Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

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.

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 locomotion					
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 locomotion 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 non-dopaminergic 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 $5\text{-}HT_{2A}$ 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

Table 2. Cocaine responses in monoamine receptor transgenic mice

Citation	Gene	Micro-dialysis	Loco-motion	Sensiti-zation	CPP	Self-administration	PPI	Adverse effects
Xu, M. *et al.* 1994	D_1 KO		Decreased					
Miner, L.L. *et al.* 1995	D_1 KO				Unaffected			
Xu, M. *et al.* 2000	D_1 KO		Eliminated					
Caine, S.B. *et al.* 2007	D_1 KO					Eliminated		
Karlsson, R.M. *et al.* 2008	D_1 KO			Eliminated				
Doherty, J.M. *et al.* 2008	D_1 KO						Eliminated cocaine-induced impairments	
Chausmer, A.L. *et al.* 2002	D_2 KO		Unaffected					
Rouge-Pont, F. *et al.* 2002	D_2 KO	Increased DA release						
Caine, S.B. *et al.* 2002	D_2 KO					Increased		
Welter, M. *et al.* 2007	D_2 KO		Decreased		Slight reduction			
Doherty, J.M. *et al.* 2008	D_2 KO						Partially eliminated cocaine-induced impairments	
Xu, M. *et al.* 1997	D_3 KO		Increased		Increased			
Carta, A.R., C.R. Gerfen & H. Steiner. 2000	D_3 KO		Decreased	Eliminated				
Katz, J.L. *et al.* 2003; Rubinstein, M. *et al.* 1997	D_4 KO		Increased					
Elliot, E.E., D.R. Sibley & J.L. Katz. 2003	D_5 KO		Decreased					
Karlsson, R.M. *et al.* 2008	D_5 KO		Unaffected	Decreased	Unaffected			
Doherty, J.M. *et al.* 2008	D_3 KO						Increased cocaine-induced impairments	

Continued

Table 2. *Continued*

Citation	Gene	Micro-dialysis	Loco-motion	Sensiti-zation	CPP	Self-administration	PPI	Adverse effects
Karasinska, J.M. *et al.* 2005	D_1/D_3 KO		Decreased		Decreased			
Rocha, B.A. *et al.* 1997	5-HT_{1B} KO					Increased		
Belzung, C. *et al.* 2000	5-HT_{1B} KO				Unaffected			
Shippenberg, T.S., R. Hen & M. He. 2000	5-HT_{1B} KO	Increased DA release				Increased		
Salomon, L. *et al.* 2007	5-HT_{2A} KO		Increased	Unaffected				
Rocha, B.A. *et al.* 2002	5-HT_{2C} KO							
Allan, A.M. *et al.* 2001	5-HT_3 over-expression				Decreased			
Witkin, J.M. *et al.* 2007	5-HT_7 KO							Increased cocaine-induced seizures and lethality
Schank, J.R. *et al.* 2006	DBH KO		Increased		Preference at 5 mg/kg, aversion at 20 mg/kg			
Jasmin, L., M. Narasaiah & D. Tien. 2006	DBH KO				Eliminated			
Gaval-Cruz, M. *et al.* 2008	DBH KO							No effect on cocaine induced seizures
Drouin, C. *et al.* 2002	α_{1b} KO		Decreased	Decreased				

on a combination of monoaminergic effects or in the absence of DAT other monoaminergic mechanisms can compensate for the absence of DAT. Again, like the circumstance with cocaine, this is not to say that DA has no role, even without DAT. Systemic AMPH still increases extracellular DA in the nucleus accumbens without DAT,[21,64] although local striatal infusion does not.[64] Furthermore, this study also demonstrated that AMPH would reduce DA cell firing in WT mice but not in DAT KO mice. This effect is probably due to reduced autoreceptor function in DAT KO mice,[65] which reveals an underlying non–DAT-mediated stimulatory effect that can be observed when autoreceptor feedback is impaired.[66] With these data, as well as the data discussed earlier for cocaine, it would seem likely that serotonergic mechanisms are involved in AMPH CPP in DAT KO mice. Consistent with this hypothesis, AMPH-induced CPP was abolished by pretreatment with a 5-HT_{1A} receptor antagonist in DAT KO mice, even though the drug did not change AMPH place preference in WT mice,[64] again suggesting that the basis of psychostimulant reward is somewhat different in mice that have experienced a lifelong deletion of the DAT gene. These results indicate that other mechanisms, most likely involving 5-HT, may not play a major role in the rewarding properties of AMPH in WT mice, although the extent of this interaction may be influenced by genetic background, as mentioned earlier, and will require further clarification.

By contrast, the acute locomotor response to AMPH was abolished in these mice under nonhabituated conditions; indeed, reductions in locomotion are often observed in DAT KO mice after administration of AMPH,[43,67] as was the case for cocaine, so that these effects in DAT KO mice are likely to be mediated by SERT, because fluoxetine produced a similar results in these mice.[43,67] Similar changes in response to AMPH are also observed in DAT KD mice.[25] NET may also have a role in these effects because NET KO has been found to increase the locomotor stimulant effects of AMPH.[17] As discussed in the preceding text, locomotor hyperactivity in DAT KO mice has been considered an animal model of AD/HD, an assertion that these "paradoxical" effects of psychostimulants support. Furthermore, these effects are associated with opposite effects of AMPH on postsynaptic signal transduction.[68]

DAT-overexpressing transgenic mice

As was demonstrated for cocaine,[23] overexpression of DAT has been shown to affect responses to AMPH in a separate transgenic line,[69] including increased AMPH CPP, AMPH-induced locomotion, and striatal DA efflux. Interestingly, there were no changes in the locomotor responses to several selective and nonselective DA reuptake blockers in that study, which may indicate that these effects are mediated by other transporters.

