Psychostimulants and monoamine transporters: upsetting the balance
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Monoamine transporters were originally associated simply with the termination of synaptic monoamine function. In addition to amine reuptake, however, the transporters can act as ion channels that affect exocytotic neurotransmitter release and can operate in reverse mode, mediating non-exocytotic amine release. Activity at the plasma membrane is controlled by trafficking, which is modulated by interaction with both substrates and inhibitors and by cytosolic kinases and phosphatases. Monoamine transporters also constitute the principal sites of action of many psychoactive drugs, including amphetamines and cocaine, as well as therapeutic drugs for the treatment of depression, addiction and attention deficit hyperactivity disorder, each modifying the balance of presynaptic neurotransmitter function.

Abbreviations
ADHD attention deficit hyperactivity disorder
AMPH amphetamine
CPP conditioned place preference
DAT dopamine transporter
KO knockout
METH methamphetamine
MDMA 3,4-methylenedioxymethamphetamine
NET norepinephrine transporter
PKC protein kinase C
SERT serotonin transporter
VMAT vesicular monoamine transporter
WT wild-type

Introduction
The serotonin transporter (SERT), dopamine transporter (DAT) and norepinephrine transporter (NET) located on the plasma membrane and the ubiquitous vesicular monoamine transporters (VMATs 1 and 2) belong to two gene families with structural similarities (each are predicted to contain 12 transmembrane domains with both amine and carboxyl termini in the cytosolic domain) but differential ionic dependence. The first family (containing SERT, DAT and NET) is dependent upon Na⁺/Cl⁻ ions and the latter (containing VMAT) on H⁺ ions. The role of plasma membrane transporters in terminating the action of their cognate amines by reuptake into the nerve terminal has been appreciated for over 40 years. However, the identification, expression cloning and genetic deletion of these proteins has led to a more detailed understanding of their function not only in relation to the normal uptake of amines but also to the reversal of transport, leading to non-exocytotic amine release, and their electrogenic role as ion channels (as summarized in Figure 1). Furthermore, the dynamic regulation of transporter density via internalisation and recycling under the control of kinases, phosphatases and other proteins, and their interaction with presynaptic receptors, has led to a reappraisal of their wider role in regulating presynaptic neuronal function. They also constitute the principal target sites for several psychoactive drugs, including amphetamines and cocaine, which are characterised by their high abuse potential and possible neurotoxicity, as well as therapeutic drugs used in the treatment of depression, attention deficit hyperactivity disorder (ADHD) and addiction to substances such as nicotine. The development of our knowledge concerning the role of these transporters has paralleled our understanding of the mechanism of action of these drugs, and has led both to new insights into the associated pathologies and to novel strategies for pharmacological intervention. This review discusses our current knowledge of the mechanism and regulation of monoamine transporters in relation to neuronal function and the ways in which drugs act on these transporters to disturb the synaptic balance.

Modes of action of monoamine transporters
The intracellular transport of cognate amines by DAT, NET and SERT is driven by the Na⁺/Cl⁻ gradient across the plasma membrane and is similar to the action of transporters for γ-aminobutyric acid (GABA), glycine and other organic amines belonging to the same gene family [1,2]. The substrate selectivity of DAT and SERT for dopamine and serotonin, respectively, is high, whereas NET efficiently transports both norepinephrine and dopamine. Although turnover rate is slow (approximately 1 transport cycle/s), the initial binding exceeds this by more than 100-fold [3*]. Hence at low amine...
concentrations, the transporter initially acts as an efficient buffer, adsorbing rather than absorbing the amine; as the amine concentration rises, the transport function becomes more significant in terminating receptor activation. Immunohistochemical evidence has generally failed to reveal significant amounts of the transporter located within the synaptic bouton; instead, it is found on the axon, soma and dendrites [4,5]. However, the importance of these transporters in restricting the concentration of amines within the synapse is demonstrated by the significant decrease in the clearance rate of amines within the terminal fields of transporter gene knockout (KO) mice [6,7]. Hence, deletion of DAT leads to a 300-fold increase in the persistence of extracellular dopamine within the striatum, but also to several adaptive changes including a marked decrease in dopamine tissue content, desensitisation and downregulation of both presynaptic and postsynaptic dopamine receptors, and a decrease in the magnitude of quantal exocytotic dopamine release. These effects show regional variability in intensity, which correlate with the normal density of DAT and so are greater within the striatum than in the hypothalamus [8].

Similar consequences have been observed following knockout of SERT and NET. These studies confirm the crucial role of the plasma membrane monoamine transporters in terminating the neurotransmitter role of their cognate amines and also indicate their contribution towards synaptic homeostasis.

The influx of amine through its transporter is coupled to ion movements that are electroneutral for SERT and electrogenic for DAT [9]. However, voltage-clamp studies of cultured dopaminergic neurons in the presence of D2 receptor antagonists showed that dopamine uptake was associated with a DAT-mediated current which was 1–2 orders of magnitude larger than that predicted by transport charge coupling [10]. The depolarising effect of this current increased the firing rate by three- to four-fold; was blocked by DAT inhibitors, including cocaine and GBR12909; and appeared to be mediated predominantly by Cl⁻ ions. A similar electrogenic role has been demonstrated for SERT expressed in Xenopus oocytes where the ratio of charge to serotonin transport was 7:1, generating a depolarising current mediated by Na⁺ ions [11*]. This
current flow was gated by an interaction between SERT and the synaptic protein syntaxin1A, which converted serotonin uptake to an electroneutral process. Although the physiological significance of this monoamine transporter-mediated ion channel function is not clear, its depolarising effect might increase neuronal excitability and hence exocytotic amine release during tonic firing. This may occur when the extracellular amine concentration is relatively low but would be swamped by the hyperpolarising effect of autoreceptor stimulation, which is activated by higher amine concentrations resulting from phasic firing [12].

In contrast to the normal inward movement of amines, transport can be reversed, causing the non-exocytotic efflux of amines into the perineuronal space. The potency of compounds to induce dopamine efflux via DAT correlates not with their potency as substrates for uptake but rather with their ability to induce current flow as described above [13]. This mechanism is particularly pertinent to the efflux of dopamine induced by amphetamines and has also been shown to be voltage-dependent and regulated by intracellular Na+ concentrations [14]. Although not dependent upon extracellular Ca2+ levels, both the dopamine efflux and the DAT-mediated current flow are attenuated by removal of intracellular Ca2+. Amphetamine (AMPH) itself induces an increase in intracellular Ca2+ that is blocked by cocaine and by thapsigargin, suggesting that the endoplasmic reticulum is the source of this Ca2+ [15]. Early studies had implicated protein kinase C (PKC) in the mechanism of DAT reversal, as AMPH-induced dopamine efflux was abolished by PKC antagonists, whereas PKC agonists induced dopamine efflux in the absence of AMPH [16]. Although the physiological significance of transporter reversal in synaptic terminals is unclear, it might constitute the mechanism for dendritic dopamine release ([17] but see [18]) and play a fundamental role in the pharmacological effects of amphetamines on all three monoamine transporters.

Modulation of monoamine transporter function

In common with neurotransmitter receptors, monoamine transporter function at the plasma membrane is regulated by substrate and inhibitor activity, but not uniformly. DAT activity is decreased and its surface density reduced by high concentration of substrates including dopamine and AMPH, whereas activity and surface density are increased by prolonged blockade by inhibitors such as cocaine and GBR12909 [19,20]. By contrast, the activity and density of SERT and NET are decreased by chronic administration of selective inhibitors, such as sertraline and desipramine, respectively [21,22], whereas serotonin increases the activity of SERT [23]. Modulation of NET by catecholamines appears to be similar, as NET activity is decreased in tyrosine hydroxylase KO mice (which express low levels of both norepinephrine and dopamine) but not in dopamine-β-hydroxylase KO animals (in which norepinephrine alone is decreased but dopamine continues to act as a substrate for NET) [24]. The common mechanism for substrate modulation appears to involve protein phosphorylation, as activity of each of the monoamine transporters is reduced and the surface density downregulated by several kinase enzymes, including PKC, mitogen-activated protein kinase and phosphatidylinositol 3-kinase, or inactivation of protein phosphatases [19,20]. However, it is unclear whether phosphorylation of the transporter itself is involved because deletion of the N-terminal 22 amino acids or mutation of the N-terminal serines, which are phosphorylated by PKC, does not prevent the loss of DAT from the plasma membrane [25]. The reduction in transporter activity is caused by trafficking of the protein into the cytosol via dynamin-dependent endocytosis. This has been elegantly demonstrated by amphetamine-induced modulation of fluorescent-tagged DAT expressed in HEK293 cells, where the loss of surface DAT activity temporally paralleled the accumulation of fluorescence within the cytosol and was prevented by cocaine [26]. Clearly, the dynamic modulation of transporter activity by endogenous and exogenous ligands, both illicit and therapeutic, is potentially of major significance in the regulation of monoamine synaptic function.

Techniques such as the yeast two-hybrid system, which identify protein-protein interactions through the activation of a gene-reporter mechanism, have demonstrated that monoamine transporters interact with several proteins other than kinases and phosphatases. Examples of such interactions include that between DAT and α-synuclein (a presynaptic protein that has been implicated in Parkinson’s disease); DAT and Hic-5, which interacts with the glucocorticoid receptor and several kinases; DAT and NET with PICK1, a protein that interacts with C kinase; and NET and SERT with syntaxin1A (a synaptic protein that can modulate trafficking and ion-flux) [7,11]. There is also evidence for the formation of homo-oligomers by each of the transporters but not hetero-oligomers [27–29]. Aggregation into oligomers is not essential for the transport function but does affect trafficking in the case of DAT [28].

Role of monoamine transporters in the actions of amphetamines

AMPH, methamphetamine (METH) and 3,4-methylenedioxymethamphetamine (MDMA) are the principal amphetamines used illicitly in Western culture, acting as substrates for all three transporters and inducing monoamine release by disruption of vesicular storage and transporter reversal. The effects of METH on locomotor activity and behaviour are mediated primarily by dopamine released via DAT, as these effects are blocked by DAT inhibitors or by deletion of the DAT gene; in
addition, there is significant release of serotonin via SERT [7]. MDMA similarly induces both dopamine and serotonin release but with reversed priority: its effects being attenuated by SERT inhibitors or by deletion of the SERT gene [30]. The role of DAT in the actions of MDMA is equivocal. Some studies have reported inhibition of dopamine release by administration of DAT inhibitors before MDMA, whereas others observed no effect [31].

Dopamine release by AMPH, as detailed above, is mediated by reversal of DAT driven by intracellular Na⁺ and Ca²⁺ and DAT-mediated ion flux. PKC might also be an obligatory intermediate, as deletion of the N-terminal 22 amino acids of DAT, which includes those serine residues known to be phosphorylated by PKC, abolishes AMPH-induced ion flux and the associated dopamine efflux, but has no effect on dopamine uptake [32]. This finding is particularly significant in the search for methods of treating amphetamine addiction, as it suggests that it may be possible to identify drugs that disrupt the critical phosphorylation sites on DAT and hence block the reinforcing effects of amphetamines without inhibiting normal dopamine uptake. However, studies using DAT KO mice have demonstrated that the rewarding effect of AMPH is not abolished when measured by the conditioned place preference (CPP) response, a behavioural model in which recognition of a drug is demonstrated by preference of the animal for a specific location within the test arena. Furthermore, systemic AMPH still increases extracellular dopamine in the nucleus accumbens in these mutant mice [33,34], suggesting that other mechanisms, most likely involving serotonin, also play a major role in the rewarding properties of amphetamines.

A major concern regarding the widespread illicit use of amphetamines is their neurotoxic potential as revealed in animal studies. Using appropriate dosage regimens, METH causes neurotoxicity within the terminal fields of both dopaminergic and serotonergic neurons, although not within cell bodies [35]. MDMA induces a similar form of neurotoxicity but exclusively in serotonergic neurones in all species except the mouse, where dopaminergic neurones are affected [30]. In the rat, dopaminergic neurotoxicity induced by METH is associated with a decrease in vesicular dopamine uptake capacity and the level of VMAT2 immunoreactivity in a striatal synaptic vesicular fraction, an effect prevented by prior administration of DAT inhibitors [36]. Furthermore, the neurotoxic potency of METH is increased in heterozygous VMAT2 KO mice [37]. This suggests that METH neurotoxicity may result from a reduction in the capacity for dopamine storage within cytosolic vesicles, leading to increased dopamine concentration within the cytosol of the nerve terminal where it can be oxidized to generate neurotoxic free radicals. A similar mechanism has been proposed to underlie serotonergic neurotoxicity induced by MDMA on the basis of promiscuous uptake of dopamine via SERT, which is enhanced at higher temperatures, such as those associated with MDMA-induced hyperthermia [38]. A role for DAT has also been implicated by the protection against MDMA-induced serotonergic neurotoxicity in the striatum of the rat afforded by infusion of a DAT antisense-oligonucleotide [39]. However, there remains considerable dispute over the role of DAT in mediating the effects of MDMA; the serotonergic neurotoxicity induced by MDMA is observed in many brain regions that express very low levels of both dopamine and DAT, whereas pretreatment with SERT inhibitors uniformly prevents the toxic outcome throughout the brain.

One clinical use of amphetamines (and subsequently methylphenidate) is in the treatment of ADHD in children. The calming effect of these drugs, which induce hyperactivity in adults, appears paradoxical. However, studies in homozygous DAT KO mice, in which extracellular dopamine levels and basal locomotor activity are significantly raised, demonstrate that both amphetamines and methylphenidate reduce locomotor activity [6]. This effect appears to be a result of the serotonergic properties of the drugs, as it can be mimicked by selective SERT inhibitors.

Role of monoamine transporters in the actions of cocaine

Cocaine inhibits all three monoamine transporters with relatively higher affinity for DAT and NET compared with SERT [40]. Recent evidence indicates that the locus of cocaine binding to DAT is distinct from that of substrates, and that cocaine induces a differential conformational change in DAT compared with substrates, rendering the transporter unable to reuptake dopamine [41,42].

Although the principal pharmacological and behavioural effects of cocaine are mediated by dopamine, recent gene KO studies have indicated multiple roles of the monoaminergic systems. Hence DAT KO mice retain the ability to both acquire and maintain cocaine self-administration in comparison to wild-type (WT) animals, indicating that the reinforcing effects of cocaine might be mediated via DAT-independent mechanisms [43]. Furthermore, cocaine increases extracellular dopamine levels in the nucleus accumbens of both DAT KO and WT mice when administered systemically but not when infused directly into the nucleus accumbens [44]. This increase in extracellular dopamine is mimicked by selective SERT inhibitors, suggesting a serotonergic mechanism. In contrast to WT animals, DAT KO mice conditioned to cocaine demonstrate a CPP response to fluoxetine and nisoxetine, selective inhibitors of SERT and NET, respectively. In combined NET/SERT KO...
mice, cocaine produces a larger CPP response than in wild-type mice [45], whereas this response is abolished in combined DAT/SERT KO mice [46]. These studies suggest that the interaction of cocaine with SERT, and possibly NET, may be necessary to initiate and maintain cocaine reward in KO mice, although dopamine clearly plays a central role in the reinforcing effects of cocaine in WT mice.

Despite the significant health consequences associated with cocaine administration, there are no effective treatments for cocaine addiction. Currently, one approach is to prevent cocaine binding to the transporters by administration of competing DAT inhibitors, effectively acting as cocaine antagonists [47]. However, the use of these ligands is limited, as many show high abuse liability. Other strategies include the development of proteins that increase cocaine degradation via hydrolysis of the benzoyl ester [48] and the use of bacteriophage expressing cocaine-binding protein, which can access the CNS and successfully block the locomotor effects of cocaine in rodents [49]. Clinical trials using anti-cocaine antibodies in cocaine addicts are currently in progress and represent a novel approach for the treatment of both the psychotropic and cardiotoxic effects of drugs of abuse [50].

Conclusions
As we more fully understand the modes of operation and functional regulation of monoamine transporters, the idea that they act simply as a synaptic vacuum cleaner has been superseded by one in which the transporters play a major role in the regulation of presynaptic function. However, there are some intriguing questions that remain unanswered. What is the basis of the difference in regulation of DAT by substrates and inhibitors compared with SERT and NET? What consequences does this have for the neurotransmitter role of dopamine compared with that of serotonin and norepinephrine? What implications does this have for the acute and chronic effects of therapeutic drugs acting on DAT, SERT and NET? The mechanism of action of amphetamines has developed in parallel with our understanding of transporter function but is not yet fully resolved. Although plasma membrane transporters, together with the vesicular transporter, play a major role in the amine-releasing properties of these drugs, it is unclear to what extent they are implicated in their neurotoxic action particularly in relation to MDMA. The role of DAT is central to the actions of cocaine but KO studies also implicate serotonin and norepinephrine in the generation of its addictive profile. Furthermore, differences in the density of transporters throughout the brain suggest regional variations in the presynaptic role of the transporters and subsequent modulation by inhibitors. Recent studies of DAT have either used cell culture or striatal tissue, and it is important to investigate the extent to which these properties are replicated throughout the brain, particularly within the limbic forebrain. Clearly, the transporters play a crucial role in maintaining the synaptic balance of monoamine release and reuptake, and the disruption of that balance which results from pharmacological intervention has major consequences particularly for emotive behaviour. Improved understanding of transporter function and modulation within the brain will be central to the development of new therapeutic drugs and our ability to counter the addictive characteristics of amphetamines and cocaine.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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- Demonstration that dopamine efflux mediated by DAT is dependent upon intracellular Na⁺ and membrane potential. The magnitude of dopamine efflux induced by AMPH correlates with the size of DAT-mediated ion flux.

- Demonstration that chelation of intracellular Ca²⁺ attenuates AMPH-induced DAT-mediated ion flux and dopamine efflux. AMPH induces a rise in intracellular Ca²⁺ that is blocked by cocaine or thapsigargin, suggesting an obligatory role for intracellular Ca²⁺ in the dopamine efflux mechanism.


