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COCAINE-CONDITIONED LOCOMOTION IN DOPAMINE TRANSPORTER, NOREPINEPHRINE TRANSPORTER AND SEROTONIN TRANSPORTER KNOCKOUT MICE

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Abstract—The behavioral effects of cocaine are affected by gene knockout (KO) of the dopamine transporter (DAT), the serotonin transporter (SERT) and the norepinephrine transporter (NET). The relative involvement of each of these transporters varies depending on the particular behavioral response to cocaine considered, as well as on other factors such as genetic background of the subjects. Interestingly, the effects of these gene knockouts on cocaine-induced locomotion are quite different from those on reward assessed in the conditioned place preference paradigm. To further explore the role of these genes in the rewarding effects of cocaine, the ability of five daily injections of cocaine to induce conditioned locomotion was assessed in DAT, SERT and NET KO mice. Cocaine increased locomotor activity acutely during the initial conditioning session in SERT KO and NET KO, but not DAT KO, mice. Surprisingly, locomotor responses in the cocaine-paired subjects diminished over the five conditioning sessions in SERT KO mice, while locomotor responses increased in DAT KO mice, despite the fact that they did not demonstrate any initial locomotor responses to cocaine. Cocaine-induced locomotion was unchanged over the course of conditioning in NET KO mice. In the post-conditioning assessment, conditioned locomotion was not observed in DAT KO mice, and was reduced in SERT KO and NET KO mice. These data reaffirm the central role of dopamine and DAT in the behavioral effects of cocaine. Furthermore, they emphasize the polygenic basis of cocaine-mediated behavior and the non-unitary nature of drug reward mechanisms, particularly in the context of previous studies that have shown normal cocaine-conditioned place preference in DAT KO mice. © 2009 Published by Elsevier Ltd on behalf of IBRO.

Key words: transgenic mice, dopamine transporter, serotonin transporter, norepinephrine transporter, cocaine, conditioned locomotion.

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Abbreviations: ANOVA, analysis of variance; CPP, conditioned place preference; DAT, dopamine transporter; GFP, green fluorescent protein; KO, knockout; NEO, neomycin gene; NET, norepinephrine transporter; SERT, serotonin transporter; 5-HT, serotonin.

Initial transgenic studies of the mechanisms underlying the rewarding effects of cocaine found that deletion of the gene for the dopamine transporter (DAT) alone did not eliminate the rewarding effects of cocaine as assessed in either the conditioned place preference (CPP) or self-administration paradigms (Rocha et al., 1998; Sora et al., 1998). Subsequent studies found that combined elimination of the serotonin transporter (SERT) and DAT eliminated the rewarding effects of cocaine in the CPP paradigm (Sora et al., 2001). However, the effects of SERT knockout (KO) are rather complex and can also increase the rewarding effects of cocaine (Sora et al., 1998; Hall et al., 2002). This should not be surprising given the diverse effects that pharmacological treatments aimed at specific serotonin (5-HT) receptor subtypes have on drug reward, including both increases and decreases in the rewarding effects of diverse classes of addictive drugs (Carboni et al., 1989; Fadda et al., 1991; Higgins et al., 1992a,b; Bisaga et al., 1993; Kostowski et al., 1993; Lu et al., 1994; McMillen et al., 1994; Tomkins et al., 1994a,b, 1995; Rompre et al., 1995; Parsons et al., 1998; Wilson et al., 1998; Fletcher and Korth, 1999; Harrison et al., 1999; Maurel et al., 1999; Tomkins and O'Neill, 2000; Fletcher et al., 2004). Indeed, under some circumstances (e.g. DAT KO mice) the selective 5-HT reuptake inhibitor fluoxetine has been shown to have rewarding effects (Hall et al., 2002).

In monoamine transporter KO mice the rewarding and reinforcing effects of cocaine have been assessed primarily with the CPP paradigm. The effects of these KOs in other paradigms have not been extensively characterized, but should not be expected to be necessarily uniform. The different methods used to assess the rewarding properties of drugs of abuse have often been superficially treated as if they are all equivalent measures of a single unitary construct, in part based on early descriptions equating locomotor stimulant effects with drug reward (Wise and Bozarth, 1987), even though the diversity of reward mechanisms has long been recognized (Wise and Leeb, 1993), especially the role of conditioned responses in the maintenance of drug-seeking behavior and sensitization (Post et al., 1981, 1987; Stewart, 1983). In fact a critical, though often overlooked, distinction has been made between two factors that contribute to cocaine sensitization, the role of conditioned drug effects and the role of neuropharmacological alterations induced by the repeated exposure to drugs of abuse (Pert et al., 1990). These two factors are sometimes described as context-dependent and context-independent sensitization and have been shown to involve different neurobiological mechanisms (Wise and Leeb,

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1993). However, these types of effects involve administration of drugs after repeated treatment and sensitization is evinced by enhanced response to the drug compared to untreated animals or animals treated chronically with saline. However, context-dependent sensitization can be clearly shown to be a conditioned response. The increase in behavioral response in this circumstance is dependent on exposure to the conditioned stimuli and results in conditioned increases in locomotion (e.g. conditioned locomotion) even without any drug treatment. The relative importance of context-dependent and context-independent sensitization for the actual mechanisms underlying addiction is a matter of some debate, and although both are certainly important, it has certainly been argued that alterations in associative processes may play critical roles in addiction (Everitt et al., 2001). However, it is important to note that sensitization to cocaine can be observed independently of conditioned locomotion (Carey and Gui, 1998; Carey and Damianopoulos, 2006). Furthermore, multiple conditioned effects of drugs of abuse can be observed independently of each other, further indicating the non-unitary bases of drug reward and drug seeking behavior. For instance, conditioned locomotor activity can be observed independently from CPP (Kosten and Miserendino, 1998).

In the initial description of the elimination of the locomotor effects of cocaine in DAT KO mice they were described as "indifferent" to cocaine (Giros et al., 1996), the implication being that lack of locomotor stimulant effects should be equated with elimination of rewarding effects. This was proven to be incorrect (Rocha et al., 1998; Sora et al., 1998), but there often remains a tacit assumption that manipulations that affect one aspect of cocaine-mediated behavior should affect other behaviors in a similar manner. One way to directly address this issue is to evaluate gene KOs that produce a particular pattern of effects on one cocaine-associated behavior, and compare them to the consequences of those gene KOs on another cocaine-associated behavior. The effects of monoamine transporter KOs on cocaine CPP have been well characterized: Cocaine CPP is unaffected in DAT KO mice (Sora et al., 1998), but increased in SERT KO and NET KO mice (Sora et al., 1998; Xu et al., 2000). In addition to producing a place preference cocaine also induces conditioned locomotion (Post et al., 1987), which has not been examined for any of these gene KOs. Therefore, to further explore the role of these genes in the rewarding effects of cocaine, the ability of repeated injections of cocaine to induce conditioned locomotion was assessed in DAT, SERT and NET KO mice.

EXPERIMENTAL PROCEDURES

Subjects

DAT (Sora et al., 1998), SERT (Bengel et al., 1998) and NET (Wang et al., 1999) KO mice have been described previously. These KO lines were used to create DAT/SERT (Sora et al., 2001) and NET/SERT (Hall et al., 2002) double KO strains. In the present experiments DAT +/+, DAT +/− and DAT −/− mice were bred from the DAT/SERT line; SERT +/+, SERT +/− and SERT −/− mice were bred from the DAT/SERT line; and NET +/+, NET +/− and NET −/− mice were bred from the NET/SERT line. Male and female mice were used, and were tested at 12–18 weeks of age. Mice were bred from double heterozygote (e.g. DAT +/− SERT +/−×DAT +/− SERT +/−) or single heterozygote (e.g. DAT +/− SERT +/+×DAT +/− SERT +/+) crosses.

Wild-type (+/+), heterozygote KO mice (+/−) and homozygote KO mice (−/−) were genotyped by PCR, using two internal primers, one targeted at the KO insertion sequence and one targeted at the wild-type (WT) gene, and one external primer, which generated two products identifying the WT and KO genes. The DAT and SERT transgenic KO insertion sequences contained a neomycin gene (NEO), while the NET KO contained a green fluorescent protein gene insert (GFP). PCR using Takara DNA polymerase (Takara Bio, Japan) was performed on DNA that was released from tail tip fragments after overnight digestion with protease K. For DAT genotyping the external primer (5′ AGT GTG TGC AGG GCA TGG TGT A 3′) and the WT primer (5′ TAG GCA CTG CTG ACG ATG ACT G 3′) produced a 500 bp band, while the external primer and the NEO primer (5′ CTC GTC GTG ACC CAT GGC GAT 3′) produced a 600 bp band. For SERT genotyping the external primer (5′ GCT CTC AGT CTT GTC TCC ATA AC 3′) and the WT primer (5′ TGC TGA CTG GAG TAC AGG CTA G 3′) produced a 620 bp band, while the external primer and the NEO primer (5′ CTC GTC GTG ACC CAT GGC GAT 3′) produced an 800 bp band. For NET genotyping the external primer (5′ GCT CTG TCC CTG TGC TTC ACG 3′) and the WT primer (5′ TGA GGC CTA AGC TGG AGC TCG 3′) produced a 601 bp band, while the external primer and the GFP primer (5′ CGG TGA ACA GCT CCT CGC CC 3′) produced a 470 bp band.

