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SPECIAL ARTICLE

Dopamine transporter mutant mice in experimental neuropharmacology

Raul R. Gainetdinov

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Abstract An opportunity to perform targeted genetic manipulations in mice has provided another dimension for modern pharmacological research. Genetically modified mice have become important tools to investigate functions of previously unexplored proteins, define mechanism of action of new and known pharmacological drugs, and validate novel targets for treatment of human disorders. One of the best examples of such use of genetic models in experimental pharmacology represents investigations involving mice deficient in the gene encoding the dopamine transporter (DAT). The dopamine transporter tightly regulates the extracellular dynamics of dopamine by recapturing released neurotransmitter into the presynaptic terminals, and genetic deletion of this protein results in profound alterations in both the presynaptic homeostasis and the extracellular dynamics of dopamine. By using this model of severe dopaminergic dysregulation, significant progress has been made in defining the major target of psychotropic drugs, understanding the mechanisms of their action, unraveling novel signaling events relevant for dopaminergic transmission, and mapping neuronal pathways involved in dopamine-related behaviors. Furthermore, DAT mutant mice provided an opportunity to model in vivo conditions of extreme dopaminergic dysfunction that could be relevant for human disorders such as ADHD, schizophrenia, and Parkinson's disease and, thus, could serve as test systems for developing novel treatments for these and related disorders.

R. R. Gainetdinov (✉)
Department of Cell Biology, Duke University Medical Center,
Durham 27710 NC, USA
e-mail: r.gainetdinov@cellbio.duke.edu

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Introduction

The basal ganglia form a forebrain system that is involved in various aspects of motor activity, emotions, reward, and affect. Disorders related to basal ganglia dysfunction comprise a spectrum of abnormalities that range from the movement disorders such as Parkinson's disease (PD) and Huntington's disease to major mental disorders including schizophrenia, bipolar disorder, attention deficit hyperactivity disorder (ADHD), and Tourette syndrome. Major monoaminergic neurotransmitter in the basal ganglia, dopamine (DA), exerts an important modulatory influence over the fast neurotransmission mediated by glutamate and GABAergic neurons and may play a critical pathophysiological role in these disorders (Hornykiewicz 1998; Carlsson et al. 2001).

Efficacy of dopaminergic transmission is controlled not only by processes governing DA release but also by regulation of extracellular DA concentrations via rapid reuptake by the plasma membrane dopamine transporter (DAT) (Kuhar et al. 1990; Amara and Sonders 1998; Torres et al. 2003). DAT is a member of family of the Na^+/Cl^--dependent transporters that also includes transporters for serotonin (SERT), norepinephrine (NET), GABA, glycine, creatine, betaine, taurine, and proline (Giros and Caron 1993; Amara and Sonders 1998; Torres et al. 2003). DAT protein contains 12 transmembrane domains with both amino- and carboxy-termini located in the cytoplasm. DAT-mediated transport of DA involves sequential binding and

cotransport of two Na^+ ions and one Cl^- ion with one molecule of DA (Amara and Sonders 1998; Torres et al. 2003; Chen et al. 2004). DAT is expressed exclusively in the dopaminergic cell bodies and terminals and can serve as a selective marker of these neurons (Ciliax et al. 1995; Hoffman et al. 1998). In the brain, DAT has highest expression in the striatum and nucleus accumbens followed by the olfactory tubercle, hypothalamic nuclei, and prefrontal cortex (Ciliax et al. 1995; Hoffman et al. 1998). In the periphery, DAT is expressed in the retina, gastrointestinal tract, lung, kidney, pancreas, and lymphocytes (Amenta et al. 2001; Gordon and Barnes 2003; Sotnikova et al. 2006a). Ultrastructural analysis has revealed that DAT is mostly localized perisynaptically rather than in the synaptic compartments (Nirenberg et al. 1997; Sesack et al. 1998), supporting earlier estimations that reuptake of dopamine occurs at a distance from release sites (Garris and Wightman 1994).

It is believed that DAT-mediated removal of DA from the extracellular space provides the most important regulatory control of the extracellular lifetime of the monoamine. Due to this major role of the DAT, compounds interacting with the DAT are proven to be very powerful tools to affect dopamine-related functions. Among them, the best known are psychostimulants amphetamine and cocaine, that potently elevate extracellular DA concentrations and, thereby, induce psychomotor and rewarding effects (Kuhar et al. 1990; Wise 1994). DAT is also a well-recognized target and gateway for dopaminergic neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine that can induce death of midbrain dopaminergic neurons (Langston et al. 1984; Uhl 1998; Miller et al. 1999).

Development of mice with abnormal DAT function provided a novel test system to unravel mechanisms of action of psychotropic drugs (Giros et al. 1996; Gainetdinov and Caron 2003). In particular, these mutants have proven to be extremely valuable in experimental protocols aimed at understanding the specificity and the mechanism of a psychostimulant drug action (Gainetdinov et al. 2002). In addition, effects of pharmacological compounds potentially active in counteracting consequences of dopaminergic dysfunction can be directly investigated in these mice. Finally, these mice provided an opportunity to model major endophenotypes of human disorders, such as ADHD, where responses to pharmacological treatments can be investigated experimentally. In this review, the recent advances in understanding mechanisms of action of pharmacological agents by using DAT mutant mice are summarized. While most of these studies employed DAT knockout (DAT-KO) and heterozygous mice (Giros et al. 1996), investigations involving mice with severely reduced DAT levels (DAT knockdown, DAT-KD; Zhuang et al. 2001) and mice with the moderately increased DAT levels (DAT overexpressing mice; Donovan et al. 1999) were also very informative.

