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Trafficking of Dopamine Transporters in Psychostimulant Actions

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Abstract

Brain dopamine (DA) plays a pivotal role in drug addiction. Since the plasma membrane DA transporter (DAT) is critical for terminating DA neurotransmission, it is important to understand how DATs are regulated and this regulation impacts drug addiction. The number of cell surface DATs is controlled by constitutive and regulated endocytic trafficking. Psychostimulants impact this trafficking. Amphetamines, DAT substrates, cause rapid up-regulation and slower down-regulation of DAT whereas cocaine, a DAT inhibitor, increases surface DATs. Recent reports have begun to elucidate the molecular mechanisms of these psychostimulant effects and link changes in DAT trafficking to psychostimulant-induced reward/reinforcement in animal models.

Keywords

review; dopamine transporter regulation; endocytosis; amphetamine; cocaine

1. Introduction

Brain dopamine (DA) has long been known to play a pivotal role in reward [1]. Much evidence supports the importance of DA, as well as several other key neurotransmitters like glutamate, in drug reward, reinforcement and addiction (e.g., [2–5]). The addiction-related actions of DA are mediated by two major midbrain projection pathways: (i) mesocorticolimbic DA neurons with cell bodies (somatodendritic regions/compartments) in the ventral tegmental area (VTA) and projecting to the medial prefrontal cortex and nucleus accumbens (NAc, or ventral striatum) and (ii) nigrostriatal DA neurons with cell bodies in the substantia nigra pars compacta (SNc) and projecting to the dorsal striatum (dSTR). Mesolimbic DA neurons and elevated DA in NAc are important for motivated drug taking, learned associations and behavioral reinforcement, all of which are essential for initiation of drug taking and development of addiction [2, 6]. Increased mesolimbic DA transmission is necessary and sufficient for cocaine reinforcement, and this system links reward and actions [7, 8]. DA in dSTR also plays a critical, but distinct, role in habit learning; this is involved in cue-induced craving, cocaine-seeking and compulsive behaviors that contribute to long-term drug taking and addiction [9–12].

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DA neurons fire in both tonic and phasic/burst firing patterns, exocytotically releasing DA from vesicles into the synaptic cleft where it diffuses and activates its receptors. DA receptors include postsynaptic D₁-like receptors (D₁Rs and D₅Rs) and D₂-like receptors (D₂Rs, D₃Rs, D₄Rs), as well as presynaptic D₂-like autoreceptors on the DA neurons themselves. Both D₁- and D₂-like classes of DA receptors are G protein-coupled receptors (GPCRs) that signal in a relatively slower manner.

The DA transporter (DAT), localized exclusively to DA neurons [13], is the primary mechanism for limiting/terminating DA neurotransmission [14]. DAT is a member of the Solute Carrier 6 gene family (*SLC6A3*) that encodes several Na⁺/Cl⁻-dependent neurotransmitter transporters (neurotransmitter:sodium symporter family) – including transporters for norepinephrine, serotonin, GABA, glycine (see review [15]). DAT, like the other mammalian members of this family of transporters, has intracellular amino- and carboxyl-termini and 12 transmembrane domains (TMs; Fig. 1). These transporters are active when present on the plasma membrane. DAT-mediated DA inward translocation (uptake) uses the neuronal electrochemical gradient to pump DA from the extracellular space back into DA neurons, thereby limiting the temporal and spatial interaction of DA with its receptors. From the neuronal cytoplasm, DA is either taken up into synaptic vesicles by the proton antiporter vesicular monoamine transporter-2 (VMAT-2) or metabolized by monoamine oxidase. Psychomotor stimulant drugs like cocaine and amphetamines produce their activating and addictive effects by interacting with DAT and thereby increasing extracellular DA concentrations [14, 16–18]. However, their acute mechanisms of action differ: cocaine blocks DAT-mediated uptake of DA whereas amphetamines are substrates for DAT (and VMAT-2) and thereby promote DAT-mediated DA efflux, or Na⁺-dependent non-vesicular DA release.