VMAT2 KO mice

Although the plasma membrane transporters for the monoamines may be of considerable importance for the actions of AMPH, the ultimate site of action is VMAT2. Gene KO of VMAT2 (heterozygous) has been reported to reduce AMPH CPP.[2] This result is surprising given the finding that VMAT2 KO produces a slight increase in the locomotor stimulant effects of AMPH.[2,27] Because of the apparent importance of both DAT and VMAT2 for the actions of AMPH and METH, we recently examined locomotor activity and sensitization in heterozygous DAT KO mice, heterozygous VMAT2 KO mice, double-heterozygous DAT/VMAT2 KO mice, and WT mice, to evaluate the roles of DAT and VMAT2 in METH-induced locomotor behavior and sensitization.[70] The acute locomotor stimulant effects of METH administration were attenuated in heterozygous DAT KO mice, whereas they were enhanced in VMAT$^{+/-}$ mice; each of these findings is consistent with previous observations with AMPH[2,27,43,67] (by contrast, SERT KO has no effect on AMPH-induced locomotion[71]). The attenuation of the acute effects of METH in DAT KO mice was observed regardless of whether it was combined with heterozygous VMAT2 KO. Although sensitization was observed in all groups, it was substantially attenuated in DAT KO mice, again regardless of whether it was combined with VMAT2 KO. These findings indicate that the heterozygous deletion of DAT produces a major reduction in acute psychostimulant effects of METH, as well as the sensitization of those effects, probably by reducing the ability of METH to enter DA terminals. The mechanism of the VMAT2 effects is less certain. VMAT2 KO reduces both basal and AMPH-stimulated levels of extracellular DA.[27] Thus, these effects may reflect, at least in part, compensatory changes in postsynaptic mechanisms in VMAT2$^{+/-}$ mice, which show increased responses to postsynaptic DA agonists[27] and increased high-affinity DA D_2 receptor function.[29]

Adverse effects of amphetamines

Although addiction is a serious problem for all psychostimulants, neurotoxicity and other adverse consequences of long-term AMPH use is an additional concern, although some AMPH produce more adverse effects than others. METH abuse presents serious health hazards, including irreversible neuronal degeneration, seizures, hyperthermia, and death in humans and experimental animals.[72] METH produces hyperthermia and dopaminergic neurotoxicity in most species examined. Clinical reports and animal studies indicate that lethality from METH closely correlates with hyperthermia, which may be the primary cause of death in cases of overdose. Animal studies suggest that DA receptor activation is crucial for both METH-induced hyperthermia[73] and lethality,[74] although at times there has been an assumption that the METH-induced hyperthermia is 5-HT receptor mediated, as are the hyperthermic effects of MDMA.[75] In a recent study, we examined hyperthermic and lethal toxic effects of METH in DAT, SERT, and DAT/SERT double-KO mice to elucidate the role of these two transporters in METH-induced hyperthermia and lethality.[76] METH caused significant hyperthermia even in mice with one DAT gene copy and no SERT copies, whereas mice with no DAT copies and one SERT gene copy showed significant but reduced hyperthermia when compared to WT mice after METH treatment. These results demonstrate that METH may exert a hyperthermic effect via DAT, or via SERT, without DAT. Double KO of both the DAT and SERT genes eliminated the hyperthermic effects of METH and revealed a hypothermic response. As might be expected given these findings, DAT gene deletion in mice strikingly increased the 50% lethal dose for METH by 1.7-fold compared to WT mice. However, hyperthermia was not solely responsible for lethality, because the mechanisms mediating hyperthermia and toxicity could be dissociated: DAT KO (SERT WT) mice exhibited hyperthermia but greatly reduced METH lethality, and the lethality was not different from DAT/SERT double-KO mice that had hypothermic responses to METH. These findings indicate that DAT may be a more critical mediator of the adverse events associated with METH overdose than SERT.

As mentioned before, a major concern regarding the widespread illicit use of AMPH and METH is their neurotoxic potential, as revealed in animal studies and as observed clinically. This includes both acute adverse events, as discussed earlier, as well as long-term effects of neuronal toxicity and other changes produces by these drugs. In animal models, METH produces dopaminergic,[77] and to a lesser extent serotonergic,[78] neurotoxicity. The neurotoxic effects of METH on DA neurons are eliminated in DAT KO mice,[79] although the effects of METH on serotonergic neurons are attenuated but still present. The neurotoxic effects of METH are enhanced in VMAT2$^{+/-}$ KO mice,[80,81] as are the neurotoxic effects of MPTP[2,82] and L-dopa.[83] Enhanced neurotoxicity was not observed after subchronic treatment with L-dopa in VMAT2$^{+/-}$ KO mice.[84] Increased dopaminergic toxicity after acute treatments with these agents may reflect a generally diminished capacity of VMAT2 to sequester toxins[85] in VMAT2$^{+/-}$ KO mice, as well as increased accumulation of oxidative metabolites resulting from elevated cytosolic DA concentrations. Although homozygous VMAT2 KO is lethal with a few days postnatally, a study that examined early postnatal ventral midbrain cultures from VMAT2$^{+/+}$, VMAT2$^{+/-}$, and VMAT2$^{-/-}$ mice found that there was an inverse relationship between VMAT2 expression and dopaminergic toxicity.[86]