Neurochemical and behavioral characteristics of DAT-KO mice

DAT-KO mice display remarkable alterations in both the intraneuronal and extracellular DA dynamics (Giros et al. 1996; Gainetdinov and Caron 2003). These alterations, highlighting a critical role of the DAT in proper maintenance of extracellular DA and its presynaptic homeostasis, have been described in detail in several review articles (Gainetdinov and Caron 2003; Torres et al. 2003) and will be covered just briefly in this essay. Disrupted clearance of released DA in these mice resulted in about a 300-fold increase in the lifetime of DA in the extracellular space, as evidenced by cyclic voltammetry measurements, and at least fivefold elevation in the basal extracellular DA levels, as detected by in vivo microdialysis (Gainetdinov et al. 1998; Jones et al. 1998a; Shen et al. 2004). Even more strikingly, a profound depletion of intraneuronal dopamine stores (20-fold) and decreased amplitude of evoked dopamine release (4-fold) was found in DAT-KO mice (Gainetdinov et al. 1998; Jones et al. 1998a). Without dopamine-uptake-mediated recycling, dopamine levels in the striatum of these mice are totally dependent on the rate of its ongoing synthesis. Inhibition of the rate-limiting enzyme in DA synthesis, tyrosine hydroxylase (TH), essentially eliminates dopamine in the striatum of mutant mice (Jones et al. 1998a; Sotnikova et al. 2005). Thus, DA levels in DAT- KO mice represent primarily a newly synthesized pool (Gainetdinov et al. 1998; Jones et al. 1998a; Benoit-Marand et al. 2000; Sotnikova et al. 2005). These observations strongly suggest that in normal situation, major DA storage pools in presynaptic striatal terminals must be controlled by the DAT-mediated DA recycling (Jones et al. 1998a; Gainetdinov and Caron 2003).

DA receptors undergo significant regulation in response to persistently increased dopaminergic tone. A loss of autoreceptor function was found in DAT-KO mice as evidenced by a marked desensitization of the major autoreceptor functions: regulation of neuronal firing rate and DA release and synthesis (Jones et al. 1999). At the same time, D1 and D2 DA receptors are down-regulated by approximately 50% in the striatum of DAT-KO mice (Giros et al. 1996), but paradoxically, some populations of postsynaptic DA receptors appear to be supersensitive (Gainetdinov et al. 1999a; Fauchey et al. 2000; Seeman et al. 2007). Importantly also, the level of the scaffolding protein postsynaptic density-95 (PSD-95) is significantly

reduced in the striatum and nucleus accumbens of DAT-KO mice. In addition, an enhanced long-term potentiation (LTP) of the cortico-accumbal (Yao et al. 2004) and hippocampal (Morice et al. 2007) glutamatergic synapses and markedly reduced long-term depression (LTD) in the hippocampus (Morice et al. 2007) were found in these mutants, indicating a role of DA in modulating synaptic plasticity in these brain areas. Intriguingly also, levels of brain-derived neurotrophic factor (BDNF) gene expression are reduced in the frontal cortex of intact DAT-KO mice (Fumagalli et al. 2003). Many of these neurochemical abnormalities have a clear gene-dose effect, and mice heterozygous for DAT deletion show intermediate alterations with, for example, extracellular DA levels elevated twofold (Jones et al. 1998a, 1999). Furthermore, in mice expressing only about 10% of DAT (DAT knockdown, DAT-KD) mice (Zhuang et al. 2001), these changes were generally more pronounced in comparison to DAT heterozygous mice, but nevertheless markedly less than that observed in DAT-KO mice (Jones et al. 1998a).

Apart from its function in the regulation of efficacy of DA transmission, DAT is also critical for the neurotoxic reactions induced by large doses of amphetamine derivatives and dopaminergic neurotoxins (Langston et al. 1984; Uhl 1998; Miller et al. 1999; Sotnikova et al. 2006a). Toxic lesions of dopaminergic neurons by using MPTP is a well-established approach in the modeling PD in experimental animals (Snyder and D'Amato 1986; Kopin 1992; Tipton and Singer 1993). MPTP-induced death of dopaminergic neurons is mediated by its reactive metabolite 1-methyl-4-phenylpyridium (MPP^+) that is transported into dopaminergic terminals via the DAT (Heikkila et al. 1984). As expected, a lack of MPTP neurotoxicity was found in DAT-KO mice (Gainetdinov et al. 1997; Bezard et al. 1999). Significantly reduced dopaminergic neurodegeneration and lethality was observed in these mutant also after neurotoxic regimen of methamphetamine administration, indicating that DAT is essential for the neurotoxic actions of amphetamine-related compounds (Fumagalli et al. 1998; Numachi et al. 2007). Thus, DAT is clearly important for the degeneration of presynaptic DA neurons primarily by allowing the entry of toxic compounds into the DA terminals. At the same time, the persistently elevated extracellular DA levels can exert deleterious effect on the DA-responsive postsynaptic medium spiny GABA neurons (Cyr et al. 2003; Fernagut et al. 2003). It has been observed that up to 30% of DAT-KO mice sporadically develop progressive neurodegenerative phenotype characterized by a reduction of their typical hyperactivity and the appearance of dyskinetic movements that eventually leads to death of affected animals (Cyr et al. 2003). In symptomatic DAT-KO mice, loss of approximately 30% of GABAergic medium spiny neurons, accompanied by an accumulation of hyperphosphorylated microtubule-associated protein tau, was observed (Cyr et al. 2003). Furthermore, up-regulation of cyclin-dependent kinase 5 (CDK5) and ΔFosB accumulation was found in the striatal neurons of affected mice, thus, suggesting a role of sustained DA receptor signaling mechanism in the development of this phenotype (Cyr et al. 2003; Sotnikova et al. 2006a). Notably, a similar type of damage to postsynaptic GABAergic neurons was recently described after administration of amphetamine-related compounds to normal animals (Krasnova et al. 2005). In addition, DAT-KO mice were found to be hypersensitive to 3-nitropropionic acid-induced injury to striatal GABA neurons (Fernagut et al. 2002). Loss of medium spiny GABAergic neurons is a hallmark of Huntington disease, known to be resulting from an expansion in the CAG repeat of the IT15 (huntingtin) gene. To investigate if excessive DA stimulation can increase vulnerability to this mutation, a double mutant mouse strain was developed by crossing DAT-KO mice to a knock-in mouse model of HD containing 92 CAG repeats. In these double mutant mice, a significant age-dependent motor abnormalities and increased huntingtin protein aggregates were found, suggesting that the excessive DA contributes to the deleterious effects of mutated huntingtin on striatal function and may be an important factor in the pathogenesis of Huntington disease (Cyr et al. 2006).