Conditioned locomotion procedure

Homozygous and heterozygous DAT, NET and SERT KO mice and WT littermate controls were divided into three experimental groups: Paired, Unpaired and Control groups (DAT KO, N=8–12 per genotype per condition; NET KO, N=8–11 per genotype per condition; SERT KO, N=9–18 per genotype per condition). Mice in each group received two injections each day, one before being placed in a locomotor activity chamber and one later in the home cage. Locomotor testing was conducted using an Optovarimax locomotor activity testing apparatus (Columbus Instruments, Columbus, OH, USA) under dark conditions in sound attenuating chambers. Mice in the Paired group received an injection of cocaine HCl (20 mg/kg SC) prior to locomotor testing for 30 min. Subjects were then returned to their home cages and 2 h later they received an injection of saline (10 ml/kg). Mice in the Unpaired group received an injection of saline prior to locomotor testing and an injection of cocaine (20 mg/kg SC) in the home cage. Mice in the control group received saline injections before locomotor testing and in the home cage. This procedure was conducted each day for 5 days; on the day following the final injections, mice were placed in the locomotor activity chambers for 20 min without any injections to assess conditioned locomotion.

Statistics

Statistical comparisons were made with analysis of variance (ANOVA) followed by Scheffe's post hoc analyses using StatView V. 5.0 (SAS Institute, Inc.). Conditioning data were initially analyzed by an overall ANOVA with the between subjects factors of Genotype (+/+, +/− and −/−) and Conditioning Group (Paired, Unpaired and Control), and the additional within-subjects factor of Conditioning Trial (days 1–5). Subsequently, the data for each genotype (+/+, +/− and −/−) were analyzed separately with the between-subjects factor of Conditioning Group (Paired, Unpaired and Control), and the within-subjects factor of Conditioning Trial (day 1–5). Data from the post-conditioning test were analyzed with the between subjects factors of Conditioning Group and Genotype

(+/+, +/− and −/−). Post hoc comparison's were made with Scheffe's test ($P<0.05$ significance level).

RESULTS

Locomotion during conditioning trials in DAT KO mice

During the conditioning trials mice receiving injections of cocaine prior to testing (Paired Group) were significantly more active than mice treated with saline prior to testing (Unpaired and Control groups) as reflected by an overall significant effect of Conditioning Group ($F[2,87]=66.2$, $P=0.0001$; Fig. 1A–C). DAT −/− mice were significantly more active under all conditions compared to DAT +/− and DAT +/+ mice as reflected by a significant effect of Genotype ($F[2,87]=71.0$, $P=0.0001$), but did not exhibit increases in locomotor activity after acute cocaine administration so that there was also a significant Genotype× Conditioning Group interaction ($F[4,87]=4.9$, $P=0.0013$).

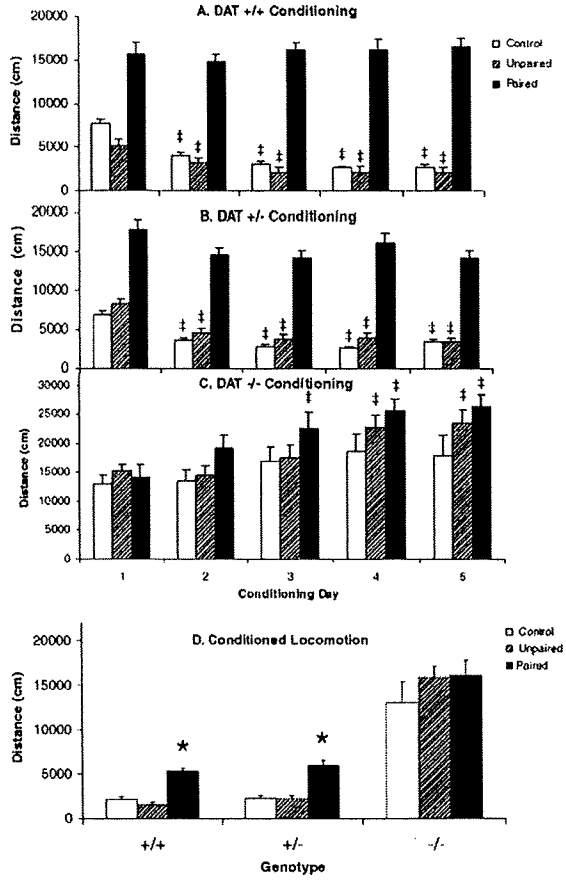

Fig. 1. Conditioned locomotion in DAT KO mice. Locomotor activity during conditioning sessions in DAT +/+ (A), DAT +/− (B) and DAT −/− (C) mice from each of the conditioning groups (Paired, Unpaired and Control) expressed in terms of distance traveled. Conditioned locomotion (D) in all groups. * Significant difference from Control conditioning group based on Scheffe's post hoc comparison ($P<0.05$). ‡ Significant difference from Trial 1 based on Scheffe's post hoc comparison ($P<0.05$). Data are represented as mean±the standard error of the mean.

Over the course of the conditioning trials locomotor activity decreased in DAT +/+ and DAT +/− saline-treated subjects, but not DAT +/+ and DAT +/− cocaine-treated subjects so that the relative magnitude of the cocaine effect increased over trials. In DAT −/− mice a different pattern of effects was observed. Unlike DAT +/+ and DAT +/− mice the activity of Control DAT −/− mice did not decrease. Furthermore, although there was no initial difference in locomotor activity between conditioning groups, over trials the activity of the cocaine-treated groups (Paired and Unpaired) increased. Note that only the Paired subjects received cocaine prior to this locomotor test, the Unpaired subjects were injected with saline. Thus, in the ANOVA there were significant effects of Conditioning Trial ($F[4,348]=8.1$, $P=0.0001$), Conditioning Trial×Conditioning Group ($F[8,348]=6.0$, $P=<0.0001$), Conditioning Trial×Genotype ($F[8,348]=30.5$, $P=0.0001$), and Conditioning Trial×Conditioning Group×Genotype ($F[16, 348]=2.0$, $P=0.012$). To further clarify the nature of these effects individual ANOVAs were performed on the data from each genotype.

DAT +/+ mice treated with cocaine prior to locomotor testing (Paired group) were significantly more active than mice treated with saline (Unpaired and Control groups) throughout all five conditioning trials (Fig. 1A; Conditioning Group: $F[2,31]=99.6$, $P=0.0001$). Over the course of the five conditioning trials the activity of mice in the Unpaired and Control groups decreased, but the activity of mice in the Paired group was unchanged compared to day 1 so that the relative difference between saline-injected and cocaine-injected animals was greater in later trials. Thus, there was a significant interaction between Conditioning Group and Conditioning Trial ($F[8,124]=5.6$, $P=0.0001$). Post hoc Scheffe's comparisons demonstrated significantly reduced locomotion in acute saline-treated groups (Unpaired and Control) for conditioning trials 2–5 compared to the first conditioning trial, but no differences between trials in the acute cocaine treated group (Paired).

A somewhat similar pattern was observed in DAT +/− mice (Fig. 1B), where there was a significant effect of Conditioning Group ($F[2,31]=147.6$, $P=0.0001$), but not a significant interaction between Conditioning Group and Conditioning Trial ($F[8,124]=1.4$, NS). In addition to decreases in locomotion in the Unpaired and Control groups, there was also a slight decrease in the activity of Paired subjects over trials. Post hoc one way ANOVA for each conditioning group revealed significant effects of Conditioning Trial in all three conditioning groups. Post hoc Scheffe's comparisons demonstrated significantly reduced locomotion in acute saline-treated groups (Unpaired and Control) for conditioning trials 2–5 compared to trial 1. In the Paired group the reduction in locomotion was much smaller than in Unpaired and Control subjects so that no individual comparisons were significant even though there was an overall effect in the ANOVA.

In contrast to the pattern of effects observed in DAT +/+ and DAT +/− mice, a completely different pattern was observed in DAT −/− mice. As has been observed previously, cocaine did not increase locomotor activity in

DAT −/− mice (Fig. 1C), although locomotion was substantially higher than the activity observed in DAT +/+ and DAT +/− mice (compare saline-treated subjects in Fig. 1A–1C). Nonetheless, there was an increase in locomotion on the second and subsequent days in cocaine-treated subjects (Paired group compared to the Control group). This increase in locomotion however was not limited to mice in the Paired group; the activity of mice in the unpaired group also increased over conditioning trials. Although there was not a significant overall effect of Conditioning Group ($F[2,25]=2.6$, NS), there was a significant effect of Conditioning Trial ($F[4,100]=21.4$, $P=0.0001$) and a significant interaction between Conditioning Group and Conditioning Trial ($F[8,100]=2.6$, $P=0.013$). In separate one way ANOVA performed on each conditioning group no effect of Conditioning Trial was found in Control subjects ($F[4,28]=1.5$, NS), but significant effects were observed in both Paired ($F[4,36]=18.9$, $P=0.0001$) and Unpaired ($F[4,36]=9.2$, $P=0.0001$) groups. Post hoc comparisons of activity versus the first testing day demonstrated significant increases in both Paired subjects on trials 3–5 compared to trial 1, and on trials 4–5 compared to trial 1 in Unpaired mice (Scheffe's post hoc comparisons).