MDMA

MDMA is another commonly abused AMPH compound that produces positive subjective feelings, produces reward, and is associated with several adverse effects including hyperthermia, lethality, and neurotoxicity.[87] The subjective state induced by MDMA is described as qualitatively different from that of other AMPH and is said to include feelings of openness and empathy.[88] Although many of its behavioral and psychological consequences have been associated with its effects on serotonergic function, MDMA increases DA and norepinephrine function as well.[89] There is evidence that MDMA CPP and self-administration depend on DA systems,[90,91] although its affinity for SERT is higher than its affinity for DAT[58] and it produces greater release of 5-HT than DA.[92] Thus, the dopaminergic effects of MDMA are likely to be indirect consequences of MDMA actions. This idea is supported by the demonstration that deletion of the SERT gene eliminates the acquisition of MDMA self-administration.[93] Some of these effects may be open to other interpretations, however. Part of the effect of SERT KO on operant responding for MDMA appeared to be due to more generalized behavioral or cognitive deficits that delayed the acquisition, and maximal response rate, of operant responding for food and water rewards. Indeed, we observed similar deficits for acquisition of cocaine self-administration in SERT KO mice.[19] However, these more general deficits in operant responding can not fully account for the effects of SERT KO on MDMA self-administration, which was abolished, as were the locomotor stimulant effects of MDMA.[71] Deletion of the SERT gene increases basal levels of 5-HT in diverse brain regions but does not affect basal DA levels.[20,94,95] Although the elevations in extracellular DA produced by MDMA in the nucleus accumbens were unaffected by deletion of the SERT gene, MDMA-induced increases in extracellular 5-HT in the prefrontal cortex were abolished,[93] as was 5-HT release in the dorsal raphe and consequent inhibition of serotonergic neurons,[96] indicating that changes in 5-HT in SERT KO mice may have the greatest relevance to the behavioral effects of MDMA discussed earlier.

In addition to its abuse potential, MDMA produces long-term changes in serotonergic neurons that have been described as neurotoxic.[92] The nature of MDMA "neurotoxicity" is a matter of debate, and although it has been suggested that this is, strictly speaking, not the case, substantial impairments in serotonergic functioning are observed.[97] Many of the long-term effects of MDMA administration, including dorsal raphe 5-HT_{1A} supersensitivity, decreased hippocampal cell proliferation, and depressive-like behavior, are all eliminated in SERT KO mice, suggesting that SERT is the primary mediator of these adverse effects as well.[96] Of course, one interpretive problem for some of these effects is that SERT KO mice, in some respects, have baseline phenotypes characteristic of WT mice chronically treated with MDMA to begin with. Other genes are important in the neurotoxic effects of MDMA as well. MDMA-induced 5-HT depletion is eliminated in MAO-B KO mice,[98] and even more interesting, in these mice DA depletion is enhanced.

Methylphenidate

Methylphenidate is a nonspecific monoamine reuptake blocker with a greater affinity for NET than cocaine, but a relatively weak affinity for SERT,[99] and the prototypical AD/HD treatment. As with other monoamine blockers, the relative importance of methylphenidate binding to different monoamine transporters for its behavioral effects is a matter of some debate. As discussed, DAT KO produces impairments in PPI that can be ameliorated by cocaine and AMPH. If this is indeed a model of AD/HD, at least in certain respects, then it should be expected that methylphenidate should also ameliorate these attentional deficits. Indeed, methylphenidate was found to ameliorate DAT KO induced PPI deficits[51] and hyperactivity.[43] In WT mice, methylphenidate produces activation of the *c-fos* in diverse brain areas that are not activated in DAT KO mice,[100] whereas DAT KO mice have activation of the *c-fos* in brain areas that are not normally activated in WT mice. This different pattern of *c-fos* activity in WT and DAT KO mice was thought to reflect dopaminergic, versus nondopaminergic, mechanisms of methylphenidate and are consistent with the different behavioral effects of methylphenidate in these mice. The locomotor-decreasing effects of methylphenidate in hyperactive DAT KO mice may also be associated with the opposite effects of AMPH on postsynaptic signal transduction compared to WT mice.[68]

Although some responses to methylphenidate are substantially altered in DAT KO mice, the rewarding effects of methylphenidate in the CPP paradigm are unaffected,[3] similar to the effects of cocaine in these mice. As discussed in a previous section, some of these effects are probably due to neurodevelopmental or compensatory alterations in DAT KO mice, because similar changes are not observed in the DAT CI mouse. The DAT CI mutant mouse has reduced binding of methylphenidate to the DAT,[101] and the rewarding effects of methylphenidate in the CPP paradigm, as well as the locomotor stimulant and stereotypical effects of methylphenidate, were all eliminated in these mice.

Methylphenidate has a low affinity for SERT, although it does bind to both DAT and NET.[99] Thus, those effects not mediated by DAT are likely to be mediated by NET. Gu *et al.*[102] recently identified a mutant mouse with a cocaine-insensitive NET. Interestingly, the triple mutation in this mouse line resulted in a substantial reduction in binding of cocaine, but it had little effect on the affinity for AMPH or methylphenidate and had relatively normal norepinephrine transport.

Monoamine receptor knockouts

With the substantial evidence for the involvement of monoamine transporters in the effects of psychostimulant drugs, it is not surprising that there is also substantial evidence for the involvement of monoaminergic receptors. As for transporters, much of this research has reflected a dopaminergic emphasis (or bias, perhaps), at least initially, both in the pharmacological literature and in transgenic studies. As can be seen in Table 3, most studies have concentrated on the rewarding and locomotor-stimulant effects of cocaine in dopaminergic receptors, with much less work examining other psychostimulant effects and other monoaminergic receptors.