In response to permanently enhanced DA tone, DAT-KO mice display a very characteristic behavioral phenotype. Most notably, DAT-KO mice show perseverative locomotor hyperactivity and stereotypy in a novel environment, have growth deficit and multiple behavioral abnormalities related to cognitive inflexibility. Particularly, deficits were detected in eight-arm maze and Morris water maze learning and memory tests, pre-pulse inhibition sensorimotor gating test and measures of behavioral lateralization. Furthermore, sleep dysregulation and specific alterations in social interaction related to behavioral inflexibility have been also reported (Giros et al. 1996; Bosse et al. 1997; Sora et al. 1998; Gainetdinov et al. 1999b; Spielewoy et al. 2000b; Ralph et al. 2001; Wisor et al. 2001; Gainetdinov and Caron 2003; Morice et al. 2005, 2007; Rodriguiz et al. 2004; Pogorelov et al. 2005). In tests assessing the rewarding values of tastants or food, DAT-KO mice develop a more positive bias towards a hedonically positive tastant (Costa et al. 2007) and enhanced resistance to extinction from food-reinforced operant behavior (Hironaka et al. 2004), thus, reflecting the role of dopamine in updating rewarding values and habit learning and memory. In the periphery, lack of DAT resulted in skeletal abnormalities (Bliziotes et al. 2000), alterations in gut motility (Walker et al. 2000) and respiratory control (Vincent et al. 2007).

DAT-KO mice as a tool to define targets and mechanisms of action of psychotropic drugs

Modulation of DA levels via an interaction or blockade of DAT has proven to be a very effective approach to affect critical functions mediated by DA in a variety of pathological conditions (Kuhar et al. 1990). Many psychostimulants (such as cocaine and amphetamine) and several antidepressants (such as bupropion) exert their actions, at least in part, via interference with DAT function. Availability of mice with altered DAT function provided in vivo test model to define the major target and/or unmask the secondary targets of psychostimulants and other drugs (Table 1).

In the striatum of DAT-KO mice, psychostimulants cocaine, methylphenidate, amphetamine, and methamphetamine did not affect DA clearance or extracellular levels (Fumagalli et al. 1998; Jones et al. 1998a, b; Gainetdinov et al. 1999b) clearly indicating the critical role of DAT in the effects of psychostimulants on dopamine dynamics in this brain area. While cocaine and methylphenidate are classical DA reuptake blockers, amphetamine derivatives have a more complex mechanism of action, involving interaction not only with the DAT but also with the vesicular storage (Sulzer et al. 1995). Amphetamine can enter the DA neuron both through the DAT and transmembrane diffusion (Seiden et al. 1993). Inside the nerve terminal amphetamine penetrates the vesicles, at least partially, via the vesicular monoamine transporter 2 (VMAT2) and as "a weak base" disrupts the pH gradient in vesicles (Sulzer et al. 1995). It is believed that this process causes the redistribution of DA from vesicles into the cytoplasm that, in turn, triggers efflux of DA from the cytoplasm into the extracellular space via outward DAT-mediated transport. It should be noted also that amphetamine derivatives can directly inhibit monoamine oxidase (MAO), thus, potentially affecting intraneuronal metabolism of DA (Seiden et al. 1993). Accordingly, in mice lacking the DAT, no effect of amphetamine on the extracellular DA dynamics in the striatum was found, but intraneuronal actions of amphetamine were still observed (Jones et al. 1998b).

Importantly, while DAT seems to be critical for effects of cocaine and amphetamine on DA levels in the striatum, both cocaine and amphetamine were still able to elevate extracellular DA in the nucleus accumbens of DAT-KO mice (Carboni et al. 2001; Budygin et al. 2004; Sotnikova et al. 2006a). These observations could provide an explanation for the fact that DAT-KO mice are able to self-administer cocaine (Rocha et al. 1998) and display conditioned place preference (CPP) for cocaine (Sora et al. 1998; Hall et al. 2002; Mateo et al. 2004; Medvedev et al. 2005) and amphetamine (Budygin et al. 2004). These results indicate that the DAT is not the sole mediator of rewarding properties of psychostimulants, and other targets of these drugs should be also considered. There is some evidence that these unexpected effects could be related to the effects of psychostimulants on the NET that may contribute to the clearance of extracellular DA in the nucleus accumbens and frontal cortex (Carboni et al. 2001; Moron et al. 2002). At the same time, several lines of evidence suggest a more important role of indirect modulation of dopaminergic neurons via 5-HT system at the level of cell bodies (Budygin et al. 2002, 2004; Mateo et al. 2004). This hypothesis received a strong support from studies employing double mutant mice lacking both the DAT and the SERT that were found to have disrupted preference for cocaine in CPP test (Sora et al. 2001), thus, directly demonstrating that the interaction with the SERT is sufficient to induce cocaine reward in hyperdopaminergic mice (Hall et al. 2002; Mateo et al. 2004).

