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MONOAMINE TRANSPORTERS: From Genes to Behavior

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■ **Abstract** Modulation of fast neurotransmission by monoamines is critically involved in numerous physiological functions and pathological conditions. Plasma membrane monoamine transporters provide one of the most efficient mechanisms controlling functional extracellular monoamine concentrations. These transporters for dopamine (DAT), serotonin (SERT), and norepinephrine (NET), which are expressed selectively on the corresponding neurons, are established targets of many psychostimulants, antidepressants, and neurotoxins. Recently, genetic animal models with targeted disruption of these transporters have become available. These mice have provided opportunities to investigate the functional importance of transporters in homeostatic control of monoaminergic transmission and to evaluate, in an in vivo model system, their roles in physiology and pathology. The use of these mice as test subjects has been helpful in resolving several important issues on specificity and mechanisms of action of certain pharmacological agents. In the present review, we summarize recent advances in understanding the physiology and pharmacology of monoamine transporters gained in mice with targeted genetic deletion of DAT, SERT, and NET.

INTRODUCTION

Monoaminergic transmission is involved in a variety of physiological, behavioral, and endocrine functions (1, 2). These systems are believed to be critically important in both the pathophysiology and the pharmacotherapeutics of a number of brain disorders, including Parkinson's disease, depression, drug abuse, schizophrenia, attention-deficit hyperactivity disorder (ADHD), and Tourette syndrome (1–3). A complex balance between the amount of neurotransmitter synthesized, stored, released, metabolized, and recaptured determines the intensity of monoaminergic signaling (3). Monoamines released into the extracellular space can undergo enzymatic degradation and dilution by diffusion; however, the major mechanism controlling extracellular monoamine dynamics has proven to be reuptake by presynaptic neurons via plasma membrane monoamine transporters (4–9). Monoamine transporters, such as that for dopamine (DAT), serotonin (SERT),

and norepinephrine (NET), that most likely localized perisynaptically (10–12) in corresponding neurons, remove neurotransmitters from outside cells and recycle it back into the releasing and/or neighboring terminals (4–9). In most cases, this uptake is neurotransmitter specific; however, under certain conditions or in distinct brain regions, monoamines can be cleared from extracellular space heterologously by multiple monoamine transporters (13–16).

The DAT, NET, and SERT are members of the family of Na^+, Cl^--dependent substrate-specific neuronal membrane transporters, which includes transporters for GABA, glycine, taurine, proline, betaine, and creatine (4–8). The putative structure of these transporters consists of 12 transmembrane domains with both the N- and C-terminal domains located within the cytoplasm. The mechanism of the transporter-mediated uptake of monoamines is believed to involve an electrogenic transport of monoamines by sequential binding and cotransport of Na^+ and Cl^- ions (4–8).

These transporters represent established targets of many psychostimulants and antidepressants, which exert their potent psychotropic action via interference with transporter function, resulting in a rise in extracellular levels of monoamines (4, 8). Some neuron-specific toxins can enter the cells through plasma membrane monoamine transporters, thereby revealing the additional functional role of transporters as a molecular gateway for neurotoxins (6, 9).

Relatively little attention has been given to another important aspect of transporter function. Several lines of evidence have suggested that drugs affecting monoamine transporters can significantly modulate presynaptic neuronal homeostasis (17–22). However, until genetic animal models with targeted disruption of these transporters became available (23–25), this homeostatic role of plasma membrane transporters was not fully appreciated.

One of the attractive applications of mice having genetic deletions of certain proteins is their use as test subjects to evaluate the role of these molecules in therapeutic or pathological actions of psychotropic drugs. Several important observations in this context were made in transporter mutant mice (26).

Another important implication of genetically altered animals is to model, in vivo, the pathological conditions underlying human diseases. Profound physiological and behavioral changes detected in these mice have provided some intriguing insights on the potential role of these proteins in human diseases (27).

In this essay, recent findings on DAT (23), SERT (24), and NET (25) knockout (KO) mice on the homeostatic function of monoamine transporters, their involvement in the psychotropic or neurotoxic actions of various drugs, and their role in aberrant behaviors are discussed.

DOPAMINE TRANSPORTER-DEFICIENT MICE

The unique role of dopamine (DA) as a mediator of such critical functions as movement, emotion, and affect determines the involvement of this neurotransmitter in a variety of pathological conditions and disorders (1–3). It is not surprising,

therefore, that mice lacking the DAT have attracted continued interest, and a considerable amount of data on transporter function and pharmacology have been gained using this model. The DAT knockout (DAT-KO) mice, generated through genetic deletion of the DAT by homologous recombination (23, 28), display a distinct behavioral phenotype. The DAT-KO mice are hyperactive (23, 28–30), dwarf (31), display cognitive (29) and sensorimotor gating (32) deficits, and sleep dysregulation (33). The mutant mice demonstrate normal social interaction (30), but females lacking the DAT show an impaired capability to care for their offspring (23), most likely due to anterior pituitary hypoplasia-related hormonal dysregulation (31). Abnormalities in skeletal structure (34) and altered regulation of gastrointestinal tract motility (35) have been also described in DAT-KO mice.

Neurochemistry

EXTRACELLULAR DA DYNAMICS Hyperactivity of central dopaminergic transmission in DAT-KO mice was first demonstrated in cyclic voltammetry experiments in mouse striatal slices, which showed a 300-fold increase in extracellular lifetime of DA released by single pulse stimulation (23, 36). As might be expected, cocaine and amphetamine did not affect DA clearance in the striatum of DAT-KO mice (23, 36, 37). Furthermore, neither serotonin and norepinephrine transporter inhibitors nor inhibitors of the DA degradative enzymes monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) were able to modulate the rate of DA clearance in the striatum of these mice (36). Thus, it has been demonstrated that within the time frame of single pulse stimulation, only diffusion may be involved in removing extracellular DA in the striatum of DAT-KO mice (36). Similar conclusions were reached when a complementary technique to study evoked DA release and the kinetics of DA elimination, carbon fiber amperometry, was used (38). In these in vivo experiments in anesthetized mice, striatal DA release was evoked by electrical stimulation of the medial forebrain bundle. Extracellular DA half-life was estimated to be at least two orders of magnitude higher in DAT-KO mice. Again, the inhibition of COMT did not significantly affect DA clearance. However, inhibition of MAO by pargyline modestly slowed down DA elimination in DAT-KO mice, suggesting that metabolism of DA by MAO may play some role, in addition to diffusion, in the clearance of DA released over the course of multiple stimulations. It has also been demonstrated that in mice lacking the DAT, low-frequency firing resulted in consistently high extracellular DA levels that could not be distinguished from DA levels achieved by high-frequency firing. These observations were interpreted to suggest that in DAT-KO mice the burst firing activity cannot be specifically translated into phasic changes in extracellular DA as happens in normal animals (38).