Conditioned locomotion in DAT KO mice

In the post-conditioning test DAT +/+ and DAT +/− mice demonstrated a typical pattern consistent with conditioned locomotion (Fig. 1D): increased locomotor activity during the post-conditioning test in Paired mice compared to both Unpaired and Control mice. This test was conducted without any drug injection so it only reflects the ability of the conditioned associations of the environment to evoke locomotion. DAT −/− mice were much more active than DAT +/+ and DAT +/− mice independent of conditioning group. Thus, there were significant effects of both Conditioning Group ($F[2,87]=12.7$, $P=0.0001$) and Genotype ($F[2,87]=126.5$, $P=0.0001$). In post hoc Scheffe's comparisons in DAT +/+ and DAT +/− Paired subjects were significantly more active than either Unpaired or Control subjects. Locomotor activity during the post-conditioning test was slightly greater in both Paired and Unpaired DAT −/− mice, compared to Control subjects, but neither comparison was significant.

Locomotion during conditioning trials in NET KO mice

During the conditioning trials mice receiving injections of cocaine prior to testing (Paired Group) were significantly more active than mice treated with saline prior to testing (Unpaired and Control groups) as reflected by an overall significant effect of Conditioning Group ($F[2,81]=373.6$, $P=0.0001$; Fig. 2A–C). Over the course of the conditioning trials locomotor activity decreased in saline-treated subjects, but not in cocaine-treated subjects, so that there was a significant Conditioning Trial×Conditioning Group interaction ($F[8,324]=6.7$, $P=0.0001$). There was no effect of Genotype ($F[2,81]=2.7$, NS), nor any significant interactions with genotype: Conditioning Group×Genotype ($F[4,81]=0.5$, NS), Conditioning trial×Genotype ($F[8,324]=1.6$, NS), and Conditioning Trial×Conditioning Group×Genotype ($F[16,324]=1.6$, NS). Thus, for all genotypes individual post hoc ANOVA identified only the effects of Conditioning Trial, Conditioning Group, and their interaction.

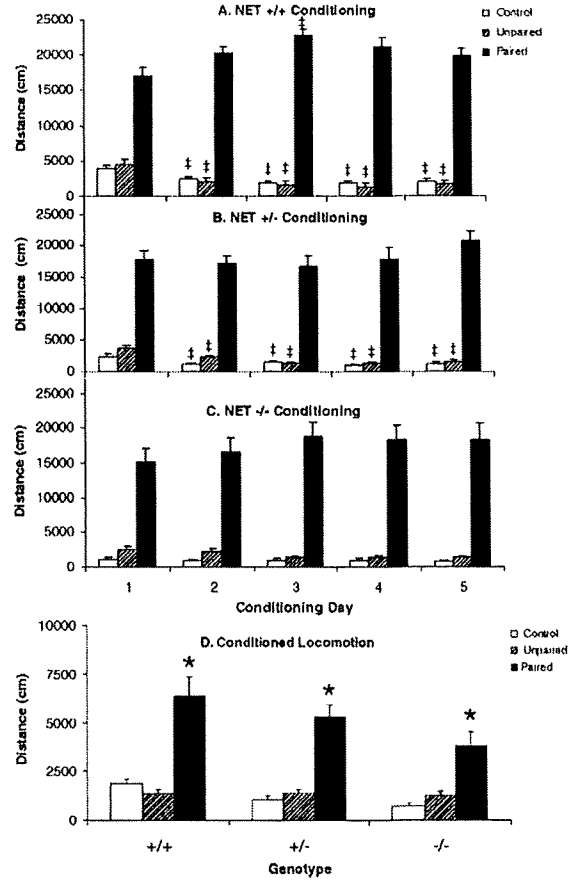

Fig. 2. Conditioned locomotion in NET KO mice. Locomotor activity during conditioning sessions in NET +/+ (A), NET +/− (B) and NET −/− (C) mice from each of the conditioning groups (Paired, Unpaired and Control) expressed in terms of distance traveled. Conditioned locomotion (D) in all groups. * Significant difference from Control conditioning group based on Scheffe's post hoc comparison ($P<0.05$). ‡ Significant difference from Trial 1 based on Scheffe's post hoc comparison ($P<0.05$). Data are represented as mean±the standard error of the mean.

NET +/+ mice treated with cocaine before testing (Paired group) were significantly more active than mice treated with saline (Unpaired and Control groups) over all conditioning trials (Fig. 2A, $F[2,25]=128.5$, $P=0.0001$). Over the course of the five conditioning trials the activity of saline-treated mice in the Unpaired and Control groups decreased, but the activity of mice in the paired group actually increased compared to day 1. These differential changes over conditioning trials resulted in a significant interaction between Conditioning Group and Conditioning Trial in the ANOVA ($F[8,100]=9.6$, $P=0.0001$). Post hoc one-way ANOVA for each conditioning group in NET +/+ mice revealed a significant effect of Conditioning Trial in Control mice ($F[4,36]=17.1$, $P=0.0001$), Unpaired mice

(F[4,36]=14.8, P=0.0001) and Paired mice (F[4,28]=4.2, P=0.0082). In both Control and Unpaired NET +/+ mice locomotor activity scores in trials 2–5 were all significantly lower than trial 1 (P<0.05 Scheffe's comparison). In Paired NET +/+ mice only trial 3 was significantly greater than trial 1 (P<0.05 Scheffe's comparison), but in no cases were decreases in activity observed in relation to trial 1.

A similar pattern was observed in NET +/− mice (Fig. 2B), where there was a significant effect of Conditioning Group (F[2,28]=141.1, P=0.0001), Conditioning Trial (F[4,112]=3.1, P=0.019) and a significant interaction between Conditioning Group and Conditioning Trial (F[8,112]=2.9, P=0.0060). Post hoc one-way ANOVA for each conditioning group in NET +/− mice revealed a significant effect of Conditioning Trial in Control mice (F[4,36]=6.6, P=0.0004), and Unpaired mice (F[4,36]=12.2, P=0.0001) but not Paired mice (F[4,40]=2.3, NS). In Control NET +/− mice locomotor activity was significantly reduced in conditioning trials 2, 4 and 5 compared to trial 1 (P<0.05, Scheffe's comparison), while in Unpaired NET +/− mice locomotor activity was significantly reduced in trials 2–5 compared to trial 1 (P<0.05, Scheffe's comparison). In Paired NET +/− mice no decreases in activity were observed.

In NET −/− mice administration of cocaine produced increases in locomotion across all conditioning trials but activity in NET −/− mice changed less across conditioning trials than activity in NET +/+ and NET +/− mice (Fig. 2C). Thus, there was a significant effect of Conditioning Group (F[2,28]=108.4, P=0.0001), but not Conditioning Trial (F[4,112]=0.3, NS), nor was there a significant interaction between Conditioning Group and Conditioning Trial (F[8,112]=1.3, NS).

Conditioned locomotion in NET KO mice

NET +/+, NET +/− and NET −/− mice demonstrated the typical pattern consistent with conditioned locomotion (Fig. 2D) as shown by a significant effect of Conditioning Group in the ANOVA (F[2,81]=73.0, P=0.0001). In addition, activity was slightly reduced in NET KO mice independent of conditioning group. Thus, there was a significant effect of Genotype (F[2,81]=5.9, P=0.0041) in the ANOVA, but not a significant Genotype×Conditioning Group interaction (F[4,81]=1.9, NS).

Locomotion during conditioning trials SERT KO mice

During the conditioning trials mice receiving cocaine prior to testing were significantly more active than mice treated with saline as reflected by an significant effect of Conditioning Group (F[2,104]=51.9, P=0.0001; Fig. 3A–C). SERT −/− mice were significantly less active under all conditions compared to SERT +/− and SERT +/+ mice as reflected by a significant effect of Genotype (F[2,104]=5.1, P=0.0078). The Genotype×Conditioning Group interaction was not significant overall (F[4,104]=1.9, NS), but there were differences between groups that emerged over repeated conditioning trials resulting in a significant Genotype×Conditioning Group×Conditioning Trial interaction (F[16,416]=2.0, P=0.012). Over the course of the conditioning trials locomotor activity decreased in saline-treated subjects of all genotypes. Locomotor activity did not decrease in SERT +/+ or SERT +/− acute cocaine-treated subjects so that the relative magnitude of the cocaine effect increased over trials, but the magnitude of locomotion in the SERT −/− mice treated with cocaine decreased so that the magnitude of the cocaine effect did not change over conditioning trials.