DAT activity can be regulated both acutely (minutes to hours) and longer-term (days) (see [18–21]). As opposed to rapid changes in stimulation-evoked DA release, regulation of DATs occurs more slowly. Nonetheless, it still represents a complementary mechanism by which DA neurotransmission can be fine-tuned. DAT regulation can occur via altered substrate/Na⁺/Cl⁻ affinity, kinetic activation, trafficking to/from the plasma membrane, and changes in protein turnover. This review will focus on DAT trafficking, which appears to be a common mechanism for acute, dynamic regulation of DAT activity and its potential contribution to psychostimulant drug actions and abuse.

2. Trafficking of newly-synthesized DAT

Like all transmembrane proteins, DAT is synthesized at the endoplasmic reticulum (ER) membrane, co-translationally translocated through the ER membrane and eventually packaged into COP (coatamer) II vesicles for its anterograde transport to the Golgi apparatus. Since DAT is proposed to be N-glycosylated in the extracellular loop 2 (EL2) (Fig. 1) [22], the transporter must pass through the Golgi to acquire this post-translational modification. From the trans-Golgi network, DAT traffics to the plasma membrane. Since somatodendritic compartments of the DA neurons are located in SNc and VTA, all of the above steps of anterograde trafficking take place in these midbrain subregions (Fig. 2). Electron microscopy studies demonstrated the presence of DAT in the ER in the soma and proximal dendrites of DA neurons in VTA and SNc [13, 23].

The molecular mechanisms of the anterograde trafficking of DAT have been investigated mostly in heterologous systems and embryonic mesencephalic primary neuronal cultures. Mutations in the TMs of DAT often result in poor expression of DAT at the plasma membrane, presumably, due to retention in the ER [24]. It is likely that such ER retention can be explained by the disruption of the integrity of the membrane spanning α -helices

yielding an unfolded molecule that is incorrectly incorporated into the ER membrane. Truncation of the carboxyl-terminal tail and site-point mutagenesis of this part of the DAT molecule demonstrated the critical role of the carboxyl-terminus in the ER export of DAT [25, 26]. For instance, deletion of the last three residues LKV, which constitute a PDZ domain binding motif, resulted in inefficient plasma membrane expression of newly synthesized DAT [27, 28] (Fig. 1). However, the function of these residues appears to be unrelated to their PDZ binding capacity [28]. Large deletions of the carboxyl-terminal tail lead to substantial retention of DAT in the ER [25, 26]. Surprisingly, a very strong, if not complete, retention of the transporter in the ER resulted from a single mutation of the conserved Gly585 to alanine [29]. This glycine does not represent a part of any known ER export motif and its function in the anterograde trafficking of DAT is not understood. It is possible that Gly585 is involved in intramolecular interactions necessary for the proper folding of DAT. A number of other residues in the carboxyl-terminal tail are also critical for the efficient exit of DAT from the ER (Fig. 1) [28–30]. Finally, it has been demonstrated that dimerization/oligomerization of DAT takes place in the ER [26]. It has been suggested that this oligomerization is necessary for the efficient anterograde transport of DAT through the ER-Golgi system [25, 26]. The molecular machinery that is responsible for the key sorting step at the ER, e.g. recruitment of DAT into forming COPII vesicles, is not defined. However, it is likely that DAT interacts with the Sec24 complex that serves as an adaptor during recruitment of various cargo into COPII vesicles [31].

Fully glycosylated, “matured” DAT is delivered from the trans-Golgi to its functional sites in the DA neurons. Electron microscopy analysis revealed the presence of DAT in the plasma membrane of axonal varicosities and between these varicosities in dSTR and NAc. Plasma membrane DAT was also observed in the distal dendrites, but not in the neuronal soma [13, 23]. Interestingly, in axonal varicosities DAT was not detected in the active zone of the synapses, but rather it was located extrasynaptically, including perisynaptically [23, 32]. Such distribution of DAT is presumably important for the spatial and temporal control of extracellular DA concentrations by DAT.