Overall, both voltammetric and amperometric studies convincingly demonstrated remarkably prolonged lifetime of extracellular DA in DAT-KO mice (36, 38). However, both investigations also revealed a significantly decreased (by 75% in voltammetric experiments and by 93% in amperometric studies) amount of

DA molecules released in response to stimulation (36, 38). To directly prove that the absence of DAT can induce elevation in extracellular DA concentrations, an alternative approach to assess basal extracellular DA level, a quantitative "no net flux" microdialysis technique was used (36, 39). These studies documented that, despite decreased levels of releasable DA, the greatly prolonged extracellular lifetime of DA in DAT-KO mice results in significantly elevated basal extracellular DA levels (fivefold). Nevertheless, these abnormal extracellular concentrations of DA were substantially lower than might be inferred from alterations in the clearance rate observed in DAT-KO mice (36, 38). It is important to underscore that extracellular DA levels in DAT-KO mice were still reflective of depolarization-dependent vesicular exocytosis. In microdialysis experiments, infusion of tetrodotoxin or Ca^{2+}-free artificial media gradually reduced levels of extracellular DA to undetectable levels (40). Altogether, these neurochemical investigations firmly establish the DAT-KO mice as a genetic model of persistent hyperdopaminergia (40).

PRESYNAPTIC HOMEOSTASIS Although profound alterations in extracellular DA dynamics in mice lacking the DAT were predictable from previous pharmacological experience, dramatic changes in the regulation of DA transmission at the level of presynaptic terminals were unexpected. Most notably, total tissue DA levels in the striatum, which generally reflect the intraneuronal vesicular storage pool of DA, were found drastically (20-fold) reduced in DAT-KO mice (36). Furthermore, these low levels of DA in the striatum of DAT-KO mice were extremely sensitive to the inhibition of tyrosine hydroxylase (TH)-mediated synthesis of DA, suggesting that they represent a newly synthesized pool of DA (36).

The striatal levels of TH protein were also markedly decreased in DAT-KO mice (36, 41). However, these reductions in DA and TH levels cannot be explained as a consequence of abnormal development or degeneration of DA neurons, due to several reasons. First, TH-positive neurons in the substantia nigra (SN), where cell bodies of nigrostriatal DA neurons are located, were only marginally decreased with no modification in the ratio of TH mRNA levels per neuron. Second, the striatal levels of the other enzyme involved in DA synthesis, DOPA decarboxylase, were not modified, and, finally, the vesicular transporter VMAT2 levels were only marginally decreased (39, 41). Furthermore, functional activity of TH in the striatum as measured by L-DOPA accumulation was in fact increased in mutant mice (36). Although the exact mechanisms responsible for such dramatic alterations in TH regulation are not clear, it is likely that the decrease in intracellular DA itself, rather than loss of dopaminergic projections, is responsible, at least in part, for this effect.

It is important to note that depleted striatal DA storage in DAT-KO mice cannot be explained by a deficit in VMAT2-mediated vesicular uptake mechanisms. Neither VMAT2 mRNA in the SN or tetrabenazine binding, VMAT2 protein levels, nor functional vesicular uptake of DA in the striatum was significantly altered in these mice (39). Thus, the depletion of the DA storage pools and the decreased

DA release in DAT-KO mice could be direct consequences of the lack of inward transport of DA through the DAT. Consequently, in the normal situation, a dependence of DA storage on recycled, rather than newly synthesized, DA must exist (Figure 1) (36, 39).

In fact, depletion of DA stores in DAT-KO mice occurred even though the DA synthesis rate was elevated (twofold) (36). Accordingly, the tissue levels of DA metabolites were either unaltered (DOPAC) or elevated (HVA) in DAT-KO mice (36). Thus, both DA synthesis and turnover are extremely high in the mutant animals, despite the low levels of striatal TH protein (36). This apparently paradoxical data may be explained by the disinhibition of TH, which is a known subject of tonic feedback inhibition by both intraneuronal and extraneuronal DA (3, 42). In the DAT-KO mice, intraneuronal DA is reduced; this could account for the disinhibition of TH. Alternatively, activation of TH in DAT-KO mice may be explained by dysregulation of autoreceptor control due to pronounced extracellular DA concentrations (3). Indeed, D2 DA receptor mRNA and binding of selective ligands were decreased by 50% in dopaminergic cell body regions in DAT-KO mice (23, 43). Neurochemical and electrophysiological studies also revealed a marked desensitization in major autoreceptor functions (43). The firing rate of DA neurons in the ventral midbrain was elevated and only slightly sensitive to DA agonist application. Striatal nerve terminal release-regulating autoreceptors were also essentially ineffective. The D2/D3 DA receptor agonist quinpirole elicited only a slight decrease in striatal DA release in DAT-KO mice, as measured by both cyclic voltammetry and in vivo microdialysis. Similarly, terminal DA autoreceptors controlling DA synthesis were also found to be nearly nonfunctional (43). This down-regulation of autoreceptor function, which is likely a consequence of persistently elevated extracellular DA, once more underscores an important homeostatic control that DAT exerts over presynaptic neuronal function.

POSTSYNAPTIC RECEPTOR RESPONSIVENESS Another important consequence of the marked alterations in extracellular DA dynamics is dysregulation in postsynaptic DA receptor responsiveness. As might be expected in the striatum of DAT-KO mice, mRNA and protein levels of the two major postsynaptic DA receptors, D1 and D2, were down-regulated by approximately 50% (23). However, some populations of postsynaptic DA receptors appear to be up-regulated. In quantitative in situ hybridization studies, decreased mRNA levels for both D1 and D2 receptors but increased levels of the D3 receptor mRNA (+40%–110%) were found in DAT-KO mice (44). Similarly, an increased density of preproenkephalin A-negative neurons that express the D3 receptor mRNA was described (44). In addition, investigations of the firing rate of DA-responsive neurons in the nucleus accumbens of DAT-KO mice have shown unaltered responsiveness of postsynaptic receptors to a microiontophoretically applied D1 receptor agonist, despite the apparent decrease in receptor numbers (45). A similar decrease in the number of D2 DA receptors that result in a reduced efficiency of coupling to G proteins (L.M. Bohn & M.G. Caron, unpublished data) is surprisingly associated with a conversion from

the normal inhibitory electrophysiological response to quinpirole to an excitatory effect (45). Another paradoxical observation in this regard was gained using an in vivo approach (40). In DA-depleted DAT-KO mice, the D1/D2 DA receptor agonist apomorphine induced more pronounced locomotor activation in comparison to wild-type controls. Thus, it appears that postsynaptic receptors are not uniformly adapted to the inactivation of DAT, with some populations becoming down-regulated but others being supersensitive.

