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SLC6 Neurotransmitter Transporters: Structure, Function, and Regulation

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***Abstract*——The neurotransmitter transporters (NTTs) belonging to the solute carrier 6 (*SLC6*) gene family (also referred to as the neurotransmitter-sodium-symporter family or Na^+/Cl^--dependent transporters) comprise a group of nine sodium- and chloride-dependent plasma membrane transporters for the monoamine neurotransmitters serotonin (5-hydroxytryptamine), dopamine, and norepinephrine, and the amino acid neurotransmitters GABA and glycine. The *SLC6* NTTs are widely expressed in the mammalian brain and play an essential role in regulating neurotransmitter signaling and homeostasis by mediating uptake of released neurotransmitters from the extracellular space into neurons and glial cells. The transporters are targets for a wide range of therapeutic drugs used in treatment of psychiatric diseases, including major depression, anxiety disorders, attention deficit hyperactivity disorder and epilepsy. Furthermore, psychostimulants such as cocaine and amphetamines have the *SLC6* NTTs as primary targets. Beginning with the determination of a high-resolution structure of a prokaryotic**

homolog of the mammalian *SLC6* transporters in 2005, the understanding of the molecular structure, function, and pharmacology of these proteins has advanced rapidly. Furthermore, intensive efforts have been directed toward understanding the molecular and cellular mechanisms involved in regulation of the activity of this important class of transporters, leading to new methodological developments and important insights. This review provides an update of these advances and their implications for the current understanding of the *SLC6* NTTs.

I. Introduction

Transport across cellular membranes of impermeant solutes such as ions, amino acids, nutrients, and signaling molecules is carried out by a large group of integral membrane proteins known as solute carriers or more commonly transporters. Transporters are present in virtually every cell and are essential for life of all prokaryotic and eukaryotic organisms. The transport processes are often energetically coupled, either directly through the hydrolysis of ATP by the transport protein itself or indirectly by the use of transmembrane ion gradients that enable transport of the substrate against its concentration gradient. If ion channels are excluded, major classes of transport proteins in humans encompass ATP-driven ion pumps (e.g., the ubiquitously expressed Na^+/K^+ ATPase), ATP binding cassette transporters (e.g., the cystic fibrosis transmembrane conductance regulator and the multidrug resistance transporter P-glycoprotein), cytochrome B-like proteins, aquaporins (water transporters), and the solute carrier superfamily (*SLC*[1]) (http://www.bioparadigms.org).

SLC transporters encompass approximately 350 transporters organized into 55 families of the SLC series (Hediger et al., 2004; for overview, see Fredriksson et al., 2008; He et al., 2009). The *SLC6* family is among the largest SLC families, containing 20 genes that encode a group of highly similar transporter proteins. These proteins perform transport of amino acids and amino acid derivatives into cells, using cotransport of extracellular Na^+ as a driving force for substrate translocation against chemical gradients (Chen et al., 2004b; Höglund et al., 2005; Bröer, 2006). The *SLC6* transporters are secondary active transporters (Chen et al., 2004b), because they use the electrochemical potential difference across the cell membrane of Na^+ as energy source for transport. In addition, the *SLC6* transporters are further classified as symporters, in that the coupled transport of Na^+ is performed in the same direction as substrate transport, although a few members also exhibit antiport activity by performing counter-transport of K^+ (Rudnick and Clark, 1993).

The majority of the *SLC6* transporters has a well defined biological function and physiological role, including known endogenous substrates, and is divided into four subclasses on the basis of sequence similarity and substrate specificity (Bröer, 2006) (Fig. 1; Table 1). This review focuses on the subset of *SLC6* neurotransmitter transporters (NTTs), which includes the transporters for the monoamine neurotransmitters serotonin [5-hydroxytryptamine (5-HT)], dopamine, and norepinephrine and the transporters for the amino acid neurotransmitters GABA and glycine (Fig. 1). Three transporters

[1]Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; 5-HTTLPR, serotonin-transporter-linked polymorphic region; ADHD, attention deficit hyperactivity disorder; AdiC, arginine-agmatine antiporter; Akt, protein kinase B; BZT, benztropine; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CI-966, 1-(2-(bis(4-(trifluoromethyl)phenyl) methox)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid; CNS, central nervous system; COPII, coat protein complex II; CP-802,079, *N*-[3-(4-chlorophenyl)-3-[4-(2-thiazolylcarbonyl)phenoxy]propyl]-*N*-methyl-glycine hydrochloride hydrate; DAT, dopamine transporter; EF1502, (*R*)-*N*-(4,4-bis(3-methyl-2-thienyl)-3-butenyl)-3-hydroxy-4-(methylamino)-4,5,6,7-tetrahydrobenzo(*d*)isoxazol-3-ol; EL, extracellular loop; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ESCRT, endosomal sorting complex required for transport; F11440, eptapirone; FRET, fluorescence resonance energy transfer; GABA, γ-aminobutyric acid; GAT, γ-aminobutyric acid transporter; GlyR, glycine receptor; GLYT, glycine transporter; GST, glutathione transferase; HEK, human embryonic kidney; JHW 007, *N*-(*n*-butyl)-(bis-fluorophenyl)methoxytropane; KO, knockout; LeuT, leucine transporter; Lu AA20465, (*R*)-4-[5-chloro-2-(4-methoxy-phenylsulfanyl)-phenyl]-2-methyl-piperazin-1-yl-acetic acid; Lu AA24530, 4-(2-((4-methylphenyl)sulfanyl)phenyl)piperidine; LY2365109, ((2-(4-benzo(1,3)dioxol-5-yl-2-*tert*-butylphenoxy)ethyl)methylamino)acetic acid; MacMARCKS, homolog of myristoylated alanine-rich C kinase substrate; MAPK, mitogen-activated protein kinase; MD, molecular dynamics; MDMA, methylenedioxymethamphetamine; NET, norepinephrine transporter; NMDA, *N*-methyl-D-aspartate; NNC-711, 1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid; nNOS, neuronal nitric-oxide synthase; NRI, norepinephrine reuptake inhibitorc; NRI, selective norepinephrine-reuptake inhibitor; NTT, neurotransmitter transporter; ORG 24598, (*R*)-(−)-*N*-[3-[(4-triflouromethyl)phenoxy]-3-phenyl-propyl]glycine; ORG 25543, 4-benzyloxy-3,5-dimethoxy-*N*-[(1-dimethylaminocyclopentyl)methyl]benzamide; PDZ, postsynaptic density 95/discs-large/zona occludens; PE, premature ejaculation; PICK, proteins that interact with C kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PMA, phorbol 12-myristate 13-acetate; PP, protein phosphatase; PSD-95, postsynaptic density-95; PTM, post-translational modifications; RG1678, (4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl)(5-methanesulfonyl-2-(2,2,2-trifluoro-1-methylethoxy)phenyl)methanone; RTI-55, (−)-2β-carbomethoxy-3β-(4-iodophenyl)tropane; SCAM, substituted cysteine accessibility method; SERT, serotonin transporter; siRNA, small interfering RNA; SKF89976A, 1-(4,4-diphenyl-3-butenyl)-3-piperidinecarboxylic acid hydrochloride; SLC, solute carrier; SNAP-5114, 1-(2-(tris(4-methoxyphenyl)methoxy)ethyl)-3-piperidinecarboxylic acid; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; SNP, single-nucleotide polymorphism; SNRI, serotonin- and norepinephrine-reuptake inhibitor; SSR130800, *N*-[3-(4-chlorophenyl)-3-[4-(2-thiazolylcarbonyl)phenoxy]propyl]-*N*-methyl-glycine hydrochloride hydrate; SSR504734, 2-chloro-*N*-((*S*)-phenyl((2*S*)-piperidin-2-yl)methyl)-3-trifluoromethyl benzamide; SSRI, selective serotonin-reuptake inhibitor; TCAs, tricyclic antidepressants; TM, transmembrane; vSGLT, *Vibrio parahaemolyticus* sodium/glucose transporter; WIN 35,428, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane; Xen2174, Sec-Gly-Val-Cys-Cys-Gly-Tyr-Lys-Leu-Cys-His-Pyl-Cys.

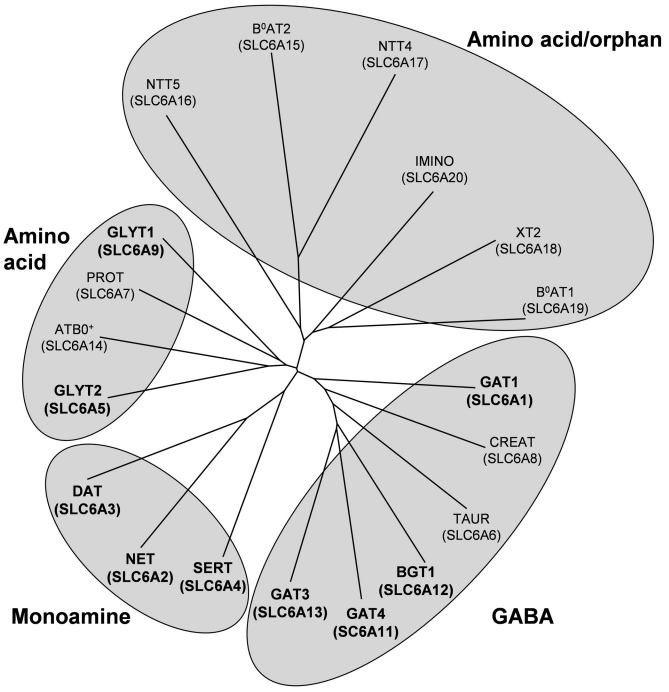

FIG. 1. Phylogenetic tree of the *SLC6* transporter family. *SLC6* transporters are divided into four subfamilies: monoamine, GABA, amino acid, and amino acid/orphan. NTTs are found in three subfamilies and are highlighted in bold. The *SLC6*A10 transporter, a putative creatine transporter, has been omitted from the tree because the gene encoding seems to be a pseudogene (see Table 1 legend).

exist for the biogenic monoamine transmitters, named according to their primary endogenous transmitter substrate the 5-HT, norepinephrine, and dopamine transporters (SERT, NET, and DAT, respectively), whereas four transporters exist for GABA (GAT1, GAT2, GAT3, and BGT1) and two transporters exist for glycine (GLYT1 and GLYT2) (Table 1; Fig. 2). All the *SLC6* NTTs are expressed in the central nervous system (CNS), where their primary physiological role is regulation of neurotransmitter homeostasis. However, some of them are also found in other tissues, where they serve important physiological functions (Table 1). Because the NTT genes were among the founding gene members of the *SLC6* gene family, alternate designations for the family are often used, such as the "neurotransmitter-sodium symporters," the "sodium-neurotransmitter symporter family," or the "Na^+/Cl^- neurotransmitter transporter" family (Nelson, 1998; Beuming et al., 2006).

A series of breakthroughs in the understanding of the structure and function of bacterial transporters related to the *SLC6* family have rejuvenated the *SLC6* NTT research field in a manner not seen since the cloning of the first transporter genes in the early 1990s (Singh et al., 2007, 2008; Zomot et al., 2007; Beuming et al., 2008; Forrest et al., 2008; Zhao et al., 2010a,b). These advances have promoted a profound increase in the understanding of the structural biology and molecular pharmacology of the *SLC6* NTTs, in addition to highlighting the importance of understanding other aspects of transporter biology, including cellular mechanisms for transporter regulation by post-translational modifications (PTMs), protein-protein interactions, and trafficking. This review provides an update of the molecular and cellular biology of the *SLC6* NTTs with particular focus on discoveries relating to transporter structure, function, and pharmacology, including the mechanisms of action for transporter drugs of clinical relevance and drugs of abuse. We also include an account on the current insights into cellular mechanisms governing the activity and availability of NTTs in the plasma membrane as well as a status of our understanding of their physiology, disease involvement, and role as drug targets.

A. Neurophysiological Role of the Solute Carrier 6 Neurotransmitter Transporters

The cognate substrates for the NTTs are all common neurotransmitters in the mammalian CNS playing essential roles in multiple aspects of brain function. The amino acid neurotransmitters GABA and glycine are inhibitory transmitters that regulate neuronal excitability throughout the CNS, whereas the monoamine neurotransmitters 5-HT, dopamine, and norepinephrine have more diverse roles and activity patterns. The monoamine NTTs seem to be exclusively expressed in their respective monoaminergic neurons, and the distribution of monoamine NTTs in the brain therefore correlates with the distribution of the respective cognate neurotransmitter systems. In neurons, monoamine NTTs are distributed in both dendrites and axons with a predominant extrasynaptic localization. For the individual GABA and glycine NTTs, the expression patterns in the brain are more complex, including differences across regions of the CNS and also localization in astrocytes (Jursky et al., 1994; Zafra et al., 1995a,b; Nirenberg et al., 1996; Zhou et al., 1998; Pickel and Chan, 1999; Schousboe, 2000; Schroeter et al., 2000; Zahniser and Doolen, 2001; Chen et al., 2004b; Bak et al., 2006). Nevertheless, the central role of all NTTs is to mediate the rapid uptake from the extracellular space of synaptically released neurotransmitter within and around synapses (Fig. 2). The uptake can be maintained against very large concentration gradients, (Zafra et al., 1995b; Nirenberg et al., 1996; Zhou et al., 1998; Pickel and Chan, 1999; Schroeter et al., 2000; Zahniser and Doolen, 2001; Chen et al., 2004b) and the kinetics of NTT-mediated transport follows the Michaelis-Menten model with substrate K_M values in the lower micromolar range and maximal turnover rates ranging from 1 to 20 substrate molecules per second (Table 2). Apart from the mandatory requirement for extracellular Na^+, NTT transport is also dependent on extracellular Cl^-. The relationship between extracellular concentrations of Na^+ and Cl^- and transport activity also follows Michaelis-Menten kinetics with K_M values in 5 to 50 mM range (Humphreys et al., 1994; Supplisson and Roux, 2002).

TABLE 1
The SLC6 transporters

Human Gene	UniProt Name[a]	Endogenous Substrate	Tissue Distribution	Link to Disease
*SLC6*A1	GAT1	GABA	Brain, peripheral nervous system	Epilepsy, schizophrenia, anxiety
*SLC6*A2	NET	Norepinephrine	Brain, peripheral nervous system, adrenal gland, placenta	Depression, orthostatic intolerance, anorexia nervosa, cardiovascular diseases
*SLC6*A3	DAT	Dopamine	Brain	Parkinson's disease, Tourette syndrome, ADHD, addiction
*SLC6*A4	SERT	Serotonin	Brain, peripheral nervous system, placenta, epithelium, platelets	Anxiety, depression, autism, gastrointestinal disorders, premature ejaculation, obesity
*SLC6*A5	GLYT2	Glycine	Brain, spinal cord	Pain, spasticity
*SLC6*A6	TAUT	Taurine	Brain, retina, liver, kidney, heart, spleen, pancreas	Taurine deficiency diseases, retinal blindness, abnormal renal development
*SLC6*A7	PROT	Proline	Brain	
*SLC6*A8	CT1	Creatine	Ubiquitous	Creatine deficiency syndrome, mental retardation, musculoskeletal disorders, cardiomyopathy
*SLC6*A9	GLYT1	Glycine	Brain, pancreas, uterus, stomach, spleen, liver, retina	Schizophrenia
*SLC6*A10[b]				
*SLC6*A11	GAT3	GABA	Brain	Epilepsy
*SLC6*A12	BGT1	Betaine, GABA	Brain, kidney	Epilepsy
*SLC6*A13	GAT2	GABA	Brain, liver, kidney	Epilepsy
*SLC6*A14	ATB0+	Neutral, cationic amino acids	Lung, trachea, salivary gland, mammary gland, stomach, pituitary gland	
*SLC6*A15	NTT73	Large neutral amino acids	Brain	
*SLC6*A16	NTT5	Unknown	Testis, pancreas, prostate	
*SLC6*A17	NTT4	Neutral amino acids	Brain	
*SLC6*A18	B0AT3	Neutral amino acids	Kidney	
*SLC6*A19	B0AT1	Neutral amino acids	Intestine	Hartnup disorder
*SLC6*A20	XTRP3	Proline, pipecolate, sarcosine	Brain, kidney, small intestine, thymus, spleen, ovary, lung	

[a] Universal Protein Resource (http://www.uniprot.org/).
[b] *SLC6*A10 has been proposed to be a pseudo gene duplicated from *SLC6*A8 because of the presence of a stop codon located in exon 4 of the gene (Eichler et al., 1997; Höglund et al., 2005).

The role of Cl^- in the transport process is not fully understood, but for most NTTs, one or more Cl^- ions are hypothesized to be cotransported with substrate and Na^+ (section II).

By mediating the rapid removal of neurotransmitters from the synapses, NTTs are major determinants in regulation of synaptic signaling. Accordingly, cellular regulation of the cell-surface density and/or catalytic activity of NTTs is an important determinant for plasticity of synaptic signaling, because dynamic changes in the number and function of transporters allows regulation of the rate by which released transmitters are removed from synapses. The NTT-mediated reuptake into presynaptic neurons is also critical for recycling of neurotransmitters, because after delivery into the cytoplasm of the neurotransmitters, they can be sequestered into synaptic vesicles by vesicular transporters. These vesicular transporters, which include the *SLC18* vesicular monoamine transporters 1 and 2 and the *SLC32* vesicular inhibitory amino acid transporter (Hediger et al., 2004), are both functionally and structurally distinct from the *SLC6* NTTs. They are secondary active transporters; instead of being Na^+-coupled symporters, however, they are proton-coupled antiporters and use the low pH in the vesicular lumen as driving force for upconcentrating neurotransmitter inside the vesicles.

Given the pivotal role of the *SLC6* NTTs for the homeostasis of five major neurotransmitters in the CNS, pharmacological modulation of NTT activity allows direct or indirect regulation of neuronal activity. Hence, compounds targeting the NTTs are important as pharmacological tools for studies of neurotransmission at the molecular, cellular and neural circuit level (section III). Moreover, four of the nine *SLC6* NTTs are established and important drug targets for treatment of a broad array of brain diseases and are targets for more than 30 drugs in clinical use (section III). In addition, the NTTs that are currently not targeted by medications are actively being pursued in drug discovery efforts (section VI). It is noteworthy that the monoamine NTTs SERT, DAT, and NET are also targets for some of the most widely used drugs of abuse, including cocaine, 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") and amphetamine/methamphetamine (section III). Despite the importance of NTTs as drug targets, surprisingly little has been known about their molecular pharmacology, including localization and structure of drug binding sites and mechanism of action. As mentioned above, recent progress provided by structures of bacterial counterparts to the *SLC6* transporters has provided a new level of insight into the structural biology of the NTTs that present new opportunities to interpret existing knowledge as well as guide new studies to understand drug modulation of individual NTT members at the molecular level (section III).

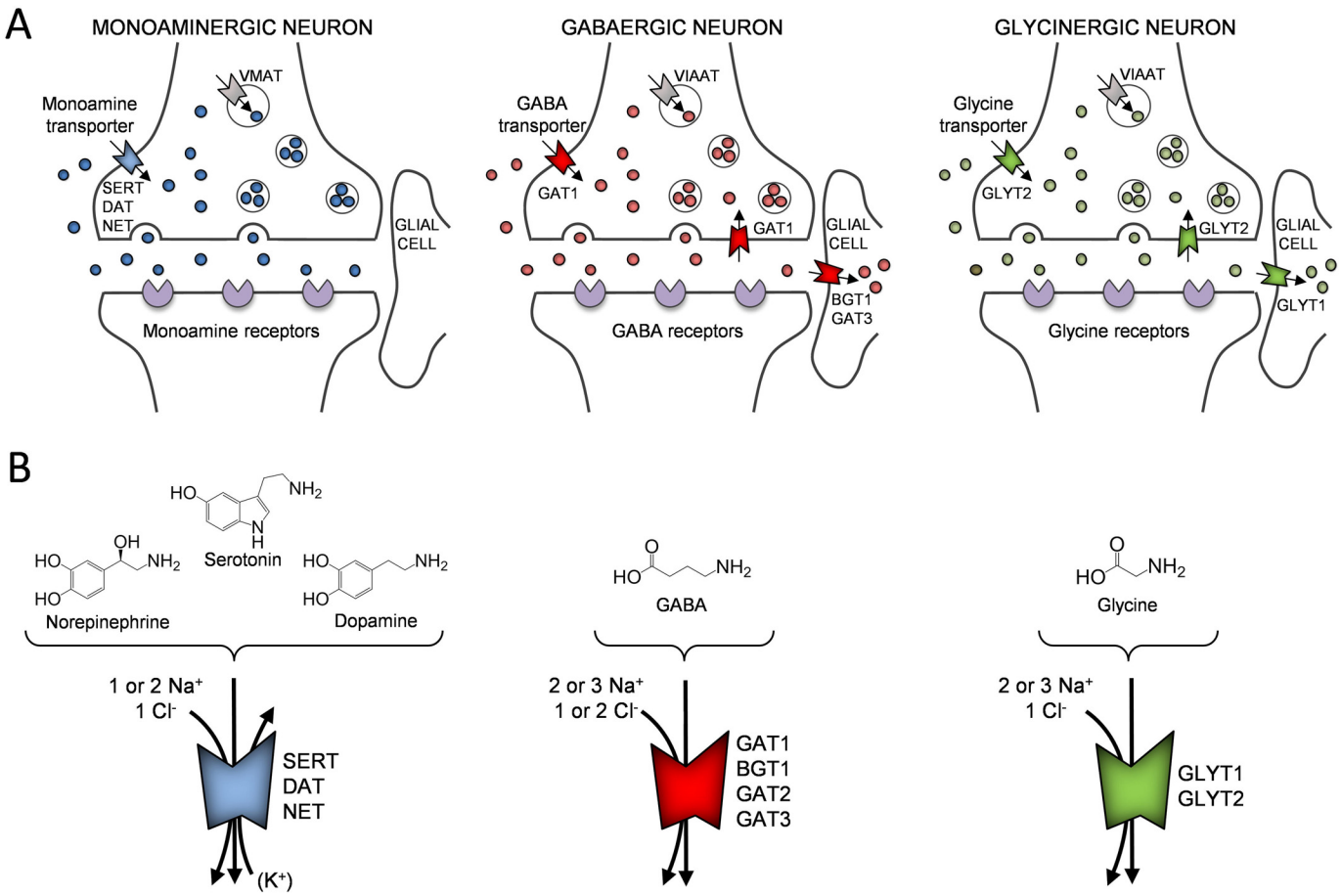

FIG. 2. Role of the *SLC6* NTTs in synaptic transmission. A, schematic representation of monoaminergic, GABAergic, and glycinergic synaptic terminals. In the presynaptic terminals of monoaminergic neurons, vesicular monoamine transporters (VMATs) belonging to the *SLC18* gene family (Eiden et al., 2004) sequester serotonin, dopamine, and norepinephrine into synaptic vesicles, whereas the vesicular inhibitory amino acid transporters (VIAATs) belonging to the *SLC32* gene family (Gasnier, 2004) sequester GABA and glycine into synaptic vesicles in GABAergic and glycinergic neurons, respectively. After vesicular release, neurotransmitters exert their effects on post- and presynaptic receptors. The *SLC6* NTTs are crucial for termination of neurotransmission by performing reuptake of the neurotransmitters from the synaptic cleft into presynaptic terminals or glial cells as well as for maintaining low tonic neurotransmitter concentrations outside synapses. The monoamine transporters (SERT, NET, and DAT) are localized to extrasynaptic sites (Torres et al., 2003b), whereas GATs and GLYTs are localized to synaptic and extrasynaptic sites in addition to glial cells (Supplisson and Roux, 2002; Conti et al., 2004; Madsen et al., 2010). B, chemical structures of the endogenous substrates for *SLC6* NTTs and ion coupling stoichiometry for neurotransmitter reuptake (see also Table 2).

II. Structure and Transport Mechanism

Before the determination of the first high-resolution X-ray crystal structure of a bacterial homolog to the mammalian *SLC6* transporters, the structural understanding of *SLC6* NTTs was based on indirect observations derived from extensive biochemical and mutagenesis studies that provided indispensable insight into transporter topology and secondary structure but limited information on the tertiary structure. This was revised in 2005 when Yamashita et al. (2005) reported a high-resolution X-ray crystallographic structure of a prokaryotic homolog to the *SLC6* transporters, the leucine transporter (LeuT) from the thermophile bacterium *Aquifex aeolicus* (Deckert et al., 1998). Although LeuT is evolutionary distant from the *SLC6* NTTs (20–25% overall sequence identity), the protein has proved to be a suitable and highly useful structural and functional template for the *SLC6* NTTs (Singh, 2008). In addition, the discovery of a remarkably structural conservation between LeuT and secondary active transporters from other transporter families, not originally thought to be structurally and mechanistically related to *SLC6* transporters (Abramson and Wright, 2009), have further strengthened the validity of using LeuT as a structural template for understanding fundamental aspects of *SLC6* NTT function. In this section, we recapitulate these recent advances and bring them into context with earlier work to provide an overview of the current understanding of the structural mechanisms underlying NTT function, including the binding of substrates and ions, their translocation, and the associated conformational states and changes. Note also that the aforementioned advances in the structural biology of other secondary active transporters and their significance for understanding the *SLC6* NTTs have been covered in several excellent reviews (Gether et al., 2006; Henry et al., 2006b; Kanner, 2006; Rudnick, 2006; Torres and Amara, 2007; Kanner and Zomot, 2008; Singh, 2008; Abramson and Wright, 2009; Gouaux, 2009; Krishnamurthy et al., 2009).

TABLE 2
The SLC6 neurotransmitter transporters

Transporter	Splice Variants	Size	K_M	Turnover Rate	Substrate-Ion Stoichiometry (Substrate/Na^+/Cl^-)
		amino acids	*μM*	s^{-1}	
Monoamine transporters					
SERT		630	0.2–1.0[a]	1.0–3.1[b]	1:1:1:1 K^+ (out)[c]
NET	3[d]	617	0.3–1.4[e]	1.7–2.5[f]	1:1:1[g]
DAT		620	0.3–5.2[h]	0.7–1.9[i]	1:2:1[j]
GABA transporters					
GAT1		599	5.0–7.0[k]	6.0–93[l,m]	1:2:1[n]
BGT1[o]		614	19–120[p]	22[m,q]	1:3:(1 or 2)[r]
GAT2[s]		602	3.0–8.0[t]	1.7–3.8[m,u]	1:2:1[v]
GAT3[w]		632	2.3–12[x]	1.5–16[m,y]	1:2:1[z]
Glycine transporters					
GLYT1	5[d]	706	20–100[aa]	18–20[bb]	1:2:1[cc]
GLYT2	3[d]	797	25–94[dd]	20[ee]	1:3:1[cc]

[a] Blakely et al., 1991; Hoffman et al., 1991; Ramamoorthy et al., 1993; Gu et al., 1994; Chang et al., 1996; Kristensen et al., 2004; Andersen et al., 2009b; human, rat, and mouse SERT.
[b] Gu et al., 1994; Sucic and Bryan-Lluka, 2002; Quick, 2003; Kristensen et al., 2004; human and rat SERT.
[c] Talvenheimo et al., 1983; porcine SERT.
[d] Chen et al., 2004b; NET splice variants differ in the C-terminal region. GLYT1 and GLYT2 splice variants differ in their N- and C-terminal regions.
[e] Pacholczyk et al., 1991; Gu et al., 1994, 1996c; Galli et al., 1995; Paczkowski et al., 1999; human, rat, and bovine NET.
[f] Bönisch and Harder, 1986; Galli et al., 1995; Sucic and Bryan-Lluka, 2002; human and rat NET.
[g] Gu et al., 1996b; human NET.
[h] Giros et al., 1991, 1992; Kilty et al., 1991; Shimada et al., 1991; Gu et al., 1994, 1996a; human and rat DAT.
[i] Gu et al., 1994; Meiergerd et al., 1994; Lin et al., 1999; Prasad and Amara, 2001; rat DAT.
[j] Gu et al., 1994; rat DAT.
[k] Guastella et al., 1990; Loo et al., 2000; human and rat GAT-1.
[l] Mager et al., 1993; Deken et al., 2000; Fesce et al., 2002; Gonzales et al., 2007; human and rat GAT1.
[m] Electrophysiological measurements; highly dependent on temperature and membrane potential.
[n] Loo et al., 2000; human GAT1.
[o] Equivalent to mouse GAT2.
[p] Lopez-Corcuera et al., 1992; Yamauchi et al., 1992; Borden et al., 1995; Matskevitch et al., 1999; human and canine BGT-1 and mouse GAT-2.
[q] Forlani et al., 2001; canine BGT1.
[r] Matskevitch et al., 1999; canine BGT1.
[s] Equivalent to mouse GAT3.
[t] Borden et al., 1992; Sacher et al., 2002; rat GAT-2 and mouse GAT-3.
[u] Sacher et al., 2002; mouse GAT3.
[v] Sacher et al., 2002; mouse GAT3.
[w] Equivalent to mouse GAT4.
[x] Borden et al., 1992; Clark et al., 1992; Karakossian et al., 2005; rat GAT-3 and mouse GAT-4.
[y] Karakossian et al., 2005; mouse GAT4.
[z] Karakossian et al., 2005; mouse GAT4.
[aa] Guastella et al., 1992; Liu et al., 1992b; Smith et al., 1992; Kim et al., 1994; Roux and Supplisson, 2000; rat GLYT1.
[bb] Supplisson and Bergman, 1997; Supplisson and Roux, 2002; rat GLYT1.
[cc] Roux and Supplisson, 2000; rat GLYT1b and GLYT2a splice variants.
[dd] Borowsky et al., 1993; Roux and Supplisson, 2000; rat GLYT2.
[ee] Supplisson and Roux, 2002; rat GLYT2.

A. Neurotransmitter Transporter Structure

1. Molecular Organization. The molecular cloning of the cDNA encoding the NTTs in the early 1990s revealed a highly similar primary structure of these transporters with an average amino acid sequence identity of 40%, suggesting that NTTs share a similar general structure (Guastella et al., 1990; Blakely et al., 1991; Hoffman et al., 1991; Pacholczyk et al., 1991; Liu et al., 1992a, 1993). Most of the NTTs contain approximately 600 amino acids, ranging from 599 (GAT1) to 632 (SERT), except that the two glycine transporters each contain approximately 700 amino acids. Sequence analysis combined with biochemical approaches suggested a membrane topology with 12 transmembrane (TM) helices with intracellular N- and C-termini and a large glycosylated loop between transmembrane helices III and IV (see section V) (Table 2). Except for NET, GLYT1, and GLYT2, only single isoforms have been identified for each NTT (Fig. 3). This membrane topology has been experimentally verified across the monoamine (Torres et al., 2003a), GABA (Kanner, 2006) and glycine transporter subfamilies by a large number of studies, mainly using specific antibodies (Melikian et al., 1994; Brüss et al., 1995; Qian et al., 1995; Nirenberg et al., 1996; Chen et al., 1997a) or site-selective biochemical labeling of Lys and/or Cys residues (Guastella et al., 1990; Pacholczyk et al., 1991; Giros and Caron, 1993; Nirenberg et al., 1996; Povlock and Amara, 1996; Chen et al., 1998; Ferrer and Javitch 1998). Another structural feature of all NTTs is a pair of Cys residues in extracellular loop 2 that form an intraloop disulfide bridge. The loop also contains sites for N-linked glycosylation (Patel et al., 1994; Olivares et al., 1995; Qian et al., 1995; Nguyen and Amara, 1996; Chen et al., 1997a, 1998; Ramamoorthy et al., 1998b; Cai et al., 2005) (section V) (Table 2).

2. Analyses of Functional Domains. In the decade after the cloning of the NTTs, substantial efforts were made to identify and characterize the regions and specific residues that contribute to core transporter functions such as substrate binding and translocation, PTMs (section IV), or interactions with intracellular proteins. These efforts also focused on probing for secondary structure elements as well as to establish proximity relationships between TM domains and specific resi-

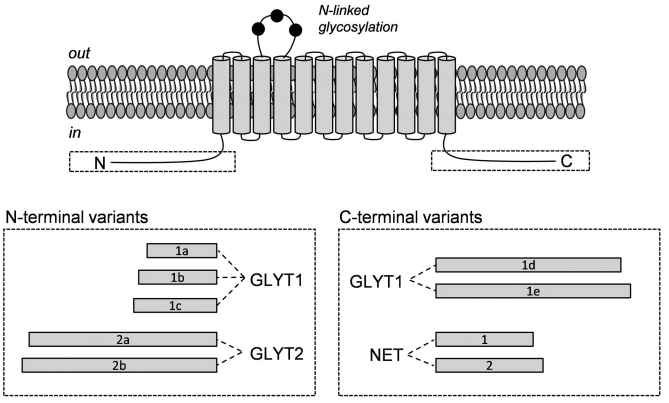

FIG. 3. Membrane topology of *SLC6* NTTs and splice variants. The NTTs contain 12 TM-spanning regions connected by intra- and extracellular loops with N and C termini located intracellularly. The extracellular loop between TM3 and TM4 contains sites for N-linked glycosylation. The genes for NET, GLYT1, and GLYT2 give rise to multiple splice variants. Five GLYT1 splice variants with variations in the N-terminal (GLYT1a, -1b, and -1c) and C-terminal (GLYT1d and -1e) and two splice variants of GLYT2 (GLYT2a and -2b) have been reported (Supplisson and Roux, 2002). For NET, two splice variants (NET1 and NET2) have been described with variations in the C-terminal region (Kitayama and Dohi, 2003).

dues. Experimental approaches mainly relied on site-directed mutagenesis in combination with recombinant expression of NTTs in heterologous expression systems. The substituted cysteine accessibility method (SCAM) (Akabas et al., 1992) in particular has been widely used, especially for DAT, SERT, and the GABA transporter GAT1, to probe for residue accessibility and secondary structure elements in TM and intra- and extracellular loop regions, as well as to define the functional role of individual residues for binding of substrate and inhibitors (section III). In SCAM studies, native residues are systematically replaced by cysteines and selectively derivatized with thiol-modifying agents (Akabas et al., 1992; Javitch, 1998; Karlin and Akabas, 1998). If the cysteine side chain is modified by agents only reactive in a hydrophilic environment, it implies that the residue is exposed at the water-accessible surface of the protein. Likewise, hydrophobic agents can be used to probe membrane-embedded or intracellular residues when the transporter resides in intact cells. SCAM analysis of entire TM regions and loop regions of SERT provided early evidence for α-helical structure of putative TM spanning domains and identified residues important for binding of substrates and inhibitors (Chen et al., 1997b; Henry et al., 2003; Keller et al., 2004b; Mitchell et al., 2004; Sato et al., 2004; Zhang and Rudnick, 2005, 2006).

DAT has also been subject to SCAM analysis, leading to insights into substrate- and cocaine-induced conformational changes in the transporter, oligomerization domains, and residues involved in substrate binding and/or binding of inhibitors such as cocaine (Ferrer and Javitch, 1998; Chen et al., 2000, 2004a; Loland et al., 2002, 2003, 2004; Hastrup et al., 2003; Uhl and Lin, 2003; Sen et al., 2005).

SCAM analysis of the GABA and glycine transporters has been carried out to a lesser extent, although several key residues for transporter function of GAT1 have been identified by mutagenesis studies (Kanner et al., 1994; Keshet et al., 1995; Mager et al., 1996; Bismuth et al., 1997; MacAulay et al., 2001; Zomot and Kanner, 2003; Zhou et al., 2004, 2006; Zomot et al., 2005; Rosenberg and Kanner, 2008; Ben-Yona and Kanner, 2009). For analysis of proximity relationships between specific residues, the primary approach has been to take advantage of the structural constraints required for construction of binding sites for divalent cations in proteins. Engineering of His and/or Cys residues to generate artificial binding sites for Zn^{2+} or Cd^{2+} in monoamine and GABA transporters has been used to establish distances between specific residues (Loland et al., 1999, 2003; Norregaard et al., 2000, 2003; MacAulay et al., 2001; Goldberg et al., 2003; Torres et al., 2003b; Volz and Schenk, 2005; Zomot et al., 2005; Henry et al., 2006b; Kanner, 2006; White et al., 2006; Kanner and Zomot, 2008). Detailed accounts on the elucidation of NTT topology and mutational analysis are provided in several reviews (Povlock and Amara, 1996; Chen and Reith, 2000, 2002; Lin and Uhl, 2002; Torres et al., 2003b; Uhl and Lin, 2003; Rudnick, 2006; Kanner and Zomot, 2008).