Persistent hyperdopaminergia and related behavioral manifestations in DAT-KO mice have provided a valuable model to test effects of drugs that could be effective in disorders that may involve dopaminergic hyperfunction (Carlsson and Carlsson 1990; Gainetdinov et al. 2001b; Arguello and Gogos 2006; Swanson et al. 2007). Pronounced locomotor hyperactivity in a novel environment of DAT-KO mice provides a very sensitive and simple test system in which the actions of pharmacological agents in regulating DA-related function can be easily assessed (Gainetdinov et al. 1999b, 2001b Spielewoy et al. 2000b). Hyperactivity of DAT-KO mice is very sensitive to manipulations with DA transmission such as inhibition of DA synthesis or blockade of DA receptors with haloperidol, clozapine, raclopride, and SCH23390 (Gainetdinov et al. 1999b; Ralph et al. 2001). However, this dopamine-dependent hyperactivity can be also effectively modulated via other neurotransmitter systems. For example, amphetamine, methylphenidate, cocaine, 3,4-methylenedioxymethamphetamine (MDMA) (Gainetdinov et al. 1999b; Spielewoy et al. 2001; Morice et al. 2004a; Powell et al. 2004) and an "endogenous amphetamine" trace amine β-phenylethylamine (Sotnikova et al. 2004) demonstrate potent stimulatory effects in normal animals, but paradoxically inhibit hyperactivity in DAT-KO mice. An inhibition of hyperactivity by amphetamine was found also in DAT-KD mice (Zhuang et al. 2001) and, at certain doses, observed in Dat heterozygous mice (Spielewoy et al. 2001). Similarly, reduced locomotor responses and attenuated sensitization to methamphetamine was observed in heterozygous DAT mutant mice (Fukushima et al. 2007). We hypothesized that the well-known SERT-mediated effects of psychostimulants on serotonin neurotransmission could be involved in their inhibitory action on DA-dependent hyperactivity (Gainetdinov et al. 1999b). In fact, various serotonergic drugs, such as the SERT inhibitor

Table 1 Major pharmacological compounds investigated in DAT-KO mice

Compounds tested	Effects found
Cocaine	Had no effect on clearance and extracellular DA in the striatum (Giros et al. 1996; Jones et al. 1998a; Rocha et al. 1998) Lack of effect on DA clearance (Budygin et al. 2002) and uptake (Moron et al. 2002), but elevated extracellular DA in the nucleus accumbens (Carboni et al. 2001; Sotnikova et al. 2006a) Induced self-administration (Rocha et al. 1998) and reward in CPP test (Sora et al. 1998; Hall et al. 2002; Mateo et al. 2004; Medvedev et al. 2005) Induced inhibition of locomotion (Gainetdinov et al. 1999b; Morice et al. 2004)
Amphetamine	Lack of effect on clearance and extracellular levels of DA in the striatum (Giros et al. 1996; Jones et al. 1998a; Gainetdinov et al. 1999b) but induced elevation of extracellular DA in the nucleus accumbens (Carboni et al. 2001; Budygin et al. 2004) Induced rewarding effects in CPP test (Budygin et al. 2004) Induced inhibition of locomotion (Gainetdinov et al. 1999b; Spielewoy et al. 2001; Beaulieu et al. 2006)
Methamphetamine	Lack of effect on extracellular DA in the striatum (Fumagalli et al. 1998) Reduced dopaminergic neurotoxicity and lethality (Fumagalli et al. 1998; Numachi et al. 2007)
Methylphenidate	Had no effect on extracellular levels of DA in the striatum (Gainetdinov et al. 1999b) Induced reward in CPP test (Sora et al. 1998) Induced inhibition of locomotion (Gainetdinov et al. 1999b)
MDMA	Inhibition of hyperactivity (Powell et al. 2004)
GBR12909	Lack of effect on extracellular DA in the nucleus accumbens (Carboni et al. 2001)
MPTP	Lack of dopaminergic neurotoxicity (Gainetdinov et al. 1997; Bezard et al. 1999)
Modafinil	Disrupted wake-promoting action (Wisor et al. 2001)
Fluoxetine	Induced inhibition of locomotion (Gainetdinov et al. 1999b; Spielewoy et al. 2001; Beaulieu et al. 2006) Induced rewarding effects in CPP test (Hall et al. 2004) Reversed deficit in PPI test (Yamashita et al. 2006)
Citalopram	No effect in PPI test (Yamashita et al. 2006)
Nisoxetine	Induced inhibition of DA uptake in the frontal cortex and nucleus accumbens (Moron et al. 2002) Had no effect on hyperactivity (Gainetdinov et al. 1999b) Induced rewarding effect in CPP test (Hall et al. 2004) Reversed deficits in PPI test (Yamashita et al. 2006)
Reboxetine	Induced increase in DA levels in the nucleus accumbens (Carboni et al. 2001)
Haloperidol	Inhibited locomotion (Gainetdinov et al. 1999b; Spielewoy et al. 2000b)
Clozapine	Inhibited locomotion (Spielewoy et al. 2000b)
Raclopride	Corrected PPI deficits, inhibited locomotion (Ralph et al. 2001)
SCH23390	Normalized perseverative pattern of locomotion, inhibited locomotion, but had no effect on PPI deficits (Ralph et al. 2001)
α methyl-p-tyrosine	Induced elimination of striatal DA (Jones et al. 1998a; Sotnikova et al. 2005) Induced rigidity and akinesia (Sotnikova et al. 2005)
5-HT precursors l-tryptophan and 5-hydroxytryptophan	Inhibited locomotor hyperactivity (Gainetdinov et al. 1999b)
5-HT agonists quipazine and 5-CT	Inhibited locomotor hyperactivity (Gainetdinov et al. 1999b; Beaulieu et al. 2006)
5-HT2A antagonist M100907	Inhibited locomotor hyperactivity, and reversed perseverative pattern of locomotion and PPI deficits (Barr et al. 2004)
(+)-MK-801	Markedly enhanced hyperactivity without effect on striatal DA release (Gainetdinov et al. 2001b)