Table 3. Psychostimulant responses in monoamine transporter transgenic mice

Citation	Gene	Drug	Micro-dialysis	Loco-motion	CPP	Self-administration	Adverse effects
Budygin, E.A. *et al.* 2004	DAT KO	AMPH			Unaffected, abolished by 5-HT_{1A} antagonist		
Salahpour, A. *et al.* 2008	DAT over-expression	AMPH	Increased	Increased	Increased		
Spielewoy, C. *et al.* 2001	DAT KO	AMPH		Decreased			
Xu, F. *et al.* 2000	NET KO	AMPH		Increased			
Takahashi, N. *et al.* 1997; Fukushima, S. *et al.* 2007	VMAT2 KO	AMPH, METH		Increased	Decreased		
Numachi, Y. *et al.* 2007	DAT KO	METH					Reduced hyper-thermia
Fumagalli, F. *et al.* 1998	DAT KO	METH					Eliminated neurotoxic effects
Fumagalli, F. *et al.* 1999; Guillot, T.S. *et al.* 2008	VMAT2 KO	METH					Enhanced neurotoxic effects
Trigo, J.M. *et al.* 2007	SERT KO	MDMA	Abolished 5-HT in PFc			Eliminated	
Bengel, D. *et al.* 1998	SERT KO	MDMA		Eliminated			
Renoir, T. *et al.* 2008	SERT KO	MDMA					Decreased hippocam-pal cell prolifera-tion was eliminated
Sora *et al.* 1998	DAT KO	Methyl-phenidate			Unaffected		
Tilley, M.R. & H.H. Gu. 2008	DAT CI	Methyl-phenidate		Eliminated	Eliminated		

PFc: prefrontal cortex.

Dopamine receptors

The studies of DAT KO mice discussed herein obviously implicate dopaminergic mechanisms in many psychostimulant effects but do not specify which DA systems are involved. Because of the belief of the importance of DA for psychostimulant effects, some of the first gene KO mice made were for dopaminergic receptor genes. Dopaminergic receptors are classified as D_1-like (D_1 and D_5) or D_2-like (D_2, D_3, and D_4) receptors on the basis of sequence homology and pharmacology.[103] DA receptors also have different distributions in the brain.[104–108] This would then indicate that transgenic manipulations of dopaminergic receptors may produce more specific effects on behavior, and the effects of psychostimulants, than monoamine transporter manipulations. However, it may also be possible that there is a greater possibility of compensation by other receptors in the absence of one.

Cocaine

D_1 KO mice. There is substantial pharmacological evidence for the involvement of DA receptors in drug reward, and in the effects of cocaine in particular. Full D_1-like agonists are self-administered by rats,[109] and administration of D_1-like antagonists decreases cocaine self-administration.[110] Of course one problem with many pharmacological agents used to study dopaminergic effects is specificity for DA receptor subtypes, so that the effects mentioned earlier may not be due to actions at D_1 receptors per se. Transgenic techniques thus presented a way to specifically address which DA receptor subtypes may be involved in the rewarding effects of cocaine. D_1 KO mice have been reported to demonstrate normal responses to the rewarding effects of cocaine in the CPP paradigm,[111] although they do show reduced voluntary ethanol consumption,[112] suggesting that deletion of the D_1 receptor does attenuate the reinforcing properties of some drugs. Interestingly, and in a manner somewhat reminiscent of the consequences of deletion of the DAT gene, the locomotor stimulant effects of cocaine, as well as locomotor sensitization, are eliminated in D_1 KO mice.[111,113,114] Indeed, this parallel may go even further, because D_1 KO mice have been reported to have locomotor-decreasing effects of cocaine.[115] Although another study did not observe this, it did observe locomotor-decreasing effects of cocaine in combined D_1–D_3 KO mice, which were hyperactive at baseline.[116] Combined D_1–D_3 KO also reduced cocaine CPP, but only at the lowest dose examined.[116] Despite the observation of normal cocaine CPP in D_1 KO mice, cocaine self-administration is virtually eliminated, most of the subjects not meeting the criteria for acquisition.[117] Again, this situation is similar to that observed in DAT KO mice in a recent study.[19] In WT mice the immediate early genes *c-fos* and *zif268* are activated by cocaine, but this does not occur in D_1 KO mice, and instead there is activation of the expression of the substance P gene.[118] D_1 KO also reversed the effect of cocaine on CREB phosphorylation, producing decreases, rather than increases, in CREB phosphorylation,[116] and a reduction in the number of pCREB immunoreactive cells were observed throughout the striatum in these mice.

D_2 KO mice. On the basis of pharmacological evidence alone, there is perhaps even more evidence for the involvement of the D_2 receptor in the rewarding effects of psychostimulants. Similar to D_1-like DA receptors, D_2-like agonists are self-administered by rats[109] and D_2-like antagonists reduce cocaine reinforcement.[119] However, despite the indications from pharmacological studies, self-administration of low to moderate doses of cocaine is unaffected, whereas self-administration of moderate to high doses of cocaine is actually increased in D_2 KO mice,[119] and D_2 KO produced only a slight reduction in cocaine CPP.[120] Those authors also found a reduction in the ability of cocaine to stimulate production of c-Fos. D_2 KO also does not affect the discriminative stimulus effect of cocaine.[121] Thus, it would appear at the very least that D_2 KO does not produce quite the same effects as D_2 antagonists in WT mice in models of drug reward. Whether this indicates that there are compensatory changes in other DA receptors, or that normally multiple receptors are involved, remains to be determined.