B. Prokaryotic Transporters As Structural Templates for the Solute Carrier 6 Neurotransmitter Transporters

Attempts to determine the tertiary structures of *SLC6* transporters has so far proven unsuccessful, mainly because of problems in obtaining sufficiently pure and stable transporter protein in quantities appropriate for protein crystallization (Tate and Blakely, 1994; Tate, 2001; Tate et al., 2003; Rasmussen and Gether, 2005). A major step forward has been the identification of prokaryotic transporter proteins with remarkable sequence homology and functional similarities to the *SLC6* transporters. In addition to the amino acid transporter LeuT, these include the tyrosine transporters TnaT (Androutsellis-Theotokis et al., 2003; Kniazeff et al., 2005) and Tyt1 (Quick et al., 2006) as well as multiple unexplored transporters from prokaryotic organisms, with the exception of *Escherichia coli*. Like LeuT, Tyt1, and TnaT originate from thermophile bacteria and have properties that allow for generation of stable proteins by heterologous expression. The first and so far only transporter among these to be crystallized was LeuT (Yamashita et al., 2005). LeuT is a Na^+-coupled amino acid transporter with specificity for the hydrophobic amino acids glycine, alanine, methionine, and leucine (Singh, 2008). The first LeuT structure was in complex with Leu and of a remarkably high resolution for a membrane protein X-ray crystal structure (1.65 Å). The structure provided unambiguous insight into central aspects of the structural biology of *SLC6* transporters, including long-sought details for how *SLC6* transporters accommodate substrate and ions. Subsequently, structures of LeuT have been solved in complex with different substrates (Singh et al., 2008) as well as competitive and noncompetitive inhib-

itors (Singh et al., 2007, 2008; Zhou et al., 2009). It is noteworthy that these LeuT structures have been established as valid templates for molecular modeling of the mammalian NTTs, and LeuT is emerging as an important model protein for biophysical and computational studies addressing the molecular dynamics underlying NTT function and pharmacology (Jørgensen et al., 2007a, 2008; Caplan et al., 2008; Celik et al., 2008a; Noskov and Roux, 2008; Noskov, 2008; Shi et al., 2008; Shaikh and Tajkhorshid, 2010; Zhao et al., 2010a,b).

1. Structure of the Leucine Transporter. The X-ray crystal structure of LeuT showed that the protein adopted a unique fold compared with the few other secondary active transporters for which structures were available, such as the glycerol-3-phosphate/phosphate antiporter GlpT (Huang et al., 2003) and the proton-coupled lactose symporter LacY (Abramson et al., 2003). The structure confirmed many predictions made for *SLC6* transporter architecture by revealing 12 TM regions connected by short intra- and extracellular loops with intracellular N and C termini (Fig. 4). The predicted secondary structure of the TM regions was confirmed, because all 12 TMs are almost exclusively α-helical, except short unwound segments in TM1 and TM6. Furthermore, several α-helical elements are also present in the intra- and extracellular loops (Fig. 4). The TMs pack as an intertwined helical bundle into a cylindrical shape that can be divided into an interior and outer part (Fig. 4). The inner ring is formed almost exclusively by TM1, TM3, TM6, and TM8 and holds a central substrate binding site (S1) in which the substrate (Leu) and two Na^+ ions are accommodated (Figs. 4 and 5). In all substrate-bound LeuT structures, this central substrate binding pocket is occluded from both the external and internal medium (Yamashita et al., 2005; Singh et al., 2007, 2008; Zhou et al., 2007, 2009; Quick et al., 2009). Access to the S1 binding pocket from the extracellular medium is blocked by an interaction network formed between side chains from TM1, TM3, TM6, and TM10 (Fig. 5). The aromatic side chains of Tyr108 on TM3 and Phe253 on TM6 form a hydrophobic lid across the top of the pocket (Figs. 5B and 8C). Just above this external "lid," the guanidium group of Arg30 on TM1 interacts via a pair of water molecules with the carboxylate group of Asp404 on TM10 (Fig. 5). In later structures of LeuT in complex with the noncompetitive inhibitor Trp, a direct interaction between the α-substituents of the inhibitor and the side chains of Arg30 and Asp404 is observed, an observation that is potentially significant for the mechanism of noncompetitive inhibition in LeuT (Singh et al., 2007; Zhou et al., 2007, 2009) (section III). Access from the substrate binding pocket toward the intracellular medium is blocked by a much larger protein layer containing a network of interactions formed mainly by tight packing of the intracellular halves of TM1, TM6 and TM8 (Yamashita et al., 2005; Singh et al., 2007, 2008; Zhou et al., 2007, 2009), resulting in a layer of ~20-Å ordered protein structure being situated directly beneath the substrate binding pocket. This structure seems to be stabilized mainly by an interaction network formed between Arg5, Asp369, Ser267, and Tyr268 (Fig. 8D). Adjacent to these residues is Trp8, which has also been suggested to stabilize the substructure. Together, these regions prevent access to the central pocket and are proposed to constitute the gating regions in LeuT; e.g., they act as external and internal gates toward the extra- or intracellular medium by undergoing structural rearrangement as the protein shuttle through a series of conformational states during the substrate translocation mechanism (Yamashita et al., 2005; Kanner, 2008; Singh, 2008) (section III). This crucial role is supported by the observation that all

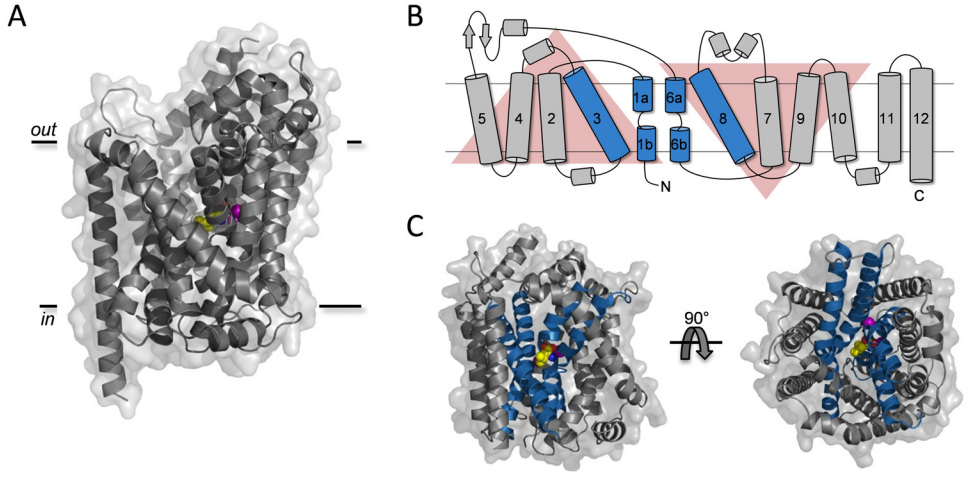

FIG. 4. X-ray crystal structure and topology of LeuT, a bacterial homolog of *SLC6* NTTs. A, structure of LeuT in the substrate-bound, outward-facing occluded conformation (Protein Data Bank ID 2A65). The substrate binding site is located at the core of the transporter, and the substrate and two Na^+ ions are shown as van der Waals spheres in yellow and purple, respectively. B, topology of LeuT with the inner ring (TM1, TM3, TM6, and TM8) that forms the substrate binding site shown in blue. The red triangles highlight the 5 + 5 inverted repeats, formed by TMs 1 to 5 and TMs 6 to 10, that are related by an apparent two-fold symmetry. C, structure of LeuT with the TM domains that form the inner ring highlighted in blue seen from the side (left; TM10 is omitted for clarity) and the top (right; EL1 and EL4 are omitted for clarity).

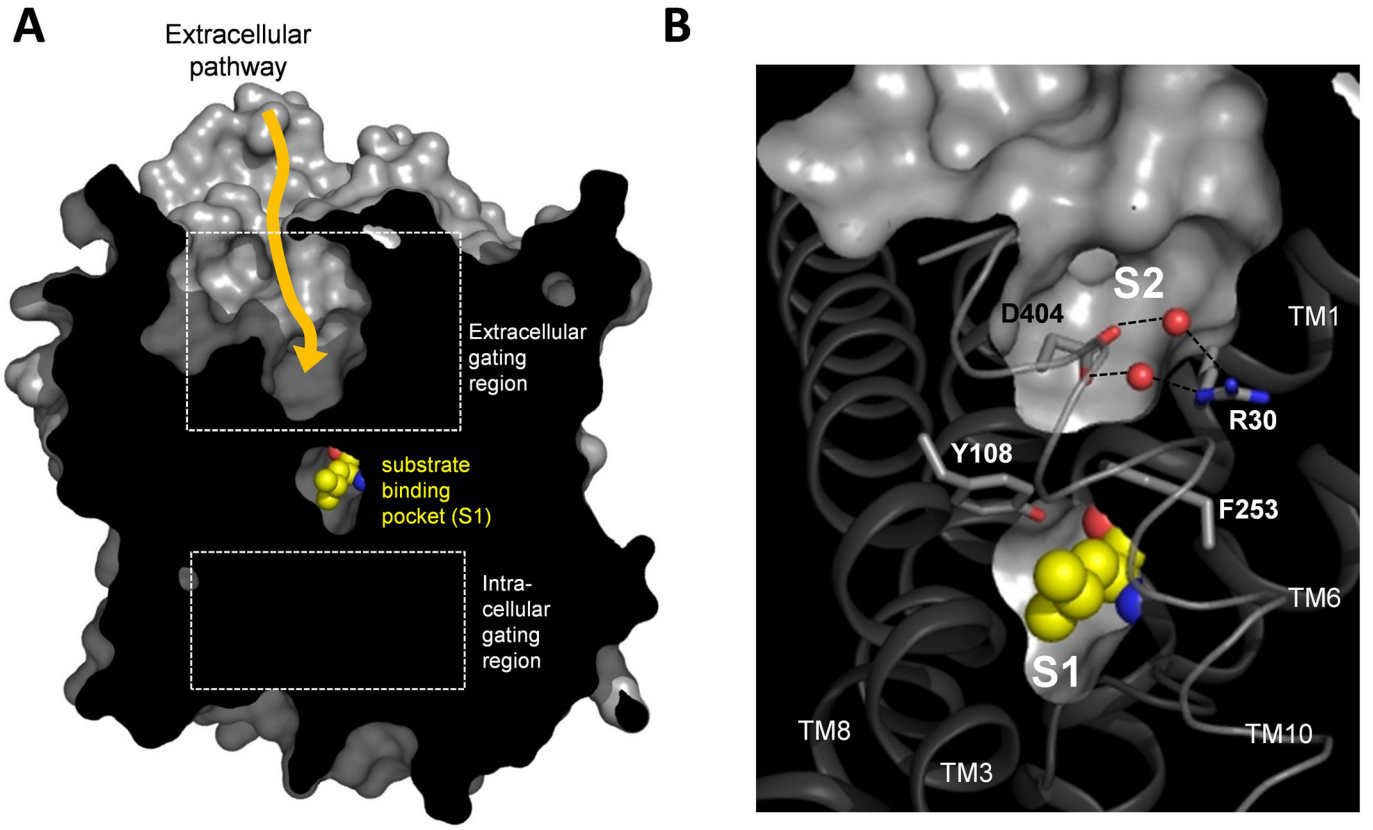

FIG. 5. Cross-sectional illustration of LeuT in an outward-facing occluded conformation. A, leucine is located in the substrate binding site in the core of the transporter and shown as van der Waals spheres in yellow. The extracellular and intracellular gating regions are highlighted with dashed lines, and the water-accessible pathway from the extracellular space to the substrate binding site is denoted with an orange arrow. B, close-up view of the S1 and S2 regions. The inner ring TM domains (TM1, TM3, TM6, and TM8), in addition to TM10, which lines the extracellular vestibule, are shown. The extracellular gate, formed by a water-mediated salt bridge between Arg30 and Asp404 in addition to the two aromatic residues Tyr108 and Phe253, separate the substrate binding site from the extracellular vestibule. Figures constructed on basis of the crystal structure of LeuT in complex with leucine (Protein Data Bank ID 2A65).

residues participating in the two networks are strictly conserved across the *SLC6* NTTs except for Asp404, which is substituted by a glutamate residue in SERT (Beuming et al., 2006), indicating important roles for both networks for the function of LeuT and the *SLC6* NTTs. A notable feature of the "occluded" substrate-bound structures is the presence of an extracellular pathway, which leads from the occluded binding pocket to the extracellular surface of LeuT (Fig. 5), whereas a similar pathway leading from the substrate binding site toward the intracellular medium is less obvious.

Although the overall LeuT structure is asymmetric, two similar structural motifs are found to be arranged by a pseudosymmetric inverted-repeat architecture: a five-helix bundle, containing TM1 to TM5, forms a V-shaped structural motif that is related by an apparent 2-fold rotational symmetry around an axis through the center of the membrane plane to a similar motif formed by TM6 to TM10 (Yamashita et al., 2005) (Fig. 4). The fold of this structural motif, denoted the "5 + 5 inverted repeat" fold, has subsequently been identified in several structures of secondary active transporters from prokaryotes, belonging to transporter families with no sequence relationship to LeuT or other *SLC6* transporters and thus were not expected to be structurally or mechanistically related (Forrest and Rudnick, 2009; for review, see Abramson and Wright, 2009). These structurally related transporters include three Na^+-coupled symporters for galactose (vSGLT; Faham et al., 2008), benzylhydantoin (Mhp1; Weyand et al., 2008), and betaine (BetP, Ressl et al., 2009), the arginine-agmatine antiporter AdiC (Gao et al., 2009, 2010) as well as the proton-coupled amino acid symporter ApcT (Shaffer et al., 2009) and the Na^+-independent carnitine-butyrobetaine antiporter CaiT (Schulze et al., 2010). Consequently, the 5 + 5 inverted repeat fold first observed in LeuT is emerging as the structural hallmark of a large family of secondary active transporters that potentially operate via a conserved structural mechanisms (Lolkema and Slotboom, 2008; Forrest and Rudnick, 2009; Krishnamurthy et al., 2009; for review, see Abramson and Wright, 2009) (section II.C).

2. Binding Pockets in the Leucine Transporter and Solute Carrier 6 Neurotransmitter Transporters. If sufficient sequence conservation is present, structures of bacterial proteins are often excellent templates for construction of three-dimensional structural models of homologous mammalian proteins. LeuT and the *SLC6*

NTTs share a remarkably high sequence similarity (55–67%) in the regions that are thought to constitute the core transport machinery, especially within the four-helix bundle, which house the central substrate binding site (S1) as well as the Na^+ binding sites (Yamashita et al., 2005; Beuming et al., 2006) (Fig. 6). Therefore, the

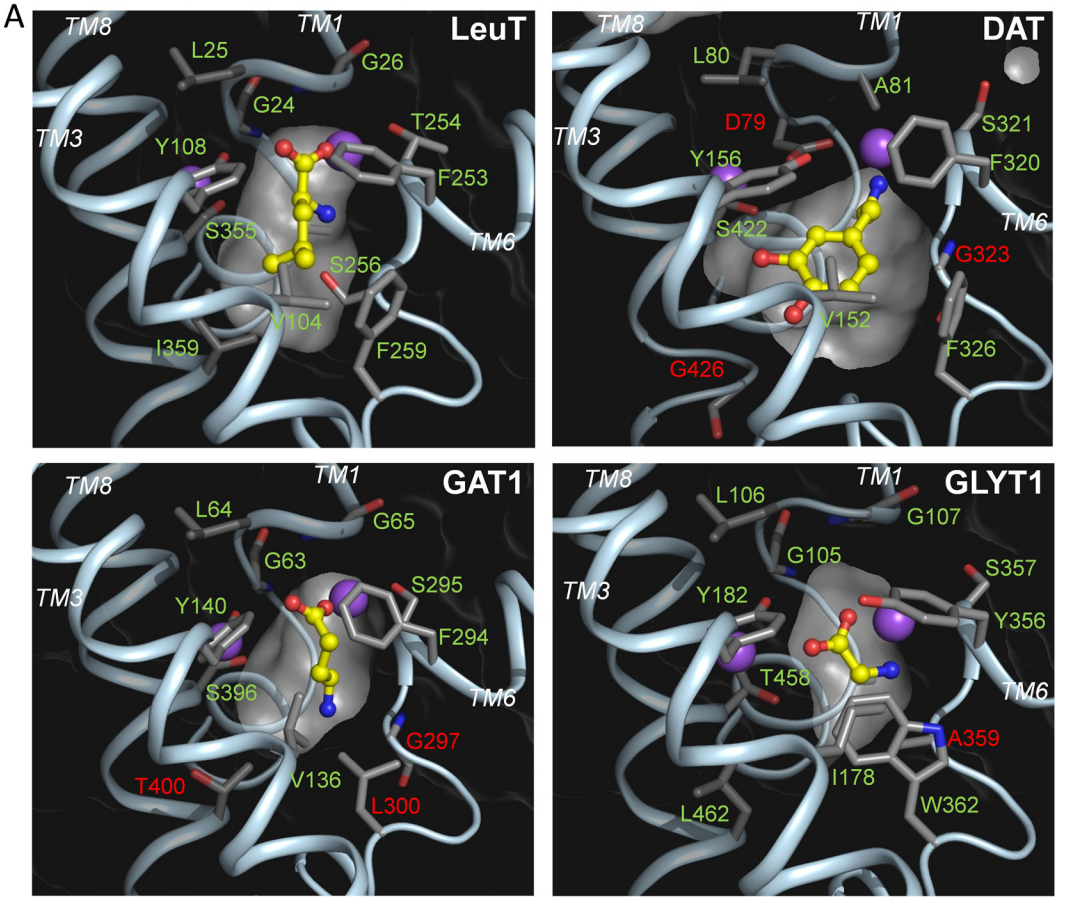

FIG. 6. Substrate binding pocket in LeuT and *SLC6* NTTs. A, cross-sectional view of the substrate binding site in LeuT (Protein Data Bank ID 2A65), DAT (Beuming et al., 2008), GAT1 (Skovstrup et al., 2010), and GLYT1 (Beuming et al., 2006). TM domains that form the substrate binding site (TM1, TM3, TM6, and TM8) are shown as blue helices, Na^+ ions as purple spheres, residue side chains as gray stick representations, and the substrates in yellow ball-and-stick representation. *SLC6* residues conserved in LeuT are indicated in green. B, alignment of amino acid sequences of the inner ring helices (TM1, TM3, TM6, and TM8) of LeuT and human SLC6 NTTs: SERT (P31645), DAT (Q01959), NET (P23975), GAT1 (P30531), BGT1 (P48065), GAT2 (Q9NSD5), GAT3 (P48066), GLYT1 (GLYT1c isoform; P48067-1), and GLYT2 (Q9Y345) (UniProt-SwissProt human accession numbers are given in parentheses). Stars indicate the positions involved in substrate binding in LeuT (Yamashita et al., 2005); green and red indicate whether LeuT residue is conserved or nonconserved, respectively, in human NTTs.

LeuT structures were predicted to provide a solid basis for construction of homology models for NTTs (Yamashita et al., 2005; Beuming et al., 2006; Henry et al., 2006b; Rudnick, 2006), which has not previously been possible because of the lack of appropriate template structures (Ravna and Edvardsen, 2001; Ravna et al., 2003; Pratuangdejkul et al., 2008). Accordingly, LeuT-based homology modeling is emerging as a valuable tool in studies of the NTT members, both in purely computational studies (Ravna et al., 2006; Huang and Zhan, 2007; Jørgensen et al., 2007a,b; Indarte et al., 2008; Xhaard et al., 2008; Kardos et al., 2010; Wein and Wanner, 2010) and as a complementary tool in functional studies (Dodd and Christie, 2007; Forrest et al., 2007, 2008; Paczkowski et al., 2007; Vandenberg et al., 2007; Zomot et al., 2007; Beuming et al., 2008; Celik et al., 2008b; Kniazeff et al., 2008; Andersen et al., 2009b, 2010; Kaufmann et al., 2009; Tavoulari et al., 2009; Field et al., 2010; Koldsø et al., 2010; Sinning et al., 2010) (section III). Modeling of DAT and SERT have so far received the most attention, which probably reflects the important role of these transporters as drug targets and results in generation of several three-dimensional models of human DAT (Beuming et al., 2006, 2008; Ravna, 2006; Indarte et al., 2008) and human SERT (Ravna et al., 2006; Forrest et al., 2007; Jørgensen et al., 2007a,b; Celik et al., 2008b; Forrest et al., 2008). However, models of NET (Paczkowski et al., 2007; Xhaard et al., 2008), GABA (Meinild et al., 2009; Kardos et al., 2010; Skovstrup et al., 2010; Wein and Wanner, 2010), and glycine transporters (Beuming et al., 2006; Edington et al., 2009) have also been generated. In general, the high degree of sequence similarity observed between the core region of LeuT and the *SLC6* NTTs produce models with very similar overall structure of the inner and outer ring regions. In contrast, the intra- and extracellular loop regions are deviating more between the *SLC6* NTTs and LeuT and are therefore more difficult to align with the LeuT template and confined with higher inaccuracy in the models (Beuming et al., 2006). Furthermore, the N- and C-termini of LeuT are much shorter and have no sequence similarity to the corresponding regions in the *SLC6* NTTs counterparts (section V) (Fig. 10), thus these cannot be modeled.

3. The S1 Binding Site in the Central Binding Pocket. In all substrate-bound LeuT structures reported so far, a single substrate molecule is accommodated in a common binding site, designated S1, that is located in the central cavity of the inner TM ring (Fig. 4). The conformations adapted by LeuT in these substrate-bound structures are highly similar and thought to represent the transporter in an "outward-facing occluded" conformational state occurring early in the translocation process. Here, the substrate and ions have triggered a conformational change that has closed access to the pocket from the external medium (section II.C). The S1 pocket surface is lined by polar, aromatic, and aliphatic amino acid side chains all four TMs (TM1, TM3, TM6, and TM8) surrounding the binding site, in addition to backbone amide groups from the unwound regions of TM1 and TM6 (Yamashita et al., 2005; Singh et al., 2008) (Fig. 6). The S1 pocket can be divided into two regions: a polar region formed exclusively by the unwound regions of TM1 and TM6 that accommodates the α-amino and α-carboxylate groups of the amino acid substrates, and a hydrophobic pocket formed by aliphatic side chains from TM1, TM3, and TM6 accommodating the hydrophobic substrate side chains (Yamashita et al., 2005; Singh et al., 2008) (Fig. 6). Within the polar region, the α-substituents of the substrate form hydrogen bonds with exposed backbone amide groups of TM1 and TM6. In addition to providing hydrogen-bonding partners for the substrate within the backbone, the helical breaks have been suggested to allow the substrate to interact directly with the ends of the helical segments, thus maximizing α-helical dipole moments (Yamashita et al., 2005). The α-amino group of the substrates interacts with backbone carbonyls from Ala22 in TM1, Phe253 and Thr254 in TM6, and with a side-chain hydroxyl from Ser256 in TM6 (Fig. 6). The α-carboxyl group of the substrate interacts with one of the two Na^+ and backbone amide nitrogen from Leu25 and Gly26 in TM1, and a phenolic hydroxyl moiety from Tyr108 in TM3 (Yamashita et al., 2005). The hydrophobic region accommodating the aliphatic substrate side chain is formed by the side chains of Val104 and Tyr108 in TM3, Phe253, Ser256 and Phe259 in TM6, and Ser355 and Ile359 in TM8 (Fig. 6). As will be discussed, this region is a major determinant for substrate specificity of the *SLC6* NTT binding pockets. It is noteworthy that Tyr108 and Phe253 are part of the predicted extracellular gate that separates the substrate from the extracellular vestibule as their aromatic side chain moieties align to form a barrier across at the top of the pocket (Figs. 5, 6, and 8C) (Singh, 2008).

4. The S2 Binding Site in the Extracellular Vestibule. It has been suggested that the extracellular pathway (often referred to as the extracellular vestibule) that forms the solvent-accessible path from the extracellular medium toward the S1 site harbors a second substrate binding site in LeuT, designated the S2 site (Shi et al., 2008). The S2 site is located at the bottom of the extracellular vestibule, separated from the S1 site by the extracellular gate (Figs. 5 and 9). Substrate binding to the S2 site was first suggested by Shi et al. (2008), who used molecular dynamics (MD) simulations in combination with biochemical experiments on reconstituted LeuT to propose that occupation of the S2 site is required to trigger conformational changes that releases substrate from the S1 to intracellular side. Although the S2 site has not been found to occupy substrates in any structure of LeuT crystallized in presence of substrates, other structures of LeuT crystallized in presence of certain LeuT inhibitors or detergents have found that these

bind in the S2 site (Zhou et al., 2007, 2009; Singh et al., 2008; Quick et al., 2009), hereby preventing conformational changes necessary for substrate translocation (Singh et al., 2007; Quick et al., 2009; Zhou et al., 2009), a mechanism that might be relevant for the mechanism of action of *SLC6* NTT inhibitors (Zhou et al., 2007, 2009) (section III.B).

5. Structural Basis for Substrate Recognition in Mammalian Neurotransmitter Transporters. Although the overall amino acid identity between mammalian NTT transporters and LeuT is 20 to 25%, the TM regions forming the S1 binding site are much more conserved with 55 to 67% sequence similarity (Fig. 6). Moreover, 7 of the 11 residues in LeuT that have direct interactions with the substrate are conserved across all nine mammalian NTTs (Fig. 6). This strongly indicates that the S1 site is structurally conserved between LeuT and *SLC6* NTTs, consistent with a large body of experimental evidence that have identified S1 residues to control substrate affinity in the *SLC6* NTTs (Beuming et al., 2006; Rudnick, 2006; Henry et al., 2007; Kanner and Zomot, 2008).

6. Structure of the S1 Pocket in Neurotransmitter Transporters and Implications for Substrate Selectivity. For the monoamine transporters, docking 5-HT and dopamine into models of the S1 site in SERT and DAT, respectively, have obtained strikingly similar poses of the substrates (Huang and Zhan, 2007; Beuming et al., 2008; Celik et al., 2008b; Indarte et al., 2008). The aromatic moieties of the substrates are accommodated in a hydrophobic region of the pocket, formed by hydrophobic and aliphatic residues in TM1, TM3, and TM6 (Fig. 6), whereas the alkylamine side chains of 5-HT and dopamine both occupy a region equivalent to the polar region in LeuT that accommodates the substrate α-carboxyl group (Fig. 6). An important feature in this region of SERT, DAT, and NET is the presence of an Asp residue close to the TM1 helical break at a position where all other NTTs contain a Gly residue (Fig. 6). The acidic side chain in this position is strictly required for the function of the monoamine NTTs (Kitayama et al., 1992; Barker et al., 1999; Celik et al., 2008b; Andersen et al., 2009b), leading to the early suggestion that this residue is involved in a critical interaction with the monoamine substrate, possibly by a direct interaction with the amino group of the substrate (Kitayama et al., 1992; Barker et al., 1999). This idea is supported by recent models of monoamine substrates docked into LeuT-based homology models of DAT (Beuming et al., 2008) and SERT (Celik et al., 2008b; Kaufmann et al., 2009) as well as mutational studies of SERT showing that the loss of affinity observed when shortening the alkyl-amine side chain of 5-HT derivatives by one methylene can be compensated for by extending the amino acid side chain by one methylene via an Asp-to-Glu mutation in this position (Barker et al., 1999; Celik et al., 2008b). Moreover, as the monoamine substrates lack a negatively charged carboxylate group, the unique presence of an Asp residue in this position in the monoamine transporters is believed to compensate for the inability of the monoamine substrates to coordinate with one of the Na^+-ions (section II.B). Sequence analysis show that the polar S1 region in LeuT accommodating the α-amino and α-carboxyl groups of the amino acid substrates are highly conserved in both GLYT1 and GLYT2 transporters, indicating a conserved binding mode of this substrate moiety (Beuming et al., 2006). By contrast, the residues forming the hydrophobic pocket surrounding the substrate side chains in LeuT are substituted with residues of larger size or different shape in GLYT1 and GLYT2 (Fig. 6). Hereby, the volume of the S1 pocket is reduced, so that only amino acids with no or small side chains such as Gly and Ala can be accommodated in the glycine transporters. In contrast, the equivalent positions in the S1 site in SERT, DAT, and NET contain amino acids with smaller-sized side chains in accordance with accommodation of larger substrates (Figure 6) (Pratuangdejkul et al., 2005; Celik et al., 2008b).

For the GABA transporters, several recent studies have employed LeuT-based homology modeling and ligand docking to generate models of the potential binding mode of GABA and other ligands in the S1 site (Kardos et al., 2010; Skovstrup et al., 2010; Wein and Wanner, 2010). The models display very similar orientations of GABA in the S1 site and show that the GABA transporter displays the highest degree of similarity with LeuT in terms of the substrate binding mode. Accordingly, when GABA is docked into models of GAT1, GAT2, and GAT3, the substrate is predicted to adopt an extended conformation, where the α-carboxyl group of GABA is accommodated equivalent to the α-carboxyl groups of the amino acid substrates in LeuT. The amino propyl chain of GABA is accommodated similarly to the alkyl side chain of Leu in LeuT, with formation of direct hydrogen bonds between the γ-amino group of GABA and the side chains of Tyr60, Ser396, Thr400, and the backbone carbonyl groups of Tyr60 and Ser396 (human GAT1 numbering) (Kardos et al., 2010; Skovstrup et al., 2010; Wein and Wanner, 2010) (Fig. 6). It has been suggested that GABA can assume a "cyclic" conformation in the binding pocket where the γ-amino group of GABA form an intermolecular interaction with the α-carboxyl group (Kanner and Zomot, 2008). This was recently supported by MD simulations of GABA binding in GAT1, although the majority of GABA-GAT1 interactions were conserved after rearrangement of the GABA molecule into a "cyclic" binding conformation (Skovstrup et al., 2010; Wein and Wanner, 2010).

7. Ion Binding Sites. A fundamental feature of all *SLC6* transporters is the cotransport of Na^+ ions with the substrate molecule. Because Na^+ is transported along the electrochemical gradient, this provides the energy required for the thermodynamically unfavorable transport of substrates. In addition, binding and, in

some cases, cotransport of Cl^-, K^+, and H^+ ions is necessary for the function of most *SLC6* transporters (Fig. 2; Table 2). Considering these similarities among the NTTs, there seem to be surprisingly large differences in terms of the transport stoichiometry between substrate and Na^+ and Cl^-. Even among the individual members of the monoamine, GABA, and glycine transporter subclasses, different ionic stoichiometries are observed (Fig. 2; Table 2). For example, the monoamine transporters DAT and NET are believed to translocate one substrate molecule with two Na^+ and one Cl^-, whereas SERT translocate 5-HT with one Na^+ and one Cl^-, whereas performing antiport of one K^+ and maybe H^+ (Keyes and Rudnick, 1982; Talvenheimo et al., 1983; Gu et al., 1994, 1996b; Quick, 2003). Similar differences are also observed among GABA transporters with variation in the number of Na^+ and Cl^- translocated per substrate molecule (Keynan and Kanner, 1988; Kavanaugh et al., 1992), whereas GLYT1 and GLYT2 cotransport glycine with two and three Na^+, respectively, as well as one Cl^- (López-Corcuera et al., 1998; Roux and Supplisson, 2000; Supplisson and Roux, 2002). Essentially, all knowledge on the structural basis for ion binding to the NTTs has been provided by studies starting from LeuT structures, because previous mutagenesis studies had identified only a few residues that seemed involved in ion dependence and selectivity (Keshet et al., 1995; Mager et al., 1996; Penado et al., 1998; Ponce et al., 2000; Kristensen et al., 2004).

a. Na^+ binding sites. In all LeuT structures, two distinct sites in the S1 pocket are unambiguously being occupied by a Na^+ ion, designated Na1 and Na2 (Yamashita et al., 2005; Zhou et al., 2007, 2009; Singh et al., 2008). Both Na^+ binding sites are believed to have a key role in stabilizing in particular the unwound structures of TM1 and TM6, in the presence of substrate (Figs. 6 and 7). Furthermore, the Na^+ ion located in Na1 interacts directly with the substrate α-carboxyl group, thereby providing a potential structural basis for the coupled Na^+ and substrate translocation. Figure 7 compares the LeuT residues that form the Na1 and Na2 sites with the corresponding residues in the mammalian NTTs. In the Na1 and Na2 sites, Na^+ is coordinated by five and eight backbone carbonyls or side-chain oxygens, respectively (Fig. 7). The residues forming the Na1 site are highly conserved across all *SLC6* NTT members, strongly indicating that an equivalent site is present in these transporters (Rudnick, 2006; Singh, 2008; Gouaux, 2009). The only major difference among these residues is found in the monoamine transporters, which contain an Asp in the position equivalent to Gly24 in LeuT. Homology models of SERT (Henry et al., 2006b; Ravna et al., 2006; Celik et al., 2008b; Forrest et al., 2008; Andersen et al., 2009b) and DAT (Huang and Zhan, 2007; Beuming et al., 2008; Jin et al., 2008b) suggest that the β-carboxyl group of Asp compensates for the inability of the monoamine substrates to interact with Na^+ in the Na1 site

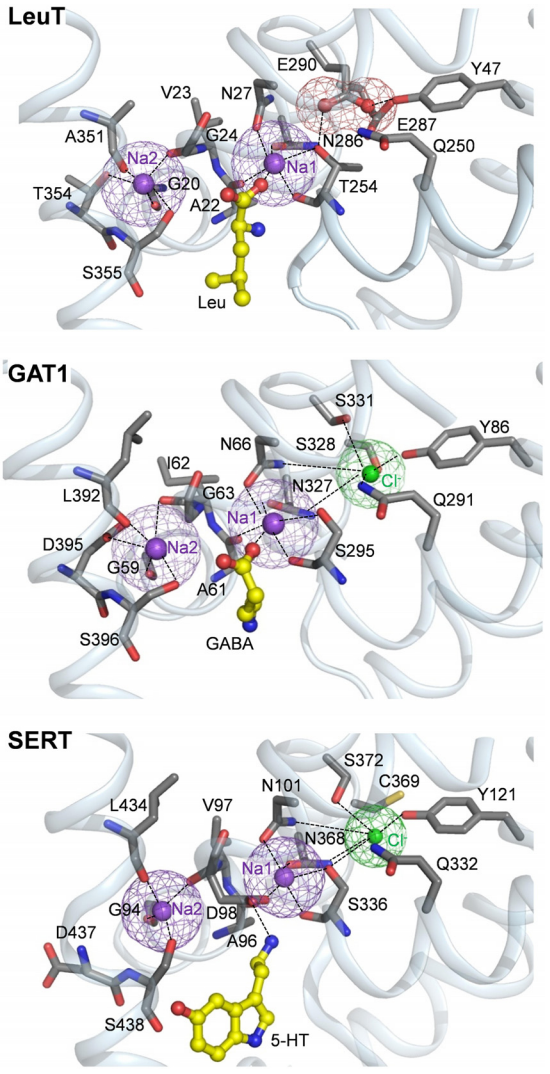

FIG. 7. Ion-binding site in LeuT and *SLC6* NTTs. Na^+ and putative Cl^- binding sites in LeuT (Protein Data Bank ID 2A65), GAT1 (Skovstrup et al., 2010), and SERT (Celik et al., 2008b). Na^+ and Cl^- ions are shown as purple and green spheres, respectively, and the substrate is shown as yellow stick-and-ball representation. Putative direct interactions between ions and specific residues are indicated. In contrast to *SLC6* transporters, LeuT is Cl^--independent. The Cl^- binding site in *SLC6* transporters has been suggested to be located at a site equivalent to negatively charged carboxylate group (highlighted with red mesh) of Glu290 in LeuT (Forrest et al., 2007; Zomot et al., 2007). Similar to leucine, GABA and glycine are believed to coordinate Na1 directly through the α-carboxylate group. In the monoamine transporters, an Asp residue located in close proximity to the Na1 has been suggested to compensate for the lacking carboxylate group in monoamine neurotransmitters.

because these lack a negatively charged carboxyl group, in contrast to GABA and glycine as mentioned previously (Figs. 5 and 7). This is supported by the observation that mutation of this Asp residue in all monoamine transporters has detrimental effects on transport function (Kitayama et al., 1992; Barker et al., 1999; Henry et al., 2003; Celik et al., 2008b; Andersen et al., 2010). Of the five residues that coordinate Na2 in LeuT, one is identical and three are highly similar to the equivalent residues in the majority of NTTs (Fig. 7). The single nonconserved residue is a Thr in LeuT and an Asp in the

NTTs, except for GLYT1, where it is a Gly. Assuming that the Asp β-carboxyl group could coordinate Na^+ similar to the β-hydroxy group in Thr present in LeuT, it is feasible that the Na2 site is also conserved in all *SLC6* NTTs, even for those that only translocate one Na^+ with the substrate (Rudnick, 2006; Singh, 2008). Although Na^+ binding sites similar to Na1 and Na2 exist in the mammalian NTTs, the precise role of occupation of these sites by Na^+ for substrate binding and translocation remains largely unknown, partly because of a lack of functional data addressing the role of each site in the individual NTT members. For example, it has been suggested that NTTs translocating one Na^+ (SERT and NET) uses only the Na1 site, whereas the transporters that translocate at least two Na^+ per substrate also use the Na2 site (Rudnick, 2006; Singh, 2008).

b. Cl^- binding site. The majority of mammalian *SLC6* transporters, including all nine members of the NTT subfamily are functionally dependent on extracellular Cl^-. In contrast, the function of prokaryotic *SLC6* homologs, including LeuT, is Cl^--independent (Zomot et al., 2007; Kanner and Zomot, 2008; Kanner, 2008; Zhao et al., 2010a). Still, the LeuT structure played an important role for the recent identification of the location of Cl^- binding sites in the mammalian NTTs (Fig. 7). In two independent studies (Forrest et al., 2007; Zomot et al., 2007), sequence analysis of mammalian *SLC6* transporters and Cl^--independent prokaryotic transporters provided hints that eventually lead to identification of Glu290 in TM7 of LeuT as a candidate position for the Cl^- binding site in mammalian transporters. It is noteworthy that the negatively charged residue is conserved in the prokaryotic transporters but not in the mammalian transporters (Forrest et al., 2007; Zomot et al., 2007). Structural analysis of LeuT-based homology models of GAT1 and SERT with Cl^- docked into the equivalent position of Glu290 in LeuT showed that this could accommodate Cl^- by coordination to the amide group of Gln291 and the hydroxyl groups of Ser331, Ser295, and Tyr86 (human GAT1 numbering; Fig. 7). Accommodated at this site, Cl^- is close to Na^+ in the Na1 site, thus supporting the idea that Cl^- is translocated along with Na^+ and substrate during transport (Rudnick and Clark, 1993). The essential role of Cl^- in the site for transport was further supported by the observation of Cl^--independent transport in GAT1, GAT3, and DAT upon introduction of a Glu or Asp residue to mimic the negative charge provided by Cl^- at this site (Zomot et al., 2007). Furthermore, by manipulation of the intracellular pH to neutralize the negative charge of the introduced acidic side chain resulted in potentiation of transporter turnover in the GAT1 mutant (observed as an increase in transport V_{max}), leading to the proposal that the role of Cl^- cotransport is to supply a negative charge that is critical for the translocation of substrate, but not for the return step (Kanner and Zomot, 2008). However, a recent study using patch-clamp electrophysiological measurements of currents associated with conformational changes in DAT to study the kinetics of the transport mechanism found that increasing intracellular Cl^- concentration, thus saturating DAT with Cl^- during the return step, facilitated transporter turnover, suggesting a variable functional role of Cl^- among the *SCL6* NTTs, which highlights the presently poorly understood role of Cl^- for the transport function (Erreger et al., 2008).