The mechanisms that control movement of newly synthesized DAT from Golgi to specific cell surface locations within the axons and distal dendrites are not known. It is possible that DAT is inserted into the plasma membrane in the soma and then moves laterally along the plane of the membrane to its functional sites where it is retained by specific interactions with the resident proteins (Fig. 2). Alternatively, DAT could be moved from the soma to distal locations by anterograde vesicles in microtubule-dependent manner and then be inserted into the plasma membrane locally at the axonal varicosities and distal dendrites. In polarized MDCK cells DAT is targeted to the apical surface, although the molecular mechanisms responsible for this targeting are not understood [33]. The role of the PDZ binding motif and the carboxyl-terminus of DAT in the plasma membrane expression and synaptic localization of DAT was studied *in vivo* in *C. elegans* [34]. In these experiments large deletions of the carboxyl-terminus interfered with the synaptic accumulation of DAT, whereas the PDZ binding motif had little, if any, impact on DAT localization at the synapse.

3. Regulation of DAT by endocytic trafficking

Transmembrane proteins, that have been delivered through the biosynthetic pathway to the plasma membrane, undergo constitutive and regulated endocytosis. After endocytosis, internalized cargo can be recycled back from endosomes to the plasma membrane, or sorted to lysosomes or other compartments. It is logical to assume that because DAT functions at the cell surface, its constitutive endocytosis is expected to be much slower than, for example, the endocytosis of VMAT-2 that functions in synaptic vesicles and, therefore, must be efficiently internalized and sorted to these vesicles. Nonetheless, early studies that

measured membrane capacitance, cell surface DAT binding and/or localization with immunofluorescence confocal microscopy in *Xenopus* oocytes and Sf9 insect cells expressing cloned human DATs (hDATs) suggested that loss of cell surface DATs, in addition to down-regulation of DAT activity, can occur within minutes after a stimulus, viz. activation of protein kinase C (PKC) by phorbol esters [35, 36]. Such findings prompted investigation of DAT endocytosis and down-regulation in mammalian cells. Two studies in hDAT-PC12 and -MDCK cells were particularly important in launching during the past decade an intensive research effort focused on elucidating the cellular and mechanistic underpinnings of DAT endocytosis [37, 38]. Both studies clearly demonstrated PKC-dependent endocytosis of DAT. In MDCK cells the endocytosis was shown to be dynamin-dependent, and the internalized DAT was shown to be degraded in lysosomes [37]. In contrast, in PC12 cells internalized DAT was mostly recycled from early and recycling endosomes back to the cell surface [38]. More recent work from Melikian and co-workers has also demonstrated constitutive endocytosis of DAT expressed in PC12 cells [39].

Development of RNA interference methods has allowed a more in-depth characterization of the pathways and molecular mechanisms of hDAT endocytosis in heterologous expression systems. DAT was shown to internalize both constitutively and in a PKC-dependent manner via clathrin-coated pits in PAE and HeLa cells [30, 40]. Thus, both types of internalization appeared to be clathrin-dependent. Cholesterol-disrupting drugs did not affect PKC-dependent DAT endocytosis, suggesting that DAT internalization is not mediated by lipid rafts [30]. However, a pool of plasma membrane DAT is also associated with cholesterol-rich rafts, which regulates its transport activity [41].

Activation of PKC resulted in the internalization of DAT into early and recycling endosomes containing Rab5, EEA.1 and transferrin receptors [30, 38]. However, mutations or deletions of conventional internalization signals, such as LL in the amino-terminus, did not result in inhibition of PKC-dependent internalization [30, 42]. Clues about the molecular mechanisms of PKC-dependent internalization of DAT were obtained from the mass-spectrometry analysis of purified DAT [43]. These experiments detected ubiquitination of DAT, which was subsequently confirmed by Western blotting. Mutagenesis of potential ubiquitin conjugation sites in DAT revealed that a cluster of three lysines in the amino-terminus is important for DAT ubiquitination [44]. Moreover, mutation of these sites resulted in diminished endocytosis of DAT in response to PKC activation [44]. siRNA screening for proteins involved in PKC-dependent DAT endocytosis identified the NEDD4-2 protein, a HECT domain containing E3 ubiquitin ligase, as an essential component of PKC-dependent DAT endocytosis [40]. Knock-down of NEDD4-2 also resulted in inhibition of DAT ubiquitination, thus implicating NEDD4-2 as an E3 ligase for DAT [40]. These data, together with DAT mutagenesis, strongly suggest that ubiquitination of the DAT amino-terminus is critical for PKC-dependent DAT endocytosis.