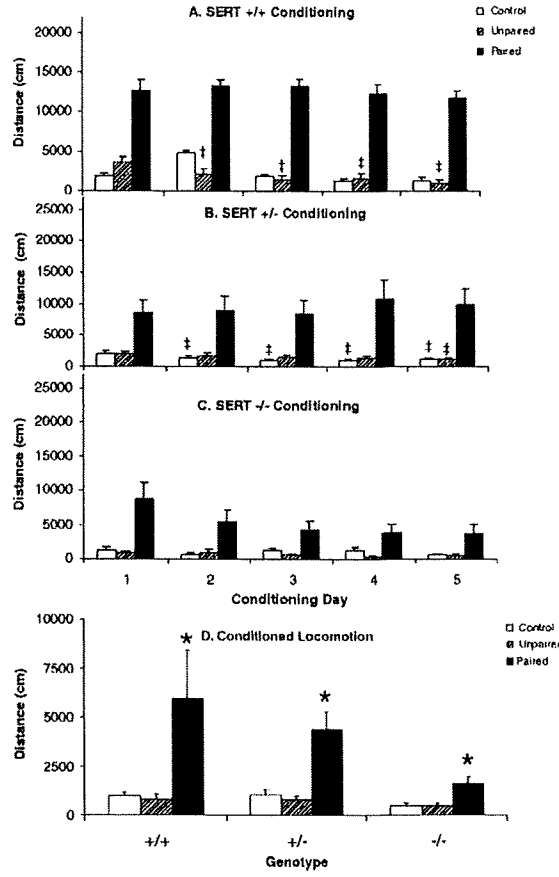

Fig. 3. Conditioned locomotion in SERT KO mice. Locomotor activity during conditioning sessions in SERT +/+ (A), SERT +/− (B) and SERT −/− (C) mice from each of the conditioning groups (Paired, Unpaired and Control) expressed in terms of distance traveled. Conditioned locomotion (D) in all groups. * Significant difference from Control conditioning group based on Scheffe's post hoc comparison (P<0.05). ‡ Significant difference from Trial 1 based on Scheffe's post hoc comparison (P<0.05). Data are represented as mean±the standard error of the mean.

SERT +/+ mice treated with cocaine prior to locomotor testing (Paired group) were significantly more active than mice treated with saline (Unpaired and Control groups) on the first and subsequent days (Fig. 3A). There was a significant effect of Conditioning Group (F[2,27]=22.5, P=0.0001), but the interaction between Conditioning Group and Conditioning Trial was not significant (F[8,108]=0.4, NS). Decreased locomotion across trials was observed in both saline-treated groups as confirmed in one-way ANOVA for the Unpaired (F[4,44]=14.8, P=0.0001) and Control (F[4,32]=3.9, P=0.011) groups. In post hoc Scheffe's comparisons in the Control group there

were no individual trials that were significantly different from trial 1, but in the Unpaired group trials 2–5 were all significantly lower than trial 1. There was no change in locomotion over trials in the Paired group ($F[4,32]=0.1$, NS).

A similar pattern was observed in SERT +/− mice (Fig. 3B), where there was a significant effect of Conditioning Group ($F[2,40]=14.1$, $P=0.0001$), but not a significant interaction between Conditioning Group and Conditioning Trial ($F[8,160]=1.5$, NS). Again, decreased locomotion across trials was observed in both saline-treated groups as confirmed in one-way ANOVA for the Unpaired ($F[4,40]=4.0$, $P=0.084$) and Control ($F[4,52]=9.2$, $P=0.0001$) groups. In post hoc Scheffe's comparisons for the Control group trials 2–5 were significantly lower than trial 1. For the Unpaired group only trial 5 was significantly lower than trial 1. There was no change in locomotion over trials in the Paired group ($F[4,68]=1.3$, NS).

In SERT −/− mice a different pattern of effects emerged. Administration of cocaine produced increases in locomotion on all conditioning trials (Fig. 2C), as shown by a significant effect of Conditioning Group ($F[2,37]=33.7$, $P=0.0001$). Locomotion decreased across trials, as shown by a significant overall effect of Conditioning Trial ($F[4,148]=5.6$, $P=0.003$), but this effect was due primarily to reductions in locomotion in the cocaine-treated group. Thus, there was a significant interaction between Conditioning Group and Conditioning Trial ($F[8,148]=3.3$, $P=0.0017$). Individual post hoc one-way ANOVA revealed a significant effect of Conditioning Trial in Paired SERT −/− mice ($F[4,40]=3.2$, $P=0.023$), but not Unpaired ($F[4,68]=1.3$, NS) or Control ($F[4,40]=2.1$, NS) SERT −/− mice.

Conditioned locomotion in SERT KO mice

SERT +/+, SERT +/− and SERT −/− mice demonstrated the typical pattern consistent with conditioned locomotion (Fig. 3D), as demonstrated by a significant overall effect of Conditioning Group ($F[2,104]=19.7$, $P=0.0001$). In addition, SERT −/− mice had reduced locomotion independent of conditioning group as demonstrated by a significant overall effect of Genotype ($F[2,104]=4.7$, $P=0.011$). This reduction in locomotor activity in SERT −/− mice compared to SERT +/+ mice was somewhat greater in Paired subjects than in Unpaired or Control subjects. The Conditioning Group×Genotype interaction was just statistically significant ($F[4,104]=2.5$, $P=0.050$). Nonetheless, for all genotypes Paired mice had significantly greater activity than Unpaired or Control mice ($P<0.05$, Scheffe's post hoc comparison).

DISCUSSION

The main conclusion that may be drawn from these experiments is that the ability of cocaine to produce conditioned locomotion is dependent on DAT, but not NET or SERT. This is consistent with a dopamine lesion study which found that 6-OHDA-induced lesions of the nucleus accumbens attenuated amphetamine conditioned locomotion (Gold et al., 1988). In addition, differences in context-independent sensitization and context-dependent sensitization were found in DAT KO, SERT KO and NET KO mice during the conditioning phase of the experiment. These are discussed below in detail but further emphasize the non-unitary structure of drug reward mechanisms, the polygenic basis of drug reward mechanisms, and the involvement of all three of these neurotransmitters in cocaine-mediated behavior, albeit to a different degree and in different circumstances.

The role of conditioned responses in drug-seeking behavior has long been recognized, including the role of conditioned responses in cocaine sensitization (Post et al., 1981, 1987; Stewart, 1983). Different underlying mechanisms are known to be involved in context-dependent sensitization and context-independent sensitization (Wise and Leeb, 1993), in particular, but this same argument can be applied to numerous cocaine-induced behaviors including acute locomotor responses, conditioned locomotion and CPP. For instance, differential sensitivity to cocaine sensitization across inbred strains of mice is not simply the result of differential acute sensitivity (Elmer et al., 1996). Different types of drug exposure experiences that enhance cocaine responses clearly have a different basis, including those relating to repeated drug exposure alone and those involving associative mechanisms. Enhanced responses after repeated cocaine treatments have both context-dependent and context-independent components, which can be dissociated, but specific conditioned responses can be further dissociated, including conditioned locomotion (Carey and Gui, 1998; Carey and Damianopoulos, 2006), which is not correlated with sensitization to cocaine (Hotsenpiller and Wolf, 2002; Tirelli et al., 2003) and persists for a longer time (Tirelli et al., 2005). Furthermore, conditioned responses can be dissociated from each other, including conditioned locomotion and CPP (Kosten and Miserendino, 1998).

Since many effects of cocaine and other psychostimulants have been thought to involve primarily dopaminergic mechanisms (Wise and Bozarth, 1987), much research has emphasized the importance of dopamine in these effects. This includes the first publication in DAT KO mice, in which these mice were described as "indifferent" to cocaine because they failed to exhibit locomotor stimulant responses after acute treatment (Giros et al., 1996). The presumption here was that all cocaine effects, including rewarding effects, could be represented in a unitary fashion by cocaine-stimulated locomotion. This was found to be incorrect by the demonstration that DAT KO mice can exhibit both cocaine CPP and cocaine self-administration (Rocha et al., 1998; Sora et al., 1998), although more recent evidence clearly demonstrates that the ability of cocaine to act as a reinforcer is substantially degraded in DAT KO mice (Thomsen et al., 2009). These and other studies also demonstrated the ability of gene KOs of other cocaine targets (e.g. SERT and NET) to modulate the rewarding effects of cocaine (Sora et al., 1998, 2001; Hall et al., 2002). Since there appears to be a somewhat differential involvement of these systems in different cocaine

effects, the present experiments examined another paradigm that examines cocaine-conditioned responses.

Although the primary aim of the present study was to examine cocaine-conditioned locomotion, analysis of behavior during the five conditioning sessions also allowed the examination of context-dependent sensitization of the acute locomotor stimulant effects of cocaine to some extent. Context-dependent sensitization could be observed during the conditioning trials but context-independent sensitization, in the unpaired subjects, obviously could not. DAT KO eliminated cocaine-conditioned locomotion, as might be expected since there was no initial locomotor stimulant response to cocaine in DAT −/− mice, but in addition locomotor activity increased slightly across conditioning trials in cocaine-treated DAT −/− mice, regardless of whether or not cocaine was paired with the testing environment. In addition, it was apparent that the activity of Control DAT −/− mice did not decrease across trials, indicating impaired between-session habituation. A previous study found that sensitization of cocaine-induced locomotion was eliminated in both DAT +/− and DAT −/− mice (Mead et al., 2002). However, the methods used in that experiment to examine cocaine sensitization are difficult to compare to the present findings or to the literature; in that study cocaine was administered i.v. after an extended period of habituation that almost normalized activity between the DAT −/− and DAT +/+ mice. Extended habituation would substantially affect the ability of the environment to act as a conditioned stimulus. In addition, the temporal differences between s.c. and i.v. drug administration would also affect the ability of different types of stimuli to act as reinforcers. Finally, the experimental conditions appeared to affect the acute locomotor stimulant effects of cocaine as well; in that study acute locomotor stimulant effects of cocaine were eliminated in DAT +/− mice, which was not observed in previous studies (Giros et al., 1996; Sora et al., 1998, 2001). The length of drug treatment may be another factor influencing sensitization in DAT KO mice as a recent study found that methamphetamine sensitization was not attenuated in DAT +/− mice, but its development was delayed (Fukushima et al., 2007).