Table 1 (continued)

Compounds tested	Effects found
Ampakines	Induced inhibition of hyperactivity without effect on striatal DA release (Gainetdinov et al. 2001b)
Morphine	Enhanced DA release in the nucleus accumbens and reward in CPP test, but disrupted hyperlocomotor responses (Spielewoy et al. 2000a)
Endocannabinoids	Inhibited locomotor hyperactivity (Tzavara et al. 2006)
Nicotine	Inhibited locomotor hyperactivity, improved spatial learning (Weiss et al. 2007a)
Ethanol	Sex-dependent differences in preference and consumption (Savelieva et al. 2002; Hall et al. 2003)
Antimanic drugs lithium and valproate	Inhibited locomotor hyperactivity (Beaulieu et al. 2004)
GSK3 inhibitors	Inhibited locomotor hyperactivity (Beaulieu et al. 2004)
ERK inhibitor SL327	Inhibited locomotor hyperactivity (Beaulieu et al. 2006)

fluoxetine, nonselective 5-HT agonists quipazine and 5-carboxamidotryptamine (5-CT), and 5-HT precursors tryptophan and 5-hydroxytryptophan were found to be effective in inhibiting hyperactivity of DAT-KO mice (Gainetdinov et al. 1999b; Beaulieu et al. 2006). It should be noted here that the concept of reciprocal roles of 5-HT and DA in psychostimulant-induced behavioral activation has been known for more than 30 years (Breese et al. 1975). Role of serotonergic tone in the hyperactivity phenotype of DAT-KO mice was indicated also by the differences in the level of hyperactivity and responses to cocaine in DAT-KO mice on C57BL/6J and DBA/2J inbred backgrounds (Morice et al. 2004) that are known to be significantly different in brain 5-HT synthesis due to a functional single nucleotide polymorphism of the neuronal 5-HT synthesis enzyme tryptophan hydroxylase 2 (TPH2) (Zhang et al. 2004).

While the inhibitory role of serotonin on dopamine-dependent hyperactivity strongly supported by several lines of evidence, it is still unclear which of the 14 known serotonin receptor subtypes could mediate this effect. Both "stimulatory" and "inhibitory" roles of 5-HT receptors as regard to behavioral activation have been described (Martin et al. 1998; Gainetdinov et al. 1999b; Rocha et al. 2002; Barr et al. 2004). The primary candidates for the "stimulatory" action are 5-HT1B and 5-HT2A receptors, while 5-HT1A and 5HT2C receptors are likely "inhibitory" receptors (Martin et al. 1998). One intriguing hypothesis suggests that that a balance between the action of 5-HT on these "stimulatory" and "inhibitory" serotonin receptors may be important for the behavioral activation (Martin et al. 1998), and thus, DA-related hyperactivity could be inhibited either by agonists of "inhibitory" receptors or antagonists of "stimulatory" 5-HT receptors. In fact, hyperactivity and other aberrant behaviors of DAT-KO mice can be potently suppressed either by direct and indirect 5-HT agonists (Gainetdinov et al. 1999b; Spielewoy et al. 2001; Morice et al. 2004; Powell et al. 2004; Beaulieu et al. 2006) or by an antagonist of "stimulatory" 5-HT2A receptors (Barr et al. 2004). To add to the complexity of the situation, a potential contribution of NE system in these effects cannot be excluded also.

Inhibitory effect of psychostimulants and serotonergic drugs in DAT-KO mice does not involve a direct modulation of dopaminergic activity, but rather depends on intact fronto-striatal glutamatergic transmission (Martin et al. 1998; Mohn et al. 1999; Gainetdinov et al. 2001a). Both DAT-KO and heterozygous mice are more sensitive to the locomotor-stimulating effect of the NMDA antagonist (+)-MK-801 with the dose–response curves shifted leftward in magnitude that is proportional to the differences in the basal levels of extracellular DA (fivefold in DAT-KO mice; twofold in DAT heterozygous mice) (Gainetdinov et al. 2001a). Furthermore, compounds that can enhance efficacy of glutamatergic transmission either via positive modulation of AMPA glutamate receptors, such as AMPAkines (Gainetdinov et al. 2001a) or via increase in glycine concentration, such as glycine transporter type 1 (Glyt1) inhibitors (unpublished observations), effectively suppress hyperactivity in DAT-KO mice. Finally, (+)-MK-801 pretreatment abolished inhibitory action of psychostimulants and serotonergic drugs in DAT-KO mice (Gainetdinov et al. 2001a). Thus, in DAT-KO mice, dopamine-related striatal responses are dependent on the intensity of fronto-striatal glutamatergic signaling, which, in turn, can be potently regulated by changes in 5-HT tone. The fact that facilitation of glutamatergic transmission can effectively counteract hyperdopaminergia-related behaviors indicates that drugs enhancing glutamate transmission may have therapeutic potential in disorders believed to be related to DA hyperfunction, such as schizophrenia and ADHD (Carlsson and Carlsson 1990; Johnson et al. 1999; Carlsson et al. 2001; Gainetdinov et al. 2001a; Arguello and Gogos 2006; Swanson et al. 2007).