Similarly to the effects discussed in the foregoing section, locomotor stimulant effects of cocaine were largely unaffected in D_2 KO mice, once differences in basal activity were taken into account,[121] although another study did find reduced locomotor-stimulant effects of cocaine,[120] which was accompanied by pronounced stereotypical grooming. DA autoreceptor function was eliminated in D_2 KO mice,[122] but cocaine-mediated DA efflux was only slightly affected in striatal synaptosomes. This outcome was observed even though DAT clearance rates were reduced by 50%,[123] which seemed to result from a change in activity because the density and affinity of DAT sites were unchanged. In any case, regardless of the mechanism, these changes are associated with substantially increased DA release in response to cocaine as measured by *in vivo* microdialysis in D_2 KO mice, or mice with a selective deletion of the long isoform of the D_2 (D_{2L}) receptor.[124]

D_3, D_4, and D_5 KO mice. There has been less pharmacological evidence of a role for other DA receptor subtypes in the effects of psychostimulants, although certainly some, and perhaps more for the D_3 receptor,[125] because more selective agents have not been available for as long. Thus, examination of genetic deletion of the other DA receptors should be especially illuminating here. Both D_3 KO mice[126] and D_4 KO mice[127,128] have increased locomotor responses to cocaine. By contrast, D_5 KO was reported to produce a reduction in cocaine-stimulated locomotion,[129] although this effect was not found in another study.[114] The basis of the effects in D_3 and D_4 KO mice was suggested to be quite different. D_3 KO mice have increased sensitivity to combined D_1 and D_2 agonists, so it was suggested that the enhanced responses to cocaine in these mice were due to increased D_1/D_2 synergy.[126] In contrast, the effects of D_4 gene deletion were suggested to be mediated by the elimination of inhibitory effects of the D_4 receptor.[116] In either case the mechanisms involved are somewhat speculative. The baseline difference in cocaine responsiveness observed in D_3 KO mice discussed in the preceding section was not large and limited to a low dose. Using a higher dose and testing in the home cage, another study found that D_3 KO mice had reduced locomotor responses to cocaine, although this appeared to be the result of stereotypical head-bobbing behavior.[130] Furthermore, in WT mice repeated cocaine treatment produced locomotor sensitization, but this was not found in D_3 KO mice, which instead showed sensitized stereotypical head bobbing.[130] The increased stereotypy observed in D_3 KO mice is also associated with increased stimulatory effects on *c-fos* and dynorphin gene expression,[130] which were thought to be indicative of enhanced D_1 stimulation in the absence of D_3. In contrast to diminished effects on cocaine locomotion, D_3 KO mice exhibit increased sensitivity to cocaine in the CPP paradigm.[126] Reduced locomotor sensitization was observed in D_5 KO mice,[114] which did not exhibit sensitization under most conditions tested.

Far less work has been done to examine other psychostimulant effects. The potency of cocaine as a discriminative stimulus was enhanced in D_4 KO mice[127] but unaffected by D_5 KO.[129] Cocaine CPP was also normal in these mice.[114] The ability of cocaine to produce conditioned locomotion is not different in D_3 KO mice compared to WT control mice,[131] although those authors found that D_3 agonists did inhibit the behavior, again suggesting compensatory actions of other DA receptors when one is eliminated. The effects of cocaine on PPI appear to involve DA D_1, D_2, and D_3 receptors.[132] D_1 KO eliminated cocaine-induced impairments in PPI, whereas D_2 KO was partially effective. By contrast, D_3 KO produced increases in cocaine-induced impairments in PPI. Finally, DA receptors may also play a role in cocaine-induced toxicity. Although D_3 KO did not affect cocaine-induced convulsions by itself, it did block the protective effects of a D_2/D_3 agonist, whereas D_2 KO was without effect.[133]

Much more work remains to be done here. Many of the effects of psychostimulants that have been identified in DAT KO mice have not been examined in DA receptor KO mice. Furthermore, as for monoamine transporter KOs, it may be necessary to examine multiple receptor KOs where there is no or little effect of single receptor gene KOs.

Amphetamines

As for cocaine, most transgenic work has concentrated on dopaminergic receptor KOs, as can be seen in Table 4. Most studies have concentrated on the rewarding and locomotor-stimulant effects of AMPH, with much less work examining other psychostimulant effects and other AMPH compounds.

D_1 KO mice. There are conflicting reports on the effects of AMPH in D_1 KO mice. An early study found that although initial locomotor responses to AMPH were unaltered in D_1 KO mice, the sensitization of these responses was diminished.[134] Another study found a slight diminution in the acute locomotor-stimulating effects and largely unaltered locomotor sensitization,[17] although one difficulty of interpretation here was high locomotor activity in saline-treated subjects, so that when this is taken into account, it could be considered that they have reduced sensitization. Contrary to these studies,

Table 4. **Psychostimulant responses in monoamine receptor transgenic mice**

Citation	Gene	Drug	Loco-motion	Sensiti-zation	CPP	PPI	Adverse effects: hyper-thermia	Adverse effects: lethality
Crawford, C.A. *et al.* 1997	D_1 KO	AMPH	Unaffected	Decreased				
Karper, P.E. *et al.* 2002	D_1 KO	AMPH		Unaffected				
McDougall, S.A. *et al.* 2005	D_1 KO	AMPH	Increased	Increased				
Ralph, R.J. *et al.* 1999	D_2 KO	AMPH				Disrupted AMPH-induced impair-ments		
Kelly, M.A. *et al.* 2008	D_2 KO	AMPH	Decreased	Unaffected				
Xu, R. *et al.* 2002	D_{2L} KO	AMPH				Unaffected AMPH-induced impair-ments		
Xu, M. *et al.* 1997	D_3 KO	AMPH	Increased					
Ralph, R.J. *et al.* 1999	D_3 KO	AMPH				Unaffected AMPH-induced impair-ments		
Ralph, R.J. *et al.* 1999	D_4 KO	AMPH				Unaffected AMPH-induced impair-ments		
Kruzich, P.J., K.L. Suchland & D.K. Grandy. 2004	D_4 KO	AMPH		Increased				
Kelly, M.A. *et al.* 2008	D_4 KO	AMPH	Increased					
Harrison, S.J. & J.N. Nobrega. 2009	D_5 KO	AMPH		Increased				
Bronsert, M.R. *et al.* 2001	5-HT_{1B} KO	AMPH	Increased	Increased				
Weinshenker, D. *et al.* 2002	DBH KO	AMPH	Increased	Unaffected				
Drouin, C. *et al.* 2002	α_{1b} KO	AMPH	Decreased	Decreased				