The mechanism underlying those remaining cocaine effects in DAT KO mice has been a matter of some speculation. Despite the fact that acute locomotor stimulatory effects are eliminated in DAT KO mice, cocaine still retains the ability to increase extracellular levels of dopamine, at least in some brain areas (Mateo et al., 2004b; Shen et al., 2004). There is some evidence that the locus of this effect may be different in DAT KO mice than that in wild-type mice. Local infusions of cocaine in either the dorsal or ventral striatum fail to increase extracellular dopamine levels (Mateo et al., 2004b; Shen et al., 2004) nor does cocaine affect DA clearance in striatal slices (Mateo et al., 2004a). Although there has been some suggestion that reuptake by NET or SERT, in the absence of DAT, might account for the effects of cocaine, neither desipramine nor fluoxetine affects DA clearance in striatal slices (Mateo et al., 2004a). However, peripheral injections of SERT blockers do increase extracellular DA in the striatum (Mateo et al., 2004b; Shen et al., 2004), effects that are not observed in WT mice. The locus of the cocaine effect might involve SERT in the VTA where local injections of cocaine or fluoxetine lead to increased release of dopamine in the nucleus accumbens (Mateo et al., 2004b). This is consistent with the ability of combined DAT-SERT KOs to eliminate cocaine CPP (Sora et al., 2001), and for fluoxetine to produce CPP in DAT KO mice (Hall et al., 2002).

Since cocaine retains its ability to elevate extracellular dopamine in DAT KO mice, albeit via different mechanisms than in WT mice, and only in some brain regions, it might be suspected that cocaine sensitization may still be possible in DAT KO mice. Indeed the present study suggests that context-independent sensitization may be enhanced, while at the same time conditioned locomotion is eliminated. Because of the profound changes in dopamine clearance in DAT KO mice (Giros et al., 1996; Jones et al., 1998) there may be substantial alterations in spatiotemporal aspects of dopamine transmission between wiring (local synaptic) and volume transmission (Gonon et al., 2000), which may alter the influence of dopamine on glutamate function. In addition to elevating extracellular levels of dopamine, cocaine also increases glutamate levels (Smith et al., 1995), an effect that is increased in animals that have developed context dependent cocaine sensitization (Pierce et al., 1996; Reid and Berger, 1996; Kalivas and Duffy, 1998). These changes are associated with increased sensitivity of dopaminergic neurons to glutamatergic stimulation (White et al., 1995; Zhang et al., 1997), and are associated with changes in glutamate receptor subunit expression in the NAC and VTA (Churchill et al., 1999). Sensitization of the glutamate response to cocaine has been found to result from context dependent, but not independent, sensitization (Bell et al., 2000) and the development, but not expression, of context-dependent sensitization can be blocked by AMPA antagonists (Li et al., 1997), and NMDA antagonists (Damianopoulos and Carey, 1995; Cervo and Samanin, 1996; Kim et al., 1996). Conditioned activity is associated with increases in nucleus accumbens glutamate and can be attenuated by AMPA antagonists (Cervo and Samanin, 1996; Hotsenpiller et al., 2001) and NMDA antagonists (Cervo and Samanin, 1996). Both NMDA and AMPA antagonists block the development of context independent sensitization as well (Li et al., 1999). Expression of a mutant NMDA receptor with impaired Ca^{2+} flux in cells containing dopamine D1 receptors (DRD1) prevents the development of context-dependent cocaine sensitization and cocaine CPP (Heusner and Palmiter, 2005). Convergent DRD1-NMDA stimulation has been suggested to play a critical role in the development of context-dependent sensitization (Valjent et al., 2005). The observed role of glutamate and glutamate–dopamine interactions in these phenomena are dependent in part upon experimental parameters and are not entirely clear by any means. However, because of the profound alterations in the dynamics of dopamine release in DAT KO mice it would appear likely that glutamatergic mechanisms would also be affected, although perhaps in such a way as to differentially affect context independent sensiti-

zation and conditioned locomotion. This possibility has not been investigated to any great degree, although glutamate manipulations do affect baseline hyperactivity in DAT KO mice (Gainetdinov et al., 2001).

Other evidence indicates that cocaine enhances glutamatergic inputs to midbrain dopamine neurons in a manner dependent on both DRD1 and glutamate AMPA receptors (Dong et al., 2004). Part of the evidence for this interaction involved the elimination of these effects in GLURA KO mice. Elimination of this gene also blocked both conditioned locomotion and CPP, without affecting acute locomotor responses to cocaine (Dong et al., 2004). This study implicates potential neuroadaptations in glutamatergic afferents to midbrain dopamine neurons in the effects of context on conditioned responses that enhance drug-seeking behavior. Changes in synaptic spine density are observed in the nucleus accumbens core in response to a cocaine treatment regimen that induced context-dependent sensitization (Li et al., 2004). Interestingly, the same dose regimen produced neither behavioral nor morphological changes when administered in the home cage, but higher doses that induced context-independent sensitization were able to increase spine density in the nucleus accumbens core. Increased spine densities were also observed in the nucleus accumbens shell, but were observed even after repeated context-independent treatment with low doses of cocaine that did not produce sensitization. Increased spine densities were also observed in the medial prefrontal cortex under both conditions, but the increases were greater after context-dependent sensitization. These data would suggest that experimental parameters have a substantial effect on the morphological consequences of cocaine treatment, which are highly dependent on experimental parameters and differ substantially across brain regions. These changes are likely to underlie changes in glutamate responsiveness to cocaine and various forms of cocaine conditioning and cocaine sensitization.

Such differential effects are necessary to explain the difference in context independent responses in DAT KO mice and conditioned locomotion. The anatomical locus most critical to conditioned locomotion appears to be different from that involved in the acute locomotor stimulant properties of cocaine. Quinolinic acid–induced lesions of the amygdala have no effect on the acute locomotor effects of cocaine, but block the development of conditioned locomotion (Brown and Fibiger, 1993). This brain region has not been investigated in DAT KO mice. Pairing of novel contextual cues with cocaine produces greater sensitization than pairing with discrete stimuli (Crombag et al., 2000), although conditioning to discrete stimuli is also observed in terms of both context dependent sensitization and conditioned locomotion (Panlilio and Schindler, 1997). Interestingly, in that study the discrete stimuli that were used to produce conditioned locomotion also acted as conditioned reinforcers in a subsequent operant circumstance in which lever processing produced presentation of the conditioned stimuli. It has been recently shown that there is a substantial overlap between striatal neurons activated by acute cocaine (e.g. c-fos) and those that are activated by chronic cocaine (e.g. FOSB), but that the number of activated neurons is a small percentage of the overall number of striatal neurons and that each environment may induce a distinct subset, or ensemble, of striatal neurons (Mattson et al., 2008). These subjects were not tested for conditioned locomotion (e.g. the effect of re-exposure to the conditioned environment without any injections), but nonetheless subjects that were returned to the cocaine-paired environment and injected with saline showed substantial elevations in c-fos and a substantial overlap with FOSB; this activation probably represents the effect of the environmental context on the neuronal ensemble that drives locomotor behavior in this circumstance, and is likely related to the changes in synaptic morphology associated with chronic cocaine treatments discussed above.

Although there has been accumulating evidence that serotonin and norepinephrine may modulate cocaine reward, and that SERT and NET may have a role in cocaine reward under some circumstances, the present experiments suggest that these effects are limited for conditioned locomotion. Both SERT KO and NET KO mice demonstrated conditioned locomotion. Although the effects appeared to be reduced, this decrease was not significant in NET KO mice and marginally significant in SERT KO mice. These effects may be the result of other factors, such as the reduced locomotion observed here in SERT KO and NET KO mice. Reduced locomotion has been described in SERT KO mice previously (Kalueff et al., 2007a,b). Generally speaking in the paradigm utilized in these studies context-dependent sensitization was not observed (e.g. increased locomotion in paired subjects). Because of the habituation of activity across trials in saline-treated subjects, the relative magnitude of cocaine effects in Paired mice was greater in trial 5 than in trial 1, which might be taken to indicate sensitization. With this in mind, the activity of paired SERT KO mice decreased across trials, similarly to saline-treated subjects, which may indicate an impairment of context dependent sensitization or even tolerance to the locomotor stimulant effects of cocaine. However, as this study was not designed to primarily examine context dependent sensitization this conclusion must be tentatively placed forward until this phenomenon can be examined in a more appropriate paradigm. There is some evidence that stimulation of dorsal raphé 5-HT1A receptors potentiates cocaine-induced locomotion, cocaine-induced dopamine release and cocaine-induced glutamate release (Szumlinski et al., 2004). There are substantial reductions in these receptors observed in SERT KO mice (Fabre et al., 2000) as well as other neuroadaptations (Mathews et al., 2004).