It has been reported that stimulation of the "inhibitory" 5-HT2C receptors in the frontal cortex can suppress

DA-related hyperactivity (Rocha et al. 2002; Filip and Cunningham 2003), suggesting that the psychostimulant-induced elevation of extracellular 5-HT in the frontal cortex may be primarily responsible for the inhibitory effect of psychostimulants in DAT-KO mice. In fact, methylphenidate, amphetamine, and cocaine are known to affect frontal cortex neurons in DAT-KO mice as evidenced by an increase in c-*fos* expression and changes in 5-HT levels (Rocha et al. 1998; Gainetdinov et al. 2001a; Trinh et al. 2003).

Recently, we performed investigation of cellular signaling mechanisms that could be involved in the paradoxical inhibitory effects of psychostimulants in hyperactive mice (Beaulieu et al. 2006). Three major striatal signaling pathways, including protein kinase A (PKA)-mediated, Akt/glycogen synthase kinase 3 (GSK-3)-mediated, and ERK-mediated, that are known to be involved in the regulation of locomotor activity by dopamine, were investigated in DAT-KO mice after treatment with psychostimulants and various 5-HT agonists. These studies revealed that inhibition of ERK signaling is a common determinant for the ability of all drugs tested to antagonize hyperactivity. In contrast, in normal animals, psychostimulants increased phosphorylation of ERK. Direct inhibition of the ERK signaling cascade in vivo using the MEK inhibitor SL327 potently suppressed activity level in DAT-KO mice and blocked the locomotor-enhancing effect of amphetamine in normal mice. Thus, it has been concluded that the inhibitory action of psychostimulants on dopamine-dependent hyperactivity may involve serotonin-mediated regulation of striatal ERK signaling (Beaulieu et al. 2006).

Polymorphism in the DAT gene is one of the most commonly replicated findings in genetic ADHD research (Cook et al. 1995; Swanson et al. 2007). Psychostimulants, such as methylphenidate and amphetamine isomers remain the most effective class of drugs to manage hyperactivity, impulsivity, and inattention symptoms of ADHD. As discussed above, DAT-KO mice have pronounced hyperactivity and significant behavioral deficits related to cognitive inflexibility. Importantly, both the analysis of the pattern of locomotor activity and cognitive tests indicated that mutant animals display perseverative pattern of activation and cognitive errors, suggesting that these mice may have impaired behavioral inhibition (Gainetdinov et al. 1999b; Barr et al. 2004). Furthermore, extreme hyperactivity in DAT-KO mice can be inhibited by psychostimulants that are used in the treatment of ADHD (Gainetdinov et al. 1999b), and this effect is mediated, at least in part, via the enhancement of 5-HT tone. It is well established that 5-HT plays a critical role in impulse regulation and inhibitory control on external stimuli-induced behavioral activation (Lucki 1998; Winstanley et al. 2005), and thus, it might be expected that modulation of 5-HT system could affect multiple other behavioral abnormalities in DAT-KO mice. At the same time, it is clear that certain behavioral deficits in these mutants may be more sensitive to modulation of NE transmission. For example, it has been shown that the NET inhibitor nisoxetine, but not the SERT inhibitor citalopram, is effective in reversing the PPI deficits related to deficient sensorimotor gating in DAT-KO mice (Yamashita et al. 2006). Overall, the DAT-KO mice display several endophenotypes of ADHD, including hyperactivity, cognitive deficits, and paradoxical inhibitory responses to psychostimulants (Gainetdinov et al. 1999b; Gainetdinov and Caron 2000, 2001). Thus, DAT-KO mice can be used as an animal model that could be instrumental to understand the mechanism of action of psychostimulants in ADHD (Russell 2007; van der Kooij and Glennon 2007).

At the same time, DAT-KO mice could provide an opportunity to investigate effects of drugs on hyperdopaminergia-related endophenotypes of other disorders. Dopaminergic theory of schizophrenia suggests that elevated dopaminergic tone is the major cause of positive symptoms of schizophrenia (Carlsson and Carlsson 1990; Martin et al. 1998; Gainetdinov et al. 2001b; Arguello and Gogos 2006). Manic states of bipolar disorder also may be related to dopaminergic hyperfunction (Ralph-Williams et al. 2003). In fact, amphetamines and dopamine precursor L-DOPA are known to provoke psychotic reactions, while essentially all clinically effective antipsychotic drugs share ability to block dopamine D2 receptors at least partially (Martin et al. 1998). Thus, DAT-KO mice may be particularly useful to investigate dopamine-related effects of antipsychotic and antimanic drugs (Gainetdinov et al. 2001b; Ralph-Williams et al. 2003). One such investigation resulted in an identification of a novel signaling pathway critical for dopamine actions. In a pilot study, it has been observed that antimanic drugs lithium and valproate are effective in suppressing hyperactivity in DAT-KO mice (Beaulieu et al. 2004). Similarly, valproate attenuated hyperactive and perseverative behaviors in DAT-KD mice (Ralph-Williams et al. 2003). Combination of behavioral and biochemical approaches in genetically altered mice revealed that in addition to classical PKA-mediated signaling pathway, DA receptors can also engage lithium-sensitive signaling cascade involving Akt and glycogen synthase kinase 3 (GSK3). Akt and GSK3 are serine/threonine kinases are best known for their roles in the regulation of glycogenesis, cell survival, and development (Frame and Cohen 2001; Scheid and Woodgett 2001). Analysis of Akt and GSK3 activity in the striatum of DAT-KO mice revealed a marked inactivation of Akt and a concomitant activation of GSK3. In normal mice, similar changes in the activity of Akt and GSK3 were induced by amphetamine (Beaulieu et al. 2004). These changes in Akt and GSK3 activity were antagonized by D2