Continued

Table 4. *Continued*

Citation	Gene	Drug	Locomotion	Sensitization	CPP	PPI	Adverse effects: hyperthermia	Adverse effects: lethality
Sallinen, J. *et al.* 1998	α_{2c} KO	AMPH	Increased	Increased				
Lahdesmaki, J. *et al.* 2004	α_{2A} KO	AMPH				Increased AMPH-induced impairments		
Ito, M. *et al.* 2008	D_1 KO	METH					Modestly attenuated METH-induced hyperthermia	Substantially attenuated METH-induced lethality
Ito, M. *et al.* 2008	D_2 KO	METH					Eliminated METH-induced hyperthermia	Substantially attenuated METH-induced lethality
Rubinstein, M. *et al.* 1997	D_4 KO	METH	Increased					
Allan, A.M. *et al.* 2001	5-HT_3 overexpression	METH			Decreased			
Risbrough, V.B. *et al.* 2006.	D_1 KO	MDMA	Increased					
Risbrough, V.B. *et al.* 2006.	D_2 KO	MDMA	Decreased					
Risbrough, V.B. *et al.* 2006.	D_3 KO	MDMA	Unaffected					
Dulawa, S.C. *et al.* 1998, 2000	5-HT_{1B} KO	MDMA				Increased		
Scearce-Levie, K., S.S. Viswanathan & R. Hen. 1999	5-HT_{1B} KO	MDMA	Decreased			Increased		
Bexis, S. & J.R. Docherty. 2005	α_{2A} KO	MDMA					Biphasic response, hypothermia followed by hyperthermia	

another study found generally increased responses to AMPH after chronic treatment in D_1 KO mice, including increased context-dependent sensitization, context-independent sensitization, and conditioned locomotion.[135] Yet another study found no differences in sensitization in D_1 KO mice.[136] It is difficult to say why these different results have been obtained.

D_2 KO mice. One complication of the study of D_2 KO mice is that the D_2 receptor is expressed both presynaptically and postsynaptically. DA autoreceptor function is eliminated in D_2 KO mice,[122] although interestingly the effects of AMPH on DA release were unaltered in that study. Interpretation of psychomotor stimulant effects in D_2 KO mice is complicated by reduced basal levels of activity.[137] However, even when this is taken into account they do appear to display diminished locomotor-stimulant effects of METH,[138] although sensitization of those responses did not appear to be affected.

D_3, D_4, and D_5 KO mice. As for cocaine, both D_3 and D_4 KO mice have increased locomotor-stimulant effects of AMPH,[126,139] although this is limited to particular doses.[140] Importantly, these changes in D_3 KO mice were not associated with changes in the stereotypical effects of AMPH,[140] as might be predicted based on the localization of that receptor compared to the D_2 receptor. D_4 KO mice also have increased locomotor responses to METH.[128] The mechanisms may be different in each case, as discussed earlier, and may or may not involve other DA receptors. Locomotor sensitization to AMPH is also enhanced in D_4 KO mice,[139] at least under some conditions. AMPH sensitization was not different from WT controls in D_5 KO mice.[141]

The PPI-impairing effects of AMPH were disrupted in D_2 KO mice but not D_3 or D_4 KO mice.[142] This pattern is slightly different from that discussed earlier for cocaine. This is a further indication that the effects of psychostimulants, though substantially overlapping, still involve some different mechanisms. The effect of AMPH was not disrupted in D_{2L} KO mice, which may suggest that these effects are mediated by the D_{2S} isoform.[143]

Adverse effects of AMPH. Evidence for the importance of DA in the adverse effects of METH was discussed earlier, including data from DAT KO mice, including evidence that the hyperthermic and lethal effects of METH were somewhat dissociable. DA antagonists reduce METH-induced hyperthermia[73] and lethality,[74] but these effects are highly dose dependent and substantially dependent on ambient temperature. In a recent study, we examined the roles of dopamine D_1 and D_2 receptors in METH-induced hyperthermia and lethal effects by using D_1 KO and D_2 KO mice.[144] This study found that both the D_1 and D_2 receptors have roles in the lethal effects of METH but differently affect the hyperthermic effects of METH. D_2 KO eliminated METH-induced hyperthermia, whereas D_1 KO produced a more modest attenuation of this response. Both KOs produced a substantial attenuation of METH-induced lethality. These data further dissociate the mechanisms underlying METH-induced lethality and METH-induced hyperthermia, even though dopaminergic mechanisms appear to be involved in both effects.

MDMA. Most research into the mechanisms underlying the effects of MDMA has concentrated on serotonergic mechanisms, but there is also evidence for direct or indirect roles of dopaminergic systems in MDMA-induced effects, although not much work has been done in this area in transgenic mice. In male D_1 KO mice the locomotor stimulant effects of MDMA were increased, whereas D_2 KO was found to reduce MDMA effects and D_3 KO was without effect.[145] There were also some changes in the pattern of activity, including reduced MDMA-induced perseverative thigmotaxis in D_2 KO mice. There was also some sex dependency of these effects, so although D_3 KO was without effect in males, there was a slight reduction in MDMA-induced hyperlocomotion in females.

