## RESULTS

### *Northern Blot Hybridization*

As shown in FIGURE 1, a single band of 4.4 kb for Dnmt2 was detected with antisense riboprobe. Our result was consistent with a previous report for the cloning and functional analysis of murine Dnmt2, which detected three transcripts of 1.6, 2.6, and 4.4 kb.[4]

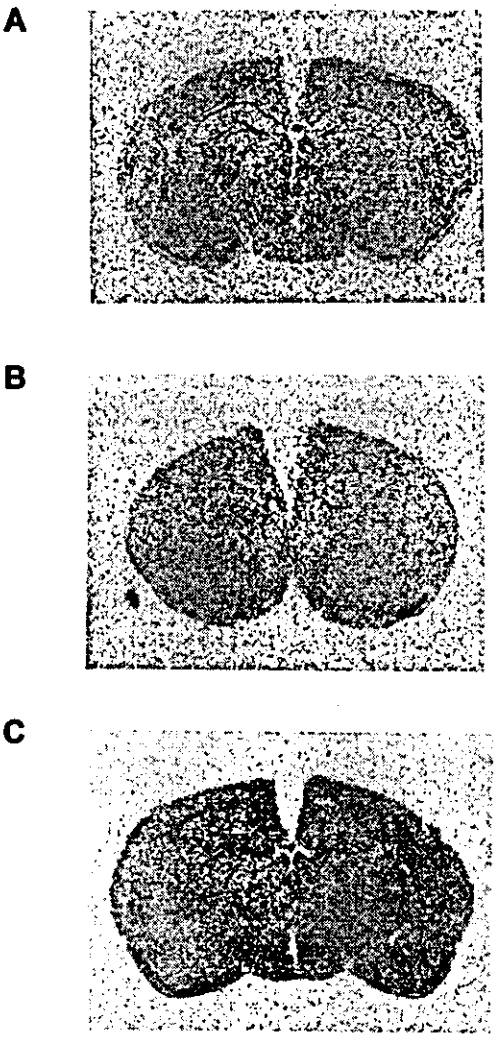


**FIGURE 2.** Representative *in situ* image of Dnmt2 and reelin. (A) Dnmt2 mRNA in rostral slice, including hippocampus; (B) reelin mRNA in caudal slice, including striatum; and (C) reelin mRNA in rostral slice, including hippocampus.

### In Situ *Hybridization*

Representative *in situ* images are shown in FIGURE 2. Strong hybridization signals of Dnmt2 and reelin were observed in brain regions: piriform cortex, hippocampus, and habenular nucleus. FIGURE 3 shows changes in Dnmt2 mRNA after MAP treatment, which significantly decreased by 27% to 39% in hippocampus dentate gyrus (DG), CA1, and CA3 24 h after MAP. No change was observed in habenular nucleus. Changes in reelin mRNA are shown in FIGURE 4, which significantly decreased by 28% in frontal cortex 3 h after MAP. Reelin mRNA was not changed by MAP in piriform cortex, striatum, hippocampus, and habenular nucleus.

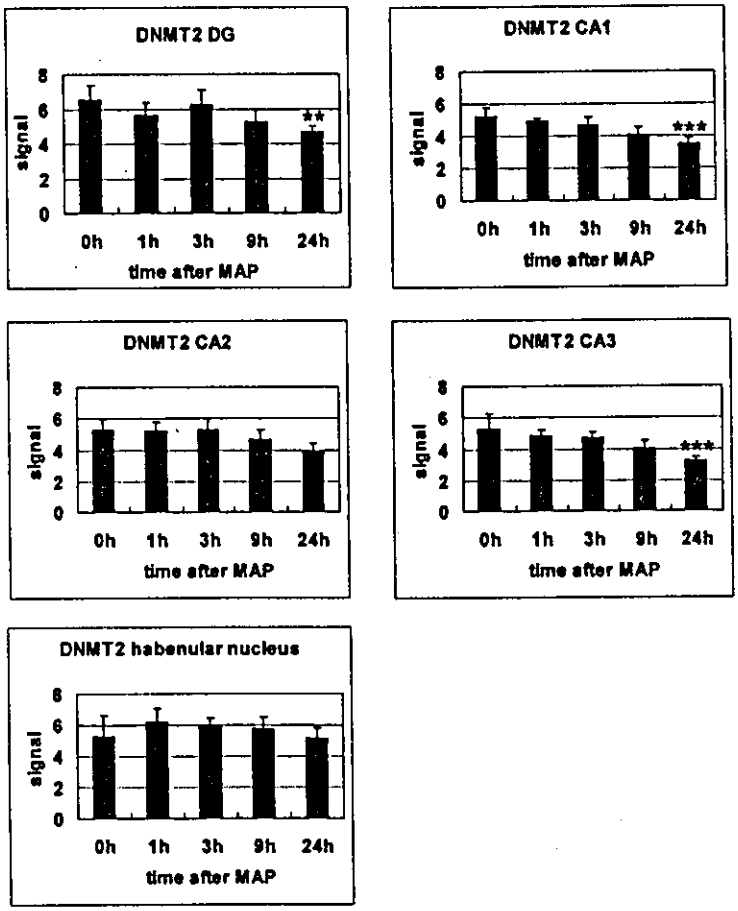


**FIGURE 3.** Dnmt2 mRNA in the rat brain: time course after single MAP treatment. Values are expressed as mean ± S.D. $^{**}P < .01$; $^{***}P < .0001$; analyzed by ANOVA.

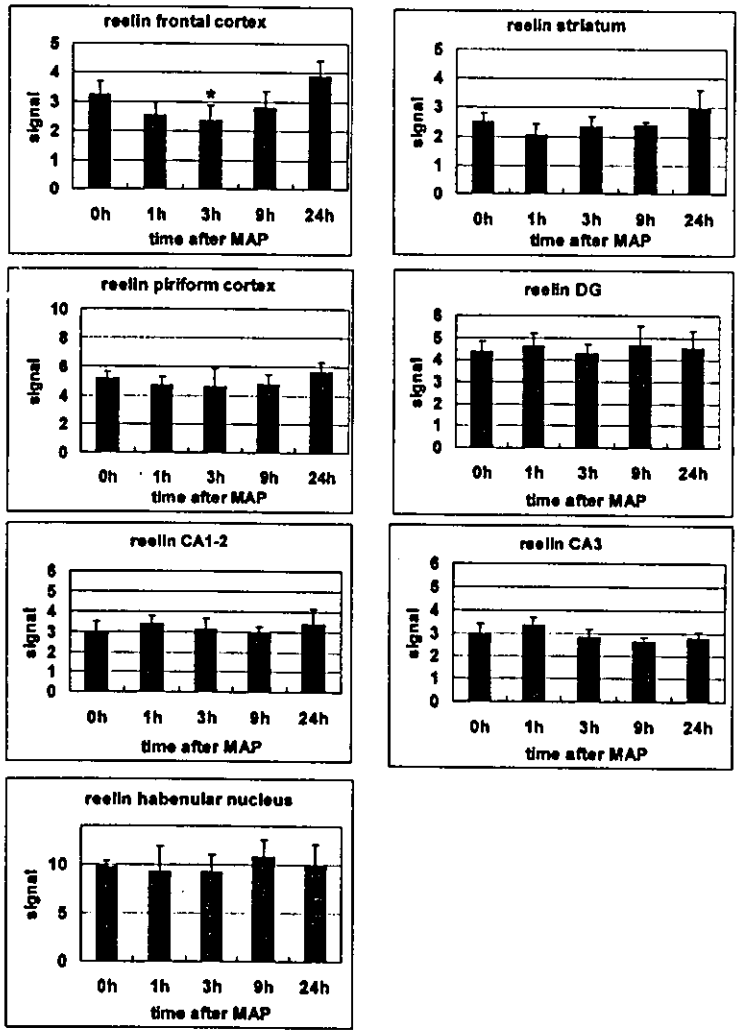


**FIGURE 4.** Reelin mRNA in the rat brain: time course after single MAP treatment. Values are expressed as mean ± S.D. $^*P < .03$; analyzed by ANOVA.

## DISCUSSION

Thus far, four mammarian Dnmts (Dnmt1, 2, 3a, and 3b) have been identified. While Dnmt1 is a maintenance methyltransferase to preserve the preexisting methylation pattern of DNA, Dnmt3a and 3b are *de novo* methyltransferase. Dnmt2, despite having all the conserved DNA methyltransferase motifs, may be involved in

cellular processes other than DNA methylation, such as DNA repair, DNA recombination, and carcinogenesis.[4] We observed decreased Dnmt2 mRNA in the hippocampus 24 h after MAP treatment, but not in the habenular nucleus. In our previous experiment, we also observed decreased Dnmt1 mRNA in the hippocampus of Fischer 344 rats, 3 h after acute MAP treatment. Hippocampus plays an important role in adaptive behavior, including drug dependence and psychosis.[1] Decreased hippocampal Dnmt2 mRNA by MAP might reflect long-term alterations in gene expression, which is responsible for the persistence of MAP-induced mental disorders.

Epigenetic differences (i.e., differences in DNA methylation status) in genomic DNA might explain the discordance for schizophrenia in monozygotic twins.[6] So far, we only have data on MAP-induced changes in Dnmt1 and Dnmt2 for mRNA. Further studies will be necessary to discover whether MAP can affect the protein expression and function of Dnmts, and whether MAP can alter DNA methylation of genes related to the pathogenesis of schizophrenia, including reelin.

We observed changes in reelin mRNA only in the frontal cortex, which was decreased by 30% 3 h after MAP treatment, but returned to baseline by 24 h after MAP. Because the MAP-induced decrease in reelin mRNA was temporary, the functional significance of this alteration remains unclear. However, our results are partially consistent with a previous report, which found a significant decrease in reelin mRNA in the frontal cortex, temporal cortex, cerebellum, caudate nucleus, and hippocampus in schizophrenic patients.[2] Our results suggest that MAP can cause a decrease in reelin mRNA exclusively in the frontal cortex, which is similar to changes observed in schizophrenic patients and a possible animal model for schizophrenia, the heterozygous reeler mouse. Because the expression of reelin is regulated by DNA methylation, it should be elucidated if MAP can alter the methylation status of the CpG island in the promoter of the mouse reelin gene, as well as its mRNA and protein expression in the rat frontal cortex. If this is the case, a MAP-induced decrease in reelin mRNA might be related to the pathogenesis of schizophrenia-like symptoms in MAP psychosis.

## ACKNOWLEDGMENT

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# Regional Differences in Extracellular Dopamine and Serotonin Assessed by *In Vivo* Microdialysis in Mice Lacking Dopamine and/or Serotonin Transporters

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Cocaine conditioned place preference (CPP) is intact in dopamine transporter (DAT) knockout (KO) mice and enhanced in serotonin transporter (SERT) KO mice. However, cocaine CPP is eliminated in double-KO mice with no DAT and either no or one SERT gene copy. To help determine mechanisms underlying these effects, we now report examination of baselines and drug-induced changes of extracellular dopamine ($DA_{ex}$) and serotonin ($5\text{-}HT_{ex}$) levels in microdialysates from nucleus accumbens (NAc), caudate putamen (CPu), and prefrontal cortex (PFc) of wild-type, homozygous DAT- or SERT-KO and heterozygous or homozygous DAT/SERT double-KO mice, which are differentially rewarded by cocaine. Cocaine fails to increase $DA_{ex}$ in NAc of DAT-KO mice. By contrast, systemic cocaine enhances $DA_{ex}$ in both CPu and PFc of DAT-KO mice though local cocaine fails to affect $DA_{ex}$ in CPu. Adding SERT to DAT deletion attenuates the cocaine-induced $DA_{ex}$ increases found in CPu, but not those found in PFc. The selective SERT blocker fluoxetine increases $DA_{ex}$ in CPu of DAT-KO mice, while cocaine and the selective DAT blocker GBR12909 increase $5\text{-}HT_{ex}$ in CPu of SERT-KO mice. These data provide evidence that (a) cocaine increases $DA_{ex}$ in PFc independently of DAT and that (b), in the absence of SERT, CPu levels of $5\text{-}HT_{ex}$ can be increased by blocking DAT. Cocaine-induced alterations in CPu DA levels in DAT-, SERT-, and DAT/SERT double-KO mice appear to provide better correlations with cocaine CPP than cocaine-induced DA level alterations in NAc or PFc.
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**Keywords:** dopamine; serotonin; monoamine transporter; cocaine reward; knockout mice; *in vivo* microdialysis

## INTRODUCTION

Cocaine increases extracellular levels of dopamine (DA), serotonin (5-HT) and norepinephrine (NE) by blocking the neural plasma membrane transporters for those neurotransmitters. Increased extracellular DA ($DA_{ex}$) levels in mesocorticolimbic DA systems have been postulated to mediate cocaine reward (Kuhar *et al*, 1991; Koob and Nestler, 1997; Bardo, 1998; Kelley and Berridge, 2002). However, homozygous dopamine transporter (DAT) knockout (KO) mice (DAT−/− mice) express intact cocaine reward in conditioned place preference (CPP) (Sora *et al*, 1998) and drug self-administration paradigms (Rocha *et al*, 1998). Cocaine reward is eliminated in double-KO mice with no DAT gene copies and either no or one copy of the SERT gene (Sora *et al*, 2001), but not in double-KO mice with neither DAT nor NET gene copies (Hall *et al*, 2002). Further, serotonin transporter (SERT) blockade with fluoxetine or norepinephrine transporter (NET) blockade with nisoxetine can yield rewarding effects in DAT-KO mice, which are never seen in wild-type animals (Hall *et al*, 2002).

We and others have postulated that the retention of cocaine reward in DAT-KO mice may be due to (a) roles for non-DA systems in normal cocaine reward and (b) adaptations to the lifelong loss of DAT found in DAT-KO mice (Kirkpatrick, 2001; Sora *et al*, 2001; Uhl *et al*, 2002). Some of these adaptive changes could come from involvement of redundant monoaminergic systems in cocaine

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reward. Since each transporter displays significant affinities for each monoamine (Faraj *et al*, 1994; Giros *et al*, 1994; Gu *et al*, 1994; Eshleman *et al*, 1999), the absence of its cognate transporter might allow a monoamine to diffuse further from its site of release and be accumulated by another transporter.

Cocaine and selective norepinephrine transporter (NET) blockers (eg reboxetine) are each reported to increase $DA_{ex}$ in NAc of DAT-KO mice, suggesting that NET could act as an alternative uptake site for DA in such animals and that NET blockade might be a mechanism for both the cocaine- and nisoxetine-induced rewards found in DAT-KO mice (Carboni *et al*, 2001; Hall *et al*, 2002). However, *in vitro* data fail to identify cocaine influences on CPu or NAc DA uptake in DAT-KO mice (Budygin *et al*, 2002; Moron *et al*, 2002). The simple idea that NET mediates cocaine reward in the absence of DAT is also incompatible with observations that cocaine reward is ablated in DAT/SERT double-KO mice that express normal levels of NET (Sora *et al*, 2001).

Roles for 5-HT systems in cocaine reward (or aversion) are also less than clear from current data (Cunningham and Callahan, 1991; Kleven *et al*, 1995; Rocha *et al*, 1997; Kleven and Koek, 1998; Lee and Kornetsky, 1998; Parsons *et al*, 1998; Shippenberg *et al*, 2000; Baker *et al*, 2001; Sasaki-Adams and Kelley, 2001). Homozygous SERT-KO mice display enhanced cocaine CPP that is increased even more in combined SERT/NET double-KO mice (Sora *et al*, 1998; Hall *et al*, 2002). SERT-KO mice, in themselves and in combination with DAT-KOs, thus provide interesting models in which to investigate 5-HT, DA, and 5-HT/DA interactions important for psychostimulant reward.

In this present study, we have therefore examined baselines and drug-induced changes of $DA_{ex}$ and $5\text{-}HT_{ex}$ in several brain regions implicated in psychostimulant effects, the NAc, CPu and prefrontal cortex (PFc) in DAT-KO, SERT-KO, and both heterozygous and homozygous DAT/SERT double-KO mice. We have studied the effects of both the nonselective blocker cocaine and the selective SERT and DAT blockers fluoxetine and GBR12909. These investigations provide insights into adaptive processes found in these mice and into 5-HT, DA, and 5-HT/DA interactions of the possible importance for cocaine reward.

## MATERIALS AND METHODS

### Animals

Mutant mice lacking DAT, SERT, and littermate wild-type mice were obtained from heterozygote crosses on 129/C57 mixed genetic backgrounds. DAT/SERT double-KO mice were obtained by intercrossing single KO lines as described previously (Sora *et al*, 2001). DNA extracted from tail biopsies was genotyped using PCR. Mice were group-housed (two to four per cage) with food and water *ad libitum* in a room maintained at $22\pm2$°C and $65\pm5$% humidity under a 12 h light–dark cycle. Male and female mice from 10–24 weeks old of each genotype group (*n*equals;4–8) were used in each experiment equally. All animal experiments were performed in accordance with the Guidelines for the Care of Laboratory Animals of the Tokyo Institute of Psychiatry.

For the CPu cocaine study, all the nine DAT × SERT genotypes were examined (DAT+/+ SERT+/+, DAT+/+ SERT+/−, DAT+/+SERT−/−, DAT+/−SERT+/+, DAT+/−SERT+/−, DAT+/−SERT−/−, DAT−/− SERT+/+, DAT−/−SERT+/−, and DAT−/−SERT−/−). For NAc and PFc cocaine studies and for fluoxetine CPu and NAc studies, the four homozygous genotypes were examined (wildtype, DAT−/−SERT+/+, DAT+/+SERT−/−, DAT−/−SERT−/−). GBR12909 effects on CPu $5\text{-}HT_{ex}$ levels were examined in wild-type and DAT+/+SERT−/− mice.

### Surgery

Mice were stereotaxically implanted with microdialysis probes under sodium pentobarbital anesthesia (50 mg/kg) in CPu (anterior +0.6 mm, lateral +1.8 mm ventral −4.0 mm from bregma), NAc (anterior +1.2 mm, lateral +1.0 mm ventral −5.0 mm from bregma) or PFc (anterior +2.0 mm, lateral +0.5 mm ventral −3.0 mm from bregma) according to the atlas of Franklin and Paxinos (1997). Probe tips were constructed with regenerated cellulose membranes that provided 50 kDa molecular weight cutoffs, outer diameters of 0.22 mm, and membrane lengths of either 1 mm (NAc) or 2 mm (CPu and PFc) (Eicom, Kyoto, Japan). Dialysis probe placements were verified histologically at the ends of each experiment (Figure 1), and experimental data were excluded if the membrane portions of the dialysis probes lay outside the central CPu, medial PFc or NAc core or shell regions, respectively.

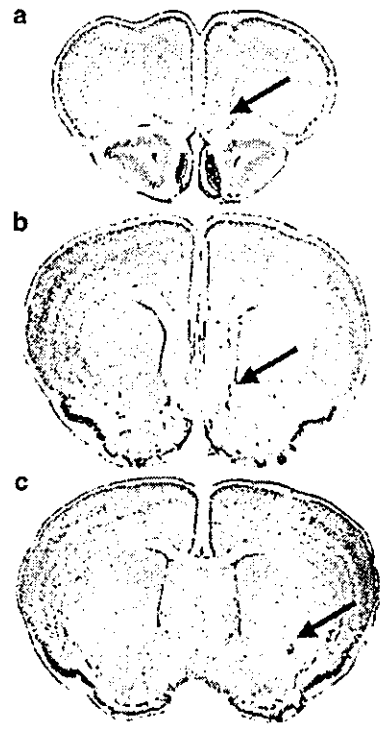


**Figure 1** Location of dialysis probes in coronal sections of PFc (a), NAc (b), and CPu (c). The arrows illustrate the implantation sites of dialysis probes.

### Microdialysis and Analytical Procedure

At 24 h after implantation, probes in freely moving mice were perfused with Ringer's solution (147 mM $Na^+$, 4 mM $K^+$, 1.26 mM $Ca^{2+}$, 1 mM $Mg^{2+}$, and 152.5 mM $Cl^-$, pH 6.5) at 1 µl/min for 180 min. $DA_{ex}$ and 5-$HT_{ex}$ baselines were obtained from average concentrations of three consecutive 10 min, 10 µl samples. These and subsequent 10 min, 10 µl dialysate fractions were analyzed using an AS-10 auto-injector (Eicom), high-performance liquid chromatography (HPLC), with a PPS-ODS reverse-phase column (Eicom) and a ECD-100 graphite electrode detector (Eicom). The mobile phase consisted of 0.1 M phosphate buffer (pH 5.5) containing sodium decanesulfonate (500 mg/l), EDTA (50 mg/l), and 1% methanol. Detection limits for DA and 5-HT were 1 fmol/sample with signal-to-noise ratios of at least 2. *In vitro* recoveries from the 1- and 2-mm membrane length probes were 10 and 15%, respectively.

### Drugs

Test drugs were dissolved in saline for systemic administration or in Ringer's solution for local infusion via microdialysis probes. After establishment of stable baselines, cocaine HCl (10 mg/kg for subcutaneous injection or 100 µM for local infusion; Dainippon, Osaka, Japan), fluoxetine (20 mg/kg, Sigma, Tokyo, Japan), GBR12909 (10 mg/kg, Sigma) or saline (10 ml/kg) was administered subcutaneously (s.c.) and dialysates collected for 3 or 2 h, respectively.

### Statistics

Baselines of $DA_{ex}$ and 5-$HT_{ex}$ were compared across genotype groups using two-way ANOVAs (DAT genotype, and SERT genotype). DA and 5-HT responses to drugs were expressed as percentages of baselines. Effects of drugs on $DA_{ex}$ and 5-$HT_{ex}$ were assessed by calculating the areas under time-response curves (AUC) for the first 120 or 180 min after drug administration. AUCs were analyzed using two-way ANOVAs (Drug, Genotype). Least significant difference tests were applied for multiple comparisons and *P*-values less than 0.05 were considered statistically significant. Statistical analyses used STATISTICA (StatSoft Inc., Tulsa, OK).

## RESULTS

### Baselines of $DA_{ex}$ and 5-$HT_{ex}$ in CPu, NAc, and PFc

The mean (±SEM) baselines of $DA_{ex}$ and 5-$HT_{ex}$ in dialysates from the CPu, NAc, and PFc in mice, who were subsequently treated with either vehicle or test drugs, are shown in Table 1. Two-way ANOVA of $DA_{ex}$ baselines confirmed that DAT-KO had significant effects on $DA_{ex}$ baselines in CPu ($F(1,91)=299.77$, $P<0.00001$) and NAc ($F(1,55)=101.49$, $P<0.00001$), but not PFc ($F(1,33)=0.07$, $P=0.79$). Dialysate DA in homozygous DAT-KO mouse CPu and NAc was approximately 10-fold higher than that in mice with either one or two copies of the DAT gene. 5-$HT_{ex}$ baselines were unaffected by DAT-KO in any region.

SERT-KO exerted significant effects on 5-$HT_{ex}$ baselines in each of these three regions ($F(2,91)=87.06$, $P<0.00001$; $F(1,55)=29.95$, $P<0.00001$; $F(1,33)=80.37$, $P<0.00001$, respectively). In CPu, NAc, and PFc, 5-$HT_{ex}$ baselines in mice with no SERT gene were six to ten times as large as that found in mice with one or two copies of SERT gene. $DA_{ex}$ baselines were unaffected by SERT-KO in any region.

Interestingly, there was a significant interaction between DAT and SERT genotype effects on basal NAc dialysate DA levels ($F(12,55)=4.33$, $P<0.05$). $DA_{ex}$ levels in NAc of mice with no DAT or SERT genes (DAT−/−SERT−/−) were higher than those of mice with no DAT genes but two SERT genes (DAT−/−SERT+/+).

### Systemic Cocaine Effects on $DA_{ex}$ in CPu, NAc, and PFc

$DA_{ex}$ level changes in CPu, NAc, and PFc following systemic cocaine administration are shown in Figure 2a, c, and e. DA responses to cocaine in the CPu of wild-type and DAT+/− mice peak at 40–60 min (Figure 2a). Cocaine also induces a slower DA response curve in the CPu of homozygous

**Table 1** The Baselines (fmol/10 min) of $DA_{ex}$ and 5-$HT_{ex}$ in CPu, NAc and PFc

| Genotype | | CPu | | | NAc | | | PFc | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DAT | SERT | *n* | DA | 5-HT | *n* | DA | 5-HT | *n* | DA | 5-HT |
| +/+ | +/+ | 18 | 73.88±4.97 | 1.82±0.15 | 16 | 17.79±1.69 | 1.19±0.08 | 10 | 2.11±0.11 | 3.05±0.36 |
| +/+ | +/− | 8 | 82.28±13.76 | 2.39±0.29 | — | — | — | — | — | — |
| +/+ | −/− | 13 | 69.82±7.12 | 16.33±2.58$^{*,+}$ | 15 | 16.23±2.23 | 15.50±4.20$^{*}$ | 9 | 2.52±0.35 | 25.55±3.18$^{*}$ |
| +/− | +/+ | 9 | 79.69±12.32 | 1.28±0.13 | — | — | — | — | — | — |
| +/− | +/− | 10 | 105.12±10.70 | 2.90±0.33 | — | — | — | — | — | — |
| +/− | −/− | 9 | 93.04±14.09 | 17.73±3.30$^{*,+}$ | — | — | — | — | — | — |
| −/− | +/+ | 15 | 687.18±58.16$^{*,\#}$ | 1.61±0.10 | 14 | 188.17±23.00$^{*}$ | 1.19±0.43 | 9 | 2.32±0.32 | 3.28±0.47 |
| −/− | +/− | 9 | 667.67±42.39$^{*,\#}$ | 2.38±0.31 | — | — | — | — | — | — |
| −/− | −/− | 9 | 548.78±31.42$^{*,\#}$ | 12.78±1.46$^{*,+}$ | 14 | 275.34±38.47$^{*,\&}$ | 13.42±2.36$^{*}$ | 9 | 2.22±0.15 | 17.06±2.61$^{*}$ |

$DA_{ex}$ or 5-$HT_{ex}$ baselines were obtained from average concentrations (fmol/10 min) of three consecutive stable samples before injections. Values are the mean (±SEM) of baselines. $^{*}P<0.0001$ compared to wild-type mice; $^{\#}P<0.0001$ compared to mice with one copy of DAT gene; $^{+}P<0.0001$ compared to mice with one copy of SERT gene; $^{\&}P<0.001$ compared to DAT−/− mice.

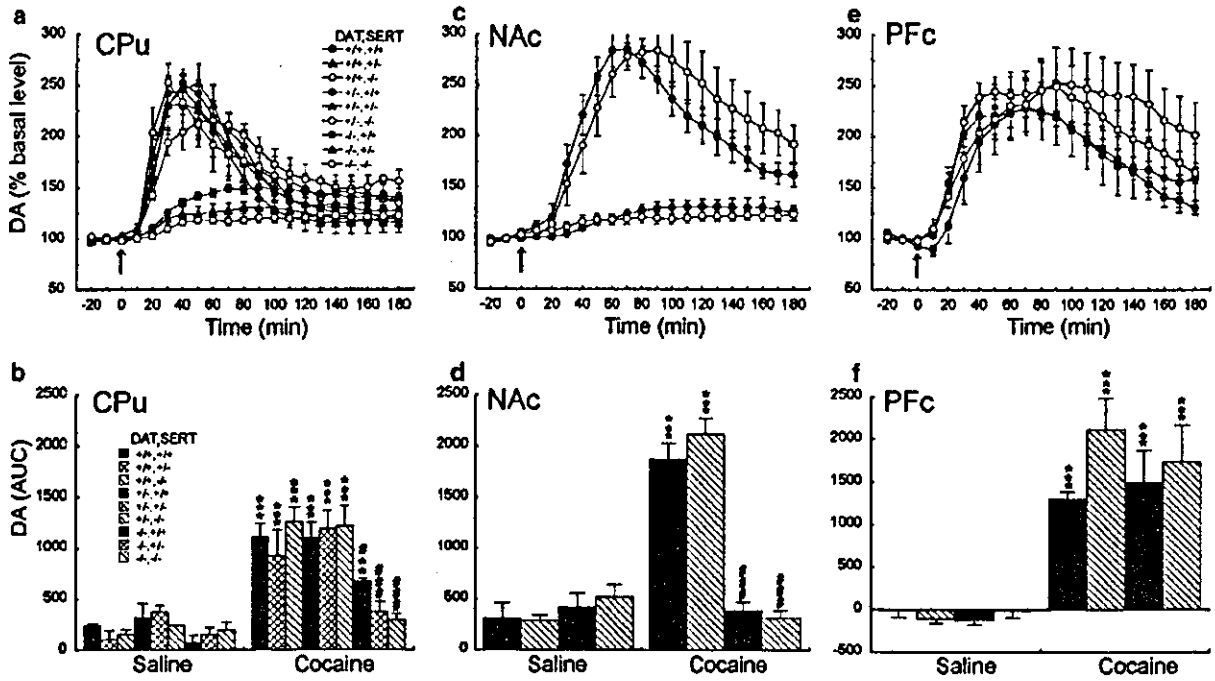


**Figure 2** (a, c, and e) Temporal pattern of DA response to cocaine (10 mg/kg, s.c.) in CPu, NAc, and PFc, respectively. Each point represents the mean (±SEM) of the percentage of $DA_{ex}$ baselines. The time of injections is indicated with an arrow. (b, d, and f) Histogram represents the mean AUC (±SEM) of DA response to saline or cocaine in CPu, NAc, and PFc during 180 min interval after injection. $^{**}P<0.01$, $^{***}P<0.001$ compared to the saline group of the same genotype; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ compared to the cocaine-treated wild-type group.

DAT-KO mice (DAT−/−SERT+/+), peaking at about 90 min (Figure 2a). This pattern is not observed in NAc, where DAT−/−SERT+/+ mice do not exhibit any larger increments in $DA_{ex}$ levels (Figure 2c). In further contrast, wild-type, DAT−/−SERT+/+, and DAT−/−SERT−/− mice each exhibit indistinguishable cocaine-induced DA responses in PFc (Figure 2e).

Drug effects on $DA_{ex}$ levels can be assessed by studying AUCs (Figure 2b, d, and f). ANOVAs of mean AUC (±SEM) for drug effects on $DA_{ex}$ levels reveal that drugs have significant effects on DA AUC in CPu ($F(1,62)=132.32$, $P<0.0001$), NAc ($F(1,34)=80.60$, $P<0.0001$), and PFc ($F(1,28)=67.59$, $P<0.0001$). Genotype and drug × genotype interactions were significant for DA AUC in CPu ($F(8,62)=5.45$, $P<0.0001$; $F(8,62)=3.41$, $P<0.01$; respectively) and NAc ($F(3,34)=23.82$, $P<0.0001$; $F(3,34)=36.09$, $P<0.0001$; respectively), but not in PFc ($F(3,28)=0.89$, $P=0.46$; $F(3,28)=0.94$, $P=0.43$; respectively). In CPu (Figure 2b), DAT-KO mice exhibit statistically significant cocaine-induced increments in $DA_{ex}$ levels, although these increases are less than those found in wild-type mice. By contrast, in DAT−/−SERT+/− and DAT−/−SERT−/− mice, the same genotypes that do not exhibit rewarding effects of cocaine also do not exhibit cocaine-induced increases in $DA_{ex}$ in CPu. No significant differences are observed in cocaine-induced DA AUC increases in CPu between the DAT+/+ and DAT+/− mice. In NAc (Figure 2d), cocaine fails to increase $DA_{ex}$ in DAT−/− SERT+/+ or in DAT−/−SERT−/− mice. There are no significant differences in cocaine-induced DA increases in NAc between wild-type and DAT+/+ SERT−/− mice. In PFc (Figure 2f), cocaine produces significant increases in $DA_{ex}$ in all genotypes.

### Systemic Cocaine Effects on 5-$HT_{ex}$ in CPu, NAc, and PFc

The temporal patterns of 5HT responses to cocaine in CPu, NAc, and PFc are shown in Figure 3a, c, and e. DAT+/+ SERT−/− and DAT+/−SERT−/− mice show gradual 5HT responses to cocaine in CPu (Figure 3a) and NAc (Figure 3c), but not in PFc (Figure 3e). 5-HT response curves produced by cocaine are observed in CPu (Figure 3a) and NAc (Figure 3c) in all genotypes except DAT−/− SERT−/− mice. The peak of 5-HT response are smaller for SERT−/− mice than for either SERT+/+ or SERT+/− mice. SERT−/− mice exhibit no 5-HT response to cocaine in PFc (Figure 3c), while wild-type mice exhibit robust increases.

Drug effects on 5-$HT_{ex}$ levels are expressed as mean AUC (±SEM) in Figure 3b, d, and f. Two-way ANOVAs of the AUC for 5-HT responses to cocaine show significant effects of Drug, Genotype, and Drug × Genotype interactions in CPu ($F(1,62)=181.49$, $P<0.0001$; $F(8,62)=5.01$, $P<0.0001$; $F(8,62)=4.88$, $P<0.0001$; respectively), NAc ($F(1,34)=31.57$, $P<0.0001$; $F(3,34)=6.44$, $P<0.0001$; $F(3,34)=8.41$, $P<0.0001$; respectively) and PFc ($F(1,28)=57.74$, $P<0.0001$; $F(3,28)=11.55$, $P<0.0001$; $F(3,28)=15.59$, $P<0.0001$; respectively). In CPu (Figure 3b) and NAc (Figure 3d), multiple AUC comparisons reveal that cocaine significantly increases 5-$HT_{ex}$ in DAT+/+SERT−/− and DAT+/−SERT−/− mice, but

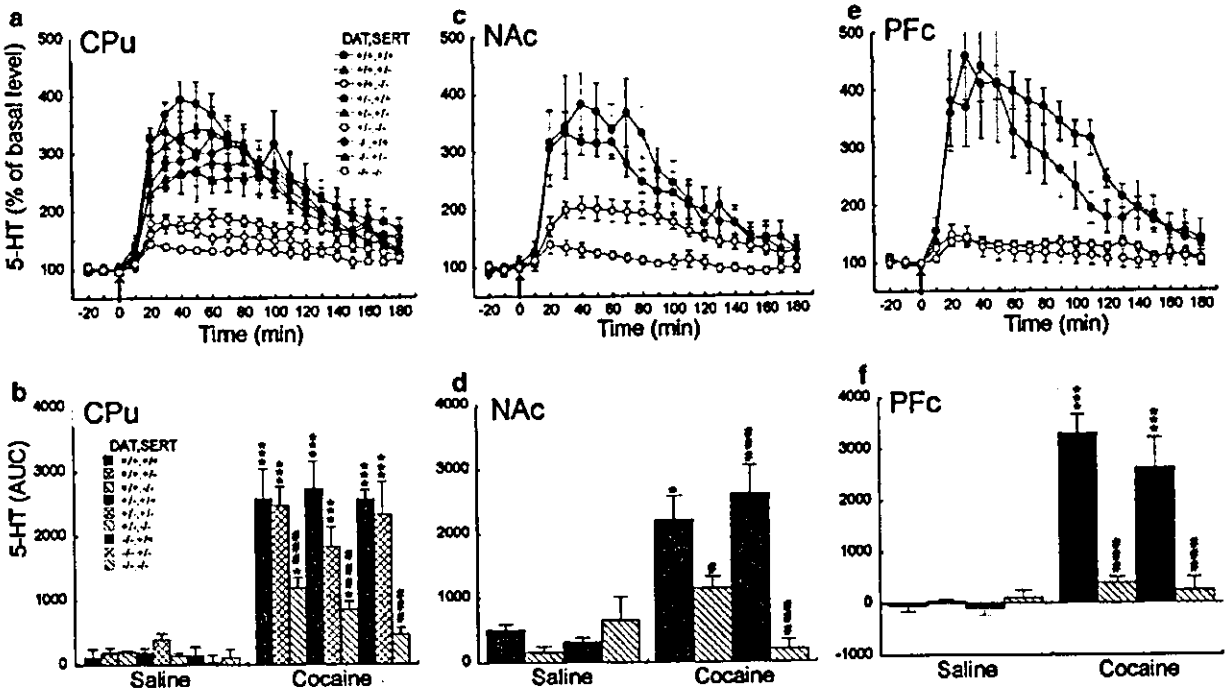


**Figure 3** (a, c, and e) Temporal pattern of 5-HT response to cocaine (10 mg/kg, s.c.) in CPu, NAc, and PFc, respectively. The time of injections is indicated with an arrow. (b) Histogram represents the mean AUC (±SEM) of 5-HT response to saline or cocaine in CPu, NAc, and PFc during the 180 min interval after injection. $^{*}P<0.05$, $^{***}P<0.001$ compared to the saline group of the same genotype; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ compared to the cocaine-treated wild-type group.

not in DAT−/−SERT−/− mice. SERT+/− mice display cocaine-induced increases in 5-$HT_{ex}$ in CPu that are similar to those found in wild-type values. PFc 5-$HT_{ex}$ levels are not altered significantly by cocaine in SERT−/− mice (Figure 3f).

### Systemic Fluoxetine Effects on $DA_{ex}$ in CPu and NAc

The temporal patterns of DA response to fluoxetine in CPu and NAc of DAT+/+ SERT+/+, DAT−/−SERT+/+, DAT+/+SERT−/−, and DAT−/−SERT−/− mice are shown in Figure 4a and c. In CPu (Figure 4a), DAT−/− SERT+/+ mice exhibit gradual DA responses to fluoxetine that display time courses similar to those of cocaine and persist for at least 3 h (Figure 4a). Two-way ANOVAs of DA AUC responses show significant effects of Drug ($F(1,33)=9.62$, $P<0.01$) and Drug × Genotype interactions ($F(1,33)=4.94$, $P<0.01$). Multiple comparisons reveal that fluoxetine significantly increases DA AUC only in the CPu of DAT−/−SERT+/+ mice (Figure 4b). In NAc, DA responses to fluoxetine display no significant effects of either Drug ($F(1,29)=0.0076$, $P=0.93$), Genotype ($F(1,29)=0.49$, $P=0.69$), genotype ($F(1,29)=0.69$, $P=0.41$) or Drug × Genotype interaction ($F(3,29)=1.55$, $P=0.22$) (Figure 4d).

### Systemic GBR12909 Effects on 5-$HT_{ex}$ in CPu

The temporal pattern of CPu 5-HT response to GBR12909 is shown in Figure 5a. DAT+/+SERT−/− mice exhibit remarkable 5-$HT_{ex}$ increases after administration of GBR12909, which are not seen in WT mice. These SERT-KO mice continue to display elevated CPu 5-$HT_{ex}$ levels for at least 3 h. Two-way ANOVA of the AUC of the DA response to GBR 12909 shows significant effects of Drug ($F(1,13)=14.43$, $P<0.01$), Genotype ($F(1,13)=7.63$, $P<0.05$), and Drug × Genotype interactions ($F(1,13)=5.74$, $P<0.05$). Multiple comparisons show that GBR12909 administration significantly increases CPu 5-$HT_{ex}$ in DAT+/+SERT−/−, but not in wild-type mice (Figure 5b).

### Local Cocaine Effects on $DA_{ex}$ and 5-$HT_{ex}$ in CPu

$DA_{ex}$ and 5-$HT_{ex}$ level changes in CPu following local cocaine infusion are shown in Figure 6a and c. Local cocaine cannot induce DA response curve in CPu of DAT−/− SERT+/+ and DAT−/−SERT−/− mice, but produces gradual 5-HT response curve in DAT+/+SERT−/− mice.

ANOVAs of mean AUC (±SEM) for DA responses reveal significant effects of Drug, Genotype, and Drug × Genotype interactions in CPu ($F(1,24)=161.46$, $P<0.0001$; $F(3,24)=48.20$, $P<0.0001$; $F(3,24)=47.30$, $P<0.0001$; respectively). Multiple AUC comparisons show that local cocaine fails to increase $DA_{ex}$ in CPu of DAT−/− SERT+/+ or in DAT−/−SERT−/− mice (Figure 6b). ANOVAs of mean AUC (±SEM) for 5-HT responses also reveal significant effects of Drug, Genotype, and Drug × Genotype interactions in CPu ($F(1,24)=43.26$, $P<0.0001$; $F(3,24)=9.55$, $P<0.0001$; $F(3,24)=9.70$, $P<0.0001$; respectively). Multiple comparisons reveal that local cocaine significantly increases 5-$HT_{ex}$ in wild-type, DAT+/+SERT−/− and DAT−/−SERT+/+ mice, but not in DAT−/−SERT−/− mice (Figure 6d). Moreover, there were no significant changes in NAc $DA_{ex}$ in DAT−/−

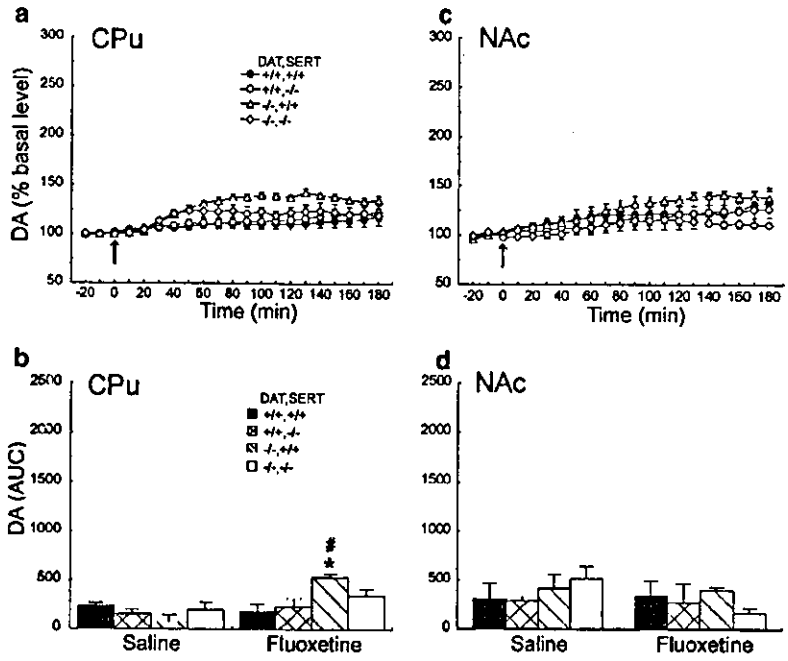


**Figure 4** (a and c) Temporal pattern of DA response to fluoxetine (20 mg/kg, s.c.) in CPu and NAc, respectively. The time of injections is indicated with an arrow. (b and d) The histogram represents the mean AUC (±SEM) of DA response to saline or fluoxetine in CPu and NAc during the 180 min interval after injection. $^{*}P<0.05$ compared to the saline group of the same genotype; $^{\#}P<0.05$ compared to the fluoxetine-treated wild-type group.

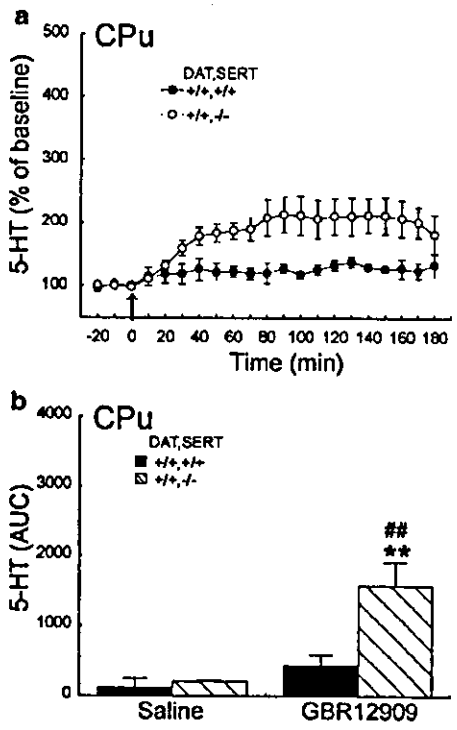


**Figure 5** (a) Temporal pattern of 5-HT response to GBR12909 (10 mg/kg, s.c.) in CPu. The time of injections is indicated with an arrow. (b) The histogram represents the mean AUC (±SEM) of 5-HT response to saline or GBR12909 in CPu during 180 min interval after injection. $^{**}P<0.01$ compared to the saline group of the same genotype; $^{\#\#}P<0.01$ compared to the GBR12909-treated wild-type group.

SERT+/+ and DAT−/−SERT−/− mice after local cocaine infusions (data not shown).

## DISCUSSION

These microdialysis results reveal parallels with and differences from the patterns of KO effects on reward elicited by cocaine and fluoxetine that we have previously reported in these mouse strains. We can thus evaluate hypotheses about the pharmacological profiles and brain localization of processes hypothesized to mediate cocaine reward with regard to their convergence or divergence with this microdialysis data.

### Differential DA Responses in to Cocaine in CPu, NAc, and PFc and Correlations with Assessments of Cocaine Reward

The current data do not provide simple correlations with models that postulate that enhanced NAc $DA_{ex}$ levels alone are necessary and sufficient for cocaine reward. Although this hypothesis has been supported by data from microinjection and lesion studies (Kuhar *et al*, 1991; Koob and Nestler, 1997; Bardo, 1998; Kelley and Berridge, 2002), many results from gene KO studies fail to support the simple hypothesis that DA alone mediates the rewarding effects of cocaine. Our current observations that cocaine does not increase $DA_{ex}$ in NAc of homozygous DAT-KO mice contrasts with the nearly-intact cocaine reward found in these animals (Rocha *et al*, 1998; Sora *et al*, 1998). These *in vivo* microdialysis data are also consistent with studies which document failure of cocaine to block DA uptake in

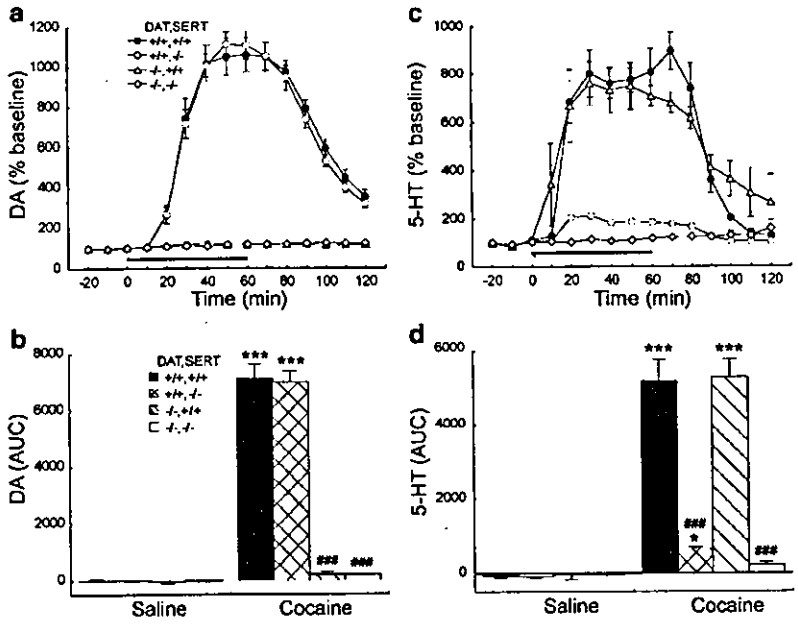


**Figure 6** (a and c) Temporal pattern of DA and 5-HT response to local cocaine infusion (100 μM) in CPu, respectively. Horizontal bar indicates the time of infusions. (b and d) The histogram represents the mean AUC (±SEM) of DA and 5-HT response to saline or cocaine in CPu during 120 min interval after injection. $^{*}P<0.05$, $^{***}P<0.001$ compared to the saline group of the same genotype; $^{###}P<0.001$ compared to the cocaine-treated wild-type group.

NAc samples taken from DAT homozygous mice in *in vitro* experiments (Budygin *et al*, 2002; Moron *et al*, 2002).

The current data also fail to provide simple correlations with models that postulate that enhanced PFc DA levels are necessary and sufficient for cocaine reward. This hypothesis has also been supported by a substantial body of lesion and microinjection data (Goeders and Smith, 1983; Goeders *et al*, 1986; Bardo, 1998; Tzschentke, 2001). Cocaine increases $DA_{ex}$ in PFc of both wild-type and homozygous DAT-KO mice that exhibit cocaine reward and DAT/SERT double homozygous KO mice that do not display cocaine reward.

Intriguingly, the current results for DA in CPu appear to provide the best fit with studies of cocaine-induced place preferences. Although intra-CPu cocaine does not affect $DA_{ex}$ levels in DAT-KO mice, systemic cocaine causes about 1.5-fold increase in peak $DA_{ex}$ concentrations in CPu dialysate from DAT-KO mice that are rewarded by cocaine, but not from DAT/SERT double homozygous KO mice that lack cocaine CPP. Systemic fluoxetine also increases CPu $DA_{ex}$ levels in homozygous DAT-KO mice in which this compound is rewarding, but not in wild-type mice or homozygous SERT-KO mice in which fluoxetine does not produce a place preference.

## Differential 5-HT Responses to Cocaine in CPu, NAc, and PFc and Correlations with Assessments of Cocaine Reward

Although cocaine-induced increases in CPu and NAc $5\text{-}HT_{ex}$ are found in SERT-KO mice that exhibit enhanced cocaine CPP, the magnitude of the increases in $5\text{-}HT_{ex}$ after cocaine administration is attenuated when it is compared with wild-type mice. Interestingly, chronic SERT blockade with fluoxetine can also potentiate cocaine reward (Cunningham and Callahan, 1991; Kleven and Koek, 1998). It is conceivable that the attenuation of cocaine-induced $5\text{-}HT_{ex}$ rise may lead mice more sensitive to the reward effect of cocaine. These sorts of data, and the current results, continue to point to possible roles for 5-HT in cocaine reward, especially in light of the more complex hypotheses of the basis of cocaine reward discussed below.

## $5\text{-}HT_{ex}$ Clearance by DAT, $DA_{ex}$ Clearance by NET, and opportunities for 'Promiscuous Uptake'

Removal of a transporter that usually provides inactivation, re-accumulation, and recycling of a released monoamine neurotransmitter provides opportunities for greater diffusion of the monoamine, documented by higher extracellular dialysate concentrations noted here. Removal of a cognate transporter also enhances the opportunities for transmitter uptake by a transporter that normally recognizes another monoamine. The presence of the same vesicular transporter in DAT-, SERT-, and NET-expressing neurons provides the opportunity for the monoamine that has been taken up by a non-cognate plasma membrane transporter to be accumulated into vesicles, and to be re-released as a 'false transmitter' (Liu and Edwards, 1997; Uhl *et al*, 2000). DA accumulation by NET-expressing neurons also provides the opportunity for DA to be subjected to β-hydroxylation to produce norepinephrine, providing a 'true' transmitter for noradrenergic neurons. It is interesting to note that elimination of monoamine transporters has different effects on basal monoamine levels in different brain regions, supporting ideas that factors that mediate DA and 5-HT clearance from synaptic clefts may differ substantially from one terminal field to another.

Many of the present and previously reported results appear to provide evidence for uptake by non-cognate transporters, and even for possible 'false transmission' in these transporter-KO mice. Cocaine and the selective DAT blocker GBR12909 produces a substantial increase in dialysate 5-HT in SERT-KO mice that is not found in wild-type animals. These findings were supported by previous reports that have documented 5-HT uptake by cultured neurons from SERT-KO mice that could be blocked by selective DAT blockers (Pan *et al*, 2001), and 5-HT-like immunoreactivity in substantia nigra and ventral tegmental area dopaminergic neurons (Zhou *et al*, 2002). False transmission may be region-dependent, with differences in the relative densities of DAT- SERT- and NET-expressing neural elements providing differential opportunities for such processes.

Moreover, our observations of virtually identical PFc $DA_{ex}$ baselines in each of these KO strains appear to support a relatively reduced prominence of DAT-mediated DA uptake in this region even in wild-type mice. These observations are compatible with the relatively sparse distribution of PFc DAT in several species (Freed *et al*, 1995; Sesack *et al*, 1998), in contrast with more prominent NET and SERT expression. They are also in accord with pharmacological and other evidence for significant NET-mediated DA uptake in rodent PFc (Di Chiara *et al*, 1992; Tanda *et al*, 1997; Yamamoto and Novotney, 1998). DA may thus be accumulated by NET in PFc of both wild-type and DAT-KO mice.

The current observations in DA response to cocaine and fluoxetine in CPu of DAT-KO mice may provide a different picture. Although systemic cocaine and fluoxetine increase significantly CPu $DA_{ex}$ in DAT-KO mice, local cocaine fails to change it. These results demonstrate that SERT does nt play a role of 'promiscuous uptake' in DA clearance. Systemic cocaine- or fluoxetine-induced DA increase in CPu of DAT-KO mice may result from DA release from activated DA neuron rather than local clearance by SERT.

## Comparisons with Other Results

Observations that CPu dialysate monoamine levels apparently provide the best parallel with the loss of cocaine CPP found in current results could be consistent with a previously underappreciated role for CPu structures in mediating some of the 'learned' features of cocaine reward that are manifest in conditioned place preference testing (White and McDonald, 2002). These structures can be critical for stimulus-response 'habit' learning, including that related to reward (Jog *et al*, 1999; Reynolds *et al*, 2001). It is conceivable that this structure may play an even greater role in DAT-KO mice that lack cocaine-induced $DA_{ex}$ elevations in NAc.

The failure of dialysis results for DA alone in NAc or PFc to parallel cocaine reward effects of various KOs and the apparent parallel in CPu should not prevent further consideration of: (a) multiple compensating contributions of monoamines to the rewarding effects of cocaine; (b) contributions of cocaine effects on monoamines in other brain regions, for example, ventral pallidum (Gong *et al*, 1996, 1997), ventral tegmental area (Roberts and Koob, 1982; Ranaldi and Wise, 2001) for cocaine reward; (c) effects of nonmonoaminergic adaptations to the retained cocaine reward in the transporter KO mouse strains that retain such reward. Monoamine actions in brain regions such as the ventral tegmental area have been postulated to be central to the rewarding actions of major drug classes, such as opiates (Wise, 1989; Garzon and Pickel, 2001) and stimulants. It is quite conceivable that monoamine actions in areas not sampled in the current studies could play roles in normal cocaine reward mechanisms, and in adaptations that may underlie the retention of cocaine reward in DAT- and in SERT-KO mice. Mice with single or multiple transporter deletions display many adaptive alterations, as assessed through behavioral, neurochemical, per- or post-synaptic receptor binding, gene expression, and other analytical approaches. None of the current data should hinder attempts to add more explanatory power for the remarkable behavioral pharmacological profiles displayed by these KO mice through use of any or all of these alternative approaches.

The current results in NAc and CPu DA response to cocaine in DAT-KO mice produced in our laboratory, while highly reproducible in our hands, differ from those obtained in reports from another line of DAT-KO mice that which showed that systemic cocaine and reboxetine (NET blocker) increased $DA_{ex}$ remarkably in NAc of DAT-KO mice (Carboni *et al*, 2001). The different DA response to cocaine in NAc and CPu between Carboni's and our DAT KOs may be due to the different DNA construction which was used to disrupt DAT gene. Moreover, our findings are consistent with other reports which demonstrated that cocaine could not affect DA clearance in NAc of DAT-KO mice via *in vitro* experiments. It is noteworthy that (1) $DA_{ex}$ baseline in NAc of DAT-KO mice is about 10 times greater than that in wild-type mice, and that (2) the capacity for DA uptake of NET is far weaker than that of DAT (Giros *et al*, 1994; Gu *et al*, 1994). These may be the reasons why NET cannot show redundancy for DAT in NAc.

In summary, the present work adds to previous data concerning the behavioral consequences of DAT and SERT deletion, by suggesting that cocaine CPP does not necessarily correlate with simple elevations of DA the NAc or PFc. It points out unanticipated correlations with $DA_{ex}$ elevations in CPu. It is interesting that the CPu findings parallel behavioral observations of the rewarding profiles of not only cocaine but also of fluoxetine in these varying mouse strains. While these correlations do not prove causation, the data support careful re-examination of CPu roles in psychostimulant reward (or reward learning) in both wild-type and DAT-KO mice, including both the dorsal and ventral CPu regions likely to be sampled with our microdialysis approaches. Another view of the current results is that the double homozygous DAT/SERT combined KO mice that failed to display either cocaine-induced $DA_{ex}$ or $5\text{-}HT_{ex}$ elevations in NAc also failed to exhibit cocaine CPP, suggesting perhaps that either $DA_{ex}$ or $5\text{-}HT_{ex}$ elevation can mediate cocaine reward and that the absence of both effects is required to eliminates the cocaine CPP. The current data also add to the growing body of evidence that may indicate uptake of released monoamines by non-cognate transporters when their cognate transporters are deleted, and provide evidence for the brain-region specificity of these processes in wild-type and in transporter KO

mice. Each of these findings adds pieces to the complex puzzle of the mediation of cocaine reward by monoaminergic brain systems.

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特集2：生物学的精神医学研究の現状と展望（3）

311-316

# 遺伝子改変マウスモデルを用いた薬物依存と統合失調症の病態研究
## ——東北大学精神・神経生物学分野における取り組みを中心に——

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**Key words**：transgenic mice, monoamine transporter, monoamine receptor, drug abuse, schizophrenia

## 1．はじめに

東北大学精神・神経生物学分野は精神神経学分野に加えて精神医学教室の流れを汲む教室として精神疾患の生物学的研究を行うために平成14年春に新しく開講された。当教室では，主に筆者らが作製したモノアミン神経伝達に関与する遺伝子ノックアウトマウス（以下KOマウス）を用いて，薬物依存と統合失調症の病態研究を行っているので紹介したい。

精神疾患の動物モデルは，身体疾患同様に複雑な臨床症状を解析可能とするために病態解明，治療法の開発に欠かすことができない。もちろん，精神疾患においてヒトと動物の種族差は身体疾患以上に大きく，精神疾患の多彩な症状のあらゆる面を表現できる完璧な動物モデルは存在しない[33]。しかし，精神疾患の一群の症状，病態メカニズム，治療薬の反応性などが現在までにさまざまな動物モデルを用いて検討されてきた。このような動物モデルの中には遺伝モデルも開発されてきたが，多くは突然変異体あるいは人為交配の経過の途上で発見されたために，関心のある遺伝子の変異モデルを得られることは偶然に頼るしかなかった。近年，標的分子の遺伝子を狙って変異を導入する分子遺伝学的手法が開発され，遺伝子変異マウスが作製可能となった[26)29)]。遺伝子変異マウスのうちで遺伝子の発現を欠損させたノックアウトマウスが作成され，新しい疾患動物モデルとして数多くの研究が行われてきた[3)18)24)28)]。本稿では，薬物依存の病態の基礎となる報酬系のメカニズム，統合失調症と関連する逆耐性現象・プレパルスインヒビションを中心に当教室で行われている研究を紹介する。

Pathophysiology of drug addiction and schizophrenia : research using transgenic animal models at Department of Psychobiology, Tohoku University Graduate School of Medicine

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## 2．薬物依存と報酬系

薬物依存形成の基礎となる報酬系の研究に動物モデルが用いられてきた。依存性薬物が標的分子にどのように作用するのかについて，これらの動物モデルを用いてさまざまなアプローチがなされてきたが，従来の薬理学的な手法では標的分子に特異的に結合する化合物を得ることは困難であった。しかし，生体内で標的分子の遺伝子に変異を起こさせる遺伝子改変動物モデルを用いることにより，従来の古典的な薬理学の手法では明らかにすることが難しかった知見が得られるようになった[31]。本稿では覚醒剤の標的分子であるモノアミントランスポーター[22]の欠損マウスを用いて得られた薬物依存と報酬系の分子メカニズムを紹介する。

コカインはモノアミントランスポーターに結合するが，報酬効果はそのうちのドーパミントランスポーター（DAT）を介していると考えられ，「DAT 仮説」が提唱されていた[21][22]。筆者らのグループは DAT が欠損しているマウスを作製し，コカインによる運動量の増加は消失しているにもかかわらず，条件づけ場所嗜好性試験，静脈内自己投与によるコカインの報酬効果は保持されていることを見出した[27]。さらにセロトニントランスポーター（SERT）あるいはノルエピネフリントランスポーター（NET）-KO マウスにおいてもコカインの報酬は減少するどころか，むしろ増加する結果が得られた。これより SERT，DAT，NET がそれぞれ単独に欠損しても，他のトランスポーターが補い，コカインの報酬が保持されることが推測された[34]。そこで DAT と SERT が共に欠損するマウスモデルを作製し，コカインの報酬が保持あるいは消失するかどうかを検討したところ，DAT の完全欠損に SERT の部分あるいは完全欠損が加わるとコカインの報酬は消失した[25]。一方，SERT が完全欠損しても DAT の発現が部分的に存在するとコカインの報酬は保持された。このことから，コカイン報酬には DAT と SERT が共に関与しているが，SERT よりも DAT がより重要な役割を果たしていると考えられ，「DAT 仮説」は最初に提唱されたものとは異なり，複雑な系であることが明らかとなった[23][34]。

そこで我々は，報酬に関する上記の結果に対応する脳内モノアミン神経伝達を解析することを目的に，脳内微少透析法を用いてコカインに対する線条体（CPu），側坐核（NAc）と前頭前野皮質（PFc）細胞外ドーパミン（DA*ex*），セロトニン（5-HT*ex*）濃度の変化を検討した。コカインの報酬に対応して DA*ex* が増加したのは，側坐核，前頭前野皮質ではなく，線条体であることがわかった[19]。DAT-KO マウスでは DAT が欠損しているにもかかわらずコカインの報酬があり，DAT/SERT ダブル KO マウスで報酬がなくなるのは，線条体において DAT-KO マウスで DA*ex* の増加があり，DAT/SERT ダブル KO マウスでは DA*ex* の増加がないからと考えられる。DAT-KO マウスで DAT が欠損しているにも関わらず DA*ex* が増加しているのは，SERT が DA 再取り込みを補完したことを示唆している。側坐核では DAT が欠損すると DA*ex* の増加が見られなかった。前頭前野皮質では DAT-KO マウス，DAT/SERT ダブル KO マウスでも DA*ex* の増加が見られ，前頭前野皮質では NET による DA 再取り込みの補完が考えられた。5-HT については，SERT-KO マウスで前頭前野皮質での 5-HT*ex* の増加が見られないが，線条体，側坐核では増加が見られ，DAT/SERT ダブル KO マウスで見られなくなったことから，線条体，側坐核では DAT が 5-HT の再取り込みを補完していると考えられた。これらの結果は，コカイン報酬には DAT と SERT が共に関与し，同族トランスポーターの補完作用が存在することを示唆している。

## 3．統合失調症の発症脆弱性モデルとしての逆耐性現象

実験動物に覚醒剤やコカインのような中枢興奮薬を反復投与すると，移所運動量や常同行動が増加し，長期断薬後も同量またはそれ以下の薬物の再投与でこの増加が再現される[17]。この逆耐性現象はヒトの薬剤性精神病や統合失調症の症状再燃

に酷似することから，これらの精神病の有力な発症脆弱性モデルとされている[11)-13)]。統合失調症患者に覚醒剤を投与して線条体後シナプスドーパミンD2受容体の占拠率をSPECTで調べた米国のグループは，線条体DA神経終末からのDA放出量が正常人の約2倍に上昇していることを報告している[6)]。この知見は，統合失調症患者のDA神経系では逆耐性現象を形成した動物と同様の変化が生じていることを初めて示し（endogenous sensitization），統合失調症動物モデルとしての逆耐性の妥当性を示しているという点で意義深い。

当教室では主にモノアミントランスポーターKOマウスを用いて逆耐性現象の形成を検討している。DATはコカイン，メタンフェタミンの標的分子であり，DATが完全欠損したDAT-KOマウスはDA再取り込み機構が欠損しているためDA*ex*が正常の10倍に増加し，表現型として自発運動量の増加，新奇環境における馴化の低下を示す[21)27)]。コカイン，メタンフェタミンの移所運動量増加作用はDAT-KOマウスでは消失し，逆に鎮静効果が現れる[30)]。DATヘテロKOマウスでは，メタンフェタミン反復投与により逆耐性現象は形成されたが，発展は野生型に比して有意に抑制されていた。これらの結果より，中枢刺激薬の移所運動量増加作用にはDATが不可欠であること，逆耐性現象の正常な発展にはDATの完全な発現が必要であることが示唆された。脳内微少透析法による検討では，DATヘテロKOマウスにおいてメタンフェタミン投与時線条体でのDA放出は野生型に比して減少していた。移所運動の逆耐性形成には腹側被蓋野から側坐核へのDA伝達が重要であると考えられているが，この結果からは逆耐性発展における線条体DA伝達の関与が示唆された。

シナプス小胞トランスポーター2（VMAT2）はモノアミン小胞上に存在し，モノアミンを小胞内に汲み上げ貯蔵する。メタンフェタミンはVMAT2を介してシナプス小胞に貯蔵されているモノアミンを細胞質に排出させ，そのモノアミンを細胞膜上のトランスポーターを介して逆流出させる[35)]。VMAT2完全欠損マウスは致死性であるため，ヘテロKOマウスでの検討を行った。VMAT2ヘテロKOマウスのメタンフェタミン，コカイン投与による急性運動増加作用は野生型に比べて増加しているが，条件付け場所嗜好性試験における覚醒剤の報酬効果は減少しており，メタンフェタミン反復投与での逆耐性現象も形成されなかった[21)32)]。この結果はメタンフェタミンが細胞膜とシナプス小胞トランスポーターの両者を標的とすることに起因している可能性があり，メタンフェタミンの逆耐性現象形成にはVMAT2遺伝子の正常な発現が必要であることが示唆された。中枢刺激薬はSERT，NETにも比較的高い親和性で結合する。SERT-KOマウスの5-HT*ex*は野生型の10倍を示す。SERT-KOマウスにおけるメタンフェタミンの移所運動量増加作用は野生型と同等であるが[1)]，低用量メタンフェタミン反復投与では逆耐性現象が形成されなかった[30)]。NET-KOマウスではコカイン投与による移所運動量増加作用は野生型に比べて増加していたが，反復投与による逆耐性は形成されなかった[36)]。これらの結果からは5-HTまたはノルエピネフリン（NE）神経伝達過剰状態では逆耐性の形成は抑制される可能性が示唆された。

従来の薬理学的研究ではD1拮抗薬により逆耐性現象が抑制される結果が報告され，逆耐性の形成にはD1受容体活性化が必須であるとされてきたが，拮抗薬の受容体特異性の問題があった。D1-KOマウスで検討された結果，コカイン，メタンフェタミンによる急性運動刺激作用は減弱していたが逆耐性現象は形成された。また，コカインの報酬効果は保たれていた[9)]。この結果からは，D1受容体の活性化は逆耐性，報酬効果形成に必須ではないかあるいはD1-KOマウスでは何らかの代償機構が働いている可能性が推察された[20)]。D1受容体familyに属するD5受容体のKOマウスの中枢刺激薬に対する検討はなされていない。D2-KOマウスではモルヒネの報酬効果は減弱しており[7)]，依存形成にはD2受容体活性化が重要であるかと思われたが，コカインの自己投与行動は保たれ，依存が形成された[3)]。D2受容体familyであるD3，D4受容体のKOマウ

スではコカインの運動刺激効果は増強しており[16][37]，これらの受容体活性化は運動刺激に対し抑制的に働くことが示唆された。D3-KOマウスではメタンフェタミンの報酬効果は増強しており[37]，D3受容体活性化は依存形成に対しても抑制的に働く可能性があるが，D2，D3，D4-KOマウスでの逆耐性現象については報告がない。DA受容体欠損マウスでは一つのサブタイプが欠損してもfamilyに属する他のサブタイプが代償する可能性もあり，今後，複数のサブタイプを欠損したダブル・トリプルKOマウスでの検討も必要と考えられる。

## 4．統合失調症の病態指標としてのプレパルスインヒビション（PPI）

突然の強力な聴覚，視覚または触覚刺激に出会うとヒトを含めさまざまな動物は，顔面や全身の筋肉のすばやく短い痙攣様の反応を示す。我々は，この生理学的現象を驚愕反応と呼び，外部からの侵害的な刺激に対する防衛的機能の一つであると考えている[5]。驚愕反応は，条件付け，感作，馴化，薬剤投与などにより増減する特徴を有するが，なかでもPPIにおける驚愕反応減弱現象は，統合失調症で障害されていることから注目を集めている[2]。PPIとは，驚愕刺激を与える直前（一般的には30～500 ms前）に，それ自体では驚愕反応を引き起こさない程度の弱い刺激（プレパルス）をあらかじめ負荷することで驚愕反応の強度が低下する現象である。他の精神生理学的パラダイムと比べ，PPIの利点は実験動物においても同様のパラダイムを問題なく適応できる点にある。

我々は，DAが過剰であるDAT-KOマウス，5-HTが過剰なSERT-KOマウス，DAと5-HT双方が過剰なDAT/SERTダブルKOマウスにおいてPPIを検討した。DAT-KOマウスでは野生型に比べてPPIが減弱していた。DAT-KOマウスでみられるPPIの減弱は4週齢から9週齢までで顕著に認められた。一方，SERT-KOマウスとDAT/SERTダブルKOマウスではPPIが正常だったので，DAT-KOによるPPIの障害はSERT-KOが加わると回復することがわかった。DAT-KOマウスにD2受容体拮抗薬を投与するとPPIの障害が回復することから[14]，DAT-KOマウスにおけるPPIの障害はDA*ex*の増加によるDA神経伝達のtonicな変化が原因であり，アンフェタミン等の間接的DA作動薬投与によるPPI障害[15]と同様の機序が働いていると推定した。これに対して，SERT-KOマウスではPPIの障害を認めなかった。SERT-KOマウスでも，DAT-KOマウスと同様に，線条体における5-HT*ex*は野生型マウスの約10倍に達する[30]。マウスにおいて，5-HT放出薬であるMDMA（3，4-メチレンジオキシメタンフェタミン）等の投与はPPIを障害すると報告されており[4]，5-HT神経伝達過剰がPPIを引き起こす可能性がある。しかし，選択的セロトニン再取り込み阻害薬であるフルオキセチンではPPIの障害を認めなかった[8]。これらの報告と我々の結果から，マウスにおいて5-HT*ex*の過剰のみではPPIの障害を認めない，あるいは，生来的な5-HT過剰による5-HT受容体の変化を含む5-HT神経伝達の適応的変化によりPPIが正常化した可能性が示唆された。MDMA等のPPI障害効果は，5-HT*ex*過剰効果だけによるものではなく，DA系神経伝達変化等の相互作用が絡んでいる可能性も考えなければならないだろう。次に，DAT/SERTダブルKOマウスであるが，野生型マウスと比較して有意なPPI障害を認めなかった。DAT/SERTダブルKOマウスのDA*ex*，5-HT*ex*はいずれも野生型マウスの約10倍である[30]。上述した，DA*ex*の増加によるDA神経伝達の過剰によりPPIの障害が起こるという仮説が正しいならば，この状態に，5-HT*ex*上昇が加わることによって，PPIの変化が引き起こされたと推察される。DA神経伝達過剰かつ5-HT神経伝達過剰の状態にあるマウスのPPIには，感覚運動情報制御におけるDA系と5-HT系の神経伝達の変化と相互作用が関与している可能性が予想された[10]。

## 5．おわりに

精神疾患の「作業仮説」を検証する上で，ノッ

クアウトマウスを含む遺伝子改変動物は有用なモデル動物であると考えられる。精神疾患は，単一の遺伝子の異常によるものではなく，複数の遺伝子が脆弱性を形成し，遺伝要因と環境要因が複雑に関与して発症すると考えられている。当教室では，複数の遺伝子が関与していると考えられる精神疾患の病態の解明に，進展の著しいヒトゲノム計画などから得られる情報を大いに活用し，ヒトとモデル動物から得られる知見を相互にフィードバックさせて取り組んで行きたい。なお，本稿では紹介できなかったオピオイド神経伝達に関する研究等は当教室のホームページ：http：//www.psychobio.med.tohoku.ac.jp をご参照いただきたい。

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