GENE-DOSE EFFECT OF THE LEVEL OF DOPAMINE TRANSPORTER EXPRESSION Interestingly, essentially all of the neurochemical alterations listed above display a clear gene-dose effect (36, 43, 46). Thus, mice heterozygous for DAT deletion displayed intermediate neurochemical profiles between mutant and wild-type mice. Particularly, in heterozygous mice, the striatal tissue level of DA is decreased by 30%, DA synthesis is modestly elevated, TH inhibition results in faster depletion of DA storage, autoreceptor regulation is partially diminished, DA clearance is prolonged by approximately twofold, and extracellular DA is twofold higher in comparison to wild-type mice (36, 43). Similar observations have been gained in mice expressing less than 10% of DAT (46). Importantly, however, the magnitude of these changes was not directly proportional to the level of DAT expression. For example, the alterations in DA homeostasis observed in mice expressing 10% of the DAT (46) were generally more pronounced than in DAT heterozygous mice, but substantially less than the full magnitude of effect observed in DAT-KO mice (36, 43).

DOPAMINE TRANSPORTER AS DETERMINANT OF THE MODE OF DA TRANSMISSION Importantly, the alterations described in the striatum of DAT-KO mice are less evident in other brain areas of these mice. For example, less pronounced depletions in tissue DA were observed in the hypothalamus and pituitary of DAT-KO mice (31), and only minimal alterations in DA and metabolite concentrations were found in the frontal cortex (R.R. Gainetdinov & M.G. Caron, unpublished data). In fact, in DAT-KO mice, the mode of striatal DA transmission closely resembles that described in the frontal cortex of normal animals, where relatively low DAT expression (47) and DA uptake rates are found (48). Several characteristics of mesocortical DA neurons are markedly different from nigrostriatal neurons (3, 49). The firing rate of mesocortical neurons is elevated, possibly indicating less activity of impulse flow-regulating autoreceptors at the level of cell bodies (49). There are few DA synthesis-modulating autoreceptors in the frontal cortex (50). Tissue DA content is disproportionally low in comparison to both the basal extracellular DA level (51) and stimulation-evoked DA release (48). Moreover, DA storage in the frontal cortex is tightly dependent on ongoing synthesis, as evidenced by an increased sensitivity to TH inhibition (49). Similar characteristics of nigrostriatal neuron homeostasis observed in mice lacking the DAT strongly suggest that a low level of DAT expression is a primary determinant of these features of mesocortical neurons in normal animals.

The DAT, as a critical mechanism terminating extracellular DA signals, can play an important role in determining the mode of extracellular transmission from more synaptically limited to "volume"-like or "nonsynaptic" (36, 52) transmission. Profound alterations in presynaptic DA neurochemistry found in DAT-KO mice highlight an additional physiological role of DAT as a key controller in the presynaptic DA homeostasis (36, 39). Different dopaminergic groups of cells expressing various levels of DAT may have markedly different profiles of transmission. In addition, factors affecting DAT expression and regulation, such as development (53, 54), aging (55), and exposure to pharmacological or environmental agents (9, 56), may induce substantial shifts between these modalities. Particularly, because DAT is subject to substantial structural and functional maturation postnatally (53, 54), a specific developmentally determined mode of DA transmission may occur at earlier ages.

Pharmacology

ESTABLISHING THE MAJOR DRUG TARGET AND UNMASKING SECONDARY TARGETS

It is well known that DAT is the major drug target for psychostimulants like cocaine, methylphenidate, and amphetamine (4, 8). Accordingly, in the striatum of DAT-KO mice, these psychostimulants are unable to affect DA clearance or extracellular levels (23, 29, 37, 57). Whereas cocaine and methylphenidate are classical inhibitors of the DAT (3, 5, 8) and their ineffectiveness could be simply explained by the absence of their primary target, a more complex picture emerges with amphetamine (58, 59). Amphetamine enters the DA terminal not only through the DAT but also by diffusion (58). Then, via VMAT2 and/or diffusion, the drug enters vesicles and disrupts the vesicular pH gradient (59). As a result, amphetamine produces a redistribution of stored monoamine from vesicles into the cytoplasm, from which it is transported into the extracellular space by reverse DAT-mediated transport (59). In addition, while inside the cell, amphetamine decreases intraneuronal metabolism of DA via direct inhibition of MAO (58). As a result of all of these actions, amphetamine markedly elevates extracellular DA concentrations in the brain. Although most of the actions of amphetamine are attributed to its effect on the DA system, it is important to underscore that it similarly affects norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) transmission (58).

Experiments in DAT-KO mice (37) clearly illustrated that although the interaction of amphetamine with the DAT is pivotal in its action, an involvement of other critical processes is important as well. Particularly, the role of vesicular storage of DA in amphetamine action has been highlighted in these mice (37). For example, while amphetamine-induced elevation of striatal DA did not occur in mice lacking DAT, the vesicle-depleting action of amphetamine was still observed (37). Furthermore, it has been suggested that amphetamine-triggered reverse transport of DA from cytoplasm to extracellular space does not occur simply due to an elevated cytoplasmic concentration of DA but requires direct action of amphetamine on the DAT (37).

High doses or chronic treatment with amphetamine and related drugs are known to induce dopaminergic and serotonergic neurotoxicity in several brain regions (60). For example, in normal animals, a neurotoxic regimen of methamphetamine administration produces massive DA outflow, free radical formation, and neuronal damage as evidenced by reactive astrogliosis and depletion of DA levels in striatal tissue. However, the same treatment failed to modify any of these parameters in the striatum of DAT-KO mice, whereas modest decreases in striatal and hippocampal 5-HT levels were observed (61). Thus, a critical role of DAT as a mediator of methamphetamine-induced dopaminergic but not serotonergic neurotoxicity has been demonstrated (61).

Similarly, a crucial role of DAT in the neurotoxic action of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been supported in mice lacking DAT (62, 63). In numerous previous studies it had been demonstrated that MPTP neurotoxicity involves selective uptake of its active metabolite, 1-methyl-4-phenylpyridinium (MPP^+) into the DA neuron via the DAT (6, 9). In DAT-KO mice treated with MPTP, markers of striatal neurotoxicity, such as depletion in DA levels and reactive astrogliosis, were virtually absent, whereas in DAT heterozygotes a partial sensitivity was observed (62). In another investigation, no apparent DA cell body loss was found in the substantia nigra of MPTP-treated DAT-KO mice (63). Thus, an absolute requirement of DAT for development of MPTP toxicity in vivo has been firmly established (9, 62, 63).