C. Structural Mechanism of Alternating Access Transport

Early conceptual models for a transport mechanism for secondary active transporters was put forward by Peter Mitchell and Walther Wilbrandt in the late 1950s; they proposed that these transporters are allosteric proteins that mediate substrate translocation by alternating between at least two conformations in which a substrate binding site is accessible to either the external or internal medium (Mitchell, 1957; Wilbrandt and Rosenberg, 1961). Later this model was refined by Oleg Jardetzky and has become widely accepted as the "alternating access" mechanism (Jardetzky, 1966; Mitchell, 1990). The model implies that transporters are capable of sealing off access to a compartment holding the substrate binding site by transient formation of impermeable barriers between the compartment and either side of the membrane (Fig. 8), likely by sequential or concerted structural rearrangement of substructures that act as physical "gates" for extra- or intracellular access to the binding pocket. For ion-coupled transporters such as the *SLC6* NTTs, the conformational equilibrium between "inward"- and "outward"-facing states is determined by binding of ions and substrate. Although other models for *SLC6* NTT transport have been proposed (Lester et al., 1996; DeFelice et al., 2001; Adams and DeFelice, 2003), the alternating access model form the groundwork for the majority of current efforts directed toward establishing the structural mechanisms of transport in the *SLC6* NTTs.

Before the LeuT structure, several studies had identified numerous residues in extra- and intracellular loops and TM regions to undergo rearrangement during transport (Chen et al., 1997a, 2000; Stephan et al., 1997; Norregaard et al., 1998; Loland et al., 1999, 2004; Smicun et al., 1999; Androutsellis-Theotokis et al., 2001; López-Corcuera et al., 2001; Ni et al., 2001; Androutsellis-Theotokis and Rudnick, 2002; Zomot and Kanner, 2003; Zhou et al., 2004). This topic is also excellently reviewed by Chen and Reith (2000), Goldberg et al., (2003), Loland et al. (2003), and Torres et al. (2003b). However, the absence of reliable transporter models impeded translation of these findings into mechanistic models (Rudnick, 2006). The increasing number of structures of prokaryotic transporters sharing the LeuT 5 + 5 inverted repeat fold is emerging as a very useful platform for elucidating the structural basis for NTT transport. Specifically, these structures provide "snapshots"

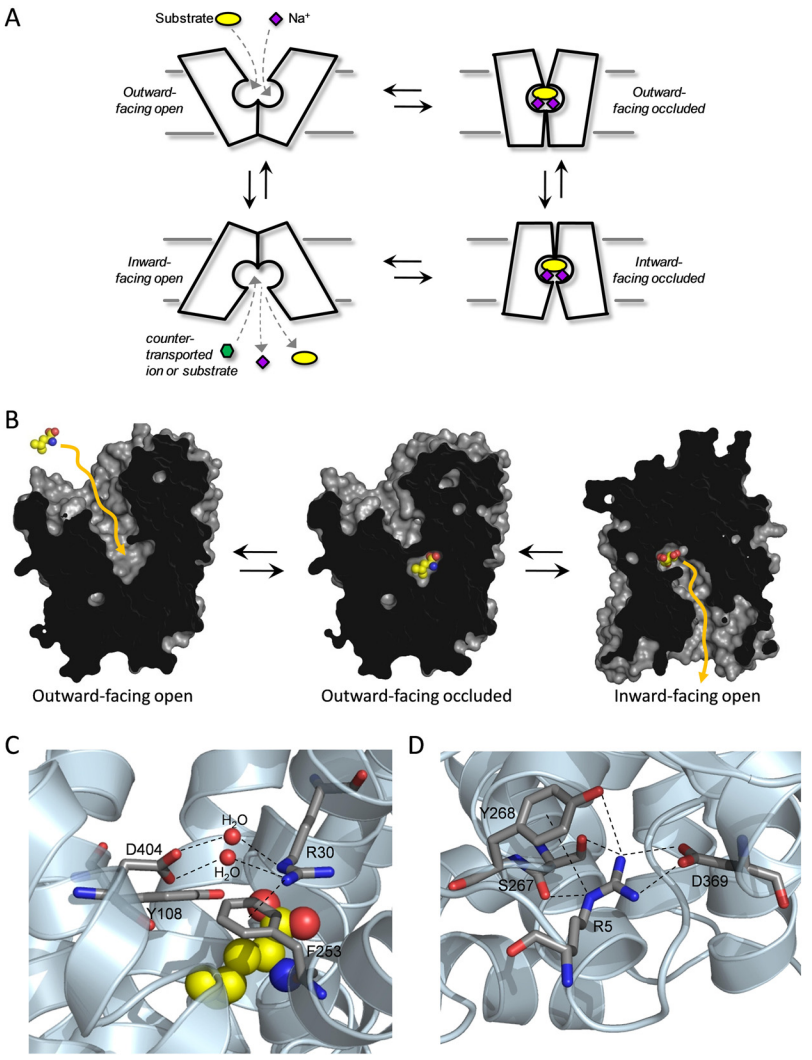

FIG. 8. Alternating access mechanism and gating regions. A, schematic representation of the conformational states the transporters have to shuttle between to move substrate from the extracellular space to the cytoplasm. B, cross-sectional illustrations of transporters with a 5 + 5 internal repeat motif crystallized in distinct conformations corresponding to different states in the alternating access mechanism. Left, LeuT in a competitive inhibitor bound outward-facing open conformation (Protein Data Bank ID 3F3A). Middle, LeuT in a substrate bound outward-facing occluded conformation (Protein Data Bank ID 2A65). Right, vSGLT in a substrate bound inward-facing open conformation (Protein Data Bank ID 3DH4). C, close-up view of the external gate in LeuT in the outward facing occluded conformation (Protein Data Bank ID 2A65). The external gate is formed by a water mediated salt-bridge between Arg30 and Asp404 and the aromatic lid, formed by Tyr108 and Phe253. The substrate is shown as *van der Waals* spheres in yellow. D, close-up view of the internal gate in LeuT (Protein Data Bank ID 2A65) formed by an ionic network among Arg5, Ser267, Tyr268, and Asp369.

of different transporter conformations, captured along what seems to be a transport cycle consistent with an alternating access mechanism (Fig. 8). The conception that transporters sharing the 5 + 5 internal repeat fold also share a general transport mechanism allows the use of these as templates for development of models of the molecular events underlying transport in the *SLC6* NTTs (Abramson and Wright, 2009; Forrest and Rudnick, 2009; Gouaux, 2009; Krishnamurthy et al., 2009). Moreover, the use of prokaryotic transporters as model proteins in biophysical, computational, and biochemical studies is emerging as a powerful approach to study the dynamics of the proteins (Forrest et al., 2007, 2008; Kniazeff et al., 2008; Zhao et al., 2010a,b). In the following section, we summarize how these recent advances are shaping current models of the *SLC6* NTT transport mechanism.

1. Structures of Prokaryotic Transporters in Various Conformational States. The classic alternating access model implies that the transporter protein shuttle through at least three principal conformational states during the transport cycle: 1) the outward-facing conformation, where the substrate binding pocket is accessible to the extracellular medium; 2) the occluded conformation, where access to the pocket is blocked from either side; and 3) inward-facing, where the pocket is open to the intracellular medium. As a close *SLC6* homolog, LeuT remains the best representative structural template for studying putative *SLC6* NTT conformations; however, the available LeuT structures represent only

outward-facing or outward-facing occluded conformations, whereas no inward-facing conformation is available. Nonetheless, some of the non-*SLC6* transporters sharing the 5 + 5 inverted repeat fold have been crystallized in both occluded and inward-facing conformations (Fig. 8), thus offering alternative templates for modeling of *SLC6* NTTs in the occluded and inward-facing conformations. These include structures of the sodium-benzylhydantoin symporter Mhp1 in occluded, outward-facing, and inward-facing conformation (Weyand et al., 2008; Shimamura et al., 2010), the arginine-agmatine symporter AdiC in occluded and outward-facing conformation (Fang et al., 2009; Gao et al., 2009, 2010), and an occluded structure of the sodium-betaine symporter BetP (Ressl et al., 2009). Furthermore, inward-facing structures are available for the sodium-galactose symporter vSGLT in the occluded (Faham et al., 2008) as well as the open form (Watanabe et al., 2010), the H^+-coupled amino acid symporter ApcT (Shaffer et al., 2009), and the carnitine-butyrobetaine antiporter CaiT (Schulze et al., 2010). It is noteworthy that virtually all of the key structural features of the transporter core formed by the 5 + 5 motif are conserved across these transporter structures (Abramson and Wright, 2009; Forrest and Rudnick, 2009; Krishnamurthy et al., 2009). Specifically, the architecture and the apparent structural role of the 10 TM helices in the 5 + 5 motif are found to be strictly maintained in these seven prokaryotic transporters, including location of substrate and ion binding pockets. This remarkable structural homology among seven different transporters from five unrelated transporter families has led to the general proposal that the core structure operates by a highly conserved mechanism, in which movements of the individual parts are determined by common molecular principles. It seems likely that this is applicable to all members of this emerging transporter superfamily, including the NTT members of the *SCL6* family (Singh, 2008; Weyand et al., 2008; Forrest and Rudnick, 2009; Krishnamurthy et al., 2009).

2. Models of Alternating Access Transport. Structural analysis of the prokaryotic transporter structures has led to construction of several models for the structural mechanism of alternating access transport by members of the 5 + 5 inverted repeat superfamily. The initial step involves binding of substrate and ions to an outward-open *apo* state in which the S1 site is accessible through an open extracellular permeation pathway (Fig. 8). Representative conformations for this state are provided by structures of LeuT bound with a competitive inhibitor (Singh et al., 2008) and of substrate-free Mhp1 (Weyand et al., 2008) and AdiC (Fang et al., 2009; Gao et al., 2009). Occupation of the S1 site triggers transition into the outward-occluded state where the substrate is trapped in S1 site, exemplified by structures of substrate-bound LeuT (Yamashita et al., 2005; Singh et al., 2008), Mhp1 (Weyand et al., 2008), and AdiC (Gao et al., 2010) (Fig. 8). Comparative analysis of the outward-open and outward-occluded structures show that the occlusion is achieved mainly by rearrangement of the extracellular parts of the TM regions that contribute to formation of the extracellular pathway [i.e., inner ring helices TM1, TM3, TM6, and TM8 as well as TM2 and TM10 from the outer ring (LeuT numbering)]. This is achieved either by formation of interaction networks between side chains across the extracellular pathway that act as an extracellular gate (as observed for LeuT, vSGLT, and AdiC) or by steric occlusion of the extracellular pathway (as observed for Mhp1 and BetP). In contrast, the subsequent transitions leading to the inward-facing states seem, not surprisingly, to require far more substantial conformational changes. Comparison of the inward- and outward-facing structures show that the extracellular pathway retained in the outward-facing occluded structures have collapsed into a compact layer, whereas a pathway that exposes the S1 site to intracellular side has formed between the intracellular halves of TM1, TM6, and TM8 of the inner ring (LeuT numbering) as observed for the inward-facing structures of ApcT, Mhp1, and vSGLT (Faham et al., 2008; Shaffer et al., 2009; Shimamura et al., 2010). After the transition into the inward-facing conformation, ions and the substrate are then released from the S1 site by diffusion through the intracellular pathway.

Several proposals have been made for the principal movements of individual elements in the transporter core during the outward-to-inward transition. Major discrepancies in these models include the type of motions of the inner ring TM1 and TM6 helices. In one model, the unwound segments in the middle of TM1 and TM6 act as flexible "hinges" around which the extra- and intracellular halves of TM1 and TM6 can move independently in sequential transitions to open and close the intra- and extracellular pathways (Yamashita et al., 2005; Singh et al., 2008; Krishnamurthy et al., 2009). In contrast, Forrest et al. (2008) and Forrest and Rudnick (2009) have proposed that the helical bundle formed by TM1 and TM6 together with TM2 and TM7 constitutes a rigid body that by a single "rocking" motion can account for the transition between the outward- and inward-facing conformations. On the basis of the structure of BetP, Ressl et al. (2009) have proposed a third type of motion for the inner ring helices during a set of sequential transitions into the inward-facing conformation via an intermediate fully occluded conformation (exemplified by the unique conformation observed for BetP), stating that TM1 and TM6 together with TM8 carry out a stepwise, iris-like rearrangement by performing concerted anticlockwise rotations.

3. Structural Correlates of Alternating Access Transport in Solute Carrier 6 Neurotransmitter Transporters. The above-mentioned models constitute a quantum leap forward in our understanding of the alternating access transport mechanism. However, many functional as-

pects of the transporters will remain difficult to correlate with the proposed structural mechanisms, because experimental data describing the protein dynamics of the transporters are lacking. In this respect, it is encouraging that 1) an increasing number of studies on transporter dynamics are emerging with focus on the fundamental ideas and concepts regarding transporter structure-function relationships proposed by these models and 2) many of these studies have focused on the *SLC6* NTTs and their bacterial homolog LeuT (Beuming et al., 2008; Kniazeff et al., 2008; Shi et al., 2008; Zhao et al., 2010a,b).

A burst of activity within the *SLC6* NTT research field has followed the growing number of bacterial transporter structures, many studies focusing on testing predictions made from the models described above, thereby often uncovering new details regarding key aspects of *SLC6* NTT structure-function relationships. MD simulations in combination with biochemical and biophysical experiments have been used to study conformational changes taking place during substrate and ion binding, and the subsequent translocation processes in both LeuT and *SLC6* (Forrest et al., 2007, 2008; Jørgensen et al., 2007a; Beuming et al., 2008; Caplan et al., 2008; Celik et al., 2008a; Kniazeff et al., 2008; Noskov and Roux, 2008; Noskov, 2008; Shi et al., 2008; Tavoulari et al., 2009; Zhao et al., 2010a,b). However, the time scale of typical MD simulations (10–100 ns) is far from the turnover rates of membrane transporters (0.1–10 s), and MD simulations are unlikely to be able to simulate the structural rearrangements during a complete translocation cycle. Still, MD simulations have played a pivotal role in studying the proposed changes of the interaction networks that form the extra- and intracellular gates (Fig. 5 and 8). Celik et al. (2008) followed the formation of the proposed extracellular gate in LeuT during a 100-ns MD simulation of LeuT in the outward-facing conformation (Fig. 5B), which is proposed to be a key event during the transition from the outward-facing open to the outward-facing occluded conformation (Fig. 8). As mentioned previously, the residues in LeuT that form the extracellular gate are conserved across all *SLC6* NTTs and have been shown to be critical for the function of several of the *SLC6* NTTs (Pantanowitz et al., 1993; Bismuth et al., 1997; Cao et al., 1998; Barker et al., 1999; Chen and Rudnick, 2000; Ponce et al., 2000; Beuming et al., 2008). In addition, MD simulation was used by Kniazeff et al. (2008) to study the effect of disrupting the interaction network that is thought to stabilize the tight packing of the intracellular halves of the inner ring helices TM1, TM6, and TM8 when the transporters reside in outward-facing states. Disruption of this tight network of interactions is predicted to be a major determinant for the transition into the inward-facing state (Yamashita et al., 2005; Singh, 2008). In the structures of LeuT, this network includes interactions between Arg5, Ser267, Tyr268, and Asp369 (Fig. 8D), all of which are strictly conserved in the *SLC6* NTTs. Specifically, Arg5 forms a salt bridge with Asp369 that is stabilized by a cation-π interaction between Tyr268 and Arg5 (Yamashita et al., 2005; Singh, 2008). The role of this network in determining the conformational equilibrium of the transporter was demonstrated in DAT by mutation of key residues in the network, which shifted the transporter into an inward-facing conformation (Loland et al., 2002; Kniazeff et al., 2008). The conclusion was supported by rescue in the mutants of transport by Zn^{2+}, which stabilizes the outward-facing conformation of DAT (Norregaard et al., 1998; Loland et al., 1999 2004; Norgaard-Nielsen and Gether, 2006). Furthermore, the mutants displayed dramatically decreased affinity for cocaine, which preferentially binds the outward-facing conformation (section III) and an increased accessibility of conformationally sensitive cysteine inserted in TM3 (Kniazeff et al., 2008). MD simulations of homology models of the DAT mutants corroborated the experimental findings by showing increased flexibility in the intracellular gating region and, moreover, that disruption of the network propagated toward the extracellular region and affected the global dynamics involved in transition of the transporter between the outward- and inward-facing conformations (Kniazeff et al., 2008). All together, these results demonstrated principal conformational changes in DAT predicted from the recent alternating access models.

Several other studies have demonstrated that transport-associated conformational changes in *SLC6* NTTs are consistent with predictions from alternating access models. These include the use of voltage-clamp fluorometry to show movement of TM1 during transport (Meinild et al., 2009) and SCAM analysis to confirm that TM8 lines the intracellular pathway of GAT1 (Ben-Yona and Kanner, 2009). Furthermore, SCAM analysis of TM5 in SERT shows that the intracellular region of this domain becomes exposed to the intracellular side when SERT is locked in an inward-facing conformation; consistent with predictions that this region lines the intracellular pathway that becomes accessible in the predicted inward-facing state (Zhang and Rudnick, 2006). It is noteworthy that Forrest et al. (2008) used SERT as a model protein for SCAM experiments to validate the principle motions of the "rocking bundle" model.

A milestone study achieving real-time monitoring of protein dynamics of specific structural elements within LeuT has been reported (Zhao et al., 2010b). Single-molecule fluorescence resonance energy transfer (FRET) (Roy et al., 2008) was used to measure time-resolved dynamic behavior of the intracellular gating region in single LeuT molecules during the application of substrates and inhibitors. The study showed molecular details of intracellular gating of LeuT that might be masked by ensemble averaging or suppressed under crystallographic conditions. The data were interpreted in the context of an allosteric model for LeuT transport developed by the same group (Shi et al., 2008). This

model incorporates the role of the S2 site into the original model proposed for alternating access transport in LeuT (Yamashita et al., 2005; Singh, 2008) (Fig. 8) and proposes that binding of a second substrate molecule to the S2 site is required to trigger the transition from the outward-facing occluded state into the inward-facing state (Shi et al., 2008).

In summary, mechanistic models based on the pool of structures obtained for prokaryotic transporters have provided a new framework for establishing hypotheses for structural dynamics of the *SLC6* NTTs. The results obtained so far have largely validated several of the proposed mechanistic principles for alternating access to be valid for the *SLC6* NTTs. Given the short time period over which these advances have been made, it must be expected that this progress will accelerate in the future years and will probably continue to be fuelled by increasingly detailed transport models for transporters of 5 + 5 structural repeat fold. However, requirements for continued progress will probably include not only development of new types of functional experiments but also structures of higher resolution, further snapshots of alternative conformations, and structures of other members of the transporter family, preferably the first structure of a *SLC6* NTT protein.

III. Pharmacology

The central role of the *SLC6* NTTs in regulation of neurotransmission makes these proteins attractive targets for pharmacological manipulation of neural activity, both in studies of basal neurophysiology and in the treatment of CNS diseases (Tables 3–5). A wide range of synthetic substrates and nontransportable inhibitors of *SLC6* NTTs have been developed, including more than 30 compounds that are currently used as drugs. In addition, a number of naturally occurring neurotoxins and psychostimulants have *SLC6* NTTs as their primary molecular targets, particularly the monoamine transporters. This section provides a summary of the pharmacology of *SLC6* NTTs with emphasis on the recent advances in deciphering the molecular mechanisms by which different *SLC6* NTT drug classes modulate transporter function. Aspects of these developments has been covered in several recent reviews that complement this section (Beuming et al., 2006; Gether

TABLE 3
Inhibitors of monoamine transporters

Inhibitor	K_i			Application	Binding Site Model
	SERT	DAT	NET		
	nM				
SERT inhibitors					
DASB[a]	1	1423	1350	PET ligand	
Sertraline[b]	0.1	420	25	Therapeutic drug	
Escitalopram[c]	3	>100,000	6514	Therapeutic drug	Andersen et al., 2010; Koldsø et al., 2010
Paroxetine[d]	0.1	490	40	Therapeutic drug	Tavoulari et al., 2009
Fluoxetine[d]	7	19,500	1020	Therapeutic drug	Tavoulari et al., 2009
Fluvoxamine[b]	2	1300	9200	Therapeutic drug	
DAT inhibitors					
GBR12935[d]	6800	22	235	Pharmacological tool	
RTI-55[e]	4	1	36	Pharmacological tool	
Cocaine[d]	410	278	910	Drug of abuse	Beuming et al., 2008
CFT WIN 35,428[d]	133	27	33	Pharmacological tool	Beuming et al., 2008
Benztropine[d]	47,000	42	292	Pharmacological tool	
JHW 007[f]	1730	25	1330	Pharmacological tool	Beuming et al., 2008
NET inhibitors					
Nisoxetine[d]	400	497	5	Pharmacological tool	
Talopram[g]	1400	44000	3	Pharmacological tool	
Talsupram[h]	430	3900	3	Pharmacological tool	
Reboxetine[i]	242	>10,000	3	Therapeutic drug	
Atomoxetine[i]	152	685	5	Therapeutic drug	
χ-Conotoxin MrIA[j]	>100,000	>100,000	1260	Pharmacological tool	Paczkowski et al., 2007
Nomifensine[b]	1010	56	16	Therapeutic drug	
Mazindol[d]	160	29	3	Therapeutic drug	
Amphetamine[k,l]	38,460	640	70	Drug of abuse	Beuming et al., 2008
MDMA[l]	2410	8290	1190	Drug of abuse	

DASB, 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)benzonitrile; GBR12935, 1-(2(diphenylmethoxy)ethyl)-4-(3-phenylpropyl)piperazine; WIN 35,428, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane.

[a] Wilson et al., 2000.
[b] Tatsumi et al., 1997.
[c] Owens et al., 2001.
[d] Eshleman et al., 1999.
[e] Carroll et al., 1995.
[f] Agoston et al., 1997.
[g] Bøgesø et al., 1985.
[h] McConathy et al., 2004.
[i] Andersen et al., 2009a.
[j] Sharpe et al., 2001.
[k] Synthetic substrates.
[l] Han and Gu, 2006.

TABLE 4
Inhibitors of GABA transporters

Inhibitor	K_i				Application	Binding Site Model
	GAT-1	BGT-1[a]	GAT-2[b]	GAT-3[c]		
			μM			
GAT-1 inhibitors						
Tiagabine[d]	0.11	>100	>100	>100	Therapeutic drug	Skovstrup et al., 2010
NNC-711[d]	1.4	1500	1200	>1600	Pharmacological tool	
SKF89976A[e]	0.13	7210	550	1990	Pharmacological tool	
CI-966[e]	0.26	300	1280	333	Pharmacological tool	
Nonselective inhibitors						
Nipecotic acid[d,f]	19	3000	530	94	Pharmacological tool	
Guvacine[d,f]	15	2300	1000	120	Pharmacological tool	
β-Alanine[d,f]	5000	660	42	36	Pharmacological tool	
Betaine[d,f]	>10,000	590	>3000	>10,000	Pharmacological tool	
(*S*)-SNAP-5114[d]	>500	250	130	50	Pharmacological tool	
(*R*)-EF1502[d]	8.9	180	170	>250	Pharmacological tool	
THPO[d]	1300	2100	1500	2200	Pharmacological tool	
Exo-THPO[g]	1000	3000	>3000	>3000	Pharmacological tool	
NNC 05-2090[h]	19	1.4	41	15	Pharmacological tool	

THPO, 4,5,6,7-tetrahydroisoxazolo(4,5-*c*)pyridin-3-ol; exo-THPO, 3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazol; NNC 05-2090, 1-[3-(9*H*-carbazol-9-yl)propyl]-4-(2-methoxyphenyl)-4-piperidinol hydrochloride.
[a] Equivalent to mouse GAT-2.
[b] Equivalent to mouse GAT-3.
[c] Equivalent to mouse GAT-4.
[d] Kvist et al., 2009.
[e] Dhar et al., 1994; human GAT-1, human BGT-1, rat GAT-2, human GAT-3.
[f] Synthetic substrates.
[g] White et al., 2002; mouse GATs.
[h] Thomsen et al., 1997; mouse GATs.

et al., 2006; Henry et al., 2006a; Kanner and Zomot, 2008; Andersen et al., 2009a; Gouaux, 2009).

A. Solute Carrier 6 Neurotransmitter Transporter Drug Classes

1. Ligands for the Monoamine Transporters. The recognition, now decades ago, of monoamine transporters as important drug targets for treatment of mood disorders initiated extensive drug discovery efforts in the pharmaceutical industry, focusing on design and synthesis of compounds targeting SERT, NET, and DAT. These endeavors have provided a plethora of ligands for these transporters, including compounds with high affinity and selectivity for each of the three transporters (Table 3). The tricyclic antidepressants (TCAs), including imipramine and clomipramine, were developed in the 1950s as the first generation of monoamine transporter drugs (Moltzen and Bang-Andersen, 2006; Andersen et al., 2009a). However, the TCAs display activity across a variety of different receptors (Gillman, 2007), and new generations of monoamine transporter inhibitors with little or no affinity for other proteins have subsequently been developed. According to their selectivity profile, these inhibitors are classified as selective serotonin-reuptake inhibitors (SSRIs), such as fluoxetine and paroxetine; selective norepinephrine-reuptake inhibitors (NRIs), such as reboxetine and atomoxetine; and selective dopamine-reuptake inhibitors, such as bupropion (Table 3). Dual-acting inhibitors with affinity for two of the three monoamine transporters include the classes of serotonin- and norepinephrine-reuptake inhibitors (SNRIs), such as duloxetine and desvenlafaxine, and NET/

TABLE 5
Inhibitors of glycine transporters

Inhibitor	K_i		Application	Binding Site Model
	GLYT1	GLYT2		
	nM			
GLYT1 inhibitors				
Sarcosine[a,b]	55,000	>100,000	Pharmacological tool	
NFPS (ALX 5407)[c]	3	>1000	Pharmacological tool	
ORG 24598[b]	32	>100,000	Pharmacological tool	
LY2365109[d]	15.8	>30,000	Pharmacological tool	
GLY2 inhibitors				
ORG 25543[e]	>100	16	Pharmacological tool	
N-Arachidonylglycine[f]	>100,000	3000	Pharmacological tool	Edington et al., 2009

NFPS, *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine.
[a] Synthetic substrate.
[b] Mallorga et al., 2003; rat GLYTs.
[c] Atkinson et al., 2001.
[d] Perry et al., 2008a.
[e] Caulfield et al., 2001.
[f] Wiles et al., 2006.

DAT inhibitors, such as nomifensine. Finally, compounds inhibiting all three monoamine transporters have also been identified, exemplified by the novel triple reuptake inhibitor tesofensine (Astrup et al., 2008).

Reflecting the central role of monoamine transporters in cocaine abuse and addiction, the tropane class of competitive inhibitors, including cocaine, has been extensively studied as monoamine transporter inhibitors. Cocaine itself is a potent and nonselective inhibitor of SERT, NET, and DAT, but several closely related analogs have been developed with improved affinity and selectivity across the monoamine transporters (Carroll, 2003; Jin et al., 2008a). Important examples include 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (WIN 35,428), which has selectivity for DAT over NET and SERT and (−)-2β-carbomethoxy-3β-(4-iodophenyl)tropane (RTI-55), which has selectivity for SERT and DAT over NET (Table 3). Substantial evidence suggests that DAT is the primary target for the rewarding properties of cocaine (Giros et al., 1996b; Chen et al., 2006). This has increased the focus on development of DAT inhibitors as potential medications for cocaine addiction, and analogs of benztropine (BZT), which generally have higher affinity and selectivity for DAT compared with cocaine, have received most attention (Newman and Kulkarni, 2002; Dutta et al., 2003; Loland et al., 2008). It is noteworthy that some of these compounds do not possess the rewarding properties associated with cocaine. Indeed, the BZT derivative *N*-(*n*-butyl)-(bis-fluorophenyl)methoxytropane (JHW 007) has been found to efficiently antagonize the behavioral effects of cocaine (Newman et al., 1995; Katz et al., 2004; Desai et al., 2005).

The use of monoamine transporters as biomarkers for imaging of monoaminergic circuits (Fowler et al., 1999; Laakso and Hietala, 2000; Huang et al., 2002; Meyer, 2007) has prompted development of several highly selective compounds for use as radiolabeled tracers in in vivo imaging techniques, such as positron emission tomography and single photon emission computed tomography (Houle et al., 2000; Wilson et al., 2000). These ligands include some of the most potent and selective compounds targeting the monoamine transporters, such as 2-(2-dimethylaminomethylphenylsulfanyl)-5-methylphenylamine (MADAM) and 3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)benzonitrile (DASB) for SERT (Houle et al., 2000; Tarkiainen et al., 2001; Meyer et al., 2004), 2β-carbomethoxy-3β-(4-chlorophenyl)-8-(2-fluoroethyl)nortropane (FECNT) for DAT (Goodman et al., 2000), and 2-[(2-methoxyphenoxy)phenylmethyl]morpholine (MeNER) and methylreboxetine for NET (Ding et al., 2003; Schou et al., 2003, 2004; Wilson et al., 2003) (Table 3).

Several synthetic substrates of pharmacological interest are available for all three monoamine transporters. Prominent examples are amphetamine and amphetamine derivates such as methamphetamine and methylphenidate, which are being used as therapeutics against attention deficit hyperactivity disorder (ADHD), in addition to the widely used drug of abuse, 3,4-methylenedioxymethamphetamine (also known as "ecstasy") (Green et al., 2003; Sulzer et al., 2005; Rothman and Baumann, 2006; Rothman et al., 2006). 1-Methyl-4-phenylpyridinium, the neurotoxic metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, is a substrate for all three monoamine transporters and has been used as tracer in functional studies of SERT, NET, and DAT (Buck and Amara, 1994; Wall et al., 1995; Scholze et al., 2000; Sitte et al., 2000, 2001). MPP^+ has been further developed into the fluorescent analog 4-(4-(dimethylamino)-styryl)-*N*-methylpyridinium (ASP+), which has enabled real-time kinetic analysis of transport mediated by SERT (Oz et al., 2010), NET (Schwartz et al., 2003, 2005), and DAT (Bolan et al., 2007; Zapata et al., 2007) in addition to high-throughput assays for the measurement of monoamine transport uptake activity (Fowler et al., 2006; Jørgensen et al., 2008). A fluorescent cocaine analog has been developed with binding properties that enabled specific labeling of DAT in living neurons for real-time monitoring of DAT trafficking by confocal microscopy imaging (Eriksen et al., 2009).

2. Ligands for the GABA Transporters. Inhibition of GABA reuptake has been recognized as a therapeutic strategy for the treatment of epileptic disorders for many years (Krogsgaard-Larsen et al., 1987, 2000). Extensive medicinal chemistry efforts have been devoted to the synthesis of GAT inhibitors with emphasis on development of compounds that discriminate between the different GAT subtypes (Høg et al., 2006). However, truly selective compounds have not yet been achieved, and only GAT1-selective inhibitors, including the antiepileptic drug tiagabine, have been identified so far (Table 4). Hence, there is a considerable lack of selective pharmacological tool compounds targeting the three remaining GAT subtypes (BGT-1, GAT2, and GAT3), and the functional role and the therapeutic potential of these subtypes remains to be established.

Pioneering work in the 1970s led to the discovery of guvacine and nipecotic acid as substrates for GABA transporters (Johnston et al., 1975; Krogsgaard-Larsen and Johnston, 1975; Krogsgaard-Larsen et al., 1975). After the cloning of the four GAT subtypes, it was realized that guvacine and nipecotic acid have selectivity for GAT1 over GAT3 with little or no affinity for BGT-1 and GAT2 (Thomsen et al., 1997; Liu et al., 2003; Kragler et al., 2005; Kvist et al., 2009). Although guvacine and nipecotic acid do not readily cross the blood-brain barrier (Krogsgaard-Larsen et al., 1987), lipophilic derivatives of these substrates represent the most important biologically active GAT inhibitors developed. Important examples include tiagabine and 1-(4,4-diphenyl-3-butenyl)-3-piperidinecarboxylic acid (SKF89976A), which are derivatives of nipecotic acid, and 1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid (NNC-711) and 1-(2-(bis(4-(trifluoromethyl)phenyl)methox)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid (CI-966), which are

derivatives of guvacine. Still, these compounds are some of the most potent and selective GAT1 inhibitors identified (Nielsen et al., 1991; Andersen et al., 1993; Borden et al., 1994; Dhar et al., 1996; White et al., 2002; Kragler et al., 2005; Kvist et al., 2009) (Table 4).

GAT inhibitors with activity on GAT2, GAT3, and/or BGT1 include (*S*)-1-(2-(tris(4-methoxyphenyl)methoxy) ethyl)-3-piperidinecarboxylic acid (SNAP-5114), which has selectivity for GAT2 and GAT3 over BGT-1 but little or no activity at GAT1 (Dhar et al., 1994; Borden, 1996; Kvist et al., 2009), and (*R*)-*N*-(4,4-bis(3-methyl-2-thienyl)-3-butenyl)-3-hydroxy-4-(methylamino)-4,5,6,7-tetrahydrobenzo(*d*)isoxazol-3-ol (EF1502), which possesses dual-acting noncompetitive inhibitory activity at the BGT-1 and GAT1 (Clausen et al., 2005; White et al., 2005) (Table 4). Assessment of these compounds in animal models of partial and generalized epilepsy indicates that BGT-1 is a potential antiepileptic drug target (White et al., 2005; Madsen et al., 2009). However, selective BGT-1 inhibitors are needed to support this hypothesis, which clearly illustrates the unmet need for subtype-selective pharmacological tool compounds.

3. Ligands for the Glycine Transporters. Several ligands are available for the two glycine transporters GLYT1 and GLYT2, including competitive inhibitors and synthetic substrates (Table 5). Their identification has been driven by their potential use in the treatment of psychotic diseases and, besides being candidates for clinical studies, the ligands have excellent properties as pharmacological tools (Lechner, 2006; for review, see Dohi et al., 2009). The prototypic GLYT1-selective substrate *N*-methyl glycine (sarcosine) has formed the basis for generations of highly potent and selective derivatives, including *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine (NFPS), (*R*)-*N*[3-phenyl-3-(4′-(4-toluoyl)phenoxy)-propyl]sarcosine [(R)-NPTS], and (*R*)-(−)-*N*-[3-[(4-trifluoromethyl)phenoxy]-3-phenyl-propyl]glycine (ORG 24598) (Bergeron et al., 1998; Atkinson et al., 2001; Aubrey and Vandenberg, 2001; Brown et al., 2001; Lowe et al., 2003; Mezler et al., 2008; Perry et al., 2008) (Table 5). For non-sarcosine GLYT1 inhibitors, several structurally diverse compounds have emerged from drug discovery programs, such as ((2-(4-benzo(1,3)dioxol-5-yl-2-*tert*-butylphenoxy) ethyl)methylamino)acetic acid (LY2365109) (Perry et al., 2008), 2-chloro-*N*-((*S*)-phenyl((2*S*)-piperidin-2-yl)methyl)-3-trifluoromethyl benzamide (SSR504734) (Depoortère et al., 2005), SSR130800 (Boulay et al., 2008), *N*-[3-(4-chlorophenyl)-3-[4-(2-thiazolylcarbonyl)phenoxy]propyl]-*N*-methyl-glycine (CP-802,079) (Martina et al., 2004), and (*R*)-4-[5-chloro-2-(4-methoxy-phenylsulfanyl)-phenyl]-2-methyl-piperazin-1-yl-acetic acid (Lu AA20465) (Smith et al., 2004), in addition to a series of cyclic tetrapeptides that was recently isolated from a soil bacteria (Terui et al., 2008). These compounds are excellent tools for studying the modulatory role of GLYT1 in neurotransmission [for example, in *N*-methyl-D-aspartate (NMDA) receptor-mediated signaling and plasticity at excitatory synapses (Bergeron et al., 1998; Kinney et al., 2003) as well as glycinergic signaling at inhibitory synapses (Bradaïa et al., 2004)].