class receptor blockade (Beaulieu et al. 2004) and were absent in mice lacking D2 dopamine receptors (Beaulieu et al. 2007b), thus, directly validating the role of D2-class receptors in the regulation of the Akt/GSK3 signaling pathway. Inhibition of this pathway either genetically or pharmacologically resulted in significant attenuation of dopamine-related behaviors, and for example, several GSK3 inhibitors, in addition to lithium, were active in suppression of DA-related hyperactivity in DAT-KO mice (Beaulieu et al. 2004) and in amphetamine-treated normal animals (Gould et al. 2004). Further investigation of this phenomenon revealed that this novel signaling mode involves recruitment of multifunctional scaffolding protein β-arrestin 2 to activated dopamine D2 receptor that forms a signaling complex consisting of β-arrestin 2, AKT, and protein phosphatase 2A (Beaulieu et al. 2005). Critical role of β-arrestin 2 in this novel GPCR signaling mode (Beaulieu et al. 2007a) has been validated by the observation that double knockout mice lacking both the DAT and β-arrestin 2 display reduced level of locomotor activity and lack of regulation of AKT/GSK3 pathway by hyperdopaminergic tone (Beaulieu et al. 2005). Taken together, these studies not only uncovered a novel mode of dopamine receptor signaling, but also provided a Biochemical explanation on how antimanic drugs can exert their effects in psychotic manifestations that can also be treated with dopaminergic antagonists (Beaulieu et al. 2007a).

In addition to hyperactivity, several other behavioral manifestations of DAT-KO mice were used as testing paradigms for pharmacological compounds. Thus, PPI deficit in DAT-KO mice can be corrected by the treatment with the D2 DA receptor antagonist raclopride, but not D1 antagonist SCH23390 (Ralph et al. 2001). Interestingly, the perseverative patterns of locomotion of DAT-KO mice was attenuated by D1, but not D2 DA receptor antagonist (Ralph et al. 2001). 5-HT2A receptor antagonist M100907 exerted potent effect on both the PPI deficit and the perseverative pattern of locomotion in DAT-KO mice (Barr et al. 2004). Investigation of morphine effects in DAT-KO mice revealed that morphine is able to further elevate the extracellular DA levels and induce an enhanced reward in CPP test without additional increase in locomotor activity. Furthermore, naloxone-induced withdrawal manifestations were blunted in DAT mutants, but morphine analgesia was not altered (Spielewoy et al. 2000a). Significant sex-dependent changes in ethanol preference and consumption in DAT-KO mice were also noted (Savelieva et al. 2002; Hall et al. 2003) that likely reflect altered hedonic mechanisms in these mutants rather than direct role of DAT in effects of alcohol (Mathews et al. 2006). One important observation suggests that cannabinoid drugs, such as selective anandamide reuptake inhibitors AM404 and VDM11 and the fatty acid amidohydrolase inhibitor AA5HT, can normalize behavioral deficits of DAT-KO mice. Intriguingly, these effects were attenuated by the transient receptor potential vanilloid 1 (TRPV1) antagonist capsazepine but not by the selective cannabinoid type 1 (CB1) receptor antagonist AM251 (Tzavara et al. 2006). Significant functional alterations in the status of nicotinic neurotransmission was found in DAT-KO mice, and nicotine was effective in normalizing behavioral abnormalities in DAT-KO mice, such as hyperactivity and cognitive deficits without tolerance and anxiogenic effects (Weiss et al. 2007a, b). These studies strongly suggest that both cannabinoids and nicotinic drugs may have therapeutic potential in conditions associated with hyperdopaminergia, such as ADHD and schizophrenia.

Finally, DAT-KO mice were instrumental in the development of a novel acute mouse model of Parkinson's disease (dopamine-deficient DAT-KO mice, DDD mice) (Sotnikova et al. 2005). Absence of DAT-mediated recycling in these mice creates a situation when intraneuronal DA storage pools are depleted and the remaining DA concentrations become entirely dependent on its de novo synthesis. Thus, acute treatment of DAT-KO mice with the irreversible tyrosine hydroxylase inhibitor α-methyl-para-tyrosine induces transient (up to 16 h) elimination of DA (Sotnikova et al. 2005). DDD mice display a striking behavioral phenotype manifested as severe akinesia and rigidity that is totally reversible by the nonselective DA agonists and L-DOPA. Thus, DDD mice represent a simple acute model of severe DA deficiency that could be used to identify compounds with potential therapeutic use for the treatment of PD. This model is particularly promising as a tool for identification of compounds that may affect movement control independently of DA (Sotnikova et al. 2005, 2006b). More than 80 compounds were tested, to date, in this model, and it has been observed that several amphetamine derivatives at high doses can reverse behavioral manifestations of DDD mice. The most effective compound was MDMA that at high doses was able to induce forward locomotion in DDD mice, and at lower doses, markedly enhance the effects of subthreshold doses of L-DOPA (Sotnikova et al. 2005). As the lack of DAT and DA in DDD mice precludes effects of amphetamines on DA transmission, targets other than dopamine should be involved. Activation of a newly identified target of amphetamines, trace amine receptor 1 (Borowsky et al. 2001; Bunzow et al. 2001; Wolinsky et al. 2006), represent an attractive potential mechanism for the observed effects, and this hypothesis is currently being investigated in detail by using mice lacking the trace amine receptor 1 (Wolinsky et al. 2006).