The wake-promoting action of psychostimulants as well as the antinarcoleptic compound modafinil was also probed in DAT-KO mice (33). Despite the widespread use of this antinarcoleptic drug in the clinic, the mechanism of its action had not been established. In DAT-KO mice, the wake-promoting action of modafinil, methamphetamine, and the DAT inhibitor GBR12909 was virtually absent. Thus, a role of DAT and DA in the antinarcoleptic action of psychostimulants and modafinil has been postulated (33).

One of the most surprizing findings made in DAT-KO mice is that the rewarding properties of cocaine are preserved in DAT-KO mice (28, 57) despite the demonstrated lack of effect of cocaine on striatal extracellular DA levels (23, 57). The ability of cocaine to block DAT and elevate extracellular DA is widely considered to be the primary determinant of its reinforcing and addictive properties (64, 65). However, DAT-KO mice were still able to self-administer cocaine (57) and display cocaine-conditioned place preference (28). These observations demonstrate that the DAT is not the sole mediator of the rewarding properties of cocaine and that other actions of cocaine should be also considered. It is well known that cocaine blocks not only DAT but can potently inhibit other monoamine transporters, such as SERT and NET (4, 8, 57). Furthermore, it has been demonstrated that DA can be captured by the NET in brain regions with low DAT expression, such as the frontal cortex (13, 16). The nucleus accumbens is the brain region that is primarily associated with reward-related behaviors (64, 65). It is known to have high densities of both DAT and NET, unlike the striatum where only DAT is present (66). The possibility has therefore been raised that the ability of cocaine to block NET

may produce the necessary rise in DA levels in the nucleus accumbens to induce cocaine reward in the DAT-KO mice (66). In fact, it has been observed that despite the lack of effect of these psychostimulants in the striatum of DAT-KO mice, both cocaine and amphetamine are able to elevate DA levels in the nucleus accumbens (66). At the same time, inhibition of the NET by reboxetine resulted in elevation of DA levels in the nucleus accumbens in microdialysis experiments (66), whereas another NET inhibitor nisoxetine was somewhat effective in blocking residual DA uptake in the nucleus accumbens synaptosomes from DAT-KO mice (16). These data have been tentatively interpreted to indicate that the ability of cocaine to block NET in the nucleus accumbens of DAT-KO mice is responsible for the elevation of DA levels and for inducing reward (66). However, in recent experiments using fast-scan cyclic voltammetry in brain slices, no such effect of locally applied cocaine or NET inhibitor desipramine on electrically evoked DA release and clearance in the shell of nucleus accumbens of DAT-KO mice was found (67). Similarly, no inhibition of residual DA uptake in nucleus accumbens synaptosomes from DAT-KO mice was observed after cocaine (16). Thus, the mechanism of this elevation of DA in the nucleus accumbens of DAT-KO mice by cocaine and amphetamine remains to be clarified. One possibility may involve an indirect modulation of DA neurons via NE (or 5-HT) inputs at the level of DA cell bodies in the ventral tegmental area leading to elevated DA release in the nucleus accumbens. It should be cautioned, however, that it is unclear whether any further elevation in DA levels could be rewarding in DAT-KO mice because extracellular levels of DA are already elevated fivefold both in the striatum and nucleus accumbens (36, 66).

There are additional reasons to believe that the 5-HT system could be primarily responsible for the unmasked mechanism of cocaine reward provided by elimination of DAT-mediated effects of the drug in DAT-KO mice. Numerous pharmacological investigations have suggested the potential involvement of 5-HT in cocaine responses (68). In DAT-KO mice, the role of 5-HT mechanisms has been suggested by analysis of cocaine analogue binding and mapping of cocaine-induced neuronal activation sites (57). Most crucial evidence for this hypothesis has been gained recently in double-mutant mice lacking both DAT and SERT (69). Unlike single mutants of DAT and SERT, these double-mutant mice do not display conditioned place preference for cocaine, strongly suggesting that, at least in this paradigm, the interaction of cocaine with the SERT is required for cocaine reward in DAT-KO mice. Future investigations of cocaine-induced behaviors in double-knockout mice lacking DAT and NET, recently developed in our laboratory, would be helpful to resolve this issue.

HYPERDOPAMINERGIA AS A MODEL TO TEST DRUG ACTIONS Neurochemical investigations have established the DAT-KO mice as a genetic model of persistent hyperdopaminergia (40). This hyperdopaminergia is revealed functionally as pronounced locomotor hyperactivity, thereby providing a simple model in which the effects of pharmacological agents in modulating DA-regulated behaviors can be assessed. Hyperactivity of these mice is extremely sensitive to treatments

diminishing DA transmission, such as inhibition of DA synthesis (29) or blockade of DA receptors with haloperidol (29, 30), clozapine (30), raclopride (32), and SCH23390 (32). Surprisingly, however, some drugs can modulate this hyperactivity without a direct effect on striatal DA function. Particularly, psychostimulants, like amphetamine, cocaine, and methylphenidate, paradoxically inhibited hyperactivity of DAT-KO mice (29, 70). A similar hypolocomotor effect of amphetamine was observed in modestly hyperactive mice with reduced expression (<10%) of DAT (46) and even in normally active DAT heterozygous mice (70). Although the lack of stimulatory action of psychostimulants in DAT-KO mice could be explained by the lack of striatal DA elevation (29, 37, 57, 66), the inhibitory effect reflects the action of these drugs on molecular targets other than the DAT. In fact, this inhibitory action of psychostimulants was found to be related to the other well-known but largely ignored target of these drugs—the SERT (29, 70). Both direct and indirect 5-HT agonists, including the selective serotonin transporter inhibitor (SSRI) fluoxetine, 5-HT agonist quipazine, and 5-HT precursors tryptophan and 5-hydroxytryptophan, but not the NET inhibitor nisoxetine, potently inhibited the hyperactivity of DAT-KO mice (29). Notably, the inhibitory role of 5-HT on DA-mediated activation and the role of this 5-HT/DA interaction in psychostimulant-induced behaviors were suggested more than 20 years ago (71).