CONCLUSION

In conclusion, it would seem that the primary mechanism by which cocaine produces conditioned locomotion is via actions at DAT. Although there is evidence that both SERT and NET gene KOs modulate cocaine-mediated behavior

during conditioning, these differences do not profoundly affect the ability of cocaine to produce conditioned locomotion. Furthermore, in DAT KO mice context-independent sensitization of cocaine-induced locomotion is observed; that is, the sensitization occurs in DAT −/− mice treated with repeated cocaine in the same or a different environment. This occurs under conditions that do not produce sensitization in other animals, and likely reflects substantial alterations in dopamine–glutamate interactions that occur in response to cocaine administration and that change in response to repeated cocaine administration. Further investigation of the function of glutamate in DAT KO mice may help illuminate the behavioral differences observed in these mice as well as those dopamine–glutamate interactions that are critical in these phenomena.

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Transgenic mice in the study of drug addiction and the effects of psychostimulant drugs

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The first transgenic models used to study addiction were based upon *a priori* assumptions about the importance of particular genes in addiction, including the main target molecules of morphine, amphetamine, and cocaine. This consequently emphasized the importance of monoamine transporters, opioid receptors, and monoamine receptors in addiction. Although the effects of opiates were largely eliminated by μ opioid receptor gene knockout, the case for psychostimulants was much more complex. Research using transgenic models supported the idea of a polygenic basis for psychostimulant effects and has associated particular genes with different behavioral consequences of psychostimulants. Phenotypic analysis of transgenic mice, especially gene knockout mice, has been instrumental in identifying the role of specific molecular targets of addictive drugs in their actions. In this article, we summarize studies that have provided insight into the polygenic determination of drug addiction phenotypes in ways that are not possible with other methods, emphasizing research into the effects of psychostimulant drugs in gene knockouts of the monoamine transporters and monoamine receptors.

Keywords: transgenic; knockout; psychostimulant; amphetamine; cocaine

Introduction

In recent years important advances have been made in developing new animal models to help identify the mechanisms of action of psychostimulant drugs underlying their behavioral and physiological effects, including the abuse liability of these drugs and other adverse consequences, in particular the toxicity and lethality associated with the use of psychostimulant drugs. Genetic mouse models are being used to identify genes that may predict risk for the development of drug abuse and addiction or to investigate under more controlled circumstances the consequence of direct manipulation of particular genes implicated in addiction from human genetic studies. Genetic mouse models have been used for estimating genetic correlations between drug-related traits[1,4] and for studying the roles of specific genes in addiction relevant behavioral and physiological traits.[5,6] Progress in this area of research has profound implications for the improved understanding and treatment of drug addiction. At this time there is a large literature on responses to psychostimulants in gene mutant mice. The largest body of literature on the genetics associated with psychostimulant-related behavioral effects has focused on drug reward and drug conditioning, including conditioned place preference (CPP) and self-administration. This work has emphasized primarily the acute rewarding effects of psychostimulants, or early stages of drug taking. Several other areas have been less well examined or, sometimes, not examined at all. There is a need for more investigation of the genetic determinants of sensitivity to psychostimulant-induced neurotoxicity and other adverse effects. Similarly, there is a great deal of work to be done in the quest for genes that influence the development and acceleration of psychostimulant dependence and phenotypes that may be associated with later stages of the addictive process, including extinction, reinstatement, reconsolidation, habit formation, and many other mnemonic aspects of responses to addictive drugs.

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So saying, transgenic models have contributed greatly to our understanding of the mechanisms underlying the actions of psychostimulant drugs. One surprising outcome of these studies has been the polygenic basis of these effects and the degree to which substantial perturbations from gene deletions may alter the normal mechanism of action of particular drugs. Thus, animals will show the same underlying behavioral phenotype, sometimes largely unaltered from the wild-type (WT) condition, but its underlying basis appears to be quite different. Several examples of this type of finding are discussed in the sections that follow, raising the important question of whether similarly large differences in underlying mechanisms exist in humans as are observed in some of these types of models.

Monoamine transporter knockouts

Psychostimulant drugs increase extracellular levels of monoamines by blocking the neuronal plasma membrane transporters (reuptake inhibitors) or by blocking the vesicular transporter (releasers). Increased extracellular dopamine (DA) levels in mesocorticolimbic DA systems have been postulated to mediate the rewarding effects of cocaine,[7] as well as other psychostimulants. The heritability of drug abuse and dependence is relatively high for psychostimulants,[8] indicating that genetic differences that determine the extent of DA release may be important determinants of addiction liability, as well as other effects of acute and chronic psychostimulant exposure. For example, we have recently shown that the number of repeat alleles of the DA transporter (DAT) gene is associated with the risk for methamphetamine (METH) psychosis.[9] This study demonstrated that the presence of nine or fewer repeat alleles of the variable number of tandem repeats in the 3′ untranslated region of DAT is a strong risk factor for a poorer prognosis of METH psychosis. Studies in transgenic mice, particularly knockout (KO) mice in which one or both of the gene alleles are deleted or inactivated, have contributed a great deal to our understanding of the mechanisms underlying psychostimulant actions. This has been particularly useful in the study of psychostimulants because they generally bind to multiple transporters and thereby affect the function of multiple monoamine systems.

Cocaine

Initial transgenic studies into the molecular mechanisms of the effects of psychostimulants, using mice lacking the monoamine transporters, were substantially influenced by the previous pharmacological literature. Prior to the development of these transgenic models, the rewarding effects of cocaine were found to be best correlated with DAT blockade on the basis of structure–activity relationships of transporter-blocking compounds with different potencies at DAT, the serotonin transporter (SERT), and the norepinephrine transporter (NET).[7] As can be seen in Table 1, most studies have concentrated on the rewarding and locomotor stimulant effects of cocaine, with much less work examining other psychostimulant effects.

DAT, SERT, and NET gene KO mice

In contrast to the hypothesis stated in the preceding paragraph, initial data in DAT KO mice demonstrated intact cocaine reward in the CPP paradigm[3] and in an initial self-administration study.[10] Hence DAT KO mice retained the ability to acquire and maintain cocaine self-administration, as well as cocaine-conditioned behavior, in ways that were not substantially different from WT mice. These data therefore indicated that the reinforcing effects of cocaine could be mediated via DAT-independent mechanisms. This is not to say that these data indicated that there was no involvement of DA in cocaine reward. In the Sora *et al.*[3] study, cocaine CPP was observed at both doses tested in WT mice, but only the higher dose produced a significant CPP in DAT KO mice. However, with the largely intact effects of cocaine in these studies, the logical next step was to examine whether other cocaine targets (e.g., SERT and NET) were involved. Further work continued to emphasize that the consequences of cocaine administration were determined by multiple interacting systems. In support of this conclusion, drawn in part from studies of mice in which multiple genes were manipulated with transgenic methods, genetic background was also found to affect the consequence of single-gene KOs. Thus, cocaine CPP was more substantially reduced in congenic DAT KO mice on either a C57BL/6[11,12] or DBA/2J[12] background, which would suggest that the expression of other genes in particular genetic backgrounds affected the consequence of the gene KO. Obviously

Table 1. Cocaine responses in monoamine transporter transgenic mice

Citation	Gene	Micro-dialysis	Loco-motion	Sensiti-zation	CPP	Self-administration	PPI	Adverse effects
Giros, B. *et al.* 1996	DAT KO		Eliminated					
Sora, I. *et al.* 1998	DAT KO		Eliminated		CPP at highest dose only			
Rocha, B.A. *et al.* 1998	DAT KO					Unaffected		
Gainetdinov, R.R. *et al.* 1999	DAT KO		Cocaine decreased locomo-tion					
Carboni, E. *et al.* 2001	DAT KO	Increased DA in NAc						
Ralph, R.J. *et al.* 2001	DAT KO						Reversed PPI deficit	
Mead, A.N. *et al.* 2002	DAT KO			Eliminated				
Morice, E. *et al.* 2004	DAT KO		Eliminated		Substantially decreased			
Shen, H.W. *et al.* 2004	DAT KO	Increased DA in striatum and PFc, but not NAc						
Mateo, Y. *et al.* 2004	DAT KO	Increased DA in NAc and striatum						
Barr, A.M. *et al.* 2004	DAT KO						Reversed PPI deficit	
Medvedev, I.O. *et al.* 2005	DAT KO		Eliminated		Substantially decreased			
Yamashita, M. *et al.* 2006	DAT KO						Reversed PPI deficit	
Thomsen, M. *et al.* 2009	DAT KO					Substantially decreased		
Hall, F.S. *et al.* 2009	DAT KO		Conditioned locomo-tion was eliminated					
Zhuang, X. *et al.* 2001; Tilley, M.R. *et al.* 2007	DAT KD		Increased locomotor by low doses of cocaine		Unaffected			

Continued.

Table 1. *Continued*

Citation	Gene	Micro-dialysis	Loco-motion	Sensiti-zation	CPP	Self-administration	PPI	Adverse effects
Chen, R. *et al.* 2006; Tilley, M.R. *et al.* 2009; Thomsen, M. *et al.* 2009	DAT CI	Failed to increase DA in NAc	Decreased under non-habituated conditions		Eliminated	Eliminated		
Hnasko, T.S. *et al.* 2007	DD TG				Unaffected			
Sora, I. *et al.* 2001	DAT/SERT KO				Eliminated			
Xu, F. *et al.* 2000	NET KO				Increased			
Kaminski, R.M. *et al.* 2005	NET KO							Reduced seizures
Hall, F.S. *et al.* 2002	NET/SERT KO				Increased			
Sora, I. *et al.* 2001	SERT KO				Increased			
Homberg, J.R. *et al.* 2008	SERT KO		Increased		Increased	Increased		
Wang, Y.M. *et al.* 1997	VMAT2 KO		Increased	Eliminated				

PFc: prefrontal cortex, NAc: nucleus accumbens.

few species comparisons are available for transgenic models, but in one rare case much more consistent effects of SERT KO are observed in rats, in which cocaine locomotion, cocaine CPP, and cocaine self-administration are all increased,[13] compared to levels in mice, as discussed in the following.