Serotonin receptors

Although the importance of dopaminergic systems in the effects of psychostimulants has been well established, data discussed earlier indicate that serotonergic systems, particularly those that interact with dopaminergic systems, also have a role. That evidence has not identified the particular parts of the serotonergic system that may be involved in psychostimulant actions and which of the many 5-HT receptor subtypes may be involved. 5-HT receptors are diverse, comprising many structurally and pharmacologically distinct mammalian 5-HT receptor subtypes, as determined from sequence homology and pharmacology,[146] which have distinctly different anatomical distributions,[147–149] and many of which are thought to modulate the effects of psychostimulants.[150] Although pharmacological evidence has been important in implicating 5-HT in the effects of many psychostimulants, because of the many 5-HT receptor subtypes the situation regarding specificity of available agents is even more of a problem than it is for dopaminergic systems. Therefore, transgenic studies have contributed substantially to our knowledge about the role of specific 5-HT receptor subtypes in the effects of psychostimulants, although some have been much more thoroughly investigated than others.

Cocaine

5-HT$_{1B}$ KO mice. On the basis of the impetus of pharmacological evidence, the 5-HT$_{1B}$ receptor has been more extensively examined in transgenic studies than other 5-HT receptor subtypes. This evidence includes data demonstrating that 5-HT$_{1B}$ receptor agonists enhance cocaine-induced reinforcement[151] and increase extracellular DA in the nucleus accumbens.[152] 5-HT$_{1B}$ KO increased the locomotor stimulant effects of cocaine,[15,153] which prompted Rocha *et al.*[15] to suggest that these mice were "presensitized" to cocaine. 5-HT$_{1B}$ KO was initially associated with accelerated acquisition of cocaine self-administration,[154] without many other changes, but was subsequently associated with increased cocaine self-administration under a variety of conditions.[15,153] Surprisingly, cocaine was reported not to produce a CPP in these mice,[155] although this appears to be yet another example in which transgenic manipulations produce divergent results in CPP and self-administration paradigms. Nonetheless, as further evidence that these effects involved interactions with dopaminergic systems, *in vivo* microdialysis studies found that basal and cocaine-evoked DA levels in the nucleus accumbens of 5-HT$_{1B}$ KO mice were increased.[156] These changes would appear to be most consistent with the self-administration studies in these mice, although there is evidence that postsynaptic changes may oppose these actions, including reduced cocaine-evoked elevation of c-Fos,[157] which may help explain the divergent effects in different models.

Other serotonin receptors. Other 5-HT receptor subtypes have been much less extensively examined in transgenic models, the initial studies beginning with 5-HT receptor subtypes localized on dopaminergic neurons and for which there was already evidence that they modulate dopaminergic function.[158] Deletion of 5-HT$_{2C}$ receptors was associated with greater release of DA in the nucleus accumbens and increased reinforcing efficacy of cocaine, including increased responding under a progressive ratio schedule.[159] In both 5-HT$_{1B}$ receptor KO mice and in 5-HT$_{2C}$ receptor KO mice, higher reinforcing efficacy of cocaine was associated with greater cocaine-stimulated DA levels in the nucleus accumbens. Thus studies of cocaine self-administration in different 5-HT receptor KO mice suggest that increased reinforcing efficacy of cocaine is ultimately associated with increased DA activity.

Some other 5-HT receptor subtypes have also been examined. The locomotor-stimulating effects of cocaine are increased in 5-HT$_{2A}$ KO mice, but they still exhibit sensitization.[160] Transgenic overexpression of the 5-HT$_3$ receptor reduces the rewarding effects of cocaine in the CPP paradigm.[161] In these data there was a slight trend for 5-HT$_3$-overexpressing mice to be more sensitive to low doses of cocaine. The 50% effective dose for locomotor-stimulating effects of cocaine was substantially reduced in these mice, which was associated with greater DA release in response to application of low doses of cocaine to striatal brain slices. The contribution of specific serotonergic receptors to the toxic or lethal effects of cocaine has not been investigated to any great degree, although a recent study has shown that 5-HT$_7$ KO increases cocaine-induced seizures and lethality.[162]

Amphetamines

Other psychostimulants have been even less examined than cocaine in 5-HT receptor KO mice. 5-HT$_{1B}$ KO mice had increased acute and sensitized locomotor effects of AMPH.[163] On the basis of comparisons between intraperitoneal and intravenous routes of administration, those authors suggested that some of the effects of 5-HT$_{1B}$ KO were due to interactions with handling stress, but not all. As for cocaine, the 50% effective dose for the locomotor-stimulating effects of METH was decreased in 5-HT$_3$-overexpressing mice.[161] Finally, MDMA does not affect PPI in WT mice but increases PPI in 5-HT$_{1B}$ KO mice,[164,165] whereas the locomotor stimulant effects of MDMA are attenuated in 5-HT$_{1B}$ KO mice.[166]

Norepinephrine system

Norepinephrine systems have been the least associated with the rewarding effects of psychostimulants of the three main monoamine systems, at least in recent years. However, as discussed in the preceding, NET KO affects several psychostimulant-induced behaviors, including psychostimulant reward. Although not extensively investigated, there is accumulating evidence for involvement of norepinephrine systems in several psychostimulant responses from recent transgenic studies. At least a part of the impetus for examining particular norepinephrine receptors comes from evidence that these receptors modulate somatodendritic DA function.[167]

Cocaine

Transgenic mice that lack the enzyme that synthesizes norepinephrine, DA β-hydroxylase (DBH), are hypersensitive to the locomotor-stimulant effects of cocaine.[168] There was also a leftward shift in the dose–response curve for cocaine CPP, with a greater CPP observed in DBH KO mice at a cocaine dosage of 5 mg/kg, as well as a pronounced cocaine conditioned place aversion at 20 mg/kg cocaine. These authors suggested that this change in responsiveness was due to profound adaptive changes in DA systems, including substantially reduced presynaptic dopaminergic responses and postsynaptic receptor supersensitivity caused by increased numbers of both D_1 and D_2 receptors in the high-affinity state. Furthermore, these effects were observed in the striatum, but not the prefrontal cortex. The reason for these differences is uncertain, as is the degree to which the adaptations may be driven, or prevented in the prefrontal cortex, by DA release from norepinephrine synapses. Another report suggested that DBH KO eliminated the rewarding effects of cocaine in the CPP paradigm,[169] but this might be due to the dose–effect relationship noted earlier. The specific receptors involved in these effects is uncertain and will remain so until more noradrenergic receptor subtypes have been investigated, but initial evidence implicates the α_{1b} receptor. Oral cocaine consumption was reduced by α_{1b} KO, and there were substantial decreases in the locomotor-stimulant effects of cocaine as well as locomotor sensitization.[170]