Importantly, this paradoxical inhibitory effect of psychostimulants on hyperactivity occurs without direct involvement of striatal DA transmission (29). Striatal control of motor behaviors involves a reciprocal functional interaction of nigrostriatal dopaminergic and frontostriatal glutamatergic pathways (2, 72). These systems are known to converge at the level of the striatal medium spiny GABA neurons (2, 72). Because a complex modulatory action of 5-HT on the glutamatergic neurons is well-known (72), a role for glutamatergic mechanisms as an intermediate in the inhibitory action of psychostimulants has been suspected (73). In fact, it has been observed that the hyperactivity of DAT-KO mice can be markedly further enhanced when N-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic transmission is blocked. Both DAT-KO and heterozygous mice were more sensitive to this effect of the NMDA antagonist (+)-MK-801 (73). Conversely, drugs that enhance glutamatergic transmission through positive modulation of AMPA glutamate receptors, such as AMPAkines (73), or increasing glycine concentration, such as glycine transporter (Glyt1) inhibitors (74), inhibited hyperactivity in DAT-KO mice. Furthermore, blockade of NMDA receptors by (+)-MK-801 effectively prevented the inhibitory effects of both psychostimulant and serotonergic drugs on hyperactivity, suggesting that intact glutamate transmission is required for the inhibitory effect of 5-HT on hyperactivity (73). These initial investigations suggested a dependence of DA-related locomotor responses on the intensity of frontostriatal glutamatergic signaling, which, in turn, can be potently modulated via serotonergic input. Therefore, because modulation by serotonin of glutamatergic transmission can counteract dopaminergic hyperactivity (29, 73), the potential therapeutic value of serotonergic and glutamatergic drugs in conditions associated with dopamine hyperfunction, such as schizophrenia and ADHD, might be contemplated.

Besides hyperactivity, the DAT-KO mice exhibit deficient sensorimotor gating as measured by prepulse inhibition (PPI) of the startle response (32), perseverative patterns of locomotion (32), and learning and memory deficits (29). To date, only few drugs have been tested in these paradigms. As might be expected, deficits in PPI were corrected by the D2 DA receptor antagonist raclopride, but not by the D1 DA receptor antagonist SCH23390 (32). In contrast, antagonism of D1 but not D2 DA receptors significantly attenuated the perseverative patterns of locomotion (32).

Another important question to be addressed in these mice is how the drugs that have a dopaminergic component as a part of their action could manifest their effects under conditions of a persistently elevated DA tone. For example, morphine was able to further elevate extracellular DA levels in the nucleus accumbens and induce enhanced reward in CPP test, but failed to increase locomotor activity in DAT-KO mice (75). In addition, whereas morphine-induced analgesia was unaffected in mutant mice, the behavioral manifestations of naloxone-induced withdrawal were blunted (75).

Dopamine Transporter-Deficient Mice as a Potential Animal Model of ADHD

Attention-deficit hyperactivity disorder (ADHD) is a common developmental disorder that manifests mostly in school-aged populations as impulsivity, hyperactivity, and inattention (76). The causes and pathophysiology of ADHD are unknown, but compelling evidence suggests an involvement of genetic factors. DA is believed to play a major role in ADHD, but a role for NE and 5-HT systems has also been suspected (76). The most commonly used pharmacotherapy for ADHD is based on the paradoxical ability of psychostimulants to produce an ameliorating (calming) effect; however, some antidepressants are also effective (77). Several studies have reported an association between a polymorphism in the noncoding regions of DAT gene and ADHD (78–80). These molecular genetic studies provide provocative evidence to anticipate that alterations in DAT-mediated processes could significantly contribute to the pathogenesis of this disorder. The functional consequence of this association is still unclear.

Several lines of evidence suggest that the observations gained in DAT-KO mice may be relevant for this disorder (29, 81, 82). As noted above, in DAT-KO mice, a fivefold increase in extracellular DA in the brain is associated with remarkable hyperactivity (36). This hyperactivity is triggered by exposure of mice to a novel environment. No corresponding rise in extracellular DA accompanies this novelty-driven hyperactivity, suggesting that these behavioral changes are regulated through more than just the DA system (29). These mice also showed impairments in tests assessing cognitive function, most likely related to poor behavioral inhibition as evidenced by both perseverative errors in eight-arm maze test and perseverative pattern of locomotion (29, 32). Most intriguingly, these mice responded to psychostimulants in the same way as individuals with ADHD. Administration of psychostimulants amphetamine, methylphenidate, or even

cocaine to the hyperactive DAT-KO mice potently inhibited their activity. Conversely, normal mice become hyperactive when given these psychostimulants (29). As described above, these mice provided a unique model to investigate a neuronal circuitry involved in the hypolocomotor action of psychostimulants (29, 73). Particularly, it has been demontrated that psychostimulants would decrease activity in DAT-KO mice by enhancing serotonin's inhibitory effects over dopaminergic hyperactivity, rather than by acting directly on the DA system (29). It should be mentioned, however, that 5-HT exerts an extremely complex set of actions on locomotor behaviors (72), and some of the 14 subtypes of 5-HT receptors known to date can induce opposite actions on locomotion. To determine which subtype(s) of 5-HT receptors are primarily involved in the hypolocomotor effect of psychostimulants represents a major challenge for future research.

Overall, the DAT-KO mice display several key characteristics of ADHD, including hyperactivity, impairments in cognitive tests, and paradoxical inhibitory responses to psychostimulants (29, 81, 82). Similarly, mice with reduced (<10%) expression of DAT demonstrate a modest hyperactivity, impaired response habituation, and paradoxical hypolocomotor reactions to amphetamine (46). In striking contrast, transgenic mice with modestly increased DAT expression (+20%–30%) show hypoactivity, which is particularly evident in a new environment (83).

One intriguing feature of ADHD is that in this developmental disorder, a significant reduction in the number of affected individuals occurs with age (76). These observations indicate that during the course of the disorder susceptibility to pathological manifestations and therapeutic responses to psychostimulants may vary. It is worthwhile mentioning that the DAT and SERT follow divergent patterns of expression in various brain areas through postnatal development (53, 54, 84), and altered responses to psychostimulants in different age groups, even in normal subjects, might occur (85).

It should be noted, however, that there are obvious caveats about this model (29, 81, 82). It is unlikely that complete functional absence of DAT occurs in ADHD patients, and as such DAT-KO mice represent an extreme case of a potential DAT dysfunction. Furthermore, multiple genes most likely contribute to this disorder and DAT-KO mice illustrate only one potential cause of these manifestations. It should be emphasized that dopaminergic dysregulation, as that observed with the DAT-KO mice, might be produced by defects in components of the system other than DAT. Moreover, dysregulation of the DA system is unlikely to be the exclusive mechanism responsible for the development of ADHD.