The opportunity to perform extreme manipulations with the efficacy of dopaminergic transmission in vivo in the same animal (from fivefold increase in intact DAT-KO mice

to essentially complete elimination in about 15 min after treatment with α-methyl-para-tyrosine that, in turn, can be reversed by L-DOPA treatment) provided effective paradigm to investigate multiple dopamine-related processes in live animal. Thus, DAT-KO and DDD mice were used to validate the role of AKT/GSK3 signaling cascade in the actions of dopamine (Beaulieu et al. 2004). Transition from extreme hyperdopaminergia to severe dopamine deficiency was instrumental to uncover the role of dopamine in the regulation of sleep–wake states that may have relevance for psychotic conditions and sleep disturbances experienced by PD patients (Dzirasa et al. 2006). By using this inducible and reversible pharmacogenetic approach, an investigation of the simultaneous activity of neuronal ensembles in the dorsolateral striatum and primary motor cortex during hyperdopaminergia and severe hypodopaminergia in the same animal was also performed (Costa et al. 2006). These experiments indicated that dysfunctional activity coordination in corticostriatal circuits, rather than changes in the overall levels of cortical and striatal activity, is critical for the dopamine-related motor dysfunctions.

Other DAT mutant mice available

DAT knock-down (DAT-KD) mice that have about 90% reduction in DAT levels display similar, but less severe, phenotype in comparison to DAT-KO mice (Zhuang et al. 2001). These mutants have about twofold increased extracellular DA levels and show a relatively mild spontaneous hyperactivity in a novel environment. DAT-KD mice display enhanced motivation, but not learning, to rewarding stimuli (Pecina et al. 2003; Cagniard et al. 2006), impaired response habituation and paradoxical hypolocomotor response to amphetamine (Zhuang et al. 2001), but not cocaine (Tilley et al. 2007). Importantly, DAT-KD mice have altered regulation of corticostriatal glutamatergic neurotransmission (Wu et al. 2007) that could contribute to the abnormal striatal information processing critical for behavioral deficits in these mutants.

Another in vivo model of DAT dysfunction involved generation of a knock-in mouse line carrying a partially functional DAT that is insensitive to cocaine (Chen et al. 2006). Like in DAT-KO mice, acute cocaine induced hypolocomotor effect in these mutants. At the same time, it has been reported that cocaine was not inducing reward in these mice as measured by CPP test (Chen et al. 2006). It should be noted, however, that only a few doses of cocaine were investigated, and a more careful investigation of the effects of cocaine involving doses that induce robust CPP in DAT-KO mice (Sora et al. 1998; Medvedev et al. 2005) is necessary to support this conclusion. Furthermore, this mutant form of DAT had also significant deficiency with regard to reuptake of endogenous DA, thus, resulting in an elevated basal DA tone and enhanced locomotor activity. Thus, these mutants, like DAT-KO and DAT-KD mice, must undergo significant homeostatic dysregulation and developmental compensations in addition to "insensitivity" to cocaine.

Recently, by using local injections of small interfering RNA (siRNA) against DAT into the ventral tegmental/substantia nigra of adult mice, an alternative approach to induce DAT deficiency has been demonstrated (Salahpour et al. 2007). siRNA-treated mice displayed about 40% reduction of DAT in the striatum, but had little changes in novelty-induced locomotor activity. At the same time, responses of DAT siRNA-treated animals to amphetamine were blunted, similarly to effects observed in DAT heterozygote animals (Salahpour et al. 2007). These studies validated effectiveness of siRNA approach to modulate expression of proteins in adult brain in vivo that permits development of animal models bearing partial deficiency in the function of targeted proteins. In the case of such critical proteins as monoamine transporters, a partial loss-of-function is sufficient to cause significant functional alterations and may have, in fact, more relevance to the genetics of human disorders, which rarely have complete loss-of-function mutations (Kalueff et al. 2007).

Mice that have modestly increased DAT expression (approximately 30% up-regulation) have been also developed, and in contrast to DAT-deficient mice, DAT-overexpressing mice show spontaneous hypoactivity in a novel environment and increased sensitivity to MPTP (Donovan et al. 1999). Recently, a transgenic mouse line expressing the Cre recombinase under the control of the regulatory elements of the DAT gene has been also generated (Turiault et al. 2007). These mice could be instrumental for future investigations of the functions of genes specifically expressed in DA neurons and/or for labeling dopaminergic cells in vivo by crossing these mice with transgenic Cre lines having particular genetic manipulations or producing fluorescent proteins.

Conclusions

Mice with genetically altered DAT function provided a powerful approach to investigate in vivo effects of pharmacological compounds in conditions of severe dopaminergic dysfunction. Numerous advances in understanding the mechanism of action of psychotropic drugs that can affect DA system either directly or indirectly at cellular and/or system levels have been made over the last decade by using this experimental model. One perspective application of DAT mutant mice is to use them as a background strain to introduce additional mutation of genes of interest

to investigate role of these genes under conditions of severe dopaminergic dysfunction. To date, DAT-KO mice were crossed with SERT-KO (Sora et al. 2001), NET-KO (Hall et al. 2004), VMAT2-KO (Fukushima et al. 2007), β-arrestin 2-KO (Beaulieu et al. 2005) mice, and knock-in mouse model of Huntington disease containing 92 CAG repeats in huntingtin gene (Cyr et al. 2006). This approach is particularly valuable for the investigation of function of proteins that do not have currently pharmacological means to affect their expression or activity.

In conclusion, these studies illustrate how mutant mice could be used to address many important questions in experimental neuropharmacology. DAT mutant mice provided unique opportunity to directly evaluate drug selectivity and specificity, investigate mechanism of their action in vivo, and assess drug efficacy in pathological models of enhanced or decreased dopaminergic transmission that may eventually bring novel treatments for several neurological and psychiatric disorders.

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