The first GLYT2-selective inhibitor was the competitive inhibitor 4-benzyloxy-3,5-dimethoxy-*N*-[(1-dimethylaminocyclopentyl)methyl]benzamide (ORG 25543) (Caulfield et al., 2001), and other GLYT2-selective inhibitors belonging to different chemical classes have subsequently been developed (Isaac et al., 2001). GLYT1 and GLYT2 have several isoforms, thus increasing Gly transporter heterogeneity in the CNS (Aragón and López-Corcuera, 2003). However, because the isoforms exclusively involve changes in the intracellular N- and C-terminal domains, which have limited contribution to transporter function, it appears unlikely that differential targeting of individual isoforms by ligands will be possible; accordingly, all Gly transporter ligands examined across isoforms show identical pharmacological properties (Mezler et al., 2008).

B. Structural Mechanisms of Drug Action

The *SLC6* NTTs are important drug targets, and much research over the past decades has therefore focused on the molecular pharmacology of these transporters. However, before the arrival of the LeuT structures, the absence of structural data on *SLC6* NTTs had slowed progress in understanding the structural and mechanistic details underlying drug modulation. Within the framework of the emerging structural models of *SLC6* NTTs, the location and the structure of drug binding sites as well as the effect of drug binding on transporter structure is now being revealed for many of the most important *SLC6* NTT drugs. In addition, studies of drug modulation of LeuT have established several important principles regarding competitive and noncompetitive inhibition that can be extended to the *SLC6* NTTs (Singh et al., 2007, 2008; Zhou et al., 2007, 2009; Quick et al., 2009). These developments have provided an indispensable basis for a range of recent studies on the structural basis and mechanism of action of several *SLC6* NTT drugs (Paczkowski et al., 2007; Beuming et al., 2008; Andersen et al., 2009b, 2010; Koldsø et al., 2010; Sinning et al., 2010; Skovstrup et al., 2010). Furthermore, understanding seemingly subtle molecular differences between different drug-induced conformations of the transporters offers new insights into the principles that determine whether a ligand becomes a substrate or a competitive- or noncompetitive inhibitor (Singh et al., 2007, 2008; Zhou et al., 2007, 2009; Quick et al., 2009).

The advances in understanding the structural mechanism of transporter function and inhibition now provides a new starting point for elucidating the molecular pharmacology and mechanism-of-action of *SLC6* NTT drugs. Moreover, homology models of *SLC6* NTT proteins derived from LeuT crystal structures allow structural interpretation of functional data on drug modula-

tion and provide important opportunities of using computational chemistry techniques to model transporter-drug interactions.

1. Location of Inhibitor Binding Pockets. So far, two binding sites have been identified in the crystal structures of inhibitor-bound LeuT: the centrally located substrate S1 binding pocket and the extracellular vestibule, which contains the putative S2 binding site (Singh et al., 2007, 2008; Zhou et al., 2007, 2009) (Fig. 9). The equivalent regions in the *SLC6* NTTs are natural candidates for harboring ligand binding sites. Indeed, re-evaluation of extensive mutational analysis shows that the majority of residues that are critical for recognition of inhibitors are located in the TM and loop regions that contribute to the formation of the extracellular permeation pathway and the substrate binding pocket (Chen and Reith, 2000, 2002; Norregaard et al., 2000; Mortensen et al., 2001; Norregaard and Gether, 2001; Goldberg et al., 2003; Gu et al., 2006; Henry et al., 2006b; Neubauer et al., 2006; Andersen et al., 2009b, 2010; Koldsø et al., 2010; Sinning et al., 2010).

2. Structural Basis for Competitive Inhibition. The central substrate binding pocket is an obvious candidate site for binding of competitive inhibitors, because inhibitor binding sites overlapping with the S1 substrate site offer a straightforward structural mechanism for competitive inhibition and are in agreement with pharmacological data showing that competitive inhibitors can be displaced in a concentration-dependent manner by substrates (Talvenheimo et al., 1979; Humphreys et al., 1988; Marcusson and Tiger, 1988; Graham et al., 1989; Apparsundaram et al., 2008). Although mutational studies on the *SLC6* NTTs have identified residues in virtually all TM domains to be important for recognition of competitive inhibitors, the majority of the most sensitive residues reside within the regions that form the extracellular permeation pathway and the S1 substrate binding pocket (Barker et al., 1994, 1998; Barker and Blakely, 1996; Mortensen et al., 1999; Adkins et al., 2001; Larsen et al., 2004; Henry et al., 2006b; Beuming et al., 2008; Severinsen et al., 2008; Walline et al., 2008; Andersen et al., 2009b, 2010; Field et al., 2010). For example, in the monoamine transporters, sensitivity toward a wide range of competitive inhibitors, including fluoxetine, paroxetine, citalopram, and imipramine, as well as psychostimulants such as cocaine and amphet-

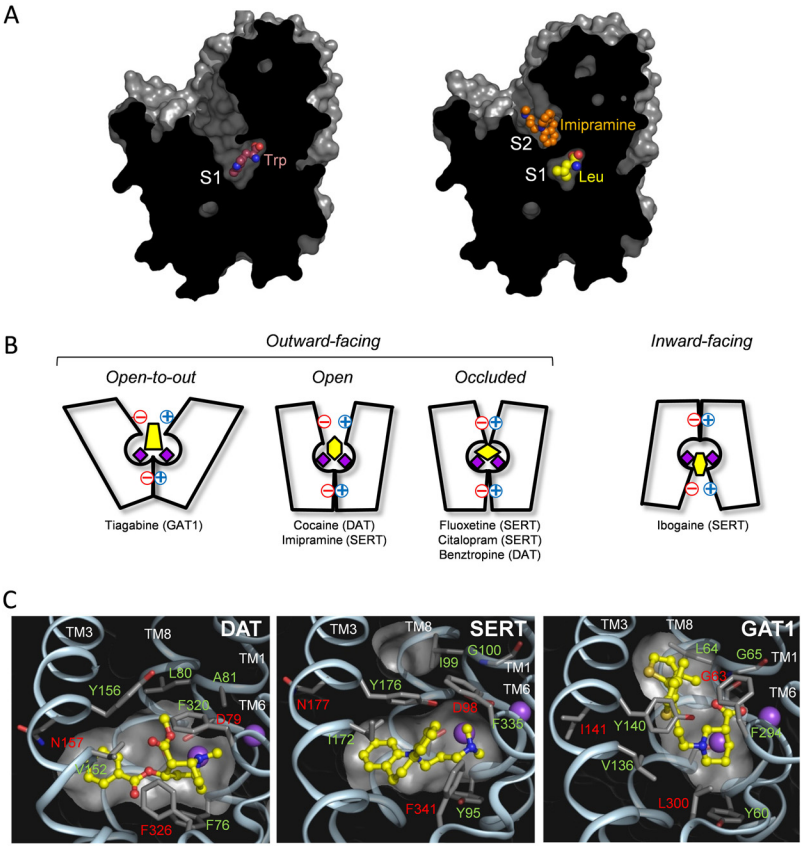

FIG. 9. Small-molecule inhibition of LeuT and *SLC6* NTTs. A, cross-sectional view of crystal structures of LeuT in complex with competitive (left, Trp; Protein Data Bank ID 3F3A) and noncompetitive (right, imipramine; Protein Data Bank ID 2Q72) inhibitors. Trp binds in the S1 site and stabilizes LeuT in an outward-facing open conformation, whereas imipramine binds in the S2 site in the extracellular vestibule and stabilizes LeuT in an outward-facing occluded conformation. B, schematic illustration of putative transporter conformations stabilized by inhibitors. C, cross-sectional illustrations of ligand-docking models of competitive inhibitor binding in *SLC6* transporters. TM domains forming the binding site (TM1, TM3, TM6, and TM8) are shown as blue helices, Na^+ ions as purple spheres, residue side chains as gray stick representations, and the inhibitors in yellow ball-and-stick representation. Conserved residues are highlighted with green labels. Left, cocaine binding in DAT (Beuming et al., 2008). Middle, imipramine binding in SERT (Sinning et al., 2010). Right, tiagabine binding in GAT1 (Skovstrup et al., 2010).

amines, can be decreased by several orders of magnitude by mutation of S1 residues (Barker et al., 1999; Henry et al., 2006b; Beuming et al., 2008; Walline et al., 2008; Andersen et al., 2009b, 2010; Koldsø et al., 2010; Sinning et al., 2010).

LeuT structures have been used as templates for construction of monoamine transporter models in complex with several competitive inhibitors, including the prototypical antidepressants imipramine (Sinning et al., 2010), escitalopram (Andersen et al., 2010; Koldsø et al., 2010), and fluoxetine (Tavoulari et al., 2009) in SERT and cocaine in DAT (Beuming et al., 2008) (Fig. 9). These experimentally validated models have revealed a common structural model for competitive inhibition of monoamine NTTs: Inhibitors compete with substrate for binding in the S1 site and thereby trap the transporter in an outward-facing occluded or outward-facing open conformational state with local structural differences in the binding site. The inhibitors are accommodated within the inner ring helices TM1, TM3, TM6, and TM8, and the essential amino group found in virtually all SERT, NET, and DAT inhibitors is coordinated by an Asp (Asp79 in DAT and Asp98 in SERT) located in the helical break of TM1 (Fig. 9). In SERT, imipramine was found to stabilize a conformation with a slightly more open external gate region compared with fluoxetine and escitalopram, probably because of the more bulky nature of the inhibitor molecule (Tavoulari et al., 2009; Andersen et al., 2010; Koldsø et al., 2010; Sinning et al., 2010). Beuming et al. (2008) have constructed experimentally validated models of the binding sites for cocaine, amphetamines, and BZTs in DAT. It is noteworthy that direct support for a binding site overlapping with that of dopamine was obtained by trapping the radiolabeled cocaine analog [^{3}H]WIN 35,428 in the transporter through cross-linking of engineered cysteines or by engineering of a Zn^{2+} binding site that was extracellular to the predicted common binding pocket within the vestibule corresponding to S2 (Beuming et al., 2008). In the same study, evidence was obtained that cocaine stabilizes a more outward-facing open conformation of DAT compared with amphetamine, MDMA, BZT, and BZT analogs, which were suggested to stabilize the transporter in an outward-facing occluded conformation (Beuming et al., 2008; Bisgaard et al., 2011). It is noteworthy that this has been linked to the reduced stimulant effect in vivo of the BZT analogs compared with cocaine (Reith et al., 2001; Katz et al., 2004; Loland et al., 2008). The precise orientation and binding mode of BZT and BZT analogs in S1 was demonstrated in a later study (Bisgaard et al., 2011).

Only a few LeuT-based homology models of GABA and glycine transporters have emerged, and the structural details underlying inhibition of GATs and GLYTs are therefore not as well characterized. However, the first model of inhibitor binding to GAT was recently constructed, thereby providing insight into the structural details of the tiagabine binding site in GAT1 (Skovstrup et al., 2010). The hydrophilic moiety of tiagabine was found to bind in the S1 substrate site, whereas the lipophilic "anchor" of tiagabine protrudes out of the extracellular lid and partially binds in the extracellular vestibule site and thereby stabilizes an outward-open conformation of GAT1 (Skovstrup et al., 2010) (Fig. 9). Hence, the tiagabine binding site overlaps with both the S1 and S2 sites, which might clarify the mixed-type mechanism of GABA uptake inhibition found in early pharmacological characterization of tiagabine (Braestrup et al., 1990). The binding site for the competitive GAT1 inhibitor SKF89976A was recently suggested to overlap the substrate binding site in GAT1, thereby stabilizing an outward-facing conformation of the transporter (Zhou et al., 2004; Dodd and Christie, 2007; Rosenberg and Kanner, 2008; Ben-Yona and Kanner, 2009), substantiating that the competitive inhibitor binding site in GATs overlaps the S1 substrate binding site as found for *SLC6* monoamine transporters.

3. Structural Basis for Noncompetitive Inhibition. LeuT has been cocrystallized with a set of noncompetitive inhibitors, including TCAs and SSRIs (Singh et al., 2007; Zhou et al., 2007, 2009). Although these compounds have very low affinity for LeuT, they are capable of inhibiting transport at high micromolar concentrations. Cocrystallization of LeuT with both TCAs and SSRIs revealed that these compounds bind in the extracellular vestibule (Fig. 9), suggesting that the equivalent vestibule in the *SLC6* NTTs have a similar function for accommodation of noncompetitive inhibitors (Zhou et al., 2007, 2009). However, it should be noted that a large body of evidence indicates that the primary high-affinity binding site for TCAs and SSRIs, including those cocrystallized with LeuT, likely occurs in the S1 site in monoamine transporters (for review, see Henry et al., 2006a; Rudnick, 2007; Singh, 2008; Gouaux, 2009). Several studies have found that TCAs and SSRIs, in addition to binding to a high-affinity binding site, also bind to a low-affinity allosteric site in SERT (Sette et al., 1983; Wennogle and Meyerson, 1983, 1985; Plenge and Mellerup, 1985; Segonzac et al., 1985; O'Riordan et al., 1990; Plenge et al., 1991; Chen et al., 2005a,b). Binding to this allosteric site markedly increases the off-rate of the inhibitor bound to the high-affinity binding site. It has been suggested that the allosteric site is situated corresponding to a suggested oligomeric interface in the transporter (Neubauer et al., 2006); however, it is tempting to speculate that the allosteric site could be situated in the S2 vestibule outside the primary S1 binding pocket. Further experimental efforts are required to investigate this possibility.

Compared with the large number of competitive inhibitors available for *SLC6* NTTs, very few inhibitors exhibit a noncompetitive mode of inhibition, in which inhibition is entirely independent of substrate concentrations. In a structural context, noncompetitive inhibitors must bind to a site whose occupation is mutually exclusive with substrate site occupation, which may be

achieved by stabilizing a distinct conformational state of the transporter in which the substrate cannot bind. An example of such a mechanism is provided by the SERT inhibitor ibogaine, which has been suggested to trap SERT in an inward-facing conformation (Zhang and Rudnick, 2006; Jacobs et al., 2007). When ibogaine promotes the inward-facing conformation, the substrate site becomes mutually exclusive with the ibogaine binding site, thereby providing an explanation for the noncompetitive mechanism of action in a structural context (Jacobs et al., 2007). Another structural mechanism underlying noncompetitive mode of inhibition has been suggested for the χ-conotoxin MrIA. The 13-amino acid χ-conotoxin acts as a noncompetitive inhibitor of NET (Sharpe et al., 2001, 2003), presumably by binding in the external permeation pathway and stabilizing the transporter in an outward-facing conformation in which access of substrate to the S1 site is efficiently blocked (Paczkowski et al., 2007). Although several noncompetitive GLYT1 inhibitors have been reported, including NFPS and Org 24598 (Aubrey and Vandenberg, 2001; Mallorga et al., 2003; Mezler et al., 2008), the structural details underlying noncompetitive inhibition of GLYTs remains elusive. However, a recent study identified EL2 and EL4 in GLYT2 to be important for recognition of the noncompetitive GLYT2 inhibitor, *N*-arachidonylglycine (Edington et al., 2009). These extracellular loop regions contribute to formation of the vestibule region, including the S2 binding site, suggesting that *N*-arachidonylglycine binds in the S2 site of GLYT2. Residues in EL2 and EL4 have previously been shown to be critical for binding of Zn^{2+}, which acts as a noncompetitive inhibitor of GLYT1 (Ju et al., 2004), suggesting that the S2 site in GLYTs forms a common noncompetitive binding site equivalent to the S2 site found in LeuT.

IV. Physiology and Pathophysiology

The use of selective *SLC6* NTT inhibitors in animal and human studies has provided comprehensive insight to the physiological and pathophysiological significance of the *SLC6* NTTs. However, more definitive insights have awaited the analyses of mice with targeted deletion (knockouts) of the individual *SLC6* NTT genes. As outlined below, several *SLC6* NTT knockout mice display distinct phenotypes that have confirmed and clarified the role of individual *SLC6* NTTs as key regulators of neurotransmitter homeostasis and provided an increased understanding of diseases related to dysfunction of their cognate neurotransmitter systems. Furthermore, an increasing number of human *SLC6* NTT polymorphisms have been identified that lead to alteration in transporter expression, function, and regulation and are associated with a wide spectrum of neurological and neuropsychiatric disorders, hereby providing further understanding of the role of *SLC6* NTTs in pathophysiological processes. Here, we will discuss in vivo genetic studies involving the *SLC6* NTTs to provide a brief overview of their impact on the understanding of correlation between genetic perturbation of *SLC6* NTTs and phenotypes in physiology and pathophysiology.

A. The Dopamine Transporter

The extensive characterization of the behavioral and neurochemical phenotypes of the DAT knockout (DAT-KO) mice (Giros et al., 1996b) has substantiated the indispensable role of DAT in regulating dopamine homeostasis (for reviews, see (Gainetdinov and Caron, 2003; Sotnikova et al., 2006; Gainetdinov, 2008). The extracellular levels of dopamine in the striatum of the mice were markedly increased (5-fold), as assessed by in vivo quantitative microdialysis (Giros et al., 1996a) and real-time measurements of dopamine clearance, and release by cyclic voltammetry in striatal slices revealed that dopamine persists up to 300-fold longer in the extracellular space (Giros et al., 1996a; Jones et al., 1998). Without DAT, the rate of dopamine clearance was similar to a clearance rate mediated by diffusion alone, which emphasizes that DAT is the main mediator of dopamine uptake (Giros et al., 1996a). It is noteworthy that with a predominant extrasynaptic localization of DAT and dopamine receptors (Nirenberg et al., 1996; Hersch et al., 1997; Sesack et al., 1998; Eriksen et al., 2009), it is being increasingly appreciated that dopamine transmission is diffusion-based "volume transmission" rather than synaptic transmission and thus that DAT rather operates to regulate dopamine's action at extrasynaptic receptors than solely removing it from the synapse (Rice and Cragg, 2008; Schmitt and Reith, 2010).

The DAT-KO mice also displayed a 95% reduction in total brain tissue content of dopamine and a 75% decrease in release of dopamine (Jones et al., 1998), consistent with deficient storage of dopamine in synaptic vesicles. Although levels of tyrosine hydroxylase were dramatically decreased in the DAT-KO mice, the synthesis of dopamine was increased by 2-fold and can therefore not account for the decrease in neuronal dopamine storage (Jones et al., 1998). This implies that reuptake of dopamine through DAT is critical for refilling of synaptic vesicles and subsequent dopamine release. In addition to DAT-KO mice, mice with reduced (Zhuang et al., 2001) or increased (Salahpour et al., 2008) DAT expression have been generated. The analyses of these mouse models have provided further support for the essential of role of DAT in dopamine homeostasis by revealing a clear gene-dose effect for the neurochemical alterations (Sotnikova et al., 2006).

The neurochemical phenotype of DAT-KO mice correlates with major behavioral phenotype usually associated with hyperdopaminergic states (Gainetdinov and Caron, 2003; Sotnikova et al., 2006; for review, see Gainetdinov, 2008) and includes locomotor hyperactivity, stereotypy, and a marked decrease in habituation

upon exposure a novel environment (Giros et al., 1996a; Jones et al., 1998; Mead et al., 2002). Moreover, DAT-KO mice display deficits in other behavioral models consistent with cognitive abnormalities (Gainetdinov, 2008). The hyperactivity of DAT-KO mice can be reversed upon treatment with amphetamine (Gainetdinov et al., 1999), which is in direct contrast to wild-type animals, in which amphetamine induces hyperactivity. Thus, certain aspects of the behavioral phenotype of the DAT-KO mouse resemble the behavioral symptoms observed in patients with ADHD (Gainetdinov et al., 1999; Gainetdinov and Caron, 2000; Spielewoy et al., 2001). It is noteworthy that the DAT gene has been implicated in ADHD genetics by both linkage and association studies (reviewed by Sharp et al., 2009). Specifically, mutation A559V in TM12 of DAT has been identified in two male siblings with ADHD (Mazei-Robison et al., 2005; Mazei-Robison et al., 2008) as well as in a patient with bipolar disorder (Grünhage et al., 2000). This mutation was found to decrease DAT-mediated dopamine efflux induced by amphetamine without affecting the normal uptake function of DAT (Mazei-Robison et al., 2005). Furthermore, it was observed that the efflux properties of the DAT A559V mutant might be regulated via the dopamine D_2 autoreceptor (Bowton et al., 2010). These data demonstrate how irregular DAT-mediated dopamine efflux potentially plays a hitherto-unappreciated role in diseases such as ADHD that are thought to be caused by dopaminergic dysfunction.

DAT mutations have also recently been linked to the rare autosomal recessive disease infantile parkinsonism-dystonia; i.e., two mutations (L368Q and P395L) were identified in two unrelated families suffering from this disease (Blackstone, 2009; Kurian et al., 2009). The disease develops during infancy with Parkinson-like symptoms (slowness of movement, muscle rigidity, rest tremor) and dystonia (sustained, abnormal muscle contractions often resulting in twisting movements). The mutants, which are situated in the outer part of TM8 (L368Q) and in the extracellular loop connecting TM7 and TM8 (P395L), were identified as loss-of function mutations that severely reduced the expression levels of mature DAT and, in the case of the Leu368Gln mutation, displayed a significant loss of dopamine binding affinity to DAT (Kurian et al., 2009). Loss of DAT function would be predicted to result in an excess of extracellular dopamine, and thus the hypodopaminergic character of the phenotype might seem surprising. However, it is possible that loss of DAT function also might lead to dopamine depletion because of impaired replenishment of the synaptic pool of dopamine. Indeed, dopamine depletion is an essential component of the disease because it has also been associated with inactivating mutations of tyrosine hydroxylase (Diepold et al., 2005).

Thus, genetic DAT models, together with DAT mutations identified in humans, represent critical tools for investigating and understanding how alteration in dopamine neurotransmission contributes to severe human disorders, ranging from rare genetic diseases to common neurological and neuropsychiatric disorders such as schizophrenia, Parkinsons' disease, and ADHD (for review, see Gainetdinov, 2008). In addition, the mice have been instrumental in delineating the mechanisms of action of psychostimulants such as cocaine and amphetamine (section VI.D).

B. The Serotonin Transporter

The SERT-KO mice, similar to DAT-KO mice, have been extensively characterized (Murphy and Lesch, 2008; reviewed by Gardier, 2009). In parallel to the observations for DAT-KO mice, SERT-KO mice display an increase in extracellular 5-HT concentration in combination with a decrease in total tissue content of 5-HT despite increased or unaltered 5-HT biosynthesis (Bengel et al., 1998). This confirms the role of SERT-mediated reuptake as the primary extracellular mechanism for clearing of released 5-HT and indicates that SERT-mediated reuptake is critical for maintaining a high intracellular 5-HT pool. At the behavioral level, SERT-KO mice are characterized by increased anxiety- and stress-related behaviors, as supported, for example, by reduced exploratory behavior in the elevated-plus maze test (Holmes et al., 2003a,b; Ansorge et al., 2004). A similar phenotype characterized by abnormal emotional behavior is observed by transient inhibition of SERT with the SSRIs during early development (Ansorge et al., 2004, 2008). Furthermore, mice overexpressing SERT display reduced extracellular 5-HT concentrations and exhibit reduced anxiety-like behaviors (Jennings et al., 2006). This suggests that a link exist between abnormal 5-HT homeostasis during development and SERT expression and adult anxiety-related behavior.

Polymorphisms in the human *SLC6A4* gene encoding SERT have also supported a central role of SERT in diseases linked to abnormalities in 5-HT homeostasis. In humans, SERT expression is modulated by variation in the length of the promoter region (5-HTTLPR). A common 44-base pair insertion/deletion polymorphism approximately 1 kilobase upstream of the transcription initiation generates two common alleles, the long (*l*) and the short (*s*) allele, the *s* allele being associated with lower transcriptional activity compared with the *l* allele (Heils et al., 1996; Lesch et al., 1996). Furthermore, two single-nucleotide polymorphisms (SNPs) within 5-HTTLPR, rs25531 and rs25532, have been found to affect transcriptional activity (Murphy and Lesch, 2008). In accordance with findings in the SERT-KO mice, the low-expressing *s* allele of the 5-HTTLPR has been associated with anxiety-related personality traits (Lesch et al., 1996; Holmes et al., 2003b) and neuropsychiatric conditions such as bipolar disorder (Cho et al., 2005; Lasky-Su et al., 2005), autism (Huang and Santangelo, 2008), obsessive-compulsive disorder (OCD) (Lin, 2007),

eating disorders (Calati et al., 2011), major depressive disorder (Clarke et al., 2010; Kiyohara and Yoshimasu, 2010), and predisposition to develop depression in response to stressful life events (Caspi et al., 2003). However, two recent meta-analyses have failed to support a correlation among 5-HTTLPR, stress, and depression (Munafò et al., 2009; Risch et al., 2009), and this gene-by-environment interaction is still the subject of debate (Risch et al., 2009; Wankerl et al., 2010). Furthermore, the 5-HTTLPR *s* allele has been suggested to predispose for poor response to SSRI treatment in depressive patients (Serretti et al., 2007). Moreover, activity of SERT as a consequence of the 5-HTTPLR *l* allele was found to be more prevalent in a group of patients with pulmonary hypertension (Eddahibi et al., 2001). However, subsequent studies in larger cohorts found only an association of the *l* allele with an earlier age at diagnosis (Willers et al., 2006) or found no significant association between the 5-HTTLPR and pulmonary hypertension (Machado et al., 2006).

Several nonsynonymous rare genetic variants have also been identified in the SERT gene. The I425V mutation was initially identified in a screen of the complete coding sequence of the SERT gene in 450 persons (Glatt et al., 2001). Later, this mutation was found in two families with OCD in which six of seven family members that were heterozygous carriers for the I425V mutation were diagnosed with OCD or obsessive-compulsive personality disorder (Ozaki et al., 2003). Expression of the I425V mutant of SERT in heterologous cell lines showed increased 5-HT uptake compared with wild-type (Kilic et al., 2003; Prasad et al., 2005), suggested to occur as result of either enhanced intrinsic transport capacity of the transporter (Kilic et al., 2003) or from altered surface expression (Prasad et al., 2005).

Genetic association studies of 120 families with a history of autism found genetic linkage at the chromosome region containing the *SLC6A4* gene, leading to identification of five SNPs that result in mutations within the coding region of SERT (G56A, I425L, F465L, L550V and K605N) (Sutcliffe et al., 2005), which all displayed gain-of-function relative to wild-type SERT (Prasad et al., 2005, 2009; Sutcliffe et al., 2005), suggesting a link between a gain-of-function SERT phenotype reuptake and autism. A G56A knock-in mouse was generated to explore the importance of this SERT phenotype in vivo, but a detailed characterization of the phenotype has not yet been reported (Veenstra-Vanderweele et al., 2009). Another recently described rare SERT variant (K201N) introduces a third glycosylation site in ECL3 of SERT (Rasmussen et al., 2009), which, according to in vitro studies, leads to enhanced glycosylation of the K201N mutant and 30% increase in SERT expression levels, suggesting that the K201N variant potentially can affect serotonergic neurotransmission in human carriers (Rasmussen et al., 2009).

C. The Norepinephrine Transporter

The NET-KO mice are similar to the DAT- and SERT-KO mice characterized by increased extracellular levels and decreased intracellular levels of norepinephrine despite increased or unaltered neurotransmitter synthesis (Xu et al., 2000; for review, see Gainetdinov and Caron, 2003). The NET-KO mice also display marked behavioral alterations, including reduced locomotor activity upon exposure to a novel environment and elevated locomotor responses to psychostimulants (Xu et al., 2000). In addition, the NET-KO mice behave like antidepressant-treated wild-type animals in the tail suspension test with no additional effects of antidepressants such as desipramine, reboxetine, and imipramine (Gainetdinov et al., 2002; for review, see Gainetdinov and Caron, 2003; Dziedzicka-Wasylewska et al., 2006). Moreover, the NET-KO mice show characteristic hemodynamic changes, such as excessive tachycardia and increased blood pressure during sympathetic activation with wakefulness and activity, whereas resting mean arterial pressure and heart rate are maintained at nearly normal levels, most likely because of increased central sympathoinhibition (Keller et al., 2004a).

Inactivation of NET in humans also results in marked hemodynamic changes. In a patient with orthostatic intolerance, sequencing of the NET gene revealed a heterozygous coding mutation converting a highly conserved Ala in TM9 into a Pro (A457P). When the patient was standing, an excessive increase in the heart rate and an abnormal high level of plasma norepinephrine was measured, which is characteristic of orthostatic intolerance. These symptoms together with A457P mutation were also observed in the mother and four of the probands siblings, including her identical twin (Shannon et al., 2000). When expressed in a heterologous cell line, the mutation resulted in a 98% loss of NET function compared with the wild-type transporter (Shannon et al., 2000). Further studies revealed that the A457P NET mutant is not expressed in the mature, fully glycosylated form and, consequently, NET surface expression is greatly reduced. Furthermore, when coexpressed with wild-type NET, the A457P mutant exerts a dominant-negative effect on wild-type NET uptake activity, probably because of transporter oligomerization (Hahn et al., 2003), providing an explanation to the striking phenotype observed in heterozygous carriers.

Another human variant of NET, a Phe528Cys mutation, was identified in a systematic SNP analysis on a set of candidate genes for blood pressure homeostasis (Halushka et al., 1999). This mutant displayed increased membrane expression of NET associated with increased norepinephrine uptake compared with the wild-type NET (Hahn et al., 2005). A recent study determined the allele frequency of F528C in a group of patients with major depression compared with healthy control subjects (Haenisch et al., 2008). Nine of 426

patients were heterozygotic for F528C, whereas only three of 642 control subjects were carriers, suggesting an association of a rare functional variant of NET with major depression (Haenisch et al., 2008).

D. Glycine Transporters

Removal of Gly from the synaptic cleft and refilling of synaptic vesicles are mediated by the glial GLYT1 and the neuronal GLYT2, respectively. The GLYT1-KO mice demonstrated that the increase in extracellular Gly levels leads to a sustained activity of the strychnine-sensitive Gly receptor (GlyR) (Gomeza et al., 2003a). The mice show severe respiratory deficits and die within the first day. Measuring respiratory activity in slices from the brain stem of the GLYT1-KO mice showed that the respiratory frequency was restored upon treatment with the GlyR antagonist strychnine. This suggests that the phenotype is due to an increased level of Gly that leads to increased activity of GlyRs (Gomeza et al., 2003a). The GLYT1-KO phenotype resembles human Gly encephalopathy (nonketotic hyperglycinemia), which is characterized by elevated levels of Gly. On the other hand, the vesicular Gly content and therefore the amplitude of GlyR-mediated miniature inhibitory postsynaptic currents was reduced in mice deficient of GLYT2 (Gomeza et al., 2003b). GLYT2-KO mice also die shortly after birth, which adds to the finding that the two GLYT transporters serve different functions and hence cannot substitute for each other in the neonatal mice. In the adult mice, however, both GLYT1 and GLYT2 contribute to the fast removal of Gly from the synapse (Eulenburg and Gomeza, 2010; Eulenburg et al., 2010). The phenotype of GLYT2-KO resembles human hyperekplexia (startle disease), a disorder that has previously been attributed to mutations in the GlyR genes. It is noteworthy that several nonsense, mis-sense, and frameshift mutations in the gene encoding human GLYT2 were recently identified in patients with hereditary hyperekplexia. Most of these mutations result in a complete loss of Gly transport function (Eulenburg et al., 2006; Rees et al., 2006).

E. GABA Transporters

GAT1 is so far the only GABA transporter that has been studied in genetic mouse models, and a mouse strain with functional knockout of GAT1 displayed elevated extracellular GABA levels in support of a key role of GAT1 in regulating GABA homeostasis in the brain (Jensen et al., 2003; Bragina et al., 2008). As would be expected from elevated GABA levels, a GAT1-KO mice, generated by homologous recombination (Cai et al., 2006), showed a marked reduction in anxiety and depression-like behaviors (Liu et al., 2007). Moreover, GAT1 deficiency was associated with reduced aggression (Liu et al., 2007) and hypoalgesia (Xu et al., 2008). In light of these phenotypic characteristics, the *GAT1* gene has recently been explored as a candidate gene for anxiety disorder. Indeed an association between GAT1 polymorphisms and anxiety disorders was observed with a constant increase in the odds ratio for disease susceptibility with an increase in panic attack severity (Thoeringer et al., 2009).

V. Cellular Regulation

The multitude of abnormal phenotypes observed in *SLC6* NTT transgenic mice and the association of human polymorphisms altering *SLC6* NTT expression and function with neurological diseases has demonstrated the importance of *SLC6* NTT regulation for normal brain function. Indeed, numerous studies have supported the idea that the activity of all of the *SLC6* NTTs are subject to dynamic modulation by a range of cellular processes. Analysis of *SLC6* NTT amino acid sequences show that their cytoplasmic domains contain numerous consensus sites for PTMs as well as protein-protein interaction motifs, suggesting that protein kinases, phosphatases, and other interacting proteins might act to modify transporter function and cellular distribution (Zahniser and Doolen, 2001; Ramamoorthy, 2002; Vaughan, 2004; for review, see Eriksen et al., 2010b). Although the *SLC6* NTTs in general have highly conserved primary structures within the TM and intracellular regions, the N- and C-terminal domains are much more diverse, with variations in both amino acid sequence and length (Fig. 10). These differences suggest a high degree of transporter-specific regulation and indicate that observations in one transporter cannot readily be extrapolated to other members of the family.

A. Post-Translational Modifications

1. Glycosylation. The *SLC6* NTTs each possess two to four consensus sites for N-linked glycosylation (N-X-S/T) within EL2 that are subject to N-linked glycosylation (Table 6) (Tate and Blakely, 1994; Olivares et al., 1995; Bennett and Kanner, 1997; Torres et al., 2003a; Li et al., 2004; Rasmussen et al., 2009). Differences in the degree of glycosylation are observed among different tissue, brain regions, and cell types and during development (Lew et al., 1991; Patel et al., 1994; Qian et al., 1995; Nguyen and Amara, 1996). Furthermore, glycosylation patterns also differ between species (Patel et al., 1993). In general, removal of the glycosylation sites by mutagenesis or by enzymatic deglycosylation leads to markedly reduced uptake activity as a consequence of a reduced number of transporters at the cell surface. This has been attributed to instability of the nonglycosylated transporter (DAT and NET) (Melikian et al., 1996; Li et al., 2004), disruption of proper trafficking to the plasma membrane (GLYT1 and GLYT2) (Olivares et al., 1995; Martínez-Maza et al., 2001), or a combination of both (GAT1) (Cai et al., 2005). In general, removal of glycosylation sites did not affect ligand binding affinities or intrinsic transporter function (Tate and Blakely, 1994; Nguyen and Am-

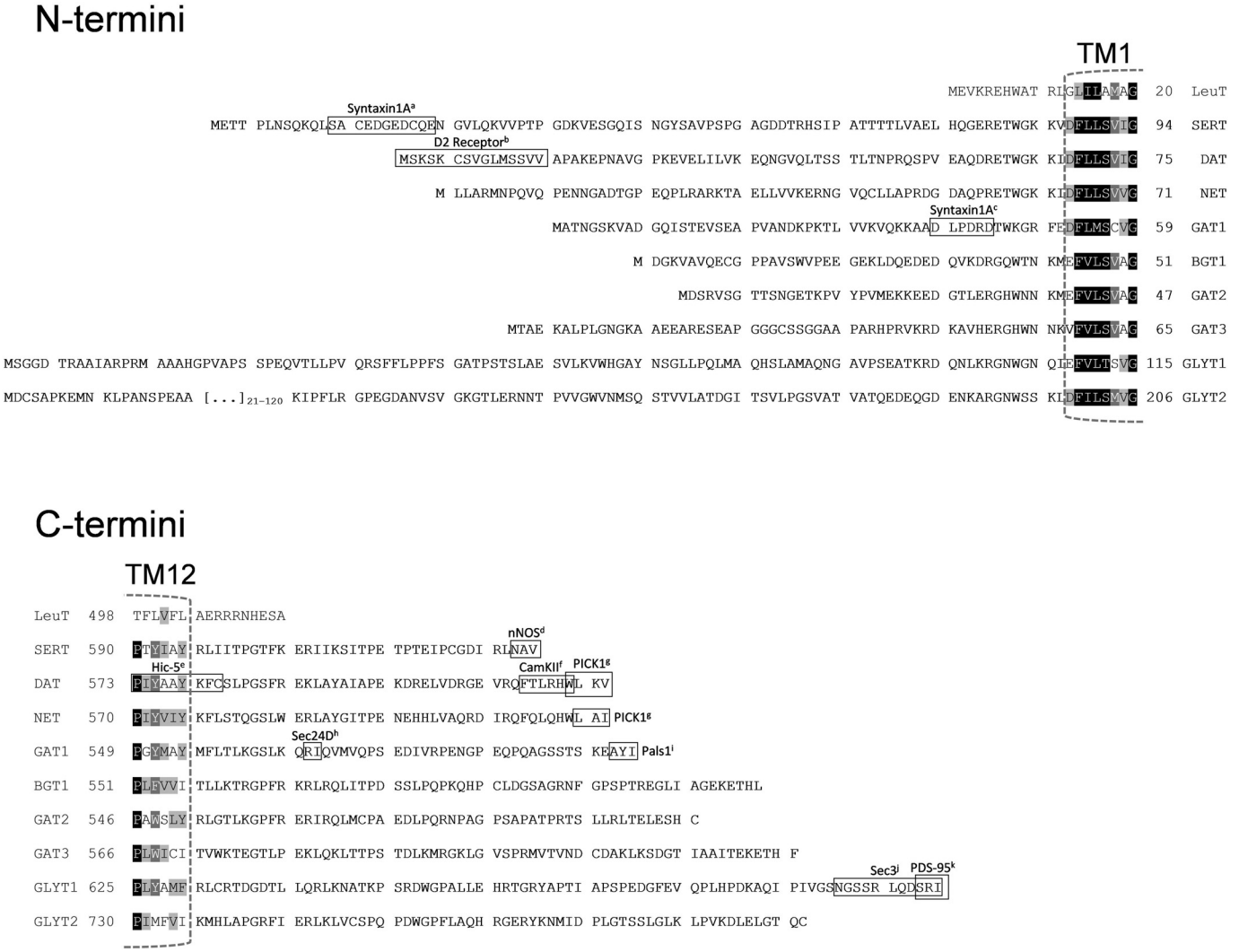

FIG. 10. N- and C-terminal sequences for the human *SLC6* NTTs. Numbering indicates the last amino acid in the N-terminal domain and the first amino acid in the C-terminal domain, respectively. Boxes indicate sites for protein-protein interaction partners. See legend to Fig. 6 for UniProt-SwissProt human accession numbers of the amino acid sequences. [a] Quick, 2003; [b] Lee et al., 2007; [c] Deken et al., 2000; [d] Chanrion et al., 2007; [e] Carneiro et al., 2002; [f] Fog et al., 2006; [g] Torres et al., 2001; [h] Farhan et al., 2007; [i] McHugh et al., 2004; [j] Cubelos et al., 2005a; [k] Cubelos et al., 2005b.

ara, 1996; Torres et al., 2003a), although reduced turnover rates have been suggested in some studies (Melikian et al., 1996; Li et al., 2004; Cai et al., 2005). For GAT1, a mechanism involving a reduced apparent affinity of the transporter for extracellular Na^+ were proposed to contribute to the reduced transport activity observed in glycosylation deficient mutants (Cai et al., 2005).

2. Phosphorylation

a. Protein kinase C-mediated phosphorylation. Numerous studies have demonstrated that the *SLC6* NTTs are phosphoproteins amenable to regulation by a number of different of kinases. However, the number of identified specific phosphorylation sites is still limited (Table 7). Protein kinase C (PKC) is the most extensively studied kinase and has a profound role in regulating the phosphorylation state of *SLC6* NTTs. Treatment of transfected cells as well as native cells or tissue expressing NTTs with PKC activators, such as the phorbol ester, phorbol 12-myristate 13-acetate (PMA), increases phosphorylation levels of DAT (Huff et al., 1997; Vaughan et al., 1997; Cowell et al., 2000; Chang et al., 2001; Foster et al., 2002; Granas et al., 2003; Lin et al., 2003; Gorentla and Vaughan, 2005), SERT (Ramamoorthy et al., 1998a; Ramamoorthy and Blakely, 1999; Jayanthi et al., 2005; Samuvel et al., 2005), NET (Jayanthi et al., 2004, 2005), GAT1 (Quick et al., 2004) and BGT1 (Massari et al., 2005). The general consequence of PKC activation is decreased transport (reduced V_{max}) as a result of redistribution of the transporter from the surface to an intracellular compartment. However, an initial rapid trafficking-independent inactivation of the transporters might occur at the plasma membrane as suggested for DAT (Mazei-Robison and Blakely, 2005) as well as SERT (Jayanthi et al., 2005).

In DAT, the N-terminal domain, which contains a cluster of five closely spaced Ser within the initial 21 N-terminal residues (Fig. 10), has been identified as a region for the majority of both basal and PKC-

TABLE 6
Post-translational modifications of SLC6 neurotransmitter transporters

Transporter	Kinase	Site	References
Phosphorylation			
SERT	PKA[a]	N- and C-terminal domains	Ramamoorthy et al., 1998a
	PKC[a]	N- and C-terminal domains	Ramamoorthy et al., 1998a
	PKG[b]	Thr276 (IL2)	Ramamoorthy et al., 2007
NET	PKC[a]	Thr258-Ser259 (IL2)	Jayanthi et al., 2006
	CamKII[c]	C-terminal domain	Uchida et al., 1998
DAT	PKC[a]	N-terminal Ser cluster (Ser2, Ser4, Ser7, Ser12, Ser13)	Foster et al., 2002; Lin et al., 2003; Granas et al., 2003; Cervinski et al., 2005; Gorentla et al., 2009
	CamKII[c]	N-terminal domain	Fog et al., 2006; Gorentla et al., 2009
	ERK1/2[a]	Thr53 (N-terminal domain)	Gorentla et al., 2009
	JNK[a]	Thr53 (N-terminal domain)	Gorentla et al., 2009
	p38 MAPK[a]	Thr53 (N-terminal domain)	Gorentla et al., 2009
	PKA[c]	N-terminal domain	Gorentla et al., 2009
	PKG[c]	N-terminal domain	Gorentla et al., 2009
	CKII[c]	N-terminal domain	Gorentla et al., 2009
	Cdk5[c]	N-terminal domain	Gorentla et al., 2009
	Akt[c]	N-terminal domain	Gorentla et al., 2009
GAT1	Tyr kinase[e]	Tyr107 (IL1), Tyr317 (IL3)	Whitworth and Quick, 2001
N-Linked glycosylation			
SERT		Asn208, Asn217	Rasmussen et al., 2009; Tate and Blakely, 1994
NET		Asn184, Asn192, Asn198	Melikian et al., 1996; Nguyen and Amara, 1996
DAT		Asn181, Asn188, Asn205	Li et al., 2004; Torres et al., 2003a
GAT1		Asn176, Asn181, Asn184	Bennett and Kanner, 1997; Cai et al., 2005
GLYT1		Asn169, Asn172, Asn182, Asn188	Olivares et al., 1995
GLYT2		Asn345, Asn355, Asn360, Asn366	Martínez-Maza et al., 2001

JNK, c-Jun NH_2-terminal kinase; CKII, casein kinase II; Cdk5, cyclin-dependent kinase 5.
[a] Phosphorylation of functional transporter, confirmed by in vitro phosphorylation.
[b] Putative. Direct transporter phosphorylation not confirmed.
[c] In vitro phosphorylation only.
[d] The corresponding residue in human DAT is a Ser.
[e] Phosphorylation of functional transporter, not confirmed by in vitro phosphorylation.

stimulated phosphorylation in native and recombinant cells (Granas et al., 2003; Cervinski et al., 2005). Furthermore, in vitro phosphorylation experiments using purified PKCα to phosphorylate purified glutathione transferase (GST) fusion proteins containing the DAT N terminus have shown that this domain is a PKC substrate (Fog et al., 2006; Gorentla et al., 2009). However, additional PKC phosphorylation sites outside the N-terminal domain might exist in DAT. Although one study indicated loss of PKC-stimulated phosphorylation after mutation of only Ser7 in the N-terminal domain of DAT (Lin et al., 2003), other studies have found residual basal and PKC-stimulated phosphorylation after mutation of the distal Ser cluster (Foster et al., 2003; Gorentla et al., 2009).

Despite the fact that PKC stimulation markedly increases DAT N-terminal phosphorylation, this seems not to be involved in the PKC-mediated endocytosis of DAT, because truncation of the DAT N terminus almost abolished detectable phosphorylation without blunting PKC-mediated down-regulation (Granas et al., 2003; Cervinski et al., 2005). These findings suggest that PKC-mediated down-regulation of DAT does not involve direct transporter phosphorylation. The N-terminal phosphorylation instead seems to be involved in amphetamine-induced reverse transport (efflux) of dopamine. Whereas N-terminal truncation as well as mutation of the five N-terminal Ser to Ala did not affect DAT trafficking, it essentially abolished amphetamine-induced efflux (Khoshbouei et al., 2004). Efflux was restored by replacing Ser7 and Ser12 with phosphor-mimicking Asp residues (Khoshbouei et al., 2004), leading to the suggestion that N-terminal phosphorylation transforms DAT from a "reluctant" to a "willing" state for dopamine efflux without affecting uptake (Khoshbouei et al., 2004).

It has been reported that Ser262 in IL2 and Ser586 and Thr616 in the C terminus of DAT are also PKC phosphor-acceptor sites, because mutation of these residues decreases PKC-stimulated DAT phosphorylation (Chang et al., 2001). Ser262 is conserved across all *SLC6* NTTs, and the corresponding residue in NET has been identified as a phosphor-acceptor site for PKC-mediated phosphorylation (Jayanthi et al., 2006). Using rat placental trophoblasts natively expressing NET, PMA-induced phosphorylation of NET has been demonstrated to occur on Ser and Thr residues (Jayanthi et al., 2006). Mutation of Thr258 and Ser259 to Ala significantly reduced transporter phosphorylation and prevented PMA-induced decrease in norepinephrine uptake and NET internalization, an effect that was not observed upon substitution of all other potential phosphorylation sites in the transporter (Jayanthi et al., 2006). In vitro phosphorylation with purified PKCε on membrane preparations containing recombinant expressed NET suggested Ser259 as the direct site of PKC phosphorylation; however, mutation of both Thr258 and Ser259 was required to impair functional down-regulation by the kinase (Jayanthi et al., 2006).

TABLE 7
Protein interaction partners for the SLC6 neurotransmitter transporters

Transporter & Interaction Partner	Site of Interaction (Terminus)	References
Monoamine transporters		
SERT		
SCAMP2	N	Müller et al., 2006
Syntaxin1A	N	Haase et al., 2001; Quick, 2003
Integrin αIIbβ3	C	Carneiro et al., 2008
Hic-5	C	Carneiro and Blakely, 2006; Carneiro et al., 2002
MacMARKS	C	Jess et al., 2002
nNOS	C	Chanrion et al., 2007
Sec23A	C	Chanrion et al., 2007
Sec24C	C	Chanrion et al., 2007
CIPP	C	Chanrion et al., 2007
α-Synuclein	N.I.	Wersinger et al., 2006b
Myosin2A	N.I.	Ozaslan et al., 2003
PP2A	N.I.	Bauman et al., 2000
NET		
14-3-3 proteins	N	Sung et al., 2005
PP2A	N	Bauman et al., 2000; Sung et al., 2005
Syntaxin1A	N	Sung et al., 2003
HIC-5	C	Carneiro et al., 2002
PICK1	C	Torres et al., 2001
α-Synuclein	N.I.	Wersinger et al., 2006a
SNAP-25	N.I.	Schroeter et al., 2000
Synaptophysin	N.I.	Schroeter et al., 2000
DAT		
D_2 Receptor	N	Bolan et al., 2007; Lee et al., 2007
Synaptogyrin	N	Egaña et al., 2009
Syntaxin1A	N	Lee et al., 2004
SCAMP2	N	Müller et al., 2006
CaMKII	C	Fog et al., 2006
Hic-5	C	Carneiro et al., 2002
PICK1	C	Torres et al., 2001
α-Synuclein	C	Lee et al., 2001; Wersinger et al., 2003
Parkin	N.I.	Moszczynska et al., 2007
PP2A	N.I.	Bauman et al., 2000
PKCβ	N.I.	Johnson et al., 2005b
NEDD4-2	N.I.	Sorkina et al., 2006
Epsin/Eps15	N.I.	Sorkina et al., 2006
GPR37	N.I.	Marazziti et al., 2007
Parkin	N.I.	Moszczynska et al., 2007
GABA transporters		
GAT-1		
Syntaxin1A	N	Beckman et al., 1998; Deken et al., 2000
ARFGAP1	C	Reiterer et al., 2008
Pals1	C	McHugh et al., 2004
Sec24D	C	Farhan et al., 2004, 2007
Glycine transporters		
GLYT1		
PSD-95	C	Cubelos et al., 2005b
Sec3	C	Cubelos et al., 2005a
Syntaxin1A	N.I.	Geerlings et al., 2000
GLYT2		
Ulip6	N	Horiuchi et al., 2000, 2005
Syntenin-1	C	Ohno et al., 2004
Syntaxin1A	N.I.	Geerlings et al., 2000, 2001

N.I., not identified; CIPP, channel-interacting PDZ protein; SNAP-25, synaptosome-associated protein 25; NEDD4-2 (neural precursor cell expressed, developmentally down-regulated 4-2); GPR37, G protein-coupled receptor 37; ARFGAP1, ADP-ribosylation factor GTPase-activating protein 1; Pals1, protein associated with Lin seven 1; Ulip6, Unc-33-like protein 6.

The specific PKC phosphorylation sites in SERT are less well characterized. In vitro phosphorylation by PKC has been reported on both N- and C-terminal SERT fusion proteins (Blakely et al., 1998), but no specific residues were identified. A study in platelets demonstrated that PKC-stimulated phosphorylation occurred at both Ser and Thr residues and suggested a biphasic effect on SERT with an initial phase in which decrease in 5-HT transport is accompanied by Ser phosphorylation and a subsequent phase in which SERT internalization is accompanied by Thr phosphorylation (Jayanthi et al., 2005). PKC-mediated phosphorylation of SERT seems to be attenuated by substrates, which has been suggested to provide an adaptive feedback mechanism for maintaining the transporter at the cell surface during periods of high transport demand (Ramamoorthy and Blakely, 1999).

GAT1 is also down-regulated after activation of PKC (Osawa et al., 1994; Sato et al., 1995b; Beckman et al., 1998, 1999; Bahena-Trujillo and Arias-Montaño, 1999; Quick et al., 2004; Wang and Quick, 2005; Cristóvão-Ferreira et al., 2009), but only one study has demonstrated increased transporter phosphorylation upon PKC stimulation (Quick et al., 2004). PKC-mediated

down-regulation is observed for GLYT1 and GLYT2 as well, but corresponding changes in the phosphorylation state of GLYT1 and GLYT2 have not been investigated (Gomeza et al., 1995; Sato et al., 1995a; Fornés et al., 2004; Morioka et al., 2008). In BGT1, Ser and Thr phosphorylation has been demonstrated in response to PKC activation, and Thr612 in the distal C terminus was identified as a likely phosphorylation site (Massari et al., 2005).

b. Phosphorylation mediated by other kinases. A number of kinases other than PKC have been suggested as mediators of phosphorylation of *SLC6* NTTs. For GAT1, Tyr phosphorylation has been shown to regulate transporter activity. In rat hippocampal cultures, GAT1 is phosphorylated at Tyr residues under basal conditions, and agents that promote Tyr phosphorylation cause an increase in surface localized protein (Law et al., 2000). Increased Tyr phosphorylation has been found to correlate with substrate-induced up-regulation of GAT1 surface levels, and mutational studies identified Tyr107 in IL1 and Tyr317 in IL3 as the phosphor-acceptor sites (Whitworth and Quick, 2001). A remarkable reciprocal relationship between this Tyr phosphorylation and PKC-mediated Ser phosphorylation was found subsequently in which activation of pathways leading to increased GAT1 Tyr phosphorylation promotes reduced Ser phosphorylation, whereas activation of PKC activity has the reverse effect, suggesting that at least two mutually exclusive phosphorylation states exist for GAT1 that determine transporter distribution between the cell-surface and intracellular compartments (Quick et al., 2004). Tyr phosphorylation might also regulate SERT function, and 5-HT uptake capacity into platelets has been shown to be positively correlated with Src-mediated Tyr phosphorylation (Zarpellon et al., 2008). Nevertheless, regulation by Tyr phosphorylation has not been reported for any of the other NTTs.

Several studies have reported SERT up-regulation by a cGMP/protein kinase G (PKG)-mediated pathway in both native and transfected cells (Miller and Hoffman, 1994; Zhu et al., 2004a,b, 2007; Prasad et al., 2005; Zhang et al., 2007). PKG-mediated increase in SERT phosphorylation has been reported (Ramamoorthy et al., 1998a), Thr276 located in IL2 being the likely phosphorylation site (Ramamoorthy et al., 2007). It is noteworthy that the corresponding residue in NET (Thr258) is part of the acceptor site for PKC-mediated phosphorylation as discussed above. No alteration in SERT surface level was observed upon PKG stimulation, and it was proposed that increased SERT activity is caused by enhanced transport capacity of the transporter (Ramamoorthy et al., 2007). However, other studies have suggested that PKG-mediated SERT phosphorylation also regulates cell-surface expression of the transporter (Zhu et al., 2004a, 2004b, 2007; Prasad et al., 2005).

Several of the *SLC6* NTTs have been found subject to regulation by mitogen-activated protein kinase (MAPK) pathways. Inhibition of p38MAPK decreases SERT phosphorylation and cell-surface levels, whereas coexpression of a constitutively active kinase, MKK3b(E), upstream of p38MAPK with SERT increases transport capacity (Zhu et al., 2004a, 2005, 2007; Prasad et al., 2005; Samuvel et al., 2005), but whether SERT is subject to direct phosphorylation by p38MAPK during these processes is unknown. For DAT, increased dopamine transport was observed in cells coexpressing DAT with the ERK1/2-(MAPK) activator MAPK kinase (Carvelli et al., 2002; Morón et al., 2003). Furthermore, decreased DAT phosphorylation and activity were observed to correlate upon inhibition of MAPK kinase (Lin et al., 2003), suggesting that MAPK-linked DAT phosphorylation is involved in regulation of DAT activity. In support of direct phosphorylation of DAT by MAPKs, a recent in vitro phosphorylation study demonstrated that the MAPKs ERK1/2, c-Jun NH_2-terminal kinase, and p38MAPK can phosphorylate Thr53 in the isolated DAT N-terminal domain (Gorentla et al., 2009). However, phosphorylation of Thr53 seems not to be involved in ERK-mediated regulation of DAT activity because mutation of Thr53 did not affect decreases in DAT activity observed upon ERK-inhibition (Gorentla et al., 2009). This finding is supported by other studies demonstrating that dopamine receptor-mediated, ERK1/2-dependent DAT up-regulation is maintained upon truncation of the first 55 N-terminal amino acids of DAT, suggesting that N-terminal phosphorylation is not involved in the regulatory mechanism underlying this effect (Bolan et al., 2007; Zapata et al., 2007).

In addition to MAPK phosphorylation, a study also demonstrated significant Ser phosphorylation on the N terminus of DAT by protein kinase A (PKA), PKG, Ca^{2+}/calmodulin-dependent protein kinase α (CaMKIIα), casein kinase II, cyclin-dependent kinase 5, and Akt (Gorentla et al., 2009). However, the identity of the specific phosphor-acceptor residues within the N terminus as well as potential functional effects of phosphorylation, were not investigated further in this study. PKA-mediated in vitro phosphorylation has also been reported for the isolated C- and N-terminal domains of SERT expressed as GST fusion proteins (Blakely et al., 1998) and stimulation of PKA activity increases SERT phosphorylation in transfected cells, but with no effect on 5-HT uptake capacity (Ramamoorthy et al., 1998a). In contrast, a study of 5-HT uptake in prefrontocortical synaptosomes showed increased transport activity upon PKA stimulation but did not investigate SERT phosphorylation level (Awtry et al., 2006). Likewise, PKA-dependent up-regulation of DAT (Batchelor and Schenk, 1998) and GAT1 (Cristóvão-Ferreira et al., 2009) has been reported but without investigation of potential correlation with changes in transporter phosphorylation level.

CaMKII has been shown to be involved in phosphorylation of several *SLC6* NTTs, but with very different

functional outcomes. For DAT, CaMKII was identified as a key mediator of amphetamine-mediated efflux (Fog et al., 2006). CaMKII phosphorylates a peptide corresponding to the first 27 amino acids of the DAT N terminus in vitro and mutation of the five Ser in the N-terminal cluster (Fig. 10) of the full-length DAT prevented CaMKII-stimulated amphetamine-mediated dopamine efflux (Fog et al., 2006). These results highlight the importance of N-terminal DAT phosphorylation in the action of amphetamine but also raise questions about importance of and potential interconnection between CaMKIIα and PKC for this process. For NET, CaMKII has been shown to in vitro phosphorylate a peptide corresponding to a segment of the NET C-terminal, and stimulation of CamKII activity in PC12 cells, which endogenously express NET, correlates with enhanced NET transport activity (Uchida et al., 1998). Likewise, for GLYT1, a study on GLYT1-expressing glia cells demonstrated reduced Gly uptake upon CaMKII inhibition, but no link to direct transporter phosphorylation was established (Gadea et al., 2002). For SERT, CaMKII activity has been found to regulate the electrophysiological properties of the transporter by modulating the interaction of SERT with syntaxin-1a (section V.G). This effect could be abolished by mutating Ser13 located in the SERT N-terminal, suggesting this residue as a CaMKII phosphorylation site, but no direct phosphorylation was established (Ciccone et al., 2008).

c. Transporter dephosphorylation. Protein phosphatases generally play a pivotal role in regulation of *SLC6* NTT phosphorylation, and tonic phosphatase activity has been suggested to maintain the transporters in a relatively dephosphorylated state. For DAT (Huff et al., 1997; Vaughan et al., 1997; Foster et al., 2002, 2003), SERT (Ramamoorthy et al., 1998a), and NET (Jayanthi et al., 2004), transporter phosphorylation increases upon inhibition of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A). For DAT (Vaughan et al., 1997), SERT (Sakai et al., 1997; Ramamoorthy et al., 1998a), and NET (Jayanthi et al., 2004), inhibition of PP1/PP2A was shown to correlate with down-regulation of transport activity. The catalytic subunit of PP2A has been found to coimmunoprecipitate with DAT, SERT, and NET from native preparations. For SERT and NET, the association with PP2A can be reduced by treatment with PKC activators as well as PP1/2A inhibitors (Blakely and Bauman, 2000; Samuvel et al., 2005). This suggests that PP2A physically associates with SERT and NET to maintain the transporters in a dephosphorylated state and that increased transporter phosphorylation occurs upon disruption of the complex. Inhibitors of p38MAPK can also reduce the association of SERT with PP2A (Samuvel et al., 2005), which, combined with the observation that both the cGMP- and p38MAPK-mediated up-regulation in SERT catalytic activity is PP1/2A-dependent (Zhu et al., 2004a, 2005), indicates that PP2A is involved in a complex regulatory mechanism for SERT (for review, see Steiner et al., 2008). In vitro dephosphorylation studies of DAT have demonstrated that PP1 can dephosphorylate both PKC-stimulated metabolically phosphorylated rDAT and in vitro phosphorylated N-terminal of DAT (Foster et al., 2003; Gorentla et al., 2009). The phosphorylation level of GATs and GLYTs and their regulation by phosphatases has so far not been investigated in greater detail.

In conclusion, although major efforts have been directed toward understanding the role of phosphorylation of *SLC6* NTTs and the processes by which phosphorylation control transporter function, more work is needed to understand the molecular basis of transporter regulation by phosphorylation. This includes identification of specific residues as phosphorylation sites, because remarkably few sites have been identified for the many kinases proposed to be involved in *SLC6* NTT phosphorylation, meaning that it remains difficult to associate the potential consequences of transporter phosphorylation with specific molecular mechanisms that can control transporter function and expression. In this respect, it is encouraging for future progress that recent advances in mass spectrometry-based phosphoproteomics (Paradela and Albar, 2008; Schreiber et al., 2008) has begun to allow parallel identification of phosphorylation sites in a wide range of neuronal membrane proteins (Trinidad et al., 2006; Yan et al., 2008).

B. Neurotransmitter Transporters in the Secretory Pathway

The passage of NTTs through the secretory pathway [i.e., through the endoplasmic reticulum (ER) and the Golgi apparatus] represents an additional step for potential regulation of NTT expression and function. ER export has been studied for several members of the *SLC6* family and from these studies, it can be concluded that, at least in heterologous expression systems, the process of ER export is regulated both by transporter oligomerization and by the cytoplasmic C terminus of the transporters.

Oligomerization (for review, see Sitte et al., 2004) was reported for several of the members in the *SLC6* family, including DAT (Hastrup et al., 2001; Sorkina et al., 2003; Torres et al., 2003a), SERT (Kilic and Rudnick, 2000; Just et al., 2004), NET (Hahn et al., 2003), GAT1 (Schmid et al., 2001; Scholze et al., 2002), and recently GLYT1 and GLYT2 (Bartholomäus et al., 2008), on the basis of application of biochemical cross-linking techniques, coimmunoprecipitations of differentially tagged transporters, and FRET. The transporters seemed to oligomerize at an early stage in the secretory pathway (Scholze et al., 2002; Sorkina et al., 2003) and apparent oligomer disruption by mutation of a leucine repeat in TM2 led to an ER retention of GAT1, even though the mutant transporter was still capable of mediating transport (Scholze et al., 2002). Therefore, it was suggested that oligomerization was required for ER export as sug-

gested also in other protein families (Reddy and Corley, 1998). However, oligomerization is not sufficient for proper ER export of the NTTs. Thus, several mutants have been identified that, despite forming oligomers, are retained within the ER (Sorkina et al., 2003; Torres et al., 2003a; Farhan et al., 2004; Miranda et al., 2004). It is noteworthy that several subtle mutations in the C terminus were shown to confer ER retention in both DAT (Sorkina et al., 2003; Bjerggaard et al., 2004; Miranda et al., 2004) and GAT1 (Farhan et al., 2004), suggesting that an intact C terminus is necessary for ER export even though it is not required for oligomerization.

The GAT1 C terminus was found to bind Sec24D, a component of the coat protein complex II (COPII) that facilitates export of membrane proteins from the ER. It is noteworthy that truncation of the GAT1 C terminus, and thereby disruption of the Sec24D interaction, caused ER retention of the transporter (Farhan et al., 2004). Of further interest, coexpression of wild-type GAT1 with C-terminally truncated GAT1, which led to formation of wild-type/mutant hetero-oligomers, caused ER retention, suggesting that all C termini within a GAT1 oligomer must be intact to allow efficient ER export (Farhan et al., 2004). The binding of GAT1 to Sec24D was suggested to be mediated via Arg566-Leu567 in the GAT1 C terminus (Farhan et al., 2007). This motif is conserved in the *SLC6* family and, remarkably, was also shown to mediate Sec24D binding in DAT and SERT (Farhan et al., 2007). Moreover, ER export of both transporters was impaired when coexpressed with a dominant-negative Sec24D (Farhan et al., 2007). In addition to the effect on ER export, the Sec24D interaction was shown to guide axonal concentration of GAT1 in hippocampal neurons (Reiterer et al., 2008), suggesting that subcellular targeting of *SLC6* NTTs seems to be determined early in the secretory pathway. In the same study, it was shown that GAT1 binds another component of the COPII coat complex, ARGFGAP1, and this interaction was also necessary for proper axonal targeting of GAT1 (Reiterer et al., 2008). Whether these mechanisms can be generalized to all NTTs remains nonetheless unknown. It is noteworthy that an additional motif important for export to the plasma membrane has been identified in the GAT1 C terminus. This motif (Val569, Met570, and Ile571) was reported to be necessary for export, because mutation to Ser-Ser-Ser led to accumulation of the transporter in the ER-Golgi Intermediate Compartment (Farhan et al., 2008). However, this motif is not found in the other members of the NTT family.

C. Endocytic Trafficking

Several studies have reported the existence of intracellular pools of both heterologously and endogenously expressed NTTs, including GAT1 (Quick et al., 2004), GLYT2 (Núñez et al., 2009), NET (Matthies et al., 2009), and DAT (Loder and Melikian, 2003; Eriksen et al., 2009). A steady-state distribution for GAT1 was estimated in cultured cortical neurons, with approximately one third of the transporter in an intracellular pool (Wang and Quick, 2005), and the steady-state distribution of DAT was, according to surface biotinylation experiments, estimated to be 60% intracellular and 40% on the cell surface (Melikian and Buckley, 1999). The existence of an intracellular pool has been explained to be mainly the result of prominent constitutive internalization of the transporters. Indeed, DAT has a high constitutive internalization rate, both when expressed in cell lines (Loder and Melikian, 2003; Sorkina et al., 2005) and for the endogenous transporter in cultured dopaminergic neurons (Eriksen et al., 2009). Likewise, endogenous GAT1 and GLYT2 were shown to constitutively internalize (Fornés et al., 2008). For DAT, GLYT2, and GAT1, the constitutively internalized transporter was suggested to sort mainly to a recycling pathway, permitting reinsertion of the transporter into the plasma membrane. Accordingly, it was proposed that the intracellular transporters represented a recruitable transporter pool permitting rapid mobilization of transporter to the surface during periods of high signaling activity. However, for DAT, it has recently been suggested that a substantial fraction of the constitutively internalized DAT is sorted to a lysosomal/degradative pathway with probably only a rather small fraction sorted to Rab4-positive "short-loop" recycling pathways (Eriksen et al., 2010a).

D. Regulated Trafficking

As described above, the most thoroughly studied mechanism in regulated trafficking of the *SLC6* NNTs is PKC activity-dependent internalization. However, as mentioned above, only for NET has an unambiguous connection between PKC-mediated transporter phosphorylation and internalization been determined (section V.A). The suggested phosphorylation sites in NET (Thr258 and Ser259) are situated in IL2, which also have been connected with PMA-induced internalization of GLYT2. Mutation of the corresponding residues, Thr419 and Ser420, in GLYT2 partially impaired PMA-mediated internalization (Fornés et al., 2004), and mutation of the nearby Lys442 abolished PMA-mediated internalization of GLYT2 without affecting constitutive internalization (Fornés et al., 2004, 2008).

For DAT, it has been proposed that PKC-mediated internalization involves ubiquitination, another post-translational modification, instead of being the result of direct PKC-mediated transporter phosphorylation (Miranda et al., 2005). Miranda et al. (2005) showed that DAT was constitutively ubiquitinated and that this was augmented upon PMA stimulation. The ubiquitination was dependent on the presence of three lysines situated in the intracellular DAT N terminus (Lys19, Lys27, and Lys35), and concomitant mutation of these residues to arginines essentially abolished both ubiquitination and phorbol ester-stimulated DAT down-regulation (Mi-

randa and Sorkin, 2007). In addition, an siRNA screen identified the ubiquitin ligase NEDD4-2 as critical for PKC-mediated DAT internalization (Sorkina et al., 2006) along with proteins associated with endosome transport (Sorkina et al., 2006), suggesting that PKC activity modulates DAT internalization through the ubiquitylation system and endosome transport processes. It is noteworthy that the ESCRT (endosomal sorting complex required for transport) machinery-associated proteins epsin and Eps15/Eps15R were also identified in the siRNA screen as important for PMA-mediated DAT endocytosis, and both epsin and Eps15 coimmunoprecipitated with DAT upon PMA stimulation (Sorkina et al., 2006). Because DAT has also been shown to colocalize with the ESCRT component hepatocyte-growth factor receptor substrate (Miranda et al., 2005), it is conceivable that ubiquitinated DAT is sorted by the ESCRT machinery. This is also in agreement with the fact that PKC activation increased DAT degradation in HeLa and Madin-Darby canine kidney cells (Daniels and Amara, 1999; Miranda et al., 2005).

A nonconventional internalization motif in the DAT C terminus (residues 587–596; FREKLAYAIA) has also been suggested to be critical for constitutive and PKC-mediated DAT internalization (Holton et al., 2005). The motif is relatively well conserved among the *SLC6* NTTs, and mutating the motif in NET also resulted in impaired internalization (Holton et al., 2005). Further studies showed that Ala substitutions of residues 587 to 590 were sufficient to abolish PKC-mediated DAT down-regulation and constitutive DAT internalization. On the basis of these results, it was suggested that the stretch of four residues is part of an endocytic braking mechanism that is relieved upon PKC stimulation (Boudanova et al., 2008). The role of this C-terminal motif in ubiquitination of the N terminus in PKC-mediated DAT endocytosis has not been elucidated and awaits further investigations. Of further interest, mitogen-activated protein kinase phosphatase, has been identified as an inhibitor of PKC-mediated DAT internalization acting downstream of the DAT ubiquitination (Mortensen et al., 2008). Although this needs further clarification, the observation should be considered together with the recent finding that PMA failed to induce internalization of endogenous DAT (Eriksen et al., 2009). Hence, variable expression of mitogen-activated protein kinase phosphatase in dopaminergic neurons might be a mean by which PKC-mediated DAT internalization is regulated.

The degree to which nonconventional trafficking motifs or ubiquitination contributes to PKC-mediated internalization of the other NTTs remains to be determined. It is noteworthy that it was shown that ubiquitination was involved in both PMA-mediated and constitutive internalization of GLYT1 (Fernández-Sánchez et al., 2009). However the ubiquitination site was identified in C terminus (Lys619), emphasizing the rather scarce homology between the individual transporters in their intracellular domains.

Other kinase pathways have been implicated in regulating trafficking of NTTs. For DAT, this includes downstream effectors of insulin signaling such as phosphatidylinositol 3-kinase (PI3K) (Carvelli et al., 2002; Lin et al., 2003) and Akt (Garcia et al., 2005), which have been suggested to promote trafficking of DAT to the surface. Hence, insulin was shown to increase dopamine uptake in a PI3K- (Lin et al., 2003) and Akt-dependent manner (Garcia et al., 2005). An in vivo role of this pathway in regulating DAT levels was supported by studies of rats depleted of insulin by the diabetogenic agent streptozotocin. In these rats, both Akt function and DAT surface expression was markedly reduced (Williams et al., 2007). Likewise, NET was shown to be up-regulated by insulin in a PI3K inhibitor-sensitive manner (Apparsundaram et al., 2001). Moreover, SERT surface trafficking seems to be regulated by p38MAPK (Samuvel et al., 2005) and possibly cGMP/PKG, perhaps via activation of adenosine receptors (Zhu et al., 2004a,b, 2007; Prasad et al., 2005). Finally, DAT trafficking might be subject to regulation by MAPK; i.e., MAPK inhibitors were shown to decrease dopamine uptake both in transfected HEK293 cells and in striatal synaptosomes. The effect may both involve alteration in DAT transport capacity and redistribution of DAT from the plasma membrane to the cytosol (Morón et al., 2003).

For GAT1, surface trafficking seems to be regulated by Tyr phosphorylation (Law et al., 2000; Whitworth and Quick, 2001), and it was recently shown that activation of adenosine A_{2A} receptors in hippocampal synaptosomes enhanced GABA uptake by opposing a constitutive PKC-mediated down-regulation of GAT1 (Cristóvão-Ferreira et al., 2009).

E. Substrate- and Inhibitor-Mediated Trafficking

There is increasing evidence supporting that substrates and inhibitors of *SLC6* NTTs are capable of regulating trafficking of these to and from the plasma membrane. For DAT, both dopamine and amphetamine were found to promote DAT internalization, with the largest effect seen for amphetamine (Saunders et al., 2000; Chi and Reith, 2003; Sorkina et al., 2003; Kahlig et al., 2004). In contrast, the DAT inhibitors cocaine and methylphenidate increased DAT surface expression (Daws et al., 2002; Little et al., 2002). It is noteworthy that in vivo observations support this scenario; i.e., methamphetamine users had less DAT in the striatum and cocaine users were reported to have an increased level of DAT (Little et al., 1993).

The molecular mechanisms underlying substrate and inhibitor induced surface regulation is still enigmatic; recently, however, it has been suggested that an inward-facing conformation of DAT is more prone to internalization than an outward-facing conformation of DAT (Sorkina et al., 2009). Thus, substrate might promote

internalization simply by increasing the fraction of transporter molecules residing in an inward-facing conformation, which might alter the interaction of the transporter with as-yet-unknown proteins. However, amphetamine-induced internalization of DAT could be promoted by intracellular application of amphetamine via a patch pipette, suggesting that DAT transport activity was not required and that an increase of intracellular amphetamine is an essential component of DAT redistribution (Kahlig et al., 2006). It has also been proposed that that CaMKII activity was necessary for amphetamine-induced internalization of DAT (Wei et al., 2007), but unlike amphetamine-induced efflux, the mechanism does not involve N-terminal phosphorylation (Cervinski et al., 2005). DAT, amphetamine, and CaMKII has furthermore been coupled to the insulin signaling pathway; i.e., amphetamine inhibits Akt through a mechanism that is dependent on DAT and CaMKII activity, and this inhibition might contribute to the amphetamine-induced DAT redistribution from the surface (Wei et al., 2007).

A biphasic effect of amphetamine on DAT trafficking has also been proposed (Johnson et al., 2005a; Chen et al., 2009; Furman et al., 2009). Preceding the amphetamine-induced internalization of DAT, a rapid increase in the level of surface-expressed transporter was observed after 1-min treatment of rat striatal synaptosomes with amphetamine (Johnson et al., 2005a). The increase in surface DAT was dependent on PKCβ activity (Furman et al., 2009). Finally, disruption of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex seems to abolish amphetamine-induced trafficking of DAT to the surface (Furman et al., 2009). For NET, amphetamine has been shown to induce endocytosis in a CaMKII- and syntaxin1A-dependent manner (Dipace et al., 2007), and recently it was shown that amphetamine-induced NET internalization also depended on the small GTPase Rab11 (Matthies et al., 2010). Furthermore, phosphorylation of Thr258 and Ser259 has been implicated in amphetamine-induced endocytosis of NET (Annamalai et al., 2010).

In SERT, 5-HT attenuates PMA-induced internalization, as demonstrated by cell-surface biotinylation experiments (Ramamoorthy and Blakely, 1999), indicating that, in contrast to DAT, the substrate stabilizes the transporter protein at the cell surface. Consistent with this observation, prolonged exposure to 5-HT led to increased surface expression of SERT (Whitworth et al., 2002). Nonetheless, recent studies on SERT have indicated a biphasic effect of 5-HT on SERT with enhanced surface expression at low 5-HT concentrations and lowered surface expression induced by higher 5-HT concentrations (Brenner et al., 2007).

GAT1 has also been shown to be regulated by extracellular substrate and inhibitor application. In the short term, substrates increase surface expression levels of GAT1 and inhibitors have the opposite effect (Bernstein and Quick, 1999). Substrate mediated up-regulation is dependent on GAT1 phosphorylation of Tyr107 and Tyr307 and is facilitated by a decreased internalization rate (Whitworth and Quick, 2001). It has been shown that the regulatory effect of GABA on GAT1 in cortical neurons is biphasic; a transporter-dependent up-regulation is observed first, followed by GABA-receptor mediated down-regulation (Hu and Quick, 2008).

F. Microdomain and Raft Localization

Similar to other membrane proteins, the *SLC6* NTTs are predicted not to be randomly distributed in the plasma membrane but are associated with intracellular protein networks and plasma membrane microdomains that ensure appropriate spatial and temporal regulation of transporter function. Several of the *SLC6* NTTs have been suggested to segregate into distinct plasma membrane domains of specific lipid composition, including so-called membrane rafts (Simons and Toomre, 2000; Tsui-Pierchala et al., 2002; Pike, 2006). Membrane rafts are believed to compartmentalize cellular processes (Simons and Toomre, 2000; Tsui-Pierchala et al., 2002; Pike, 2006), and association to membrane rafts might be important for the function of NTTs. Membrane raft association was first suggested for SERT (Magnani et al., 2004) and for NET (Jayanthi et al., 2004), where membrane raft association of NET may be critical for internalization of the transporter in response to PKC activation (Jayanthi et al., 2004). For DAT, application of fluorescence correlation spectroscopy and fluorescence recovery after photobleaching in heterologous cells supported raft and cytoskeleton association of DAT (Adkins et al., 2007). Functional analysis indicated that the association of DAT with rafts might regulate transport capacity (Adkins et al., 2007) in a fashion similar to that observed for SERT (Magnani et al., 2004). Raft association has also recently been proposed for GLYT1, but the functional significance was not assessed (Liu et al., 2009).

G. Protein-Protein Interactions

For most of the *SLC6* NTTs, several interactions between the transporters and specific intracellular scaffolding, cytoskeletal, anchoring, and signaling proteins, including other membrane proteins, have been reported that seem to regulate transporter function (for review, see Eriksen et al., 2010) (Table 7; Fig. 10). In recent years, a growing body of experimental evidence has substantiated the critical importance of these interactions in regulating *SLC6* NTT function, including their cellular trafficking, catalytic properties, and the action of drugs targeting the transporters.

1. Soluble N*-Ethylmaleimide-Sensitive Factor Attachment Protein Receptors and Synaptic Vesicle Proteins.* One interaction that seems to be general for all *SLC6* NTTs is an interaction with the SNARE protein

syntaxin1A (Beckman et al., 1998; Geerlings et al., 2000, 2001; Haase et al., 2001; Sung et al., 2003; Lee et al., 2004). Syntaxin1A is a neuronal membrane protein belonging to the family of Gln-SNAREs involved in the vesicle docking process during exocytosis of synaptic vesicles (Sudhof, 2004). An additional role of syntaxin1A has emerged with the findings that syntaxin1A binds to and regulates the activity and cell surface availability of several neuronal membrane proteins. Compelling evidence suggests that the N termini of *SLC6* NTTs interact directly with syntaxin1A, possibly through an interaction with the SNARE motif H3 (Deken et al., 2000; Quick, 2003; Lee et al., 2004). For DAT, the interaction site is contained within the first 33 residues of the N terminus (Binda et al., 2008), whereas residues 30 to 54 of the GAT1 N-terminal domain were shown to interact directly with syntaxin1A (Deken et al., 2000) (Fig. 10). Further characterization identified three Asp in the GAT1 N terminus (Asp40, Asp43, and Asp45) as critical for syntaxin1A binding. Likewise, mutation of negatively charged residues between positions 11 and 30 of SERT disrupted syntaxin1A binding (Quick, 2003). Knockdown of syntaxin1A by antisense oligonucleotide or inactivation by cleavage of syntaxin1A with botulinum toxin C1 (BoTx C1) have substantiated that syntaxin1A plays a role in regulating the level of *SLC6* NTTs at the cell surface (Beckman et al., 1998; Deken et al., 2000; Geerlings et al., 2000, 2001; Quick, 2002; Sung et al., 2003; Cervinski et al., 2010). In addition, the interaction with syntaxin1A has been shown to reduce the catalytic activity of NET and GAT (Deken et al., 2000; Sung et al., 2003). For GAT1, the interaction with syntaxin1A was reported to be regulated by GABA as prolonged incubation with substrate increased the catalytic activity of GAT1 when in the presence of syntaxin1A. Furthermore, the syntaxin1A interaction with GAT1 was found to decrease after transport activity. This suggests that syntaxin1A is involved in a regulatory mechanism in which GABA transport capacity can be up-regulated after periods with high transport activity (Quick, 2002). Finally, the electrophysiological properties of the transporters have been found to be influenced by the interaction with syntaxin1A. For SERT, interaction with syntaxin1A abolished the transport-associated current that is a result of an electrogenic nonstoichiometric transport mode as well as the Na^+ leak current observed in absence of substrate, resulting in a electroneutral transport mode in which substrate and ion transport are strictly coupled (Quick, 2003). Likewise, studies performed in *Caenorhabditis elegans* suggested that the interaction of DAT with the *C. elegans* syntaxin1A homolog changes the electrophysiological properties of the transporter (Carvelli et al., 2008).

By use of the mating-based split ubiquitin system, the synaptic vesicle protein synaptogyrin-3 has been identified as a potential interacting partner for DAT (Egaña et al., 2009). The synaptogyrin-3/DAT interaction was further confirmed by FRET experiments and GST-pull-downs and coimmunoprecipitations that further showed that the interaction was mediated between the N terminus of DAT and the cytoplasmic N terminus of synaptogyrin. In the catecholaminergic cell lines PC12 and MD9D, which both contain vesicular monoamine transporter-positive vesicles, overexpression of synaptogyrin-3 with DAT increased DAT uptake capacity, whereas siRNA-mediated knockdown of synaptogyrin-3 expression reduced DAT activity, effects that both were absent in non-neuronal HEK293 cells. Furthermore, DAT activity was reduced by 40% in the presence of the vesicular monoamine transporter 2 inhibitor reserpine in PC12 cells but not in HEK293 cells. Because the DAT N terminus was found to be capable of pulling down purified synaptic vesicles, the authors suggest the challenging hypothesis that the interaction between synaptogyrin-3 and DAT physically couples DAT to synaptic vesicles and that this coupling leads to an increase in DAT activity and presumably a more efficient filling of synaptic vesicles (Egaña et al., 2009). Synaptogyrin-3 neither bound NET nor affected NET function, suggesting that the interaction is DAT-specific. Another SNARE protein, soluble N-ethylmaleimide-sensitive factor attachment protein-25, and the synaptic vesicle membrane protein synaptophysin were found to colocalize with NET in axonal varicosities; however, a direct interaction was not confirmed (Schroeter et al., 2000).

2. Ca^{2+}/Calmodulin-Dependent Protein Kinase. There is compelling evidence that the DAT C terminus, but not the C termini of NET and SERT, binds the α subunit of the serine/threonine protein kinase CaMKII. This interaction was suggested to facilitate CaMKII-dependent phosphorylation of N-terminal Ser that in turn stimulates amphetamine-induced dopamine efflux (Fog et al., 2006). The in vivo role of this interaction was studied by in vivo chronoampermetry measurements showing that CaMKII activity was required for amphetamine-induced dopamine efflux in mouse striatum (Fog et al., 2006). It is noteworthy that amphetamine-induced dopamine efflux might also be regulated by the interaction of DAT with syntaxin1A (Binda et al., 2008). Amphetamine was found to increase the interaction between DAT and syntaxin1A in a CaMKII-dependent manner and, thus, a mechanism was proposed in which amphetamine activation of CaMKII strengthens the DAT/syntaxin1A interaction, resulting in a mode of DAT in which efflux is possible (Binda et al., 2008). It is noteworthy that CaMKII-dependent regulation of a syntaxin1A/transporter interaction has also been demonstrated for SERT (Ciccone et al., 2008) and NET (Dipace et al., 2007).

3. Postsynaptic Density 95/Discs-Large/Zona Occludens Domain Proteins. Although the *SLC6* NTTs differ in their extreme C terminus, they all contain putative PDZ (postsynaptic density 95/discs-large/zona occludens) binding sequences. PDZ domains represent one of the most widespread protein recognition domains in the human ge-

nome and are characterized by binding short linear consensus motifs mostly located at the C terminus of their interaction partners (Nourry et al., 2003; Kim and Sheng, 2004). Most PDZ domain-containing proteins play key roles in regulating targeting and trafficking of their interaction partners and are critical in protein scaffolding processes, where they organize proteins into supramolecular complexes and bring different cellular components in close proximity to ensure spatial and temporal control of cellular processes.

The synaptic PDZ domain-containing protein PICK1 was initially identified as a binding partner to the PDZ recognition motif in the extreme C termini of DAT and NET (Torres et al., 2001) (Fig. 10). This interaction was proposed to enhance DAT surface targeting and induce a clustering phenotype in transfected cells (Torres et al., 2001). However, the PICK1 interaction does not seem to play a role in ER export and surface targeting of the transporter. DAT C-terminal residues are important for appropriate membrane targeting of DAT, but mutations in DAT were identified that specifically disrupted PDZ domain interactions without affecting surface targeting, and mutations were identified that disrupted surface targeting without affecting PICK1 binding (Bjerggaard et al., 2004). Thus, the functional significance of the DAT/PICK1 interaction remains to be settled.

Additional PDZ domain-containing proteins have been identified as *SLC6* NTT interaction partners mainly by use of yeast two-hybrid systems. Syntenin-1 was found to interact with the C terminus of GLYT2 (Ohno et al., 2004). Native syntenin-1 and GLYT2 colocalize in neuronal tissue and can be immunoprecipitated together (Ohno et al., 2004). Removal of the PDZ binding motif in GLYT2 result in reduced synaptic localization in neuronal cells (Armsen et al., 2007), suggesting that the syntenin-1/GLYT2 or another PDZ domain-containing protein interaction are involved in recruitment to and/or stabilization of GLYT2 at synaptic sites. It is noteworthy that syntenin-1 also binds to syntaxin1A, but GLYT2 and syntaxin1A cannot bind to syntenin-1 simultaneously, indicating that syntenin-1 does not participate directly in syntaxin1A-mediated trafficking of GLYT2 (Ohno et al., 2004).

Using a proteomics approach, neuronal nitric-oxide synthase (nNOS) was identified as a PDZ domain-containing protein interacting with the PDZ binding motif in the C terminus of SERT (Chanrion et al., 2007) (Fig. 10). Coexpression of SERT with nNOS decreased 5-HT uptake capacity in transfected cells by decreasing SERT cell surface expression, and this effect could be abolished by removing the PDZ binding motif in SERT. Furthermore, 5-HT transport in synaptosomes from nNOS knockout mice was enhanced compared with wild-type animals. Inhibition of the SERT/nNOS interaction in wild-type animals by injection of a SERT C-terminal peptide fused to the cell membrane penetrating HIV-1 Tat peptide also increased SERT acitivty in synaptosomes (Chanrion et al., 2007).

The PDZ domain of Pals1, a member of the membrane-associated guanylate kinase family, binds the C terminus of GAT1 (Fig. 10). Coexpression of GAT1 with Pals1 increased GABA uptake because of an increase in total GAT1 protein, which led to the proposal that Pals1 might slow the turnover time for GAT1 and thereby increase GAT1 cell-surface levels (McHugh et al., 2004). Another member of the membrane-associated guanylate kinase family, PSD-95, was found to interact with GLYT1 and stabilize the transporter at the cell surface (Cubelos et al., 2005b). Gly is a coagonist at the NMDA receptor, a well characterized interaction partner for PSD-95, and the GLYT1/PSD-95 interaction was proposed to bring GLYT1 and NMDA receptors together to enable tight regulation of extracellular Gly concentration around NMDA receptors. This hypothesis correlates with the observation that GLYT1, in addition to its presence in glial cells, is present at postsynaptic sites of glutamatergic neurons within complexes containing both GLYT1 and NMDA receptors (Cubelos et al., 2005b). Finally, BGT1 was found to bind the PDZ protein LIN7 (Massari et al., 2005). It is noteworthy that the interaction with LIN7 was regulated in a PKC-dependent manner; i.e., PKC activation promoted phosphorylation of Thr612 in the LIN7 association motif, causing decreased LIN7 binding to BGT1 concomitantly with transporter internalization (Massari et al., 2004).

4. Hydrogen Peroxide-Inducible Clone-5. The LIM (Lin11, Isl-1, and Mec-3) domain-containing focal adhesion protein hydrogen peroxide-inducible clone-5 (Hic-5) is another scaffolding protein found to interact with the C terminus of DAT, NET, and SERT (Carneiro et al., 2002; Carneiro and Blakely, 2006). On the basis of results from differential extraction and sedimentation procedures with human and mouse platelets, it was suggested that the interaction of Hic-5 with SERT facilitates SERT internalization by promoting interaction with the actin cytoskeleton (Carneiro and Blakely, 2006). Furthermore, the Hic-5/SERT association was linked to the PKC-mediated regulatory pathway, because stimulation of PKC positively modulated the Hic-5/SERT interaction (Carneiro and Blakely, 2006). The protein MacMARCKS (homolog of myristoylated alanine-rich C kinase substrate) has also been proposed to be involved in PKC-mediated internalization of SERT via interaction with the SERT C terminus (Jess et al., 2002). MacMARKS is a PKC substrate and coexpression of MacMARKS and rat SERT in HEK293 cells altered the PKC-dependent regulation of SERT transport activity compared with cells expressing SERT alone (Jess et al., 2002).

5. Proteins in the Early Secretory Pathway. As mentioned before, the GAT1 C terminus binds Sec24D, a component of the COPII coat complex that facilitates transport from ER to Golgi (Farhan et al., 2004) (section

V.B). The binding of Sec24D, which depends on Arg566 and Leu567 in the GAT1 C terminus, was shown to facilitate concentrative ER export of GAT1 (Farhan et al., 2007). This motif is conserved in the *SLC6* family and consistently the corresponding motif in DAT and SERT was shown to mediate Sec24D binding with these transporters (Farhan et al., 2007). The C terminus of GLYT1 interacts directly with another component of the secretory pathway, namely Sec3, which is part of the exocyst complex implicated in targeting post-Golgi secretory vesicles to the plasma membrane. Accordingly, coexpression of Sec3 increased the uptake activity of GLYT1, suggesting that the exocyst complex augment insertion of GLYT1 into the plasma membrane (Cubelos et al., 2005a).

6. The Dopamine D_2 Receptor. Two independent studies have shown that the dopamine D_2 receptor short variant, which is the primary autoinhibitory receptor expressed in presynaptic dopaminergic neurons, interacts with DAT and causes an increase in dopamine uptake by increasing in DAT surface expression (Bolan et al., 2007; Lee et al., 2007) (Fig. 10). The potential biological significance of physical and functional coupling between D_2 receptors and DAT activity seems highly plausible considering the shared attenuating role of DAT and D_2 receptors in control of dopaminergic transmission. However, the molecular mechanism underlying the effect of D_2 receptors on the function of DAT is still not fully understood. A direct interaction between the N terminus of DAT and the third intracellular loop of the D_2 receptors was suggested to be responsible for enhanced DAT transport capacity (Lee et al., 2007). Furthermore, up-regulation of DAT activity by coexpression of D_2 receptors has been found to be dependent on receptor activation as well as downstream ERK1 signaling (Bolan et al., 2007). Further studies are required to fully understand the significance of the interaction between DAT and the D_2 receptor. It is highly interesting, however, that a correlation between schizophrenia and potential loss of the DAT/D_2 receptor interaction recently has been reported. Coimmunoprecipitation of DAT with D_2 receptors was reduced to 60% in samples from the striata of patients with schizophrenia compared with the control group, and in samples from patients with bipolar disease, no difference was observed (Lee et al., 2009).

7. α-Synuclein. DAT, NET, and SERT have all been found to interact with α-synuclein, a presynaptic protein that is implicated in the pathogenesis of Parkinson's disease (Lee et al., 2001; Wersinger and Sidhu, 2005; Wersinger et al., 2006a,b; Jeannotte and Sidhu, 2007). Coexpression studies revealed that α-synuclein modulated transporter activity by regulating cell surface expression; however, it is not clear whether the regulation is stimulatory or inhibitory. Recent studies in human neuronal cell lines revealed ~50% reduction in DAT activity upon siRNA knockdown of α-synuclein (Fountaine and Wade-Martins, 2007), suggesting that under native conditions, endogenous α-synuclein promotes transporter activity. It is noteworthy that it was shown that parkin, a protein believed to be involved in a familial form of Parkinson's disease, disrupts the α-synuclein/DAT interaction and abolish α-synuclein-induced enhancement of DAT transport in transfected cells (Moszczynska et al., 2007). It was suggested that this mechanism could account for the protective effect of parkin with respects to dopaminergic cell death in Parkinson's disease (Moszczynska et al., 2007). The importance of these interaction effects remains to be determined in vivo. So far, α-synuclein KO mice have failed to show any alterations in DAT function (Dauer et al., 2002; Chandra et al., 2004).

Additional *SLC6* NTT interacting proteins are listed in Table 7. In general, the plethora of proteins so far found to interact with the *SLC6* NTTs clearly implies that the transporter function and subcellular distribution is tightly regulated by protein-protein interactions. However, the in vivo significance and mechanistic role of most of these interactions is still far from fully understood and require further validation and exploration.

VI. Therapeutic Applications of Solute Carrier 6 Neurotransmitter Transporter Drugs

The *SLC6* NTTs have been important drug targets for several decades. Industrial drug development efforts have primarily focused on SERT, NET, and DAT, and an array of drugs targeting these transporters have been developed and is currently used against diseases such as major depression, anxiety disorders, ADHD, and obesity. Moreover, an inhibitor of GAT1, tiagabine, was approved as an antiepileptic drug in the 1990s, whereas the first drug targeting the glycine transporters has yet to be approved.

Despite being important drug targets for a number of years, the pharmaceutical industry is still focusing on developing novel drugs that target *SLC6* NTTs. This includes improvement of drugs targeting the monoamine transporters as well as exploring the therapeutic potential of GLYT inhibitors, which are currently investigated in late-stage clinical trials as a new strategy for the treatment of schizophrenia. It is noteworthy that the GABA transporters do not seem to be pursued as drug targets. Substantial side-effects such as tremor, ataxia, dizziness, and somnolence have been observed during administration of selective GAT1 inhibitors such as tiagabine (Beghi, 2004; LaRoche and Helmers, 2004; Foster and Kemp, 2006), and in the GAT1-KO mouse (Chiu et al., 2005) (section IV.E), suggesting that these side-effects are directly linked to GAT1 inhibition. Compounds selectively targeting individual GABA transporter subtypes other than GAT1 are not presently available (section III), and the therapeutic potential of blocking specific subtypes is therefore not well estab-

lished. However, compounds with broad-range inhibitory activity across the nonGAT1 subtypes display anticonvulsant activity (Dalby and Nielsen, 1997; Dalby et al., 1997; Dalby, 2000; White et al., 2005; Madsen et al., 2009), indicating that inhibition of other GAT subtypes than GAT1 might offer a strategy for the treatment of epilepsy. This hypothesis will have to await the development of more selective inhibitors of the different GABA transporter subtypes.

Below, we provide an overview of compounds that are currently in use or under investigation as human therapeutics. Novel compounds targeting *SLC6* NTTs are frequently reported in the literature, but only compounds that are currently undergoing late stage clinical trials or very recently approved are included. The overview is based on primary literature, patents, and the Investigational Drugs Database (iddB) and is summarized in Table 8.

A. Solute Carrier 6 Neurotransmitter Transporter Drugs in Current Therapeutic Use

After the discovery of the *SLC6* NTTs and their role in neurotransmission processes, the therapeutical scope for pharmacological modulation of NTT activity has been expanded to cover a large number of neurological and psychiatric disorders. Accordingly, intense drug discovery efforts have successfully developed a multitude of drugs with the *SLC6* NTTs as primary targets, including drugs acting at the monoamine transporters and at the GABA transporters. In addition to being therapeutic drug targets, the monoamine transporters are also the primary targets for psychostimulant drugs of abuse.

1. Monoamine Transporter Drugs. As described in section IV, the monoamine neurotransmitter systems are crucially involved in control of human behavior and emotional states, and diseases related to dysfunctions in monoaminergic activity are typically of affective and behavioral nature, such as depression, anxiety disorders (including OCD), and ADHD.

a. Depression and anxiety. Competitive inhibitors of the monoamine transporters SERT and NET are used as treatments for major depression and anxiety diseases. By inhibiting transmitter reuptake, they increase extracellular 5-HT and/or norepinephrine concentrations that, if maintained over a period of days to several weeks, lead to symptom relief (Brown and Gershon, 1993; Nemeroff and Owens, 2002). The prototypical TCA imipramine was the first *SLC6* NTT drug to be approved after the seminal discovery of its antidepressant effects in the 1950s (Azima and Vispo, 1958; Kuhn, 1958). Imipramine is a dual-acting inhibitor of SERT and NET and has additional blocking effect at G protein-coupled neurotransmitter receptors, including muscarinic, histaminergic, and α-adrenergic receptors, as well as at cardiac sodium channels, causing several side effects and intoxication risks (Iversen, 2000). Subsequently, a large series of closely related derivatives of imipramine has been developed, which differ in their selectivity between SERT and NET as well in their activity at other CNS targets. The realization that the clinical effect of TCAs can be attributed mainly to inhibition of monoamine transporters led to the development of a second generation of inhibitors with improved monoamine transporter selectivity (Kaiser and Setler, 1981). These include SSRIs such as paroxetine, fluoxetine, fluvoxamine, sertraline, and citalopram, which are highly selective for SERT over NET/DAT and have little or no affinity for other CNS or non-CNS targets. As a consequence, side-effect profiles were much improved compared with those of the TCAs (Waitekus and Kirkpatrick, 2004). Later generations of monoamine transporter inhibitors include the SNRIs, such as duloxetine and venlafaxine (Muth et al., 1986; Wong et al., 1988), which inhibit SERT and NET with high affinity but do not act on other CNS targets. The SNRIs have been suggested to display improved antidepressant efficacy and a faster onset of action compared with SSRIs (Tran et al., 2003). NRIs such as reboxetine and atomoxetine are also in use as antidepressants but are prescribed primarily for treatment of ADHD (Wong et al., 1982; Melloni et al., 1984; Zhou, 2004). Outside the area of mood disorders, monoamine transporter inhibitors such as the NET/DAT in-

TABLE 8
Compounds in development with novel targets or indications

Indication and compound	Type	Development Stage	Company
Depression			
Vilazodone	SERT inhibitor; 5-HT_{1A} agonist	Phase III	Merck
Lu AA21004	SERT inhibitor; 5-HT_{1A} agonist; 5-HT_3 antagonist	Phase III	H. Lundbeck A/S
Lu AA24530	SERT inhibitor; 5-HT_{2C} agonist; 5-HT_3 antagonist	Phase III	H. Lundbeck A/S
Premature ejaculation			
Dapoxetine	SSRI	Launched	Johnson and Johnson
Schizophrenia			
RG1678	GLYT1 inhibitor	Phase II	Roche
Obesity			
Bupropion; naltrexone	NET/DAT inhibitor; opioid receptor antagonist	Phase III	Orexigen
Bupropion; zonisamide	NET/DAT inhibitor; SERT/DAT inhibitor	Phase II	Orexigen
Tesofensine	SERT/NET/DAT inhibitor	Phase II	NeuroSearch A/S
Pain			
Bicifadine	SNRI	Phase II	DOV Pharmaceutical
Xen2174	NRI	Phase II	Xenome

hibitor bupropion are approved for nicotine addiction and obesity (Jorenby, 2002; Hainer et al., 2006) (section VI.B).

b. Attention deficit hyperactivity disorder. Amphetamine and amphetamine analogs such as methylphenidate, dextroamphetamine, and dextromethamphetamine are used for treatment of ADHD, which is thought to involve dysregulation of the dopaminergic system. In addition, these compounds are used for treatment of narcolepsy. Whereas methylphenidate is a nontransported competitive inhibitor, amphetamines are substrates, and uptake of these into the synaptic terminals induces a reversal in the direction of neurotransmitter transport, leading to a release of monoamine neurotransmitters into the extracellular space (Seiden et al., 1993; Sulzer et al., 1995, 2005; Jones et al., 1998). Modafinil is also used for treatment of ADHD, and recent evidence suggests that also this compound act through its inhibitory activity at DAT (Zolkowska et al., 2009).

c. Drugs of abuse. Cocaine and the amphetamines, including methamphetamine and MDMA, are widely used as recreational drugs. Whereas amphetamines inhibit the monoamine NTTs by acting as substrates for the transporters in addition to promoting transmitter efflux trough the monoamine transporters, cocaine is a nontransportable and nonselective inhibitor of SERT, NET, and DAT (Eshleman et al., 1999). Although amphetamines and cocaine have an effect on all monoamine transporters, the rewarding properties and abuse potential of the psychostimulants is believed to be mediated through DAT (Giros et al., 1996b; Wise, 1996; Torres et al., 2003b; Chen et al., 2006).

2. GABA Transporters

a. Epilepsy. GABA transporter inhibitors are in general anticonvulsants and have shown therapeutic potential as antiepileptic agents (Dalby, 2000; Czuczwar and Patsalos, 2001; Dalby and Mody, 2001; Gadea and López-Colomé, 2001; Schousboe et al., 2004; Meldrum and Rogawski, 2007). Covering a wide range of neurological disorders characterized by recurrent seizure episodes, epilepsy is caused by abnormally excessive excitatory activity. Studies of patients with epilepsy have shown that levels of extracellular GABA correlate with ability to control seizures (Rothman et al., 1993; Petroff et al., 1996). Inhibition of synaptic GABA uptake increases synaptic GABA concentrations and promotes enhanced strength and duration of inhibitory signals (Semyanov et al., 2003, 2004; Gether et al., 2006). Inhibition of GABA reuptake strengthens the inhibitory inputs in neuronal circuits (Sivilotti and Nistri, 1991; for review, see Macdonald and Olsen, 1994; Sieghart, 1995; Pirker et al., 2000), and a single GABA transporter inhibitor is approved for treatment of epilepsy in the form of the GAT1 selective inhibitor tiagabine. In addition, GAT2, GAT3, and BGT-1 are currently considered potential drug targets for treatment of epilepsy as well as for treatment of other neurological disorders, including anxiety and pain.

B. Novel Principles for Treatment of Mood Disorders

Monoamine transporter inhibitors such as SSRIs and SNRIs are by far the most prescribed antidepressants (Waitekus and Kirkpatrick, 2004). However, a late onset of action and a large fraction of nonresponding or refractory patients represent limitations of monoamine transporter inhibitors as antidepressant treatment (Hollon et al., 2006). Considerable efforts are ongoing to develop novel antidepressants that overcome these limitations and also comprise a decreased number of side effects, including inadvertent weight gain, sexual dysfunction, and sleep disturbances (Kent, 2000). In these efforts, monoamine transporter inhibition is maintained as a central requirement for drug candidates but with the addition of activity at other CNS targets (Csermely et al., 2005; Millan, 2006, 2009; Hopkins, 2008). This principle is known as polypharmacy, polypharmacology, or, more recently network pharmacology, which contests the conventional "one drug, one target" principle (Csermely et al., 2005; Hopkins, 2008) and has been thoroughly explored in the area of mood disorders (Roth et al., 2004; Millan, 2006, 2009; Wong et al., 2008). This can be achieved in at least two principally different ways: by combining two or more compounds with diverse effects into one formulation or by developing one compound that possess all the desired pharmacological properties. The former has been achieved by a combination of an SSRI, fluoxetine, and an atypical antipsychotic, olanzapine, which target 5-HT_2 and dopamine D_2 receptors, into one drug (Symbyax; Eli Lilly & Co., Indianapolis, IN) for the treatment of bipolar depression and treatment-resistant depression (Shelton, 2006; Deeks and Keating, 2008; Dodd and Berk, 2008). The latter is currently being pursued with 4-(2-((4-methylphenyl)sulfanyl)phenyl)piperidine (Lu AA24530), a monoamine transporter inhibitor with antagonistic effect on 5-HT_{2C} and 5-HT_3 receptors, which has just completed phase II clinical trials as a novel antidepressant treatment (http://clinicaltrials.gov/ct2/show/NCT00599911).

It has been hypothesized that the late onset of action of SSRIs is due to negative feedback circuitry, mediated via 5-HT_{1A} autoreceptors (Hjorth et al., 2000); therefore, compounds that are inhibitors of SERT and agonists of 5-HT_{1A} receptors have been pursued as improved antidepressants (Adell et al., 2005; Dawson and Bromidge, 2008). Two compounds, vilazodone and Lu AA21004 (Fig. 11), both possessing combined SERT inhibitory and 5-HT_{1A} agonist activity, are currently in late-stage clinical trials for depression and generalized anxiety disorder. Vilazodone is a potent SERT inhibitor and a potent partial agonist of the 5-HT_{1A} receptor (de Paulis, 2007; Dawson and Watson, 2009); the compound has shown efficacy in depression comparable with that of SSRIs, but it remains to be established whether vilazodone

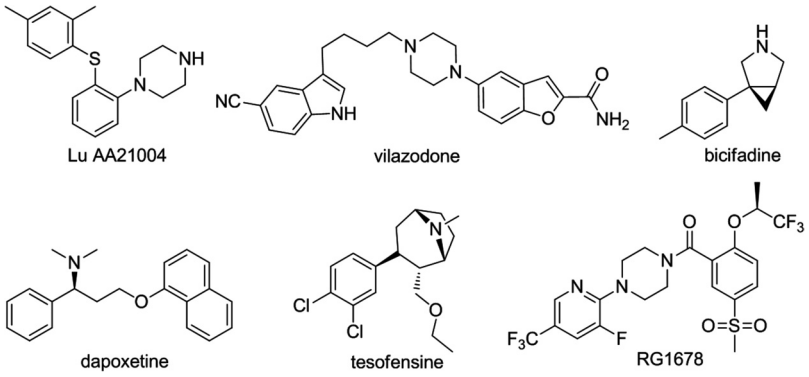

FIG. 11. Chemical structures of novel compounds targeting *SLC6* NTTs in current clinical development.

provide faster onset than other SSRIs (de Paulis, 2007). Lu AA21004 is a SERT inhibitor, a full 5-HT_{1A} receptor agonist, and a 5-HT_{3A} receptor antagonist that currently is in phase III clinical trials for treatment of depression (Bang-Andersen et al., 2011). Likewise, other companies have also pursued dual SERT and NET inhibitors and 5-HT_{1A} receptor agonists (Hatzenbuhler et al., 2006, 2008). Other multiple target strategies for development of monoamine transporters inhibitors with additional activity as antidepressants (Millan, 2006, 2009) include combined NRI activity and 5-HT_{2A} receptor antagonism (Heffernan et al., 2008), SSRI activity and neurokinin 1 receptor antagonists (Ryckmans et al., 2002a,b) or histamine H_3 receptor antagonist (Barbier et al., 2007). However, so far no data from clinical trials have been reported for such compounds. Triple inhibitors acting at SERT, NET, and DAT are also being pursued as improved treatment of depression, anxiety, and addiction (Skolnick et al., 2003, 2006; Liang et al., 2008), in addition to their potential in treating obesity as discussed in section VI.C.2.

C. Novel Indications for Drugs Targeting Solute Carrier 6 Transporters

1. Premature Ejaculation. Male sexual dysfunction is a common side effect associated with monoamine transporter inhibitors, in particular SSRIs, and includes polymorphic alterations in libido, arousal, erection, and orgasm as well as erectile dysfunction (Ferguson, 2001; Clayton et al., 2002; Labbate et al., 2003; Nurnberg et al., 2003; Balon, 2006). Although SSRI treatment seems to negatively affect all steps in the male sexual response cycle (Corona et al., 2009), the effect of delayed ejaculation is considered a potential treatment of premature ejaculation (PE), illustrated by the off-label usage of antidepressants for this condition (Waldinger et al., 2004; Fallon, 2008). PE is the most common male form of sexual dysfunction (Giuliano and Hellstrom, 2008) and was historically considered a psychological disorder but has recently been suggested to have a neurobiological component related to disturbance in monoaminergic neurotransmission (Waldinger, 2006; Giuliano, 2007). The general side-effect profile and long half-life of conventional SSRIs make these nonoptimal for PE treatment and have prompted development of short-acting SSRIs as treatments for PE. One example hereof is dapoxetine (Table 8; Fig. 11), an SSRI with rapid absorption and shorter half-life than conventional SSRIs; after favorable performance in several clinical trials, dapoxetine has been approved (Hellstrom, 2009).

2. Obesity. Obesity is associated with numerous medical complications including type 2 diabetes, cardiovascular disease, hypertension, depression, coronary artery disease and stroke, and therefore represents a serious health problem (Flegal et al., 2007). All three monoamines are implicated in regulation of appetite and energy homeostasis (Ramos et al., 2005), and the SNRI sibutramine has been one of the most widely used pharmacological treatments against obesity (Finer, 2002). Unfortunately, long-term sibutramine treatment are associated with severe cardiovascular side effects (James et al., 2010), and it has therefore been withdrawn from several markets, including Europe and the United States. The future potential of this compound remains uncertain, but a recent study found that coadministration of the 5-HT_{1A} receptor agonist eptapirone (F11440) attenuates the sibutramine-induced side-effects without compromising the effect on weight loss in animal models (Thomas et al., 2009), suggesting coadministration of 5-HT_{1A} receptor agonists with sibutramine as a potential weight loss therapy (Thomas et al., 2009). Sibutramine is rapidly metabolized in vivo to its desmethyl and didesmethyl congeners, which are potent inhibitors of all three monoamine transporters (Glick et al., 2000), indicating that combined SERT, NET, and DAT inhibition might offer an effective treatment against obesity. The potential of triple uptake inhibitors as weight-loss therapy has been demonstrated by the triple SERT/NET/DAT inhibitor tesofensine (Fig. 11), which produces significant weight loss in humans, presumably through a mechanism that involves indirect stimulation of α_1 adrenoceptor and dopamine D_1 receptor pathways (Axel et al., 2010), and is currently in clinical development for the treatment of obesity (Astrup et al., 2008). The combination of bupropion (NET/DAT inhibitor) with zonisamide, an anticonvulsant modulator of voltage-

gated Na^+ and Ca^{2+} ion channels that has been found to also modulate SERT and DAT activity, has further implied that modulation of all three monoamine transporters is beneficial in the pharmacological treatment of obesity. When administered alone, bupropion and zonisamide each induce modest weight loss (Gadde et al., 2001, 2003; Anderson et al., 2002), whereas an additive effect is achieved when they are administered in combination (Gadde et al., 2007). Bupropion has also shown promising results in combination with the opioid receptor antagonist naltrexone, which blocks the inhibitory feedback loop through pro-opiomelanocortin neurons, believed to limit sustained weight loss. The bupropion/naltrexone combination therapy is currently in late-stage phase III trials as a novel treatment for obesity (Greenway et al., 2009).

3. Schizophrenia. Compounds that block the NMDA receptors induce schizophrenia-like psychotic symptoms, indicating that glutamatergic hypofunction is involved in the etiology of schizophrenia (Tsai and Coyle, 2002). The use of NMDA receptor agonists in the treatment of schizophrenia is associated with severe side-effects, including brain damage and seizures, and indirect modulation of NMDA receptors is considered a more promising strategy (Tsai and Coyle, 2002). Gly is a co-agonist of NMDA receptors; by controlling the synaptic clearance of Gly, GLYT1 is involved in the regulation of glutamatergic neurotransmission (Tsai et al., 2004; Gabernet et al., 2005). Selective inhibitors of GLYT1 enhance glutamatergic signaling by increasing the Gly occupancy at NMDA receptors; hence, such compounds might be beneficial in the pharmacological treatment of patients with schizophrenia (Kinney et al., 2003; Sur and Kinney, 2004). Several selective GLYT1 inhibitors have been identified (section III), and some of these are currently in clinical development against schizophrenia, including (4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl)(5-methanesulfonyl-2-(2,2,2-trifluoro-1-methylethoxy)phenyl)methanone (RG1678) (Fig. 11), which recently entered phase II trials (Pinard et al., 2010). Existing pharmacological treatments generally reduce the positive symptoms (such as hallucinations, delusions, and disturbance of thought) of schizophrenia, whereas GLYT1 inhibitors reduce the negative symptoms (such as apathy and social incompetence) and cognitive symptoms (impairment of attention, memory, and executive functions) (Tsai and Coyle, 2002; Thomsen, 2006).

4. Pain. TCAs and dual-acting SNRIs are widely used in the treatment of pain (Micó et al., 2006). This establishes an important role for 5-HT and norepinephrine in modulation of pain, although the mechanisms by which antidepressant drugs modulate nociceptive signals remain unclear. Bicifadine (Fig. 11) is a potent inhibitor of SERT and NET and is currently in development against neuropathic pain (Krieter et al., 2008). It is noteworthy that bicifadine also possess inhibitory activity against DAT (Basile et al., 2007), which indicates that inhibition of all three monoamine transporters might provide a novel and efficacious strategy for the treatment of neuropathic pain. Tapentadol [(-)-(1*R*,2*R*)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol] combines NRI activity with μ-opioid receptor agonism in a single molecule. Although tapentadol has low affinity for NET, both mechanisms of action contribute to the broad-spectrum analgesic properties of the compound (Schröder et al., 2011), and tapentadol is approved for the treatment of moderate to severe acute pain in adults.

The χ-conotoxin MrIA is a 13-residue peptide isolated from the venom of the mollusk-hunting marine snail *Conus marmoreus*. MrIA is selective and noncompetitive inhibitor of NET (Sharpe et al., 2001, 2003) that has been shown to suppress neuropathic pain upon intrathecal administration to rodents (McIntosh et al., 2000). Further development of MrIA led to the identification of an analog with increased in vivo stability, Xen2174 (Sec-Gly-Val-Cys-Cys-Gly-Tyr-Lys-Leu-Cys-His-Pyl-Cys), which has proved effective against both nociceptive and neuropathic pain (Nielsen et al., 2005; Brust et al., 2009). Xen2174 have recently entered phase II clinical trials against postoperative pain (Brust et al., 2009).

D. New Therapeutic Concepts and Future Trends

As described above, the majority of current efforts in developing drugs targeting *SLC6* NTTs are centered on concepts derived from the development of drugs for treatment of mood disorders (i.e., inhibitors of the monoamine NTTs). Novel approaches reside in tweaking of selectivity profiles; where previous efforts were primarily aimed at developing selective inhibitors of SERT, compounds selectively targeting NET and dual-acting SERT/NET inhibitors have been developed more recently. The most recent trends are related to generation of compounds with added activity at neurotransmitter receptor systems, such as a combination of SERT inhibitory activity and activity at the 5-HT receptor system. Such efforts are clearly reinforced by the increasing knowledge of the structural basis and molecular principles of *SLC6* NTT inhibition, which with great certainty will provide immense advance the ability of rational design for the generation of novel compounds with tailor-made activity profiles.

With increasing knowledge of the structural mechanisms underlying *SLC6* NTT transport, we envision that principally novel approaches of inhibiting transporters will aim at targeting specific conformational states of the transporters. Several studies have indicated that structurally related compounds can trap the transporters in different conformational states during the transport cycle and that such differences in drug molecular mechanism of action can lead to dramatically different physiological outcomes. Thus, with sufficient knowledge of the structure and the functional role of the different conformational states adopted during the transport cycle, rational design of novel compounds that target specific transporter states will become within reach. Initial

studies along such lines have recently appeared, with model compounds such as ibogaine that targets inward-facing conformations SERT and with DAT inhibitors belonging to the benztropine class that target occluded conformations of DAT (Jacobs et al., 2007; Loland et al., 2008) (section III).

The pharmacological concept of inhibiting protein-protein interactions, which has been subject to increased focus in other areas (Arkin and Wells, 2004; Wells and McClendon, 2007), is also relevant for novel therapeutic approaches for *SLC6* NTT drug development. Rather than targeting the entire function of a given *SLC6* NTT, modulation of protein-protein interactions between the transporter and its intracellular interaction proteins potentially allows subtle perturbation of specific pathways linked to transporter activity. Furthermore, as the role of protein interactions for function and regulation of the *SLC6* NTTs are becoming increasingly better understood (Torres et al., 2003b; Eriksen et al., 2010b) along with the realization of the importance of these in intracellular signaling pathways, perturbation of these may lead to discovery of new aspects of *SLC6* NTTs that can be therapeutically exploited.

VII. Conclusions and Outlook

Over the years, there have been a number of landmark events in the history of the *SLC6* NTTs: first, this includes the realization in the 1960s that the clinical effects of TCAs are related to inhibition of transmitter reuptake mediated by monoamine transporters. This established neurotransmitter transporters as drug targets and initiated development in the 1970s of selective inhibitors that remain some of the most important drugs for treatments of psychiatric diseases today, most importantly mood disorders, and furthermore helped establish the role of neurotransmitter transport systems for monoamine, glycine, and GABA homeostasis in the brain. Second, the cloning of the cDNA encoding neurotransmitter transporters in the beginning of the 1990s was another landmark that provided the first insight into the structure-function relationship of these proteins and established the *SLC6* transporter family. Third, the identification of the first structure of a bacterial homolog to the *SLC6* NTTs in 2005 opened the way to an improved understanding of membrane transport at the molecular level and revolutionized our understanding of the structural details of the transporters. An unanticipated, but perhaps even more important outcome of this structure was the realization that the *SCL6* NTTs are part of a much larger family than previously expected of secondary active transporters, which is defined by protein structure and mechanism rather than gene sequence.

The monoamine transporters SERT, NET, and DAT have received the most intense attention because of the early discovery of their relevance for development of therapeutic drugs, as well as being targets for drugs of abuse. A range of transgenic animal models generated for these transporters have provided important information on the role of monoamine neurotransmission for normal brain function and in pathological conditions that have further reinforced their role as pivotal drug targets in the treatment of mood disorders. The GABA and glycine transporters are following similar paths, and glycine transporters in particular are emerging as prosperous drug targets. Finally, conceptually novel principles for targeting *SLC6* NTTs are being actively pursued, most importantly by development of compounds that are specifically designed to simultaneously target transporters as well as other proteins.

Although the *SLC6* NTTs have been highly successful drug targets for almost 4 decades and in recent years their pharmacology, structure, and function have been elucidated in great detail, these topics still represent opportunities for future progress. In the coming years, the advent of novel compounds modulating transporter activity by new principles such as target-specific states of the transporter cycle is to be expected. Such endeavors will be made possible by new structural breakthroughs, most pertinent being the structure of a *SLC6* NTT. In concert, this will provide a distinctive platform from which new discoveries will be made.

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References

Abramson J, Smirnova I, Kasho V, Verner G, Kaback HR, and Iwata S (2003) Structure and mechanism of the lactose permease of *Escherichia coli*. *Science* **301:**610–615.

Abramson J and Wright EM (2009) Structure and function of Na^+-symporters with inverted repeats. *Curr Opin Struct Biol* **19:**425–432.

Adams SV and DeFelice LJ (2003) Ionic currents in the human serotonin transporter reveal inconsistencies in the alternating access hypothesis. *Biophys J* **85:**1548–1559.

Adell A, Castro E, Celada P, Bortolozzi A, Pazos A, and Artigas F (2005) Strategies for producing faster acting antidepressants. *Drug Discov Today* **10:**578–585.

Adkins EM, Barker EL, and Blakely RD (2001) Interactions of tryptamine derivatives with serotonin transporter species variants implicate transmembrane domain I in substrate recognition. *Mol Pharmacol* **59:**514–523.

Adkins EM, Samuvel DJ, Fog JU, Eriksen J, Jayanthi LD, Vaegter CB, Ramamoorthy S, and Gether U (2007) Membrane mobility and microdomain association of the dopamine transporter studied with fluorescence correlation spectroscopy and fluorescence recovery after photobleaching. *Biochemistry* **46:**10484–10497.

Agoston GE, Wu JH, Izenwasser S, George C, Katz J, Kline RH, and Newman AH (1997) Novel *N*-substituted 3α-[bis(4′-fluorophenyl)methoxy]tropane analogues: selective ligands for the dopamine transporter. *J Med Chem* **40:**4329–4339.

Akabas MH, Stauffer DA, Xu M, and Karlin A (1992) Acetylcholine-receptor channel structure probed in cysteine-substitution mutants. *Science* **258:**307–310.

Andersen J, Kristensen AS, Bang-Andersen B, and Strømgaard K (2009a) Recent advances in the understanding of the interaction of antidepressant drugs with serotonin and norepinephrine transporters. *Chem Commun (Camb)* **(26:)**3677–3692.

Andersen J, Olsen L, Hansen KB, Taboureau O, Jørgensen FS, Jørgensen AM,

Bang-Andersen B, Egebjerg J, Strømgaard K, and Kristensen AS (2010) Mutational mapping and modeling of the binding site for (*S*)-citalopram in the human serotonin transporter. *J Biol Chem* **285:**2051–2063.
Andersen J, Taboureau O, Hansen KB, Olsen L, Egebjerg J, Strømgaard K, and Kristensen AS (2009b) Location of the antidepressant binding site in the serotonin transporter: importance of Ser-438 in recognition of citalopram and tricyclic antidepressants. *J Biol Chem* **284:**10276–10284.
Andersen KE, Braestrup C, Grønwald FC, Jørgensen AS, Nielsen EB, Sonnewald U, Sørensen PO, Suzdak PD, and Knutsen LJ (1993) The synthesis of novel GABA uptake inhibitors. 1. Elucidation of the structure-activity studies leading to the choice of (*R*)-1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid (tiagabine) as an anticonvulsant drug candidate. *J Med Chem* **36:**1716–1725.
Anderson JW, Greenway FL, Fujioka K, Gadde KM, McKenney J, and O'Neil PM (2002) Bupropion SR enhances weight loss: a 48-week double-blind, placebo-controlled trial. *Obes Res* **10:**633–641.
Androutsellis-Theotokis A, Ghassemi F, and Rudnick G (2001) A conformationally sensitive residue on the cytoplasmic surface of serotonin transporter. *J Biol Chem* **276:**45933–45938.
Androutsellis-Theotokis A, Goldberg NR, Ueda K, Beppu T, Beckman ML, Das S, Javitch JA, and Rudnick G (2003) Characterization of a functional bacterial homologue of sodium-dependent neurotransmitter transporters. *J Biol Chem* **278:**12703–12709.
Androutsellis-Theotokis A and Rudnick G (2002) Accessibility and conformational coupling in serotonin transporter predicted internal domains. *J Neurosci* **22:**8370–8378.
Annamalai B, Mannangatti P, Arapulisamy O, Ramamoorthy S, and Jayanthi LD (2010) Involvement of threonine 258 and serine 259 motif in amphetamine-induced norepinephrine transporter endocytosis. *J Neurochem* **115:**23–35.
Ansorge MS, Morelli E, and Gingrich JA (2008) Inhibition of serotonin but not norepinephrine transport during development produces delayed, persistent perturbations of emotional behaviors in mice. *J Neurosci* **28:**199–207.
Ansorge MS, Zhou M, Lira A, Hen R, and Gingrich JA (2004) Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science* **306:**879–881.
Apparsundaram S, Stockdale DJ, Henningsen RA, Milla ME, and Martin RS (2008) Antidepressants targeting the serotonin reuptake transporter act via a competitive mechanism. *J Pharmacol Exp Ther* **327:**982–990.
Apparsundaram S, Sung U, Price RD, and Blakely RD (2001) Trafficking-dependent and independent pathways of neurotransmitter transporter regulation differentially involving p38 mitogen-activated protein kinase revealed in studies of insulin modulation of norepinephrine transport in SK-N-SH cells. *J Pharmacol Exp Ther* **299:**666–677.
Aragón C and López-Corcuera B (2003) Structure, function and regulation of glycine neurotransporters. *Eur J Pharmacol* **479:**249–262.
Arkin MR and Wells JA (2004) Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. *Nat Rev Drug Discov* **3:**301–317.
Armsen W, Himmel B, Betz H, and Eulenburg V (2007) The C-terminal PDZ-ligand motif of the neuronal glycine transporter GlyT2 is required for efficient synaptic localization. *Mol Cell Neurosci* **36:**369–380.
Astrup A, Madsbad S, Breum L, Jensen TJ, Kroustrup JP, and Larsen TM (2008) Effect of tesofensine on bodyweight loss, body composition, and quality of life in obese patients: a randomised, double-blind, placebo-controlled trial. *Lancet* **372:**1906–1913.
Atkinson BN, Bell SC, De Vivo M, Kowalski LR, Lechner SM, Ognyanov VI, Tham CS, Tsai C, Jia J, Ashton D, et al. (2001) ALX 5407: a potent, selective inhibitor of the hGlyT1 glycine transporter. *Mol Pharmacol* **60:**1414–1420.
Aubrey KR and Vandenberg RJ (2001) *N*[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy-)propyl]sarcosine (NFPS) is a selective persistent inhibitor of glycine transport. *Br J Pharmacol* **134:**1429–1436.
Awtry TL, Frank JG, and Werling LL (2006) In vitro regulation of serotonin transporter activity by protein kinase A and nicotinic acetylcholine receptors in the prefrontal cortex of rats. *Synapse* **59:**342–349.
Axel AM, Mikkelsen JD, and Hansen HH (2010) Tesofensine, a novel triple monoamine reuptake inhibitor, induces appetite suppression by indirect stimulation of α_1 adrenoceptor and dopamine D_1 receptor pathways in the diet-induced obese rat. *Neuropsychopharmacology* **35:**1464–1476.
Azima H and Vispo RH (1958) Imipramine; a potent new antidepressant compound. *Am J Psychiatry* **115:**245–246.
Bahena-Trujillo R and Arias-Montaño JA (1999) [^{3}H]γ-aminobutyric acid transport in rat substantia nigra pars reticulata synaptosomes: pharmacological characterization and phorbol ester-induced inhibition. *Neurosci Lett* **274:**119–122.
Bak LK, Schousboe A, and Waagepetersen HS (2006) The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem* **98:**641–653.
Balon R (2006) SSRI-associated sexual dysfunction. *Am J Psychiatry* **163:**1504–1509.
Bang-Andersen B, Ruhland T, Jørgensen M, Smith G, Frederiksen K, Jensen KG, Zhong H, Nielsen SM, Hogg S, Mørk A, and Stensbøl TB (2011) Discovery of 1-[2-(2,4-dimethylphenylsulfanyl)phenyl]piperazine (Lu AA21004): a novel multimodal compound for the treatment of major depressive disorder. *J Med Chem* **54:**3206–3221.
Barbier AJ, Aluisio L, Lord B, Qu Y, Wilson SJ, Boggs JD, Bonaventure P, Miller K, Fraser I, Dvorak L, et al. (2007) Pharmacological characterization of JNJ-28583867, a histamine H_3 receptor antagonist and serotonin reuptake inhibitor. *Eur J Pharmacol* **576:**43–54.
Barker EL and Blakely RD (1996) Identification of a single amino acid, phenylalanine 586, that is responsible for high affinity interactions of tricyclic antidepressants with the human serotonin transporter. *Mol Pharmacol* **50:**957–965.
Barker EL, Kimmel HL, and Blakely RD (1994) Chimeric human and rat serotonin transporters reveal domains involved in recognition of transporter ligands. *Mol Pharmacol* **46:**799–807.
Barker EL, Moore KR, Rakhshan F, and Blakely RD (1999) Transmembrane domain I contributes to the permeation pathway for serotonin and ions in the serotonin transporter. *J Neurosci* **19:**4705–4717.
Barker EL, Perlman MA, Adkins EM, Houlihan WJ, Pristupa ZB, Niznik HB, and Blakely RD (1998) High affinity recognition of serotonin transporter antagonists defined by species-scanning mutagenesis. An aromatic residue in transmembrane domain I dictates species-selective recognition of citalopram and mazindol. *J Biol Chem* **273:**19459–19468.
Bartholomäus I, Milan-Lobo L, Nicke A, Dutertre S, Hastrup H, Jha A, Gether U, Sitte HH, Betz H, and Eulenburg V (2008) Glycine transporter dimers: evidence for occurrence in the plasma membrane. *J Biol Chem* **283:**10978–10991.
Basile AS, Janowsky A, Golembiowska K, Kowalska M, Tam E, Benveniste M, Popik P, Nikiforuk A, Krawczyk M, Nowak G, et al. (2007) Characterization of the antinociceptive actions of bicifadine in models of acute, persistent, and chronic pain. *J Pharmacol Exp Ther* **321:**1208–1225.
Batchelor M and Schenk JO (1998) Protein kinase A activity may kinetically upregulate the striatal transporter for dopamine. *J Neurosci* **18:**10304–10309.
Bauman AL, Apparsundaram S, Ramamoorthy S, Wadzinski BE, Vaughan RA, and Blakely RD (2000) Cocaine and antidepressant-sensitive biogenic amine transporters exist in regulated complexes with protein phosphatase 2A. *J Neurosci* **20:**7571–7578.
Beckman ML, Bernstein EM, and Quick MW (1998) Protein kinase C regulates the interaction between a GABA transporter and syntaxin 1A. *J Neurosci* **18:**6103–6112.
Beckman ML, Bernstein EM, and Quick MW (1999) Multiple G protein-coupled receptors initiate protein kinase C redistribution of GABA transporters in hippocampal neurons. *J Neurosci* **19:**RC9.
Beghi E (2004) Efficacy and tolerability of the new antiepileptic drugs: comparison of two recent guidelines. *Lancet Neurol* **3:**618–621.
Bengel D, Murphy DL, Andrews AM, Wichems CH, Feltner D, Heils A, Mössner R, Westphal H, and Lesch KP (1998) Altered brain serotonin homeostasis and locomotor insensitivity to 3,4-methylenedioxymethamphetamine ("Ecstasy") in serotonin transporter-deficient mice. *Mol Pharmacol* **53:**649–655.
Bennett ER and Kanner BI (1997) The membrane topology of GAT-1, a (Na^+ + Cl^-)-coupled γ-aminobutyric acid transporter from rat brain. *J Biol Chem* **272:**1203–1210.
Ben-Yona A and Kanner BI (2009) Transmembrane domain 8 of the γ-aminobutyric acid transporter GAT-1 lines a cytoplasmic accessibility pathway into its binding pocket. *J Biol Chem* **284:**9727–9732.
Bergeron R, Meyer TM, Coyle JT, and Greene RW (1998) Modulation of *N*-methyl-D-aspartate receptor function by glycine transport. *Proc Natl Acad Sci USA* **95:**15730–15734.
Bernstein EM and Quick MW (1999) Regulation of γ-aminobutyric acid (GABA) transporters by extracellular GABA. *J Biol Chem* **274:**889–895.
Beuming T, Kniazeff J, Bergmann ML, Shi L, Gracia L, Raniszewska K, Newman AH, Javitch JA, Weinstein H, Gether U, et al. (2008) The binding sites for cocaine and dopamine in the dopamine transporter overlap. *Nat Neurosci* **11:**780–789.
Beuming T, Shi L, Javitch JA, and Weinstein H (2006) A comprehensive structure-based alignment of prokaryotic and eukaryotic neurotransmitter/Na^+ symporters (NSS) aids in the use of the LeuT structure to probe NSS structure and function. *Mol Pharmacol* **70:**1630–1642.
Binda F, Dipace C, Bowton E, Robertson SD, Lute BJ, Fog JU, Zhang M, Sen N, Colbran RJ, Gnegy ME, et al. (2008) Syntaxin 1A interaction with the dopamine transporter promotes amphetamine-induced dopamine efflux. *Mol Pharmacol* **74:**1101–1108.
Bisgaard H, Larsen MA, Mazier S, Beuming T, Newman AH, Weinstein H, Shi L, Loland CJ, and Gether U (2011) The binding sites for benztropines and dopamine in the dopamine transporter overlap. *Neuropharmacology* **60:**182–190.
Bismuth Y, Kavanaugh MP, and Kanner BI (1997) Tyrosine 140 of the γ-aminobutyric acid transporter GAT-1 plays a critical role in neurotransmitter recognition. *J Biol Chem* **272:**16096–16102.
Bjerggaard C, Fog JU, Hastrup H, Madsen K, Loland CJ, Javitch JA, and Gether U (2004) Surface targeting of the dopamine transporter involves discrete epitopes in the distal C terminus but does not require canonical PDZ domain interactions. *J Neurosci* **24:**7024–7036.
Blackstone C (2009) Infantile parkinsonism-dystonia: a dopamine "transportopathy". *J Clin Invest* **119:**1455–1458.
Blakely RD and Bauman AL (2000) Biogenic amine transporters: regulation in flux. *Curr Opin Neurobiol* **10:**328–336.
Blakely RD, Berson HE, Fremeau RT Jr, Caron MG, Peek MM, Prince HK, and Bradley CC (1991) Cloning and expression of a functional serotonin transporter from rat-brain. *Nature* **354:**66–70.
Blakely RD, Ramamoorthy S, Schroeter S, Qian Y, Apparsundaram S, Galli A, and DeFelice LJ (1998) Regulated phosphorylation and trafficking of antidepressant-sensitive serotonin transporter proteins. *Biol Psychiatry* **44:**169–178.
Bøgesø KP, Christensen AV, Hyttel J, and Liljefors T (1985) 3-Phenyl-1-indanamines. Potential antidepressant activity and potent inhibition of dopamine, norepinephrine, and serotonin uptake. *J Med Chem* **28:**1817–1828.
Bolan EA, Kivell B, Jaligam V, Oz M, Jayanthi LD, Han Y, Sen N, Urizar E, Gomes I, Devi LA, et al. (2007) D_2 receptors regulate dopamine transporter function via an extracellular signal-regulated kinases 1 and 2-dependent and phosphoinositide 3 kinase-independent mechanism. *Mol Pharmacol* **71:**1222–1232.
Bönisch H and Harder R (1986) Binding of ^{3}H-desipramine to the neuronal noradrenaline carrier of rat phaeochromocytoma cells (PC-12 cells). *Naunyn Schmiedebergs Arch Pharmacol* **334:**403–411.
Borden LA (1996) GABA transporter heterogeneity: pharmacology and cellular localization. *Neurochem Int* **29:**335–356.
Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, and Gluchowski C (1994) Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. *Eur J Pharmacol* **269:**219–224.
Borden LA, Smith KE, Gustafson EL, Branchek TA, and Weinshank RL (1995)

Cloning and expression of a betaine/GABA transporter from human brain. *J Neurochem* **64:**977–984.
Borden LA, Smith KE, Hartig PR, Branchek TA, and Weinshank RL (1992) Molecular heterogeneity of the γ-aminobutyric acid (GABA) transport system. Cloning of two novel high affinity GABA transporters from rat brain. *J Biol Chem* **267:** 21098–21104.
Borowsky B, Mezey E, and Hoffman BJ (1993) Two glycine transporter variants with distinct localization in the CNS and peripheral tissues are encoded by a common gene. *Neuron* **10:**851–863.
Boudanova E, Navaroli DM, Stevens Z, and Melikian HE (2008) Dopamine transporter endocytic determinants: carboxy terminal residues critical for basal and PKC-stimulated internalization. *Mol Cell Neurosci* **39:**211–217.
Boulay D, Pichat P, Dargazanli G, Estenne-Bouhtou G, Terranova JP, Rogacki N, Stemmelin J, Coste A, Lanneau C, Desvignes C, et al. (2008) Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia. *Pharmacol Biochem Behav* **91:**47–58.
Bowton E, Saunders C, Erreger K, Sakrikar D, Matthies HJ, Sen N, Jessen T, Colbran RJ, Caron MG, Javitch JA, et al. (2010) Dysregulation of dopamine transporters via dopamine D_2 autoreceptors triggers anomalous dopamine efflux associated with attention-deficit hyperactivity disorder. *J Neurosci* **30:**6048–6057.
Bradaïa A, Schlichter R, and Trouslard J (2004) Role of glial and neuronal glycine transporters in the control of glycinergic and glutamatergic synaptic transmission in lamina X of the rat spinal cord. *J Physiol* **559:**169–186.
Braestrup C, Nielsen EB, Sonnewald U, Knutsen LJ, Andersen KE, Jansen JA, Frederiksen K, Andersen PH, Mortensen A, and Suzdak PD (1990) (*R*)-*N*-[4,4-bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic acid binds with high-affinity to the brain γ-aminobutyric acid uptake carrier. *J Neurochem* **54:**639–647.
Bragina L, Marchionni I, Omrani A, Cozzi A, Pellegrini-Giampietro DE, Cherubini E, and Conti F (2008) GAT-1 regulates both tonic and phasic $GABA_A$ receptor-mediated inhibition in the cerebral cortex. *J Neurochem* **105:**1781–1793.
Brenner B, Harney JT, Ahmed BA, Jeffus BC, Unal R, Mehta JL, and Kilic F (2007) Plasma serotonin levels and the platelet serotonin transporter. *J Neurochem* **102:**206–215.
Bröer S (2006) The SLC6 orphans are forming a family of amino acid transporters. *Neurochem Int* **48:**559–567.
Brown A, Carlyle I, Clark J, Hamilton W, Gibson S, McGarry G, McEachen S, Rae D, Thorn S, and Walker G (2001) Discovery and SAR of org 24598–a selective glycine uptake inhibitor. *Bioorg Med Chem Lett* **11:**2007–2009.
Brown AS and Gershon S (1993) Dopamine and depression. *J Neural Transm Gen Sect* **91:**75–109.
Brüss M, Hammermann R, Brimijoin S, and Bönisch H (1995) Antipeptide antibodies confirm the topology of the human norepinephrine transporter. *J Biol Chem* **270:**9197–9201.
Brust A, Palant E, Croker DE, Colless B, Drinkwater R, Patterson B, Schroeder CI, Wilson D, Nielsen CK, Smith MT, et al. (2009) χ-Conopeptide pharmacophore development: toward a novel class of norepinephrine transporter inhibitor (Xen2174) for pain. *J Med Chem* **52:**6991–7002.
Buck KJ and Amara SG (1994) Chimeric dopamine-norepinephrine transporters delineate structural domains influencing selectivity for catecholamines and 1-methyl-4-phenylpyridinium. *Proc Natl Acad Sci USA* **91:**12584–12588.
Cai G, Salonikidis PS, Fei J, Schwarz W, Schülein R, Reutter W, and Fan H (2005) The role of N-glycosylation in the stability, trafficking and GABA-uptake of GABA-transporter 1. Terminal N-glycans facilitate efficient GABA-uptake activity of the GABA transporter. *FEBS J* **272:**1625–1638.
Cai YQ, Cai GQ, Liu GX, Cai Q, Shi JH, Shi J, Ma SK, Sun X, Sheng ZJ, Mei ZT, et al. (2006) Mice with genetically altered GABA transporter subtype I (GAT1) expression show altered behavioral responses to ethanol. *J Neurosci Res* **84:**255–267.
Calati R, De Ronchi D, Bellini M, and Serretti A (2011) The 5-HTTLPR polymorphism and eating disorders: a meta-analysis. *Int J Eat Disord* **3:**191–199.
Cao Y, Li M, Mager S, and Lester HA (1998) Amino acid residues that control pH modulation of transport-associated current in mammalian serotonin transporters. *J Neurosci* **18:**7739–7749.
Caplan DA, Subbotina JO, and Noskov SY (2008) Molecular mechanism of ion-ion and ion-substrate coupling in the Na^+-dependent leucine transporter LeuT. *Biophys J* **95:**4613–4621.
Carneiro AM and Blakely RD (2006) Serotonin-, protein kinase C-, and Hic-5-associated redistribution of the platelet serotonin transporter. *J Biol Chem* **281:** 24769–24780.
Carneiro AM, Cook EH, Murphy DL, and Blakely RD (2008) Interactions between integrin αIIbβ3 and the serotonin transporter regulate serotonin transport and platelet aggregation in mice and humans. *J Clin Invest* **118:**1544–1552.
Carneiro AM, Ingram SL, Beaulieu JM, Sweeney A, Amara SG, Thomas SM, Caron MG, and Torres GE (2002) The multiple LIM domain-containing adaptor protein Hic-5 synaptically colocalizes and interacts with the dopamine transporter. *J Neurosci* **22:**7045–7054.
Carroll FI (2003) 2002 Medicinal Chemistry Division Award address: monoamine transporters and opioid receptors. Targets for addiction therapy. *J Med Chem* **46:**1775–1794.
Carroll FI, Kotian P, Dehghani A, Gray JL, Kuzemko MA, Parham KA, Abraham P, Lewin AH, Boja JW, and Kuhar MJ (1995) Cocaine and 3β-(4′-substituted phenyl)tropane-2β-carboxylic acid ester and amide analogs. New high-affinity and selective compounds for the dopamine transporter. *J Med Chem* **38:**379–388.
Carvelli L, Blakely RD, and DeFelice LJ (2008) Dopamine transporter/syntaxin 1A interactions regulate transporter channel activity and dopaminergic synaptic transmission. *Proc Natl Acad Sci USA* **105:**14192–14197.
Carvelli L, Morón JA, Kahlig KM, Ferrer JV, Sen N, Lechleiter JD, Leeb-Lundberg LM, Merrill G, Lafer EM, Ballou LM, et al. (2002) PI 3-kinase regulation of dopamine uptake. *J Neurochem* **81:**859–869.
Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, et al. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301:**386–389.
Caulfield WL, Collie IT, Dickins RS, Epemolu O, McGuire R, Hill DR, McVey G, Morphy JR, Rankovic Z, and Sundaram H (2001) The first potent and selective inhibitors of the glycine transporter type 2. *J Med Chem* **44:**2679–2682.
Celik L, Schiøtt B, and Tajkhorshid E (2008a) Substrate binding and formation of an occluded state in the leucine transporter. *Biophys J* **94:**1600–1612.
Celik L, Sinning S, Severinsen K, Hansen CG, Møller MS, Bols M, Wiborg O, and Schiøtt B (2008b) Binding of serotonin to the human serotonin transporter. Molecular modeling and experimental validation. *J Am Chem Soc* **130:**3853–3865.
Cervinski MA, Foster JD, and Vaughan RA (2005) Psychoactive substrates stimulate dopamine transporter phosphorylation and down-regulation by cocaine-sensitive and protein kinase C-dependent mechanisms. *J Biol Chem* **280:**40442–40449.
Cervinski MA, Foster JD, and Vaughan RA (2010) Syntaxin 1A regulates dopamine transporter activity, phosphorylation and surface expression. *Neuroscience* **170:** 408–416.
Chandra S, Fornai F, Kwon HB, Yazdani U, Atasoy D, Liu X, Hammer RE, Battaglia G, German DC, Castillo PE, et al. (2004) Double-knockout mice for α- and β-synucleins: effect on synaptic functions. *Proc Natl Acad Sci USA* **101:**14966–14971.
Chang AS, Chang SM, Starnes DM, Schroeter S, Bauman AL, and Blakely RD (1996) Cloning and expression of the mouse serotonin transporter. *Mol Brain Res* **43:**185–192.
Chang MY, Lee SH, Kim JH, Lee KH, Kim YS, Son H, and Lee YS (2001) Protein kinase C-mediated functional regulation of dopamine transporter is not achieved by direct phosphorylation of the dopamine transporter protein. *J Neurochem* **77:**754–761.
Chanrion B, Mannoury la Cour C, Bertaso F, Lerner-Natoli M, Freissmuth M, Millan MJ, Bockaert J, and Marin P (2007) Physical interaction between the serotonin transporter and neuronal nitric oxide synthase underlies reciprocal modulation of their activity. *Proc Natl Acad Sci USA* **104:**8119–8124.
Chen F, Larsen MB, Neubauer HA, Sánchez C, Plenge P, and Wiborg O (2005a) Characterization of an allosteric citalopram-binding site at the serotonin transporter. *J Neurochem* **92:**21–28.
Chen F, Larsen MB, Sánchez C, and Wiborg O (2005b) The *S*-enantiomer of *R,S*-citalopram, increases inhibitor binding to the human serotonin transporter by an allosteric mechanism. Comparison with other serotonin transporter inhibitors. *Eur Neuropsychopharmacol* **15:**193–198.
Chen JG, Liu-Chen S, and Rudnick G (1997a) External cysteine residues in the serotonin transporter. *Biochemistry* **36:**1479–1486.
Chen JG, Liu-Chen S, and Rudnick G (1998) Determination of external loop topology in the serotonin transporter by site-directed chemical labeling. *J Biol Chem* **273:** 12675–12681.
Chen JG and Rudnick G (2000) Permeation and gating residues in serotonin transporter. *Proc Natl Acad Sci USA* **97:**1044–1049.
Chen JG, Sachpatzidis A, and Rudnick G (1997b) The third transmembrane domain of the serotonin transporter contains residues associated with substrate and cocaine binding. *J Biol Chem* **272:**28321–28327.
Chen N and Reith ME (2000) Structure and function of the dopamine transporter. *Eur J Pharmacol* **405:**329–339.
Chen N, Ferrer JV, Javitch JA, and Justice JB Jr (2000) Transport-dependent accessibility of a cytoplasmic loop cysteine in the human dopamine transporter. *J Biol Chem* **275:**1608–1614.
Chen N, Rickey J, Berfield JL, and Reith ME (2004a) Aspartate 345 of the dopamine transporter is critical for conformational changes in substrate translocation and cocaine binding. *J Biol Chem* **279:**5508–5519.
Chen NH and Reith ME (2002) Structure-function relationships for biogenic amine neurotransmitter transporters, in *Neurotransmitter Transporters: Structure, Function, and Regulation, 2nd ed* (Reith ME ed) pp 53–109, Humana Press, Totowa, NJ.
Chen NH, Reith ME, and Quick MW (2004b) Synaptic uptake and beyond: the sodium- and chloride-dependent neurotransmitter transporter family SLC6. *Pflugers Arch* **447:**519–531.
Chen R, Furman CA, Zhang M, Kim MN, Gereau RW 4th, Leitges M, and Gnegy ME (2009) Protein kinase Cβ is a critical regulator of dopamine transporter trafficking and regulates the behavioral response to amphetamine in mice. *J Pharmacol Exp Ther* **328:**912–920.
Chen R, Tilley MR, Wei H, Zhou F, Zhou FM, Ching S, Quan N, Stephens RL, Hill ER, Nottoli T, et al. (2006) Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. *Proc Natl Acad Sci USA* **103:**9333–9338.
Chi L and Reith ME (2003) Substrate-induced trafficking of the dopamine transporter in heterologously expressing cells and in rat striatal synaptosomal preparations. *J Pharmacol Exp Ther* **307:**729–736.
Chiu CS, Brickley S, Jensen K, Southwell A, Mckinney S, Cull-Candy S, Mody I, and Lester HA (2005) GABA transporter deficiency causes tremor, ataxia, nervousness, and increased GABA-induced tonic conductance in cerebellum. *J Neurosci* **25:**3234–3245.
Cho HJ, Meira-Lima I, Cordeiro Q, Michelon L, Sham P, Vallada H, and Collier DA (2005) Population-based and family-based studies on the serotonin transporter gene polymorphisms and bipolar disorder: a systematic review and meta-analysis. *Mol Psychiatry* **10:**771–781.
Ciccone MA, Timmons M, Phillips A, and Quick MW (2008) Calcium/calmodulin-dependent kinase II regulates the interaction between the serotonin transporter and syntaxin 1A. *Neuropharmacology* **55:**763–770.
Clark JA, Deutch AY, Gallipoli PZ, and Amara SG (1992) Functional expression and CNS distribution of a β-alanine-sensitive neuronal GABA transporter. *Neuron* **9:**337–348.
Clarke H, Flint J, Attwood AS, and Munafò MR (2010) Association of the 5- HTTLPR genotype and unipolar depression: a meta-analysis. *Psychol Med* **40:**1767–1778.
Clausen RP, Moltzen EK, Perregaard J, Lenz SM, Sanchez C, Falch E, Frølund B, Bolvig T, Sarup A, Larsson OM, et al. (2005) Selective inhibitors of GABA uptake:

synthesis and molecular pharmacology of 4-*N*-methylamino-4,5,6,7-tetrahydrobenzo[*d*]isoxazol-3-ol analogues. *Bioorg Med Chem* **13:**895–908.
Clayton AH, Pradko JF, Croft HA, Montano CB, Leadbetter RA, Bolden-Watson C, Bass KI, Donahue RM, Jamerson BD, and Metz A (2002) Prevalence of sexual dysfunction among newer antidepressants. *J Clin Psychiatry* **63:**357–366.
Conti F, Minelli A, and Melone M (2004) GABA transporters in the mammalian cerebral cortex: localization, development and pathological implications. *Brain Res Rev* **45:**196–212.
Corona G, Ricca V, Bandini E, Mannucci E, Lotti F, Boddi V, Rastrelli G, Sforza A, Faravelli C, Forti G, et al. (2009) Selective serotonin reuptake inhibitor-induced sexual dysfuntion. *J Sex Med* **6:**1259–1269.
Cowell RM, Kantor L, Hewlett GH, Frey KA, and Gnegy ME (2000) Dopamine transporter antagonists block phorbol ester-induced dopamine release and dopamine transporter phosphorylation in striatal synaptosomes. *Eur J Pharmacol* **389:**59–65.
Cristóvão-Ferreira S, Vaz SH, Ribeiro JA, and Sebastião AM (2009) Adenosine A2A receptors enhance GABA transport into nerve terminals by restraining PKC inhibition of GAT-1. *J Neurochem* **109:**336–347.
Csermely P, Agoston V, and Pongor S (2005) The efficiency of multi-target drugs: the network approach might help drug design. *Trends Pharmacol Sci* **26:**178–182.
Cubelos B, Giménez C, and Zafra F (2005a) The glycine transporter GLYT1 interacts with Sec3, a component of the exocyst complex. *Neuropharmacology* **49:**935–944.
Cubelos B, González-González IM, Giménez C, and Zafra F (2005b) The scaffolding protein PSD-95 interacts with the glycine transporter GLYT1 and impairs its internalization. *J Neurochem* **95:**1047–1058.
Czuczwar SJ and Patsalos PN (2001) The new generation of GABA enhancers. Potential in the treatment of epilepsy. *CNS Drugs* **15:**339–350.
Dalby NO (2000) GABA-level increasing and anticonvulsant effects of three different GABA uptake inhibitors. *Neuropharmacology* **39:**2399–2407.
Dalby NO and Mody I (2001) The process of epileptogenesis: a pathophysiological approach. *Curr Opin Neurol* **14:**187–192.
Dalby NO and Nielsen EB (1997) Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin. *Epilepsy Res* **28:**63–72.
Dalby NO, Thomsen C, Fink-Jensen A, Lundbeck J, Søkilde B, Man CM, Sørensen PO, and Meldrum B (1997) Anticonvulsant properties of two GABA uptake inhibitors NNC 05-2045 and NNC 05-2090, not acting preferentialy on GAT-1. *Epilepsy Res* **28:**51–61.
Daniels GM and Amara SG (1999) Regulated trafficking of the human dopamine transporter. Clathrin-mediated internalization and lysosomal degradation in response to phorbol esters. *J Biol Chem* **274:**35794–35801.
Dauer W, Kholodilov N, Vila M, Trillat AC, Goodchild R, Larsen KE, Staal R, Tieu K, Schmitz Y, Yuan CA, et al. (2002) Resistance of α-synuclein null mice to the parkinsonian neurotoxin MPTP. *Proc Natl Acad Sci USA* **99:**14524–14529.
Daws LC, Callaghan PD, Morón JA, Kahlig KM, Shippenberg TS, Javitch JA, and Galli A (2002) Cocaine increases dopamine uptake and cell surface expression of dopamine transporters. *Biochem Biophys Res Commun* **290:**1545–1550.
Dawson LA and Bromidge SM (2008) 5-HT_1 receptor augmentation strategies as enhanced efficacy therapeutics for psychiatric disorders. *Curr Top Med Chem* **8:**1008–1023.
Dawson LA and Watson JM (2009) Vilazodone: a 5-HT_{1A} receptor agonist/serotonin transporter inhibitor for the treatment of affective disorders. *CNS Neurosci Ther* **15:**107–117.
de Paulis T (2007) Drug evaluation: Vilazodone–a combined SSRI and 5-HT_{1A} partial agonist for the treatment of depression. *IDrugs* **10:**193–201.
Deckert G, Warren PV, Gaasterland T, Young WG, Lenox AL, Graham DE, Overbeek R, Snead MA, Keller M, Aujay M, et al. (1998) The complete genome of the hyperthermophilic bacterium *Aquifex aeolicus*. *Nature* **392:**353–358.
Deeks ED and Keating GM (2008) Olanzapine/fluoxetine: a review of its use in the treatment of acute bipolar depression. *Drugs* **68:**1115–1137.
DeFelice LJ, Adams SV, and Ypey DL (2001) Single-file diffusion and neurotransmitter transporters: Hodgkin and Keynes model revisited. *BioSystems* **62:**57–66.
Deken SL, Beckman ML, Boos L, and Quick MW (2000) Transport rates of GABA transporters: regulation by the N-terminal domain and syntaxin 1A. *Nat Neurosci* **3:**998–1003.
Depoortère R, Dargazanli G, Estenne-Bouhtou G, Coste A, Lanneau C, Desvignes C, Poncelet M, Heaulme M, Santucci V, Decobert M, et al. (2005) Neurochemical, electrophysiological and pharmacological profiles of the selective inhibitor of the glycine transporter-I SSR504734, a potential new type of antipsychotic. *Neuropsychopharmacology* **30:**1963–1985.
Desai RI, Kopajtic TA, Koffarnus M, Newman AH, and Katz JL (2005) Identification of a dopamine transporter ligand that blocks the stimulant effects of cocaine. *J Neurosci* **25:**1889–1893.
Dhar TG, Borden LA, Tyagarajan S, Smith KE, Branchek TA, Weinshank RL, and Gluchowski C (1994) Design, synthesis and evaluation of substituted triarylnipecotic acid-derivatives as GABA uptake inhibitors: identification of a ligand with moderate affinity and selectivity for the cloned human GABA transporter GAT-3. *J Med Chem* **37:**2334–2342.
Dhar TGM, Nakanishi H, Borden LA, and Gluchowski C (1996) On the bioactive conformation of the GABA uptake inhibitor SKF89976-A. *Bioorg Med Chem Lett* **6:**1535–1540.
Diepold K, Schütz B, Rostasy K, Wilken B, Hougaard P, Güttler F, Romstad A, and Birk Møller L (2005) Levodopa-responsive infantile parkinsonism due to a novel mutation in the tyrosine hydroxylase gene and exacerbation by viral infections. *Mov Disord* **20:**764–767.
Ding YS, Lin KS, Garza V, Carter P, Alexoff D, Logan J, Shea C, Xu Y, and King P (2003) Evaluation of a new norepinephrine transporter PET ligand in baboons, both in brain and peripheral organs. *Synapse* **50:**345–352.
Dipace C, Sung U, Binda F, Blakely RD, and Galli A (2007) Amphetamine induces a calcium/calmodulin-dependent protein kinase II-dependent reduction in norepinephrine transporter surface expression linked to changes in syntaxin 1A/transporter complexes. *Mol Pharmacol* **71:**230–239.
Dodd JR and Christie DL (2007) Selective amino acid substitutions convert the creatine transporter to a γ-aminobutyric acid transporter. *J Biol Chem* **282:**15528–15533.
Dodd S and Berk M (2008) Olanzapine/fluoxetine combination for treatment-resistant depression: efficacy and clinical utility. *Expert Rev Neurother* **8:**1299–1306.
Dohi T, Morita K, Kitayama T, Motoyama N, and Morioka N (2009) Glycine transporter inhibitors as a novel drug discovery strategy for neuropathic pain. *Pharmacol Ther* **123:**54–79.
Dutta AK, Zhang S, Kolhatkar R, and Reith ME (2003) Dopamine transporter as target for drug development of cocaine dependence medications. *Eur J Pharmacol* **479:**93–106.
Dziedzicka-Wasylewska M, Faron-Górecka A, Kuśmider M, Drozdowska E, Rogóz Z, Siwanowicz J, Caron MG, and Bönisch H (2006) Effect of antidepressant drugs in mice lacking the norepinephrine transporter. *Neuropsychopharmacology* **31:**2424–2432.
Eddahibi S, Humbert M, Fadel E, Raffestin B, Darmon M, Capron F, Simonneau G, Dartevelle P, Hamon M, and Adnot S (2001) Serotonin transporter overexpression is responsible for pulmonary artery smooth muscle hyperplasia in primary pulmonary hypertension. *J Clin Invest* **108:**1141–1150.
Edington AR, McKinzie AA, Reynolds AJ, Kassiou M, Ryan RM, and Vandenberg RJ (2009) Extracellular loops 2 and 4 of GLYT2 are required for *N*-arachidonylglycine inhibition of glycine transport. *J Biol Chem* **284:**36424–36430.
Egaña LA, Cuevas RA, Baust TB, Parra LA, Leak RK, Hochendoner S, Peña K, Quiroz M, Hong WC, Dorostkar MM, et al. (2009) Physical and functional interaction between the dopamine transporter and the synaptic vesicle protein synaptogyrin-3. *J Neurosci* **29:**4592–4604.
Eichler EE, Budarf ML, Rocchi M, Deaven LL, Doggett NA, Baldini A, Nelson DL, and Mohrenweiser HW (1997) Interchromosomal duplications of the adrenoleukodystrophy locus: a phenomenon of pericentromeric plasticity. *Hum Mol Genet* **6:**991–1002.
Eiden LE, Schäfer MK, Weihe E, and Schütz B (2004) The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. *Pflugers Arch* **447:**636–640.
Eriksen J, Bjørn-Yoshimoto WE, Jørgensen TN, Newman AH, and Gether U (2010a) Postendocytic sorting of constitutively internalized dopamine transporter in cell lines and dopaminergic neurons. *J Biol Chem* **285:**27289–27301.
Eriksen J, Jørgensen TN, and Gether U (2010b) Regulation of dopamine transporter function by protein-protein interactions: new discoveries and methodological challenges. *J Neurochem* **113:**27–41.
Eriksen J, Rasmussen SG, Rasmussen TN, Vaegter CB, Cha JH, Zou MF, Newman AH, and Gether U (2009) Visualization of dopamine transporter trafficking in live neurons by use of fluorescent cocaine analogs. *J Neurosci* **29:**6794–6808.
Erreger K, Grewer C, Javitch JA, and Galli A (2008) Currents in response to rapid concentration jumps of amphetamine uncover novel aspects of human dopamine transporter function. *J Neurosci* **28:**976–989.
Eshleman AJ, Carmolli M, Cumbay M, Martens CR, Neve KA, and Janowsky A (1999) Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. *J Pharmacol Exp Ther* **289:**877–885.
Eulenburg V and Gomeza J (2010) Neurotransmitter transporters expressed in glial cells as regulators of synapse function. *Brain Res Rev* **63:**103–112.
Eulenburg V, Becker K, Gomeza J, Schmitt B, Becker CM, and Betz H (2006) Mutations within the human GLYT2 (SLC6A5) gene associated with hyperekplexia. *Biochem Biophys Res Commun* **348:**400–405.
Eulenburg V, Retiounskaia M, Papadopoulos T, Gomeza J, and Betz H (2010) Glial glycine transporter 1 function is essential for early postnatal survival but dispensable in adult mice. *Glia* **58:**1066–1073.
Faham S, Watanabe A, Besserer GM, Cascio D, Specht A, Hirayama BA, Wright EM, and Abramson J (2008) The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na^+/sugar symport. *Science* **321:**810–814.
Fallon B (2008) 'Off-label' drug use in sexual medicine treatment. *Int J Impot Res* **20:**127–134.
Fang Y, Jayaram H, Shane T, Kolmakova-Partensky L, Wu F, Williams C, Xiong Y, and Miller C (2009) Structure of a prokaryotic virtual proton pump at 3.2 Å resolution. *Nature* **460:**1040–1043.
Farhan H, Korkhov VM, Paulitschke V, Dorostkar MM, Scholze P, Kudlacek O, Freissmuth M, and Sitte HH (2004) Two discontinuous segments in the carboxyl terminus are required for membrane targeting of the rat γ-aminobutyric acid transporter-1 (GAT1). *J Biol Chem* **279:**28553–28563.
Farhan H, Reiterer V, Korkhov VM, Schmid JA, Freissmuth M, and Sitte HH (2007) Concentrative export from the endoplasmic reticulum of the γ-aminobutyric acid transporter 1 requires binding to SEC24D. *J Biol Chem* **282:**7679–7689.
Farhan H, Reiterer V, Kriz A, Hauri HP, Pavelka M, Sitte HH, and Freissmuth M (2008) Signal-dependent export of GABA transporter 1 from the ER-Golgi intermediate compartment is specified by a C-terminal motif. *J Cell Sci* **121:**753–761.
Ferguson JM (2001) The effects of antidepressants on sexual functioning in depressed patients. *J Clin Psychiatry* **62:**22–34.
Fernández-Sánchez E, Martínez-Villarreal J, Giménez C, and Zafra F (2009) Constitutive and regulated endocytosis of the glycine transporter GLYT1b is controlled by ubiquitination. *J Biol Chem* **284:**19482–19492.
Ferrer JV and Javitch JA (1998) Cocaine alters the accessibility of endogenous cysteines in putative extracellular and intracellular loops of the human dopamine transporter. *Proc Natl Acad Sci USA* **95:**9238–9243.
Fesce R, Giovannardi S, Binda F, Bossi E, and Peres A (2002) The relation between charge movement and transport-associated currents in the rat GABA cotransporter rGAT1. *J Physiol* **545:**739–750.
Field JR, Henry LK, and Blakely RD (2010) Transmembrane domain 6 of the human serotonin transporter contributes to an aqueously accessible binding pocket for

serotonin and the psychostimulant 3,4-methylene dioxymethamphetamine. *J Biol Chem* **285:**11270–11280.
Finer N (2002) Sibutramine: its mode of action and efficacy. *Int J Obes Relat Metab Disord* **26 (Suppl 4):**S29–S33.
Flegal KM, Graubard BI, Williamson DF, and Gail MH (2007) Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA* **298:**2028–2037.
Fog JU, Khoshbouei H, Holy M, Owens WA, Vaegter CB, Sen N, Nikandrova Y, Bowton E, McMahon DG, Colbran RJ, et al. (2006) Calmodulin kinase II interacts with the dopamine transporter C terminus to regulate amphetamine-induced reverse transport. *Neuron* **51:**417–429.
Forlani G, Bossi E, Perego C, Giovannardi S, and Peres A (2001) Three kinds of currents in the canine betaine-GABA transporter BGT-1 expressed in *Xenopus laevis* oocytes. *Biochim Biophys Acta* **1538:**172–180.
Fornés A, Núñez E, Alonso-Torres P, Aragón C, and López-Corcuera B (2008) Trafficking properties and activity regulation of the neuronal glycine transporter GLYT2 by protein kinase C. *Biochem J* **412:**495–506.
Fornés A, Núñez E, Aragón C, and López-Corcuera B (2004) The second intracellular loop of the glycine transporter 2 contains crucial residues for glycine transport and phorbol ester-induced regulation. *J Biol Chem* **279:**22934–22943.
Forrest LR and Rudnick G (2009) The rocking bundle: a mechanism for ion-coupled solute flux by symmetrical transporters. *Physiology* (*Bethesda*) **24:**377–386.
Forrest LR, Tavoulari S, Zhang YW, Rudnick G, and Honig B (2007) Identification of a chloride ion binding site in Na^+/Cl^--dependent transporters. *Proc Natl Acad Sci USA* **104:**12761–12766.
Forrest LR, Zhang YW, Jacobs MT, Gesmonde J, Xie L, Honig BH, and Rudnick G (2008) Mechanism for alternating access in neurotransmitter transporters. *Proc Natl Acad Sci USA* **105:**10338–10343.
Foster AC and Kemp JA (2006) Glutamate- and GABA-based CNS therapeutics. *Curr Opin Pharmacol* **6:**7–17.
Foster JD, Pananusorn B, and Vaughan RA (2002) Dopamine transporters are phosphorylated on N-terminal serines in rat striatum. *J Biol Chem* **277:**25178–25186.
Foster JD, Pananusorn B, Cervinski MA, Holden HE, and Vaughan RA (2003) Dopamine transporters are dephosphorylated in striatal homogenates and *in vitro* by protein phosphatase 1. *Mol Brain Res* **110:**100–108.
Fountaine TM and Wade-Martins R (2007) RNA interference-mediated knockdown of α-synuclein protects human dopaminergic neuroblastoma cells from MPP^+ toxicity and reduces dopamine transport. *J Neurosci Res* **85:**351–363.
Fowler A, Seifert N, Acker V, Woehrle T, Kilpert C, and de Saizieu A (2006) A nonradioactive high-throughput/high-content assay for measurement of the human serotonin reuptake transporter function in vitro. *J Biomol Screen* **11:**1027–1034.
Fowler JS, Volkow ND, Wang GJ, Ding YS, and Dewey SL (1999) PET and drug research and development. *J Nucl Med* **40:**1154–1163.
Fredriksson R, Nordström KJ, Stephansson O, Hägglund MG, and Schiöth HB (2008) The solute carrier (SLC) complement of the human genome: phylogenetic classification reveals four major families. *FEBS Lett* **582:**3811–3816.
Furman CA, Chen R, Guptaroy B, Zhang M, Holz RW, and Gnegy M (2009) Dopamine and amphetamine rapidly increase dopamine transporter trafficking to the surface: live-cell imaging using total internal reflection fluorescence microscopy. *J Neurosci* **29:**3328–3336.
Gabernet L, Pauly-Evers M, Schwerdel C, Lentz M, Bluethmann H, Vogt K, Alberati D, Mohler H, and Boison D (2005) Enhancement of the NMDA receptor function by reduction of glycine transporter-1 expression. *Neurosci Lett* **373:**79–84.
Gadde KM, Franciscy DM, Wagner HR 2nd, and Krishnan KR (2003) Zonisamide for weight loss in obese adults: a randomized controlled trial. *JAMA* **289:**1820–1825.
Gadde KM, Parker CB, Maner LG, Wagner HR 2nd, Logue EJ, Drezner MK, and Krishnan KR (2001) Bupropion for weight loss: an investigation of efficacy and tolerability in overweight and obese women. *Obes Res* **9:**544–551.
Gadde KM, Yonish GM, Foust MS, and Wagner HR (2007) Combination therapy of zonisamide and bupropion for weight reduction in obese women: a preliminary, randomized, open-label study. *J Clin Psychiatry* **68:**1226–1229.
Gadea A and López-Colomé AM (2001) Glial transporters for glutamate, glycine, and GABA: II. GABA transporters. *J Neurosci Res* **63:**461–468.
Gadea A, López E, Hernández-Cruz A, and López-Colomé AM (2002) Role of Ca^{2+} and calmodulin-dependent enzymes in the regulation of glycine transport in Müller glia. *J Neurochem* **80:**634–645.
Gainetdinov RR (2008) Dopamine transporter mutant mice in experimental neuropharmacology. *Naunyn Schmiedebergs Arch Pharmacol* **377:**301–313.
Gainetdinov RR and Caron MG (2000) An animal model of attention deficit hyperactivity disorder. *Mol Med Today* **6:**43–44.
Gainetdinov RR and Caron MG (2003) Monoamine transporters: from genes to behavior. *Annu Rev Pharmacol Toxicol* **43:**261–284.
Gainetdinov RR, Sotnikova TD, and Caron MG (2002) Monoamine transporter pharmacology and mutant mice. *Trends Pharmacol Sci* **23:**367–373.
Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, and Caron MG (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science* **283:**397–401.
Galli A, DeFelice LJ, Duke BJ, Moore KR, and Blakely RD (1995) Sodium-dependent norepinephrine-induced currents in norepinephrine-transporter-transfected HEK-293 cells blocked by cocaine and antidepressants. *J Exp Biol* **198:**2197–2212.
Gao X, Lu F, Zhou L, Dang S, Sun L, Li X, Wang J, and Shi Y (2009) Structure and mechanism of an amino acid antiporter. *Science* **324:**1565–1568.
Gao X, Zhou L, Jiao X, Lu F, Yan C, Zeng X, Wang J, and Shi Y (2010) Mechanism of substrate recognition and transport by an amino acid antiporter. *Nature* **463:**828–832.
Garcia BG, Wei Y, Moron JA, Lin RZ, Javitch JA, and Galli A (2005) Akt is essential for insulin modulation of amphetamine-induced human dopamine transporter cell-surface redistribution. *Mol Pharmacol* **68:**102–109.
Gardier AM (2009) Mutant mouse models and antidepressant drug research: focus on serotonin and brain-derived neurotrophic factor. *Behav Pharmacol* **20:**18–32.
Gasnier B (2004) The SLC32 transporter, a key protein for the synaptic release of inhibitory amino acids. *Pflugers Arch* **447:**756–759.
Geerlings A, López-Corcuera B, and Aragón C (2000) Characterization of the interactions between the glycine transporters GLYT1 and GLYT2 and the SNARE protein syntaxin 1A. *FEBS Lett* **470:**51–54.
Geerlings A, Núñez E, López-Corcuera B, and Aragón C (2001) Calcium- and syntaxin 1-mediated trafficking of the neuronal glycine transporter GLYT2. *J Biol Chem* **276:**17584–17590.
Gether U, Andersen PH, Larsson OM, and Schousboe A (2006) Neurotransmitter transporters: molecular function of important drug targets. *Trends Pharmacol Sci* **27:**375–383.
Gillman PK (2007) Tricyclic antidepressant pharmacology and therapeutic drug interactions updated. *Br J Pharmacol* **151:**737–748.
Giros B and Caron MG (1993) Molecular characterization of the dopamine transporter. *Trends Pharmacol Sci* **14:**43–49.
Giros B, el Mestikawy S, Bertrand L, and Caron MG (1991) Cloning and functional characterization of a cocaine-sensitive dopamine transporter. *FEBS Lett* **295:**149–154.
Giros B, el Mestikawy S, Godinot N, Zheng K, Han H, Yang-Feng T, and Caron MG (1992) Cloning, pharmacological characterization, and chromosome assignment of the human dopamine transporter. *Mol Pharmacol* **42:**383–390.
Giros B, Jaber M, Jones SR, Wightman RM, and Caron MG (1996a) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **379:**606–612.
Giros B, Jaber M, Jones SR, Wightman RM, and Caron MG (1996b) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature* **379:**606–612.
Giuliano F (2007) 5-Hydroxytryptamine in premature ejaculation: opportunities for therapeutic intervention. *Trends Neurosci* **30:**79–84.
Giuliano F and Hellstrom WJ (2008) The pharmacological treatment of premature ejaculation. *BJU Int* **102:**668–675.
Glatt CE, DeYoung JA, Delgado S, Service SK, Giacomini KM, Edwards RH, Risch N, and Freimer NB (2001) Screening a large reference sample to identify very low frequency sequence variants: comparisons between two genes. *Nat Genet* **27:**435–438.
Glick SD, Haskew RE, Maisonneuve IM, Carlson JN, and Jerussi TP (2000) Enantioselective behavioral effects of sibutramine metabolites. *Eur J Pharmacol* **397:**93–102.
Goldberg NR, Beuming T, Soyer OS, Goldstein RA, Weinstein H, and Javitch JA (2003) Probing conformational changes in neurotransmitter transporters: a structural context. *Eur J Pharmacol* **479:**3–12.
Gomeza J, Hülsmann S, Ohno K, Eulenburg V, Szöke K, Richter D, and Betz H (2003a) Inactivation of the glycine transporter 1 gene discloses vital role of glial glycine uptake in glycinergic inhibition. *Neuron* **40:**785–796.
Gomeza J, Ohno K, Hülsmann S, Armsen W, Eulenburg V, Richter DW, Laube B, and Betz H (2003b) Deletion of the mouse glycine transporter 2 results in a hyperekplexia phenotype and postnatal lethality. *Neuron* **40:**797–806.
Gomeza J, Zafra F, Olivares L, Giménez C, and Aragón C (1995) Regulation by phorbol esters of the glycine transporter (GLYT1) in glioblastoma cells. *Biochim Biophys Acta* **1233:**41–46.
Gonzales AL, Lee W, Spencer SR, Oropeza RA, Chapman JV, Ku JY, and Eskandari S (2007) Turnover rate of the γ-aminobutyric acid transporter GAT1. *J Membr Biol* **220:**33–51.
Goodman MM, Kilts CD, Keil R, Shi B, Martarello L, Xing D, Votaw J, Ely TD, Lambert P, Owens MJ, et al. (2000) ^{18}F-labeled FECNT: a selective radioligand for PET imaging of brain dopamine transporters. *Nucl Med Biol* **27:**1–12.
Gorentla BK, Moritz AE, Foster JD, and Vaughan RA (2009) Proline-directed phosphorylation of the dopamine transporter N-terminal domain. *Biochemistry* **48:**1067–1076.
Gorentla BK and Vaughan RA (2005) Differential effects of dopamine and psychoactive drugs on dopamine transporter phosphorylation and regulation. *Neuropharmacology* **49:**759–768.
Gouaux E (2009) Review. The molecular logic of sodium-coupled neurotransmitter transporters. *Philos Trans R Soc Lond B Biol Sci* **364:**149–154.
Graham D, Esnaud H, Habert E, and Langer SZ (1989) A common binding site for tricyclic and nontricyclic 5-hydroxytryptamine uptake inhibitors at the substrate recognition site of the neuronal sodium-dependent 5-hydroxytryptamine transporter. *Biochem Pharmacol* **38:**3819–3826.
Granas C, Ferrer J, Loland CJ, Javitch JA, and Gether U (2003) N-terminal truncation of the dopamine transporter abolishes phorbol ester- and substance P receptor-stimulated phosphorylation without impairing transporter internalization. *J Biol Chem* **278:**4990–5000.
Green AR, Mechan AO, Elliott JM, O'Shea E, and Colado MI (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacol Rev* **55:**463–508.
Greenway FL, Whitehouse MJ, Guttadauria M, Anderson JW, Atkinson RL, Fujioka K, Gadde KM, Gupta AK, O'Neil P, Schumacher D, et al. (2009) Rational design of a combination medication for the treatment of obesity. *Obesity* **17:**30–39.
Grünhage F, Schulze TG, Müller DJ, Lanczik M, Franzek E, Albus M, Borrmann-Hassenbach M, Knapp M, Cichon S, Maier W, et al. (2000) Systematic screening for DNA sequence variation in the coding region of the human dopamine transporter gene (DAT1). *Mol Psychiatry* **5:**275–282.
Gu H, Wall SC, and Rudnick G (1994) Stable expression of biogenic amine transporters reveals differences in inhibitor sensitivity, kinetics, and ion dependence. *J Biol Chem* **269:**7124–7130.
Gu HH, Ahn J, Caplan MJ, Blakely RD, Levey AI, and Rudnick G (1996a) Cell-specific sorting of biogenic amine transporters expressed in epithelial cells. *J Biol Chem* **271:**18100–18106.
Gu HH, Wall S, and Rudnick G (1996b) Ion coupling stoichiometry for the norepi-

nephrine transporter in membrane vesicles from stably transfected cells. *J Biol Chem* **271:**6911–6916.
Gu HH, Wu X, and Han DD (2006) Conserved serine residues in serotonin transporter contribute to high-affinity cocaine binding. *Biochem Biophys Res Commun* **343:**1179–1185.
Guastella J, Brecha N, Weigmann C, Lester HA, and Davidson N (1992) Cloning, expression, and localization of a rat brain high-affinity glycine transporter. *Proc Natl Acad Sci USA* **89:**7189–7193.
Guastella J, Nelson N, Nelson H, Czyzyk L, Keynan S, Miedel MC, Davidson N, Lester HA, and Kanner BI (1990) Cloning and expression of a rat brain GABA transporter. *Science* **249:**1303–1306.
Haase J, Killian AM, Magnani F, and Williams C (2001) Regulation of the serotonin transporter by interacting proteins. *Biochem Soc Trans* **29:**722–728.
Haenisch B, Linsel K, Brüss M, Gilsbach R, Propping P, Nöthen MM, Rietschel M, Fimmers R, Maier W, Zobel A, et al. (2008) Association of major depression with rare functional variants in norepinephrine transporter and serotonin1A receptor genes. *Am J Med Genet B Neuropsychiatr Genet* **150B:**1013–1016.
Hahn MK, Mazei-Robison MS, and Blakely RD (2005) Single nucleotide polymorphisms in the human norepinephrine transporter gene affect expression, trafficking, antidepressant interaction, and protein kinase C regulation. *Mol Pharmacol* **68:**457–466.
Hahn MK, Robertson D, and Blakely RD (2003) A mutation in the human norepinephrine transporter gene (SLC6A2) associated with orthostatic intolerance disrupts surface expression of mutant and wild-type transporters. *J Neurosci* **23:**4470–4478.
Hainer V, Kabrnova K, Aldhoon B, Kunesova M, and Wagenknecht M (2006) Serotonin and norepinephrine reuptake inhibition and eating behavior.*Ann NY Acad Sci* **1083:**252–269.
Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, and Chakravarti A (1999) Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. *Nat Genet* **22:**239–247.
Han DD and Gu HH (2006) Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol* **6:**6.
Hastrup H, Karlin A, and Javitch JA (2001) Symmetrical dimer of the human dopamine transporter revealed by cross-linking Cys-306 at the extracellular end of the sixth transmembrane segment. *Proc Natl Acad Sci USA* **98:**10055–10060.
Hastrup H, Sen N, and Javitch JA (2003) The human dopamine transporter forms a tetramer in the plasma membrane: cross-linking of a cysteine in the fourth transmembrane segment is sensitive to cocaine analogs. *J Biol Chem* **278:**45045–45048.
Hatzenbuhler NT, Baudy R, Evrard DA, Failli A, Harrison BL, Lenicek S, Mewshaw RE, Saab A, Shah U, Sze J, et al. (2008) Advances toward new antidepressants with dual serotonin transporter and 5-HT_{1A} receptor affinity within a class of 3-aminochroman derivatives. Part 2. *J Med Chem* **51:**6980–7004.
Hatzenbuhler NT, Evrard DA, Harrison BL, Huryn D, Inghrim J, Kraml C, Mattes JF, Mewshaw RE, Zhou D, Hornby G, et al. (2006) Synthesis and biological evaluation of novel compounds within a class of 3-aminochroman derivatives with dual 5-HT_{1A} receptor and serotonin transporter affinity. *J Med Chem* **49:**4785–4789.
He L, Vasiliou K, and Nebert DW (2009) Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genomics* **3:**195–206.
Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, and Bruford EA (2004) The ABCs of solute carriers: physiological, pathological, and therapeutic implications of human membrane transport proteins. *Pflugers Arch* **447:**465–468.
Heffernan GD, Coglan RD, Manas ES, and McDevitt RE (2008) Dual acting norepinephrine reuptake inhibitors and 5-HT_{2A} receptor antagonists I: synthesis and activity of novel 3-(phenylsulfonyl)-1*H*-indoles, in *236th American Chemical Society National Meeting*; 17–21 Aug 2008; Philadelphia. American Chemical Society, Washington, DC.
Heils A, Teufel A, Petri S, Stöber G, Riederer P, Bengel D, and Lesch KP (1996) Allelic variation of human serotonin transporter gene expression. *J Neurochem* **66:**2621–2624.
Hellstrom WJ (2009) Emerging treatments for premature ejaculation: focus on dapoxetine. *Neuropsychiatr Dis Treat* **5:**37–46.
Henry LK, Adkins EM, Han Q, and Blakely RD (2003) Serotonin and cocaine-sensitive inactivation of human serotonin transporters by methanethiosulfonates targeted to transmembrane domain I. *J Biol Chem* **278:**37052–37063.
Henry LK, Defelice LJ, and Blakely RD (2006a) Getting the message across: a recent transporter structure shows the way. *Neuron* **49:**791–796.
Henry LK, Field JR, Adkins EM, Parnas ML, Vaughan RA, Zou MF, Newman AH, and Blakely RD (2006b) Tyr-95 and Ile-172 in transmembrane segments 1 and 3 of human serotonin transporters interact to establish high affinity recognition of antidepressants. *J Biol Chem* **281:**2012–2023.
Henry LK, Meiler J, and Blakely RD (2007) Bound to be different: neurotransmitter transporters meet their bacterial cousins. *Mol Interv* **7:**306–309.
Hersch SM, Yi H, Heilman CJ, Edwards RH, and Levey AI (1997) Subcellular localization and molecular topology of the dopamine transporter in the striatum and substantia nigra. *J Comp Neurol* **388:**211–227.
Hjorth S, Bengtsson HJ, Kullberg A, Carlzon D, Peilot H, and Auerbach SB (2000) Serotonin autoreceptor function and antidepressant drug action. *J Psychopharmacol* **14:**177–185.
Hoffman BJ, Mezey E, and Brownstein MJ (1991) Cloning of a serotonin transporter affected by antidepressants. *Science* **254:**579–580.
Høg S, Greenwood JR, Madsen KB, Larsson OM, Frølund B, Schousboe A, Krogsgaard-Larsen P, and Clausen RP (2006) Structure-activity relationships of selective GABA uptake inhibitors. *Curr Top Med Chem* **6:**1861–1882.
Höglund PJ, Adzic D, Scicluna SJ, Lindblom J, and Fredriksson R (2005) The repertoire of solute carriers of family 6: identification of new human and rodent genes. *Biochem Biophys Res Commun* **336:**175–189.
Hollon SD, Stewart MO, and Strunk D (2006) Enduring effects for cognitive behavior therapy in the treatment of depression and anxiety. *Annu Rev Psychol* **57:**285–315.
Holmes A, Lit Q, Murphy DL, Gold E, and Crawley JN (2003a) Abnormal anxiety-related behavior in serotonin transporter null mutant mice: the influence of genetic background. *Genes Brain Behav* **2:**365–380.
Holmes A, Murphy DL, and Crawley JN (2003b) Abnormal behavioral phenotypes of serotonin transporter knockout mice: parallels with human anxiety and depression. *Biol Psychiatry* **54:**953–959.
Holton KL, Loder MK, and Melikian HE (2005) Nonclassical, distinct endocytic signals dictate constitutive and PKC-regulated neurotransmitter transporter internalization. *Nat Neurosci* **8:**881–888.
Hopkins AL (2008) Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol* **4:**682–690.
Horiuchi M, El Far O, and Betz H (2000) Ulip6, a novel unc-33 and dihydropyrimidinase related protein highly expressed in developing rat brain. *FEBS Lett* **480:**283–286.
Horiuchi M, Loebrich S, Brandstaetter JH, Kneussel M, and Betz H (2005) Cellular localization and subcellular distribution of Unc-33-like protein 6, a brain-specific protein of the collapsin response mediator protein family that interacts with the neuronal glycine transporter 2. *J Neurochem* **94:**307–315.
Houle S, Ginovart N, Hussey D, Meyer JH, and Wilson AA (2000) Imaging the serotonin transporter with positron emission tomography: initial human studies with [^{11}C]DAPP and [^{11}C]DASB. *Eur J Nucl Med* **27:**1719–1722.
Hu J and Quick MW (2008) Substrate-mediated regulation of γ-aminobutyric acid transporter 1 in rat brain. *Neuropharmacology* **54:**309–318.
Huang CH and Santangelo SL (2008) Autism and serotonin transporter gene polymorphisms: a systematic review and meta-analysis. *Am J Med Genet B Neuropsychiatr Genet* **147B:**903–913.
Huang X and Zhan CG (2007) How dopamine transporter interacts with dopamine: insights from molecular modeling and simulation. *Biophys J* **93:**3627–3639.
Huang Y, Hwang DR, Narendran R, Sudo Y, Chatterjee R, Bae SA, Mawlawi O, Kegeles LS, Wilson AA, Kung HF, et al. (2002) Comparative evaluation in nonhuman primates of five PET radiotracers for imaging the serotonin transporters: [11C]McN 5652, [11C]ADAM, [11C]DASB, [11C]DAPA, and [11C]AFM. *J Cereb Blood Flow Metab* **22:**1377–1398.
Huang Y, Lemieux MJ, Song J, Auer M, and Wang DN (2003) Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. *Science* **301:**616–620.
Huff RA, Vaughan RA, Kuhar MJ, and Uhl GR (1997) Phorbol esters increase dopamine transporter phosphorylation and decrease transport V_{max}. *J Neurochem* **68:**225–232.
Humphreys CJ, Levin J, and Rudnick G (1988) Antidepressant binding to the porcine and human-platelet serotonin transporters. *Mol Pharmacol* **33:**657–663.
Humphreys CJ, Wall SC, and Rudnick G (1994) Ligand-binding to the serotonin transporter: equilibria, kinetics, and ion dependence. *Biochemistry* **33:**9118–9125.
Indarte M, Madura JD, and Surratt CK (2008) Dopamine transporter comparative molecular modeling and binding site prediction using the $LeuT_{Aa}$ leucine transporter as a template. *Proteins* **70:**1033–1046.
Isaac M, Slassi A, Silva KD, Arora J, MacLean N, Hung B, and McCallum K (2001) 5,5-Diaryl-2-amino-4-pentenoates as novel, potent, and selective glycine transporter type-2 reuptake inhibitors. *Bioorg Med Chem Lett* **11:**1371–1373.
Iversen L (2000) Neurotransmitter transporters: fruitful targets for CNS drug discovery. *Mol Psychiatry* **5:**357–362.
Jacobs MT, Zhang YW, Campbell SD, and Rudnick G (2007) Ibogaine, a noncompetitive inhibitor of serotonin transport, acts by stabilizing the cytoplasm-facing state of the transporter. *J Biol Chem* **282:**29441–29447.
James WP, Caterson ID, Coutinho W, Finer