SEROTONIN TRANSPORTER-DEFICIENT MICE

Behavior and Pharmacology

Mice lacking the serotonin transporter (SERT-KO) were developed by homologous recombination (24). Despite evidence that an excess of 5-HT during

development may disrupt several critical processes of embryogenesis, only minor developmental abnormalities (24), such as abnormal gastrointestinal motility (86), were observed in the mutants. The role of SERT in locomotor and rewarding effects of several psychostimulants has been probed in SERT-KO mice. It has been reported that the ability of SERT-specific amphetamine derivative (+)-3,4-methylenedioxymethamphetamine (MDMA) (87) to induce locomotor activation was disrupted in mutant mice but *d*-amphetamine at high doses induced hyperactivity similarly in both SERT-KO and normal mice (24). Rewarding properties of cocaine were preserved in these mice as reflected by pronounced, and even increased, conditioned place preference to cocaine, in comparison to wild-type mice (28). Most intriguingly, in double-knockout mice lacking both DAT and SERT, no place preference for cocaine was observed, highlighting the contribution of SERT-mediated effects of cocaine to the reward process (69). Thus, these results suggest that in the absence of DAT the action of cocaine at the SERT may be sufficient to induce cocaine reward. However, it should be underscored that elimination of DAT creates a situation when extracellular levels of DA are abnormally elevated fivefold (36), which is in fact higher than what maximal doses of cocaine can produce in wild-type mice. This genetic unmasking indicates that SERT-mediated effects may play an important role in the effects of psychostimulants, and certainly these serotonergic mechanisms deserve closer scrutiny as potential ways to modulate reward mechanisms. In contrast, in double-knockout mice lacking both SERT and NET, rewarding properties of cocaine in CPP test were enhanced, suggesting that the actions of cocaine on the NET system may result, in fact, in aversive effects (88).

Extracellular 5-HT Dynamics, Presynaptic Homeostasis, and 5-HT Receptor Responsiveness

Neurochemical studies on 5-HT neuron homeostasis performed in SERT-KO mice in general recapitulate the observations gained in DAT-KO mice with respect to DA transmission (36, 39). In initial studies, a lack of high-affinity [^{3}H]5-HT uptake in brain synaptosomes from SERT-KO mice was found (24), but in primary neuronal cultures from embryonic SERT-KO mice, [^{3}H]5-HT uptake, although very weak, was observed (15). In vivo microdialysis studies have shown that disrupted uptake of 5-HT in SERT-KO mice results in a substantial increase in extracellular levels of 5-HT (five- to sixfold) (89, 90). A marked reduction (60%–80%) in 5-HT tissue levels was found in several brain regions of SERT-KO mice (24, 89, 90), suggesting deficient intraneuronal storage of 5-HT. Furthermore, 5-HT synthesis was disinhibited in SERT-KO mice (89).

The consequences of altered neurotransmission in SERT-KO mice on the responsiveness of 5-HT receptors have been extensively documented. In electrophysiological studies (91), a marked desensitization of both pre- and postsynaptic 5-HT1A receptors was found in SERT-KO mice, whereas only presynaptic receptors were affected in the heterozygous mice. Accordingly, 5-HT1A binding

sites, mRNA, and protein levels were significantly decreased in certain, but not all, serotonergic brain areas (92). Altered hypothermic and neuroendocrine responses to the 5-HT1A agonist 8-OH-DPAT were also noted in SERT-KO mice (92). Similar alterations were also found with respect to 5-HT1B receptors (90). Quantification of [^{35}S]GTP-γ-S binding evoked by potent 5-HT1A and 5-HT1B receptor agonists revealed a decrease in receptor coupling in the dorsal raphe nucleus and the substantia nigra, but not in other brain areas in SERT-KO mice (90). Accordingly, a decrease in brain 5-HT turnover rate after administration of the 5-HT1A agonist ipsapirone and an increased 5-HT release in the substantia nigra by 5-HT1B/1D antagonist GR 127935 were disrupted in SERT-KO mice (90). In addition, the density of postsynaptic 5-HT2A receptors in SERT mutants was decreased in some brain areas (93). These data convincingly demonstrate that autoreceptor function is remarkably desensitized or down-regulated in SERT-KO mice, whereas postsynaptic receptor regulation may be more complex, depending on the brain area and the receptor subtype.

NOREPINEPHRINE TRANSPORTER-DEFICIENT MICE

Behavior and Pharmacology

Using homologous recombination, mice lacking the NET (NET-KO) have recently become available (25, 94). The homozygous NET-KO mice display both lower body weight and locomotor activity in a new environment. Similar to wild-type mice treated with antidepressants, untreated NET-KO mice exhibit prolonged escape attempts in either tail suspension test or the forced swim test, two commonly used tests for behavioral screening of antidepressants (25). No additional effect of NET-specific antidepressant desipramine in the tail suspension test was found in mutant mice, but, interestingly, antidepressants affecting primarily SERT and DAT (paroxetine and bupropione, respectively) were also ineffective (25). In the warm water tail immersion pain assay, a greater degree of morphine analgesia was found in NET-KO mice; the effect apparently mediated by enhanced NE stimulation of α2-adrenoreceptors (95). Interestingly, desipramine did not affect basal level of analgesia in the NET-KO mice, but was still able to produce significant inhibitory action on the locomotor activity of these mutants, suggesting that not all the effects of this drug are mediated exclusively via interaction with the NET (95). Both cocaine and amphetamine were more effective in stimulating locomotion of NET-KO mice (25). Moreover, chronic cocaine treatment produced no significant enhancement of cocaine responses in NET-KO mice, while producing a robust sensitized effect in wild-type controls. In addition, somewhat increased rewarding properties of cocaine were observed in NET-KO mice (25), and an even more pronounced CPP to cocaine was observed in SERT/NET double-knockout mutant mice (88). These enhanced responses to psychostimulants have been accompanied with suppression of striatal dopaminergic function and

postsynaptic D2/D3 DA receptor supersensitivity (25). Intriguingly, D2/D3 DA receptor supersensitivity has been previously found in experimental animals treated chronically with either noradrenergic or serotonergic antidepressants, and this supersensitivity has been hypothesized to be a common final pathway of antidepressant action (96).

Extracellular NE Dynamics, Presynaptic Homeostasis, and Adrenergic Receptor Responsiveness

Like DAT-KO and SERT-KO mice, the NET-KO mice demonstrated profound alterations in extracellular monoamine dynamics, presynaptic neuron homeostasis, and postsynaptic receptor regulation (25). In the fast-scan cyclic voltammetry experiments performed in the bed nucleus of the stria terminalis, pars ventralis, the release of NE in response to electrical stimulation was reduced by approximately twofold and the rates of clearance following stimulation were at least sixfold slower in the NET-KO mice (25). The extracellular NE levels in the cerebellum were elevated twofold in mutant mice as evidenced by quantitative microdialysis (25). In NE-enriched brain regions such as prefrontal cortex, hippocampus, cerebellum, and spinal cord tissue, concentrations of NE, reflective of intraneuronal NE storage, were approximately 55%–70% lower in mutant mice (25, 95). The synthesis rate of NE, assessed in the hippocampus, was augmented approximately 1.7-fold over that of wild-type littermates (25). As a result of elevated extracellular levels of NE, a significant decrease (30%) in postsynaptic α1-adrenergic receptor binding in hippocampus of NET-KO mice was observed (25). However, in the binding assessment of α2-adrenergic receptor density in the spinal cord, no significant alterations were found in these mutants (95).