Several proteins present in clathrin-coated pits and possessing ubiquitin-binding domains have been proposed to recruit ubiquitinated cargo in coated pits. siRNA screening revealed the importance of such proteins (Eps15 and epsin) in DAT endocytosis, suggesting that ubiquitinated DAT can be recruited to coated pits by means of its interaction with these proteins [40]. Simultaneous alanine substitution of 10 residues in the carboxyl-terminus of DAT inhibited PKC-dependent DAT internalization [45]. However, the role of these residues is not defined.

In HeLa and MDCK cells, PKC activation also results in degradation of DAT in lysosomes, presumably by mechanisms involving the interaction of ubiquitinated DAT with ESCRT complexes in multi-vesicular bodies (MVBs) and incorporation of DAT into internal

vesicles of MVBs [37, 43]. In fact, localization of DAT in MVBs has been observed within the soma of SNc DA neurons [23].

Many questions concerning the molecular mechanisms of PKC-dependent down-regulation remain unanswered. First, how does activation of PKC lead to NEDD4-2-mediated ubiquitination of DAT? Does NEDD4-2 bind to DAT directly and is it indeed *the* E3 ligase for DAT? Evidence against this notion is the lack of a PxY motif, which typically mediates the interaction of NEDD4-2 with its substrates, in the cytoplasmic tails of the transporter. However, the interaction of NEDD4-2 with DAT could be mediated by another motif or an intermediate adaptor. Finally, the most critical questions are whether activation of PKC in DA neurons also results in DAT ubiquitination and endocytosis via mechanisms similar to those described in non-neuronal cells, and whether this occurs in the brain *in vivo* via activation of PKC by physiological and/or pharmacological stimuli. PKC-regulated endocytosis of DAT in rat striatal synaptosomes has been demonstrated using a reversible biotinylation technique [46]. A reduction in [³H]DA uptake by phorbol ester treatment of dissociated embryonic mesencephalic neuronal cultures was also observed [47, 48]. However, to date, the molecular details of PKC's effects on DAT trafficking in neurons have not been elucidated.

Constitutive endocytosis of DAT is even less well understood at the molecular level than the PKC-dependent internalization. Residues 587–596 in the carboxyl-terminal tail of DAT have been demonstrated to mediate the constitutive internalization of DAT [45]. Nonetheless, it is not known exactly how this sequence functions. In addition, mutations of some of these residues resulted in the retention of DAT in the ER [30], thus making it difficult to analyze their role in internalization. Expression of HA-tagged DAT in neuronal cultures demonstrated constitutive internalization of DAT in both axonal and somatodendritic compartments [40]. DAT can be found co-localized with Eps15 in cultured neurons, which is indicative of its presence in clathrin-coated pits [40]. Development of new experimental tools is, however, necessary before the mechanisms of constitutive and PKC-dependent endocytosis of DAT can be defined in DA neurons.

4. Regulation of DAT trafficking by intracellular signaling and protein interactions

In addition to PKC-dependent endocytosis, a number of other mechanisms and signaling pathways have been proposed to regulate DAT trafficking. Activity of MAPK/ERK1/2 appears to be important for maximal plasma membrane localization of DAT since inhibition of ERK1/2 activity results in down-regulation of DAT in heterologous expression systems, primary cultures of DA neurons and striatal synaptosomes [47, 49, 50]. Interestingly, MAPK phosphatase MKP-3 appears to have a negative role in regulation of PKC-dependent endocytosis of DAT [48]. However, it was not determined which step of endocytic trafficking is affected and by which mechanisms MAPK signaling networks regulate endocytic trafficking of DAT. Because inhibitors of tyrosine kinases cause down-regulation of DAT, it is possible that receptor tyrosine kinases, such as TrkB, are the upstream activators of the MAPK pathway in DA neurons [47]. Another possibility is that ERK/MAPK, as well as PKC, can be activated by GPCRs present in DA neurons, such as DA D_{2/3}Rs [51, 52].

Endocytosis, surface expression levels of DAT and subcellular distribution of DAT have also been proposed to be regulated by specific proteins interacting with DAT. For instance, interactions of DAT with the PDZ domain containing protein PICK1 and the scaffold protein HIC5 positively regulated plasma membrane levels of DAT [27, 53]. Over-expression of α -synuclein and its interaction with DAT resulted in up-regulation of surface

DATs [54], although more recent studies suggest that α -synuclein is also capable of down-regulating DAT activity [55]. In contrast to the effect of wild-type α -synuclein on DAT localization, expression of α -synuclein mutants found in Parkinson's patients resulted in up-regulation of DAT [56]. Interaction of the amino-terminal tail of DAT with another PDZ-domain containing protein, Piccolo, slowed down DAT endocytosis in both non-neuronal cells and DA neurons [57]. It was suggested that Piccolo reduced clathrin-mediated endocytosis of DAT through its ability to interact with and sequester phospholipid PI(4,5)P₂, thus leading to abolished recruitment of several clathrin associated proteins and reduced endocytosis. Furthermore, interaction of DAT with an orphan GPCR, GPR37, has been proposed to be necessary for constitutive endocytosis of DAT, although the mechanism of GPR37-mediated regulation is unknown [58].

5. Psychostimulant drugs – Cross-talk with DAT trafficking

Most of the evidence that addictive drugs can alter DAT trafficking comes from studies using psychostimulants that interact directly with DAT, *viz.* amphetamine, methamphetamine and cocaine. Not surprisingly, DAT substrates like amphetamines regulate DAT cell surface number in an opposite manner to DAT inhibitors like cocaine. Thus, acute exposure to amphetamine or methamphetamine reduces surface DATs whereas cocaine increases them (Fig. 3). These studies are discussed below, along with the few recent reports that link altered DAT trafficking to psychostimulant addictive behaviors in animal models. However, how altered DAT trafficking impacts psychostimulant abuse/addiction remains largely an intriguing question for future investigation.

Some addictive drugs like nicotine do not bind to DAT but affect its function indirectly. Acute systemically administered nicotine increased the maximal velocity ($V_{\max}$) of rat striatal *ex vivo* [³H]DA uptake [59]. However, two different approaches (cell surface biotinylation and subcellular fractionation) revealed no change in DAT localization, supporting the conclusion that this regulation of DAT activity occurs via a trafficking-independent mechanism.

Psychostimulants can also differentially influence trafficking of synaptic vesicle proteins like VMAT-2. One hour after systemic administration of high dose methamphetamine, VMAT-2 was redistributed away from the synaptic vesicle-enriched fraction [60]. In contrast, cocaine administration induced redistribution of VMAT-2 into synaptic vesicles.

5.1 Amphetamines and DAT trafficking

Animal studies were the first to provide evidence that amphetamines can result in a longer-term, reversible down-regulation of DAT function, measured *ex vivo* after drug washout and consistent with a reduced number of cell surface DATs. For example, both acute systemic administration of and *in vitro* exposure to methamphetamine reduced subsequent [³H]DA uptake into well-washed rat dSTR synaptosomes [61, 62]. It is intriguing that this effect was regionally specific in that it was not detected in synaptosomes prepared from NAc [63]. We observed a similar regional difference in substrate-induced reductions in DAT function when we used *in vivo* voltammetry to measure the effects of DA locally and repetitively applied every 2–3 min [64]. Loss of functional cell surface DATs would be expected to produce opposing effects on the psychostimulant actions of amphetamines. Thus, there would be fewer DATs to clear extracellular DA, but this would also reduce the DAT-mediated DA efflux that is thought to largely mediate the psychostimulant activation and rewarding properties of amphetamines. Computational modeling of amphetamine-stimulated DA efflux has recently demonstrated the predominant impact of DAT down-regulation, *e.g.* it showed that amphetamine-induced DAT trafficking is necessary for prolonged DAT-mediated efflux of DA [65].

Heterologous cell expression systems have provided direct evidence that amphetamines alter DAT trafficking, specifically by reducing the number of cell surface transporters. Initially, immunofluorescence confocal microscopy and biotinylation were used to study amphetamine-induced redistribution of epitope-tagged DATs from the plasma membrane to the cytosol in hDAT-HEK 293 cells [66]. The internalization of DAT induced by amphetamine was inhibited by cocaine and a dominant negative mutant of dynamin. The magnitude of amphetamine-induced down-regulation of surface DAT is typically considerably smaller than that in response to phorbol esters. Confocal imaging combined with patch-clamp measurements of DAT-associated currents demonstrated that the ability of amphetamine to diminish DAT function results from the loss of *active* transporters from the plasma membrane [67]. Exposure to amphetamine also resulted in a significant decrease in cell surface DATs, as measured by biotinylation.

Interestingly, more recent work from this group has shown that amphetamine acts intracellularly to regulate DAT trafficking [68]. Using a mutant hDAT to which amphetamine still binds but which has impaired uptake activity, as well as using direct intracellular application of amphetamine, they confirmed that amphetamine first must be taken up by the DAT before it can affect internalization of DAT. Their results also distinguished amphetamine from DA, which had no effect. Insulin opposes amphetamine-induced DAT internalization, and protein kinase B (Akt) is required for this effect [69]. Calmodulin kinase II (CaMKII) signaling is also involved because amphetamine reduces Akt activity by a CaMKII-dependent mechanism [70]. Exactly how Akt regulates DAT endocytosis and recycling is unknown.

PKC inhibitors blocked methamphetamine-induced reductions in DAT activity in striatal synaptosomes, as well as amphetamine-induced reductions in DAT activity in hDAT-oocytes [62, 64]. The amphetamine-induced reductions in DAT function were assumed to reflect altered trafficking because amphetamine exposure also reduced cell surface DAT binding. However, amphetamine-induced DAT endocytosis in hDAT-PC12 cells was recently shown to be independent of PKC [71]. This work utilized an hDAT with mutations of carboxyl-terminus residues 587–590 (required for PKC-stimulated DAT internalization); nonetheless, the mutated DAT still internalized when exposed to amphetamine.

Recently, the presynaptic scaffolding protein Piccolo has been shown to play a role in methamphetamine-induced DAT internalization, extracellular DA levels in NAc, locomotor sensitization and conditioned reward in mice [57]. The results of this comprehensive study suggest that Piccolo is a homeostatic mechanism triggered by repeated exposure to methamphetamine. Thus, increased expression of Piccolo, such as that which occurred in NAc of mice treated repeatedly with methamphetamine, would be expected not only to reduce methamphetamine-accelerated loss of functional DATs from the cell surface, but also to oppose the behavioral plasticity associated with psychostimulant drug abuse.

In addition to the considerable evidence that more prolonged exposure to amphetamines reduces the number of DATs at the cell surface, brief exposure was found to produce the opposite effect. Within 30 s – 1 min after amphetamine, DAT surface expression in rat striatal synaptosomes was transiently elevated, as measured with membrane-impermeable biotin [72]. Reversible biotinylation experiments suggested this observation was due to an increased insertion of DATs into the plasma membrane, rather than decreased endocytosis. This rapid up-regulation was specific to amphetamine and not observed following brief exposure to DA.

5.2. Cocaine and DAT trafficking

Studies in postmortem brain samples from chronic cocaine users or cocaine overdose victims have found increased maximal number of DAT binding sites ($B_{\max}$), and in one study using cryoprotected tissue elevated [^3H]DA uptake $V_{\max}$ [73, 74]. However, it is unknown if these compensatory-type changes reflect cocaine-induced altered DAT trafficking. Thus, the possibility that exposure to cocaine up-regulates surface DATs was explored more systematically by two groups using hDAT-expressing cells. In stably hDAT-transfected N2A cells, exposure to cocaine not only increased uptake $V_{\max}$ and binding $B_{\max}$, but also cell surface DATs (but not total DATs) [75]. These latter results from immunofluorescence and biotinylation experiments suggest that cocaine can alter DAT trafficking. Similarly, exposure to methylphenidate (Ritalin®), another DAT inhibitor, increased DAT $B_{\max}$, but DAT surface expression was not measured. hDAT-HEK cell biotinylation experiments also showed a cocaine-induced increase in DAT surface expression [76]. Results of experiments assessing *ex vivo* and *in vivo* DAT function in rat striatum after systemic cocaine injection were also consistent with cocaine-induced DAT up-regulation, but altered trafficking was not addressed specifically.

Further questions remain about when and how quickly cocaine-induced changes in DAT trafficking in brain occur. For example, in several studies *in vitro* preincubation of hDAT-expressing cells or rat striatal synaptosomes with cocaine produced no change in the percentage of DATs at the cell surface, as measured by noncleavable biotin or immunofluorescence assays [37, 46, 66, 72]. Also, chemically different DAT inhibitors like cocaine and benztropine stabilize distinct conformations of DA, and these correlate with the ability of rats to discriminate cocaine from saline [77]. Thus, it will be interesting to determine if structurally distinct DAT inhibitors differentially alter DAT trafficking.

The most relevant, but challenging, question is how cocaine-induced regulation of DAT trafficking/function relates to cocaine addiction. Only one study has addressed potential changes in cell surface DATs using the rat self-administration model of drug reinforcement. Interestingly, rats that self-administered cocaine, but not yoked saline controls, for 10 days followed by a 3-wk abstinence showed a higher level of surface DATs, increased DAT-PP2Ac association and reduced serine phosphorylation of DAT in dSTR [78]. Similar changes were not observed in NAc, leading the authors to conclude that chronic cocaine self-administration is associated with persistent, regionally distinct alterations in DAT trafficking and regulatory cascades. Whether such differential dysregulation of DAT trafficking contributes to or protects from relapse and reinstatement of drug taking remains to be investigated.

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Figure 1. Molecular determinants of DAT involved in the regulation of its intracellular trafficking

Schematic structure of DAT based on the structure of the homologous leucine transporter [79] is shown. Three putative sites of N-glycosylation in EL2 are shown in “black”. Residues in the carboxyl-terminus important for the ER exit of DAT are shown in “orange”. The PDZ domain binding motif is shown in “black”. The region of DAT proposed to be important for the constitutive endocytosis is shown in “green”. Lysine residues that are known to be ubiquitinated are shown in “blue”.

Figure 2. Hypothetic model of DAT trafficking in DA neurons

Newly-synthesized DAT is N-glycosylated in the Golgi apparatus (G), and transported from the Golgi to the plasma membrane in the somatodendritic compartment of DA neurons. DAT travels to distal axons by lateral movement in the plane of the plasma membrane or by transport vesicles. In the somatodendritic compartment the activation of PKC results in NEDD4-2-mediated ubiquitination of DAT. PKC activation can facilitate NEDD4-2-mediated ubiquitination of DAT either by phosphorylating DAT or DAT-interacting proteins, or by activating NEDD4-2. Ubiquitinated DAT is recruited into clathrin-coated pits (CCP) by means of interaction with UBD-containing proteins, such as Eps15/Eps15R and epsin, that are bound to AP-2 and clathrin in coated pits. After internalization via clathrin-coated vesicles (CCV) to early and recycling endosomes (EE/RE), DAT is sorted in MVB to lysosomes (Lys), presumably by the mechanism mediated by ESCRT complexes. In the distal axonal processes, DAT is internalized and recycled in a manner similar to that in neuronal soma, although there is likely no sorting to late endosomes within the axonal varicosities lacking late endosomes and lysosomes. SV, synaptic vesicles.

Figure 3. Effects of amphetamines and cocaine on the endocytic trafficking of DAT

(A) DAT-mediated transport of amphetamines into the cell is required for amphetamine-induced elevation in intracellular Ca^{2+} , activation of PKC and CaMKII, and inhibition of Akt activity. All or some of these events result in acceleration of DAT internalization and/or reduction of DAT recycling, ultimately leading to a slower (30–120 min) down-regulation of the surface pool of DATs and accumulation of DATs in early and recycling endosomes (*EEs/REs*). Amphetamine also causes rapid, transient (≤ 1 min) up-regulation of the surface pool of DATs by unknown mechanisms, which may involve DAT transport from *REs* to the plasma membrane.

(B) Inhibition of DAT by cocaine results in partial redistribution of DATs from endosomes to the plasma membrane by unknown mechanisms.