In any case, the observation of intact reward under at least some conditions in DAT KO mice suggested the necessity of examination of the role of the other main targets of cocaine in cocaine reward. Because manipulations of serotonin (5-HT) systems can modulate the rewarding effects of both cocaine and amphetamine (AMPH),[14,15] 5-HT was initially considered to be the most likely candidate. However, cocaine CPP was not reduced in SERT KO mice,[16] nor in NET KO mice[17]; indeed, the opposite was found: both SERT KO and NET KO mice exhibited increased rewarding effects of cocaine, effects that were even more pronounced in mice with deletion of both genes.[18] The failure of any single monoamine transporter gene KO strain to eliminate cocaine reinforcement and reward thus left open several possible roles for these transporters in cocaine reward in WT and DAT KO mice,[5] including the possibility of substantial compensatory changes and the possibility that, under normal circumstances in WT mice, multiple monoamine systems are involved in the rewarding effects of cocaine. Supporting the compensation hypothesis, SERT blockade with fluoxetine or selective NET blockade with nisoxetine was shown to produce rewarding effects in DAT KO mice, effects that are not seen in WT mice.[18] Thus, absence of DAT throughout development could produce changes in other monoamine systems that alter the reinforcing effects of SERT and NET blockade in DAT KO mice. This does not necessarily mean that SERT does not, or can not, have a role in the rewarding effects of cocaine in WT mice. Indeed, both of the foregoing hypotheses are consistent with our findings that combined deletion of DAT and SERT eliminate cocaine CPP.[16]

In contrast to findings in the CPP paradigm, our line of DAT KO mice failed to consistently self-administer cocaine.[19] This finding was in apparent contrast to a previous report that a different line of DAT KO mice did self-administer cocaine.[10] Several factors might have contributed to the differences between these studies. The initial Rocha *et al.*[10] study

examined self-administration under only a few basic circumstances and those authors suggested that although DAT KO mice could self-administer cocaine, a more detailed analysis would be needed to determine whether other differences did exist. The Thomsen *et al.*[19] study was much more detailed and could be summarized thus: DAT KO mice can self-administer cocaine, but the rewarding effects of cocaine are substantially reduced so that even those mice that do learn to self-administer cocaine under initial conditions fail to do so under more demanding conditions, such as increasing the amount of work required to receive cocaine reinforcement by increasing the fixed ratio schedule or under a progressive ratio. This finding is also consistent with our DAT/SERT double-KO study,[16] which found that although combined DAT/SERT deletion eliminated cocaine CPP, the contribution of DAT and SERT was not equal; cocaine CPP was impaired in $DAT^{-/-}SERT^{+/-}$ mice, but not in $DAT^{+/-}$ $SERT^{-/-}$ mice, suggesting a greater overall role of DAT than of SERT. However, there may be contributions of other factors to the differences between the two lines of DAT KO mice in self-administration studies. For instance, cocaine increased extracellular DA levels in the caudate putamen and prefrontal cortex, but not the nucleus accumbens in our line of DAT KO mice,[20] but in the other line of DAT KO mice cocaine and AMPH increased extracellular DA in the medial part of the nucleus accumbens.[21] It is difficult to say why these differences occurred on the basis of our present knowledge, although one is tempted to speculate that differences in genetic background might contribute, as has been shown to be the case for μ opioid receptor KO mice.[22]

DAT-overexpressing transgenic mice

Another DAT transgenic strain that produced overexpression of DAT emphasizes the importance of DAT in the rewarding effects of cocaine.[23] These mice demonstrated increased cocaine CPP, but interestingly, there was no effect on cocaine-induced locomotion.

DAT knockdown mice

As discussed previously, there are substantial compensatory changes in DAT KO mice (see Gainetdinov and Caron[24] for review). Another line has been created in which DAT expression is reduced by 90% (termed DAT knockdown [KD]), which ameliorated some of the effects of complete DAT KO, although DAT KD mice were still hyperactive, had reduced DA clearance, and had slightly elevated extracellular DA levels.[25] The DAT KD mutant line was produced by insertion of a targeting sequence into the promoter region of the DAT gene, resulting in a reduction in DAT expression to approximately 10% of WT levels. Nonetheless, all these changes were less pronounced than those seen in complete DAT KO mice. In contrast to what is observed in complete DAT KO mice, DAT KD mice show enhanced locomotor stimulant effects of low doses of cocaine, whereas there were no effects on cocaine CPP.[26]

Vesicular monoamine transporter 2 KO mice

Vesicular monoamine transporter 2 (VMAT2) is a proton-dependent transporter that accumulates monoamine neurotransmitters, including DA, 5-HT, norepinephrine, and histamine, from neuronal cytoplasm into synaptic vesicles. Normal vesicular monoamine release through calcium-dependent vesicle fusion with presynaptic membranes thus depends on normal function of VMAT2. Homozygous VMAT2 deletion is lethal within a few days postnatal, but heterozygous VMAT2 deletion results in a substantial reduction in presynaptic stores of neurotransmitters.[27] Surprisingly, given these results, $VMAT2^{+/-}$ mice have increased locomotor responses to acute cocaine[27] but do not exhibit cocaine sensitization with repeated administration, which was interpreted as reflecting a "presensitized" state. No changes in DAT function were observed in $VMAT2^{+/-}$ mice *in vitro*, although substantial changes were observed in $VMAT2^{-/-}$mice *in vitro*[28] and *ex vivo*,[2] but high-affinity DA D_2 receptors are elevated in $VMAT2^{+/-}$ mice,[29] as is seen in sensitized animals.[30]

DAT cocaine-insensitive mice

Although the DAT KO mouse has been useful in the study of psychostimulants, because of the changes that appear to occur in these mice an important recent development has been a transgenic manipulation that does not produce such dramatic changes in dopaminergic function. The amino acid residues in transmembrane domain 2 of mouse DAT are important for high-affinity cocaine binding. Another transgenic line has been created in which the mutations in these residues have been engineered, creating a DAT protein that is 80-fold less

sensitive to cocaine inhibition (termed DAT cocaine insensitive [CI]) but relatively normal DA reuptake, and consequently fewer compensatory changes than those observed in DAT KO or DAT KD mice.[31] Although there were small baseline differences in DA uptake kinetics, cocaine failed to increase extracellular DA levels or modify DA cell firing in DAT CI mice.[31] Increased locomotion in a novel environment was observed in these mice and, as typical of DAT KO mice under some circumstances, cocaine reduced locomotion in DAT CI mice.[31] However, several cocaine effects were eliminated in this transgenic strain, including cocaine CPP,[31,32] cocaine self-administration,[33] and cocaine-induced stereotypical behavior,[34] indicating the primacy of DAT in many cocaine actions, including cocaine reward. Because cocaine did not elevate extracellular DA in the nucleus accumbens of the DAT CI mouse line,[31] these findings seem to support the notion that cocaine-induced increases in extracellular DA in the nucleus accumbens are critical for cocaine reward and that in WT mice DAT inhibition is the primary mechanism underlying the rewarding effects of cocaine.

DAT/SERT double-KO mice

Some of the preceding studies suggest that nondopaminergic mechanisms are (or can be) involved in the rewarding effects of cocaine. As mentioned previously, cocaine CPP is eliminated in double-KO mice with no DAT gene copies and either no or one copy of the SERT gene.[16] These results in DAT/SERT double-KO mice suggest that the blockade of DAT and SERT are both involved in cocaine reward,[35] at least under some circumstances, although they do not necessarily indicate that DA does not have a primary role. Indeed, in distinct contrast to WT mice, pharmacological inhibition of SERT increased extracellular DA in the nucleus accumbens[36] and caudate putamen[20] of DAT KO mice to a similar extent as cocaine, which was suggested to result from adaptations in 5-HT regulation of dopaminergic neuronal activity in the ventral tegmental area of these mutant mice. Several pieces of evidence support this hypothesis. Local-infusion cocaine, fluoxetine, or nisoxetine into the dorsal or ventral striatum do not increase extracellular DA levels,[20,36] but local injections of cocaine or fluoxetine in the ventral tegmental area increase extracellular DA concentrations in the nucleus accumbens.[36] This could certainly be the basis for the novel CPP induced by fluoxetine in these mice that was discussed earlier. These studies indicate that there are interactions between DAT and SERT that are important determinants of the rewarding effect of psychostimulant drugs, such as cocaine, under at least some conditions.