NET-KO mice were used to demonstrate heterologous uptake of catecholamines in certain brain areas. Particularly, in the frontal cortex synaptosomes from NET-KO mice, no inhibitory effect of cocaine and nisoxetine on uptake of DA was found, whereas effectiveness of cocaine in the nucleus accumbens was somewhat reduced (16). Thus, it has been demonstrated that DA uptake in brain regions with low levels of the DAT may occur via NET (16). Similar conclusions were reached recently when voltammetric analyses of catecholamine clearance in the frontal cortex (97) and bed nucleus of the stria terminalis (98) were performed.

Taken together, these results support and extend the observations made in the DAT-KO and SERT-KO mice, where disruption of transporters is found to exert similar changes in homeostasis of corresponding neurons.

CONCLUSIONS

Neurochemical investigations performed in transporter mutant mice clearly illustrate that elimination of the active transport process results in a fundamental shift in the mode of neuronal transmission (23–25, 36, 39, 89) (Table 1). Absence

TABLE 1 Alterations in monoamine homeostasis in DAT-KO, SERT-KO, and NET-KO mice

Neurochemical parameters of respective monoamines	DAT-KO mice	SERT-KO mice	NET-KO mice
Extracellular clearance rate	Prolonged (300–fold) (23, 36, 38)	Not tested	Prolonged (sixfold) (25)
Amplitude of stimulated release	Decreased by 75%–90% (23, 36, 38)	Not tested	Decreased by 55% (25)
Basal extracellular levels	Elevated fivefold (36)	Elevated five- to sixfold (89, 90)	Elevated twofold (25)
Tissue content (storage)	Decreased by 95% (36)	Decreased by 65%–80% (24, 89, 90)	Decreased by 55%–75% (25, 95)
Synthesis rate	Elevated twofold (36)	Elevated (89)	Elevated by 70% (25)
Autoreceptor function	Disrupted (43)	Disrupted (90–92)	Not tested
Postsynaptic receptors	Down-regulated, but some population is supersensitive (23, 40, 44)	Down-regulated, but not uniformly (90–93)	Down-regulated (25)

of transporter function results in expected disruption of extracellular monoamine clearance and prolonged extracellular lifetime of monoamines. The most remarkable (300-fold) protraction of extracellular lifetime was found for DA in the striatum of DAT-KO mice (36). Currently, data on 5-HT clearance in the SERT-KO mice are not available to make a direct comparison. In the NET-KO mice (25), the clearance rate of NE was prolonged by only sixfold. The difference in the magnitude of alterations in clearance of monoamines observed in DAT-KO and NET-KO mice is essentially due to differences in the intrinsic clearance rates of NE and DA in normal animals because the residual clearance of NE and DA in the two respective mutants is essentially identical (25, 36). Furthermore, the assessment of DA clearance was performed in the striatum of DAT-KO mice where the highest levels of DAT are normally expressed, whereas measurements of NE dynamics were performed in the bed nucleus stria terminalis, one of the many areas expressing modest levels of NET (10, 12, 98). Thus, regional differences in transporter expression per neuron may contribute to these differences.

As a result of the protracted clearance, extracellular levels of the respective monoamines are elevated in all three mutant strains (25, 36, 89, 90), but the degree of elevation is quite variable. For example, a fivefold increase in extracellular DA was observed in DAT-KO mice (36), five- to sixfold elevation of 5-HT levels in SERT-KO mice (89, 90), but only a twofold elevation in extracellular

NE levels was found in NET-KO mice (25). Importantly, these extracellular levels of monoamines are substantially lower than could be predicted from alterations in clearance of monoamines. It is worth mentioning in this regard, that the actual amount of monoamine released per pulse was decreased for both DA and NE in mutant mice (25, 36). In addition, metabolic enzymes and potentially, additional transporter systems could contribute to the clearance of monoamines, and this contribution could vary for each neurotransmitter in each particular region.

In general, a concept of a diffusion-mediated extrasynaptic mode of neurotransmission, termed volume, paracrine, or nonsynaptic transmission (11, 52, 99), may be a suitable framework to describe the extracellular fate of monoamines in mice lacking plasma membrane monoamine transporters (36, 39). Volume transmission has been postulated for neuropeptides (52, 99) and also for classical neurotransmitters, including DA in the frontal cortex (11, 47, 48, 97) and 5-HT in many regions of the brain (11). Levels of transporter expression per neuron, alongside with the synaptic organization of the anatomical area and the proximity of release sites and receptors, could be an important contributor to the mode of transmission for a given neurotransmitter in a given brain area (36, 52).

One of the most striking observations made in mice lacking monoamine transporters is the depletion of intraneuronal storage of monoamines. These observations indicate a tight dependence of the intraneuronal storage on the monoamine uptake system (36, 39). Furthermore, monoamine synthesis in mutant mice is not decreased, but in fact seems to be elevated, strongly indicating that the contribution of newly synthesized monoamines to maintenance of storage is negligible. It is tempting to speculate that depleted monoamine storage in these mice may change several critical intracellular processes and, for example, may account for the altered regulation of monoamine synthesis (36) in the mutant mice.

There are several reports describing depletions in monoamine content after chronic administration of drugs interacting with monoamine transporters (17–22, 87). However, the depletions induced by drugs like amphetamines or cocaine were, as a rule, interpreted as a consequence of neurotoxicity induced be these drugs (60). Nevertheless, several observations have shown that these depletions do not necessarily reflect damage or loss of DA neurons (17, 18). For example, it has been reported that in chronic methamphetamine abusers, reductions in striatal DA, TH, and DAT levels, but not VMAT2 or DOPA decarboxylase levels, occur, demonstrating that DA depletion following methamphetamine in these subjects does not necessarily reflect the destruction of DA neurons, but might be a consequence of a chronically diminished DA reuptake process (18). Modest decreases in 5-HT content are found in brain tissues following chronic treatment with drugs interacting with the SERT (19, 20, 87). Similar decreases in brain NE levels are observed following chronic treatment with NET inhibitors (21, 22). These observations, along with the findings from transporter mutant mice, suggest that caution should be taken in the interpretation of monoamine depletion produced by the

drugs that interfere with monoamine transporters. Direct depletion of monoamine storage due to diminished monoamine transporter function may also account for these effects. The relative inefficiency of most current monoamine transporter inhibitors to significantly affect monoamine storage may be due to the relatively low potency or short-term duration of action of these drugs. It might be proposed that depletion of monoamines by monoamine transporter inhibitors may take place only following long-term effective blockade of reuptake. The studies employing new generations of extremely potent monoamine transporter inhibitors (100, 101) could potentially resolve this issue. It would be of interest to explore how these homeostatic changes can contribute to adverse or beneficial effects of treatments with monoamine transporter inhibitors.