Surprisingly, few studies have addressed the aversive effects of cocaine, which are often presumed to involve noradrenergic mechanisms. However, a recent report has found that the aversive effects of cocaine are eliminated in DBH KO mice.[171] With regard to lethal or toxic effects, again, not much has been done, but DBH KO had no effect on cocaine-induced seizures.[172]

Amphetamines

DBH KO mice are hypersensitive to the locomotor-stimulant effects of AMPH and exhibited a leftward shift in the dose–response curve for AMPH, including exhibiting stereotypical behavior at much lower doses of AMPH than is observed in WT mice.[173] However, this may have resulted from alterations in DA receptor function because these mice were less sensitive to a D_1 agonist and more sensitive to a D_2 agonist. Sensitization of AMPH responses was unaltered in these mice. Indeed, norepinephrine may have a more general modulating effect upon dopaminergic function and the effects of psychostimulants. Recent pharmacological studies have suggested that stimulation of the α_{1b} receptor increases psychostimulant effects, whereas stimulation of the α_2 adrenergic receptor inhibits those effects.[174] This supposition has been supported by transgenic studies. α_{1b} KO produces substantial decreases in the locomotor stimulant effects of AMPH as well as sensitization of those responses,[170] whereas the locomotor-stimulant effects of AMPH are enhanced in α_{2c} KO mice and reduced by transgenic overexpression of the α_{2c} receptor.[175] Consistent with the evidence for the involvement of both SERT- and NET-mediated responses underlying the retention of CPP in DAT KO mice,[18] there is evidence in α_{1b} KO mice for compensatory involvement of 5-HT systems. In α_{1b} KO mice a 5-HT_{2A} antagonist blocked the locomotor-stimulant effects of AMPH and the sensitization of those effects.[176] Under normal circumstances these two receptors have been suggested to be mutually inhibitory, even though they are individually behaviorally activating, and one mechanism of sensitization has been suggested to be the decoupling of these receptors producing increased DA activity.[177]

Again, little work has been done on the role of noradrenergic system genes in other psychostimulant effects. Adrenergic receptors may contribute to the effect of AMPH on sensorimotor gating in the PPI model. Consistent with some of the other effects discussed earlier, α_{2A} KO mice have increased

PPI-disrupting effects of AMPH.[178] α_{2A} KO also alters the effects of MDMA on temperature.[179] Finally, with regard to adverse effects of amphetamines, elimination of norepinephrine in DBH KO mice increases the effect of METH on DA release, oxidative stress, and neurotoxicity.[180]

Discussion

From the data presented here it is clear that there is accumulating evidence from transgenic, and especially gene KO studies, for the role of monoaminergic transporter and receptor genes in the actions of psychostimulants, and by implication addiction. This review has been limited in two major ways: to discussion of the effects of transgenic manipulations of monoamine transporter and receptor genes and to the effects of psychostimulants. There is substantial evidence that monoamine gene manipulations also affect the actions of addictive drugs that do not act directly through monoamine transporters or receptors, such as morphine and ethanol, and similarly, there is a substantial body of work demonstrating that transgenic manipulations of genes other than those discussed here affect the actions of psychostimulants. However, what is evident from the transgenic work discussed here is that there is a complex emerging picture of interactive gene effects, even when considering just the monoaminergic genes, that is important in determining the effects of psychostimulants.

An additional point that has been substantially sidestepped in this review is the relationship of these transgenic "models" to human addiction. One of the conclusions that has become most evident in recent genomewide association studies of addiction[181–187] is that the genes that underlie addiction in humans seem to rather rarely include the classes of genes discussed here, monoamine transporters and receptors. Instead, the allelic variation in the actual human population that seems to underlie addiction involves a higher proportion of other classes of genes, including many involved in signal transduction and synaptic plasticity.[188] This realization will be important for developing animal models of addiction, and as the sophistication of these approaches develops, for modeling the specific allelic variants that may underlie human addiction.

This is not to say that the extensive studies discussed here have not contributed a great deal to the study of addiction. First, these transgenic models indicate genes that may be involved in addiction in humans (this may or may not be the case depending upon the actual allelic variation that exists in these genes in humans). Second, they indicate genes that, when manipulated, produce substantial changes in observable phenotypes that are relevant for addition, and the many diverse actions of psychostimulants, and may therefore contribute to the development of addiction therapeutics. Thus, in these ways the use of transgenic techniques has substantially improved our understanding of addiction genetics and provides insight into the polygenic determination of drug addiction phenotypes in ways that would not be possible with other methods. The complex picture that has emerged from this research fits with recent polygenic descriptions of genetic influences on human addiction developed from genomewide association studies, and no matter how much we may desire simple answers to our questions, we must accept the complex reality that is evident in these data.

Acknowledgments

This study was supported in part by Grant-in-Aid for Health and Labour Science Research (Research on Pharmaceutical and Medical Safety) from the Ministry of Health, Labour, and Welfare of Japan; by Grants-in-Aid for Scientific Research (B), Scientific Research on Priority Areas—System study on higher-order brain functions and Research on Pathomechanisms of Brain Disorders; Core Research for Evolutional Science and Technology (CREST) from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and the National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health (F.S.H.).

Conflicts of interest

The authors declare no conflicts of interest.

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