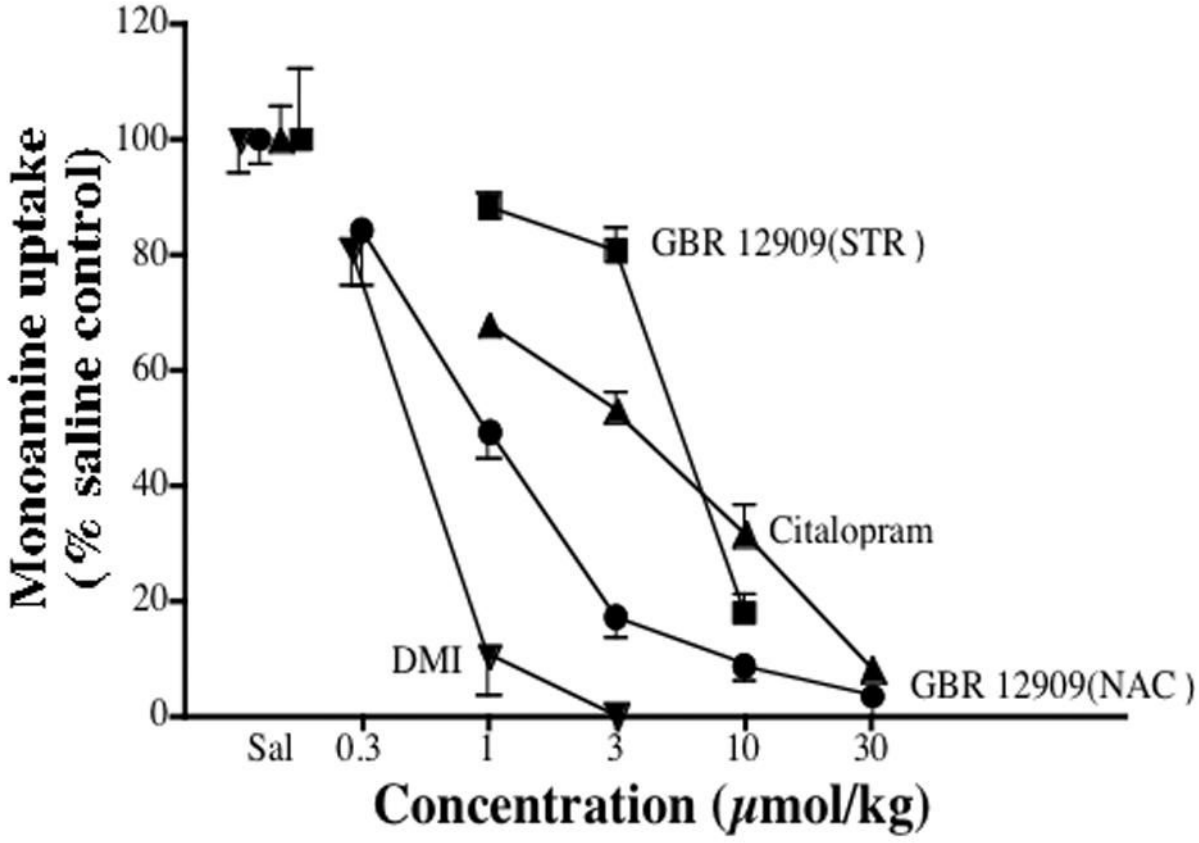


**Figure 1.**
Effects of selective monoamine uptake blockers on monoamine uptake in various brain regions. Rats (n=3–4/group) were injected with saline or the indicated drug doses 5 min before sacrifice. Points are mean values calculated as percent of the values for rats injected with saline on same day and vertical lines are the S.E.M.

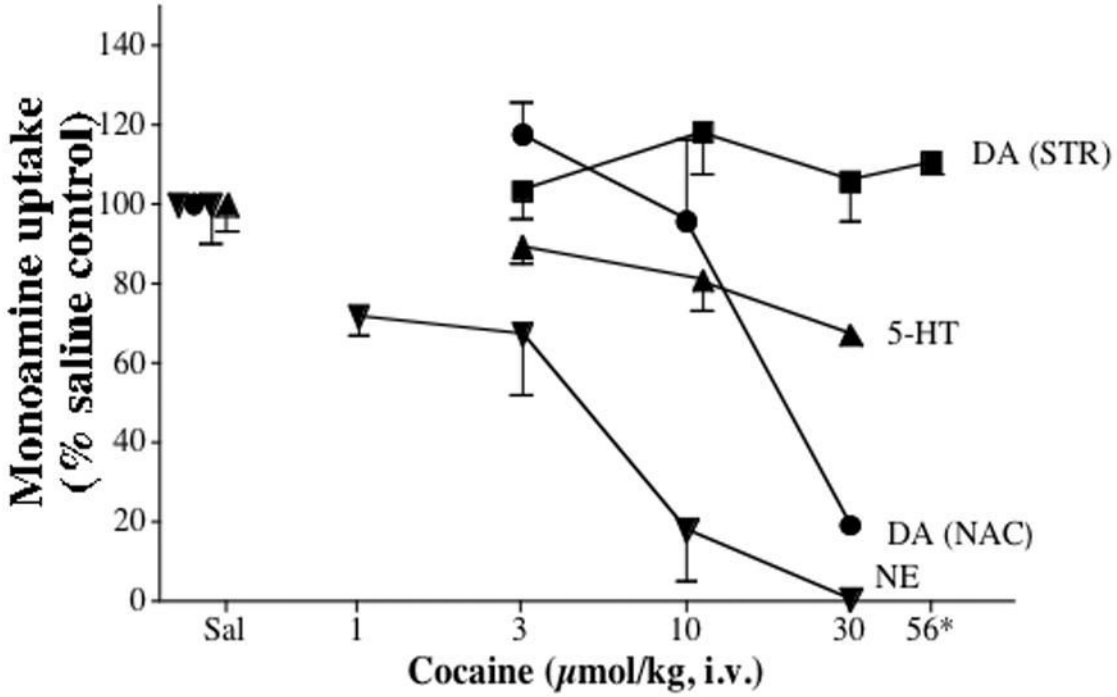


**Figure 2.**

Effects of cocaine on monoamine uptake in various brain regions. Rats (n=3–4/group) were injected with saline or cocaine 5 min before sacrifice. Vertical lines are S.E.M. *For 56 μmol/kg, rats were injected with diazepam (2 mg/kg, i.v., over 50 sec) 5 min before the cocaine injection, administered over 2 min.

## Table

Time course of cocaine effects on [$^3$H]dopamine uptake in striatum.

| Sacrifice Time (min after cocaine) | 1 | 2 | 5 | 15 | 30 |
|---|---|---|---|---|---|
| Uptake (% saline control) | 113.2 | 131.1 | 105.8 | 160.8* | 120.9 |

Rats were injected with cocaine (30 μmol/kg, i.v.) and sacrificed at designated time points.

* Significant increase ($P < 0.05$)