Furthermore, because pharmacological effects or adverse actions of many drugs interacting with monoamine transporters involve chronic treatment, an important lesson learned from these mice relates to the remarkable plasticity in the regulation of receptor function in response to persistently elevated monoaminergic tone. The persistently elevated monoamine levels result in down-regulation and functional desensitization of presynaptic autoreceptors in both DAT-KO (43) and SERT-KO (90–92) mice. With regard to postsynaptic receptors, a more complex picture seems to emerge. In general, studies in all three mutant mice depict a down-regulation of major postsynaptic receptors (23, 25, 44, 90–93). However, this down-regulation was not uniform, and some populations of receptors were found to be up-regulated (44, 90) or not affected (90). Although this nonuniform pattern in postsynaptic receptor regulation is not well understood, it is possible that the relative localization of postsynaptic receptors (synaptic versus extrasynaptic) may determine these differences.

An interesting aspect of CNS function revealed by the studies of monoamine transporter mutant mice is the subtle interplay between neurotransmitter systems. Genetic manipulation of one system can create a situation that results in the unmasking of subtle, but nonetheless important, contributions of another neurotransmitter system. For example, the contribution of 5-HT and/or NE systems to the rewarding properties of psychostimulants had been suspected but never demonstrated in such a direct way (28, 57, 69, 88).

In summary, protracted clearance of monoamine from synaptic cleft, elevated monoamine extracellular levels, depletion of intraneuronal stores of transmitter, and disinhibition of neuronal amine synthesis can be considered as hallmarks of neuronal systems without active monoamine reuptake. Thus, the expression level of transporters in monoaminergic cell groups may determine both the profile of presynaptic monoaminergic homeostasis and the mode of extracellular monoamine transmission. The factors that affect transporter function such as development, aging, and pharmacological or environmental interventions may produce substantial shifts between these modalities. It would be of considerable interest to determine whether the regulatory control that the monoamine transporters exert over monoaminergic signaling could extend to all neurotransmitter systems with transporters.

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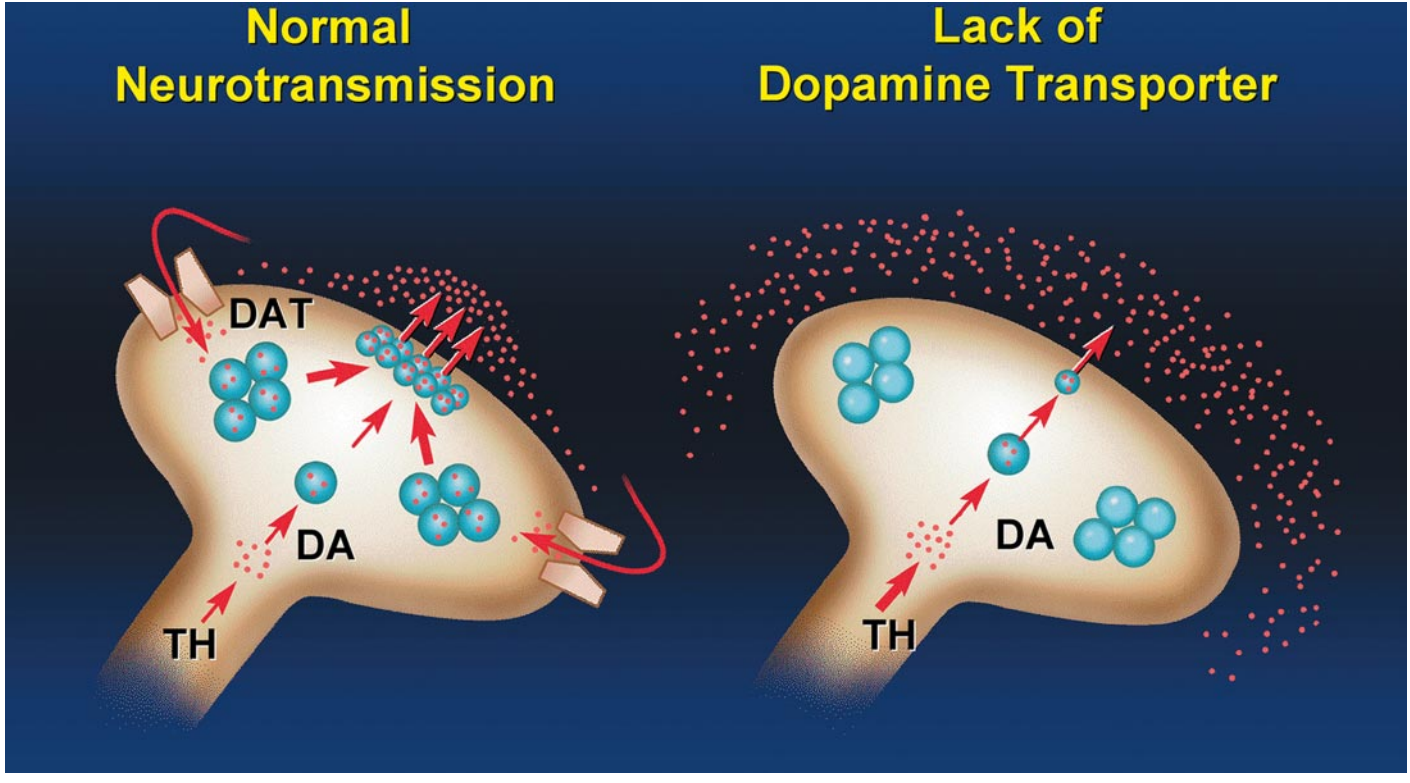

Figure 1 Hypothetical model of striatal dopaminergic terminal in normal and DAT-deficient mice. Elimination of the DAT results in fivefold elevated extracellular levels of DA. Lack of inward transport in DAT-KO mice also results in 20-fold decreased intracellular DA stores. These low intracellular levels of DA are extremely sensitive to inhibition of DA synthesis, suggesting that they represent the newly synthesized DA pool. Note that synthesis rate is increased in DAT-KO mice, but cannot restore the deficiency in DA storage in DAT-KO mice (36, 39). Whether in DAT-KO mice only a few vesicles contain substantial amount of DA to account for the remaining (5%) levels of stored DA or whether all vesicles contain 5% of the normal content has not been formally tested and requires further investigations.