Dopamine-deficient mice

Supporting these conclusions, another study has shown similarly important DA–5-HT interactions in another transgenic model, the DA-deficient (DD) mouse model in which tyrosine hydroxylase, the rate-limiting enzyme for catecholamine biosynthesis, has been inactivated selectively in DA neurons but not other catecholaminergic neurons.[37] In these mice inhibition of SERT with fluoxetine produced a CPP,[37] just as it did in DAT KO mice, indicating adaptive changes in 5-HT systems under these even more extreme circumstances. In both the DAT KO and DD models SERT appears to be an important mediator of cocaine reward, but these effects are still likely to involve DA. Both cocaine and fluoxetine CPP were blocked by inhibition of DA cell firing by the DA D_2 receptor agonist quinpirole in DD mice.[37] Those authors suggested that in DD mice cocaine increases 5-HT levels, activating DA neurons, which are still found in DD mice,[38] releasing another (unknown) neurotransmitter, perhaps one of the neuropeptides colocalized with DA. They further suggested that the proposed paradoxical excitatory effects of 5-HT in DD mice result from the hyperdopaminergic state produced by the daily L-dopa administration without which these mice would die, and which may be similar to the hyperdopaminergic state characterized in DAT KO mice.

Behavioral sensitization

The studies discussed in the foregoing sections addressed drug reward primarily as assessed by the CPP and self-administration paradigms. Other models thought to address important aspects of addiction have been less well-studied in monoamine transporter KO mice, including behavioral sensitization. Behavioral sensitization is a phenomenon whereby repeated intermittent exposure to psychostimulant drugs elicits progressive enhancement of behavioral responses, which persists for extended periods after withdrawal from the drug.[39] It is most common to examine sensitization of the locomotor

stimulant effects of drugs, such as cocaine, which are thought to reflect the underlying alterations in neuronal plasticity associated with changes in mesolimbic DA functioning that mediate drug-seeking behavior.[40,41] DAT KO mice are profoundly hyperactive in a novel environment but do not demonstrate acute locomotor stimulant effects of cocaine,[3,42] at least when injected after a period of habituation to the environment. This is also true of C57BL/6J and DBA/2J congenic DAT KO lines.[12] Under these conditions $DAT^{+/-}$ mice show normal baseline locomotion and normal locomotor stimulant effects of cocaine. By contrast, when tested under nonhabituated conditions decreased locomotion is observed after administration of cocaine in $DAT^{-/-}$mice.[43] Habituation appears to be a critical factor in determining these effects; in animals that were substantially habituated prior to drug administration both the acute locomotor effects of cocaine and sensitization of those effects were almost completely eliminated in both $DAT^{+/-}$ and $DAT^{-/-}$ mice.[44] In the same experiment normal acute locomotor effects and sensitization were observed in NET KO mice. One of the more important implications of this later study was that, at least under some conditions, heterozygous DAT KO is sufficient to reduce the locomotor stimulant effects of cocaine. This is important because the heterozygous condition, which produces a 50% reduction in DAT levels in comparison to WT mice, much more closely models the range of variance in DAT levels observed in humans[45] than the homozygous condition.

Although consistent with some other results, the study by Mead *et al.*[44] is difficult to compare with much of the literature on locomotor sensitization because it involved extended periods of habituation (12 h) and an intravenous route of administration. Sensitization involves two primary components, a context-dependent component (e.g., conditioning) and a context-independent component resulting from adaptations to repeated drug exposure that occur even if given in a context in which limited learning about the drug occurs, such as a familiar home cage environment. Such an extended period of habituation as was used in the Mead *et al.*[44] study is likely to eliminate most context dependent aspects of sensitization. Another way to approach sensitization is to specifically examine the ability of the environment, after repeated exposure to the drug, to elicit locomotion after reexposure to the environment without the drug, which is termed "conditioned locomotion." We recently examined conditioned locomotion in DAT KO, SERT KO, and NET KO mice.[46] This study found that conditioned locomotion was eliminated in DAT KO mice, but not SERT KO or NET KO mice, although small diminutions in the conditioned responses were observed in each case. In addition, repeated exposure to cocaine, either during the conditioning trials or in the home cage, resulted in sensitization of locomotor responses in the testing environment in DAT KO mice. This effect occurred in DAT KO mice that did not show acute locomotor stimulant responses to cocaine, as well as in animals given saline before locomotor testing but that received cocaine later in the home cage. Thus, even though the conditioned component was eliminated, long-term adaptations to repeated cocaine exposure were observed in DAT KO mice that may have been stronger than those observed in WT mice.

Prepulse inhibition

The hyperdopaminergia of DAT KO mice, judged in terms of extracellular DA levels in the striatum or DA-associated behaviors, such as hyperactivity, have led to the suggestion that DAT KO mice can be used as animal models of schizophrenia[47] and attention-deficit/hyperactivity disorder (AD/HD).[48] There is evidence to support both views to a certain extent. The paradoxical inhibitory effects of several psychomotor stimulants, including cocaine, on the profound locomotor hyperactivity observed in DAT KO mice have already been mentioned.[43] DAT KO mice also have deficits in prepulse inhibition (PPI) of the acoustic startle reflex, a model of sensorimotor gating,[49,50] which are also reversed by treatment with several psychostimulants, including cocaine.[51] PPI deficits in DAT KO mice can also be reversed by D_2 antagonists[50] or 5-HT_{2A} antagonists,[49] further supporting the idea of interactions between DA and 5-HT systems being fundamentally important in these mice. However, the underlying deficit in DAT KO mice is likely to involve alterations in the balance between ventral striatal and prefrontocortical activity. In part, this results from an oddity of DA function in the prefrontal cortex whereby uptake is normally mediated by NET rather than DAT.[52,53] One consequence of this situation is that in the absence of DAT in DAT KO mice

there are profound alterations in extracellular DA concentrations in the ventral striatum, whereas the prefrontal cortex remains substantially unaffected,[20] thereby potentially altering the balance of activity between the prefrontal cortex and the ventral striatum. This would appear to have dramatic effects on responses to cocaine, which impairs PPI in WT mice but normalizes PPI in DAT KO mice.[51] This study went on to show that, consistent with the previous argument regarding the normal mechanisms of DA reuptake in the prefrontal cortex, the selective NET blocker nisoxetine, normalized PPI in DAT KO mice as well. By contrast, the selective SERT blocker citalopram was without effect, although fluoxetine did reverse DAT KO impairments in PPI. This difference between the effects of citalopram and fluoxetine was suggested to potentially derive from different affinities of fluoxetine and citalopram for NET and 5-HT_{2A} receptors, both of which reversed DAT KO impairments in PPI as discussed in the foregoing text.

Adverse effects of cocaine

Adverse effects of cocaine are observed in humans, including lethality related to cardiac events[54] and seizures.[55] The mechanisms underlying the toxic and lethal effects of cocaine have not been extensively examined using transgenic models, however. With the previous discussion, and the complexity of genetic effects involved in other cocaine actions, it would be important to understand the mechanisms underlying these adverse effects. In the only known such study to date, cocaine-induced seizures were substantially reduced in NET KO mice,[56] although this did not appear to be solely the result of prevention of cocaine actions at NET, because the sensitivity to other seizure-inducing drugs, which do not presumably act at NET, were also reduced.

Amphetamines

It is important to consider separately the effects of different psychostimulant drugs because they have different mechanisms of action, despite many similarities. AMPH and METH are prototypical psychostimulant drugs that induce enhanced arousal and euphoria acutely, and psychosis and addiction chronically, but their mechanisms are quite different from those of cocaine. None of the AMPH are terribly selective in their binding affinities for the three monoamine transporters, although both AMPH and METH are less potent at binding SERT, whereas methylenedioxymethamphetamine (MDMA) has a slightly higher affinity for SERT than for DAT.[57,58] AMPH produce increases in extracellular DA that are dependent on reverse transport via DAT[59] and have similar actions via the other plasma membrane monoamine transporters.[58] This involves cytosolic accumulation of monoamines after inhibition of VMAT2.[60] Because of these mechanisms of action gene KOs of the monoamine transporters have been used to investigate the pharmacological mechanisms underlying the actions of psychostimulants.[2,3,27,42] However, homozygous deletion of the VMAT2 gene was lethal within a short time after birth.[2,61] Consequently, most studies of VMAT2 KO mice have been done in heterozygous KO mice, although another VMAT2 mutant exists that produces a 95% reduction in VMAT2 levels in the homozygous condition and is viable.[62] As mentioned previously, gene KO of monoamine transporters produces substantial changes in baseline neurotransmission. For example, homozygous deletion of the DAT gene produces five- to 10-fold increases in extracellular DA concentrations in the striatum as measured by *in vivo* microdialysis,[20,63] whereas heterozygous deletion of DAT was not found to increase extracellular DA[20] or to produce a more modest twofold elevation of DA in the striatum.[63] Thus, transgenic studies in these KO strains must be interpreted in the context of these baseline alterations.

As can be seen in Table 2, most studies have concentrated on the rewarding and locomotor stimulant effects of AMPH, with much less work examining other psychostimulant effects and less examination of other AMPH compounds.

DAT KO mice

Study of DAT KO mice has demonstrated that the rewarding effect of AMPH is not abolished in the CPP paradigm after deletion of the DAT gene.[64] Interestingly, extinction was substantially reduced in DAT KO mice in this study so that they demonstrated persistent CPP over an extended period (40 days), whereas WT mice showed preference only on the first day of testing. Because AMPH can not access dopaminergic terminals via DAT in these mice, it must be presumed that the rewarding effects of AMPH, like cocaine, are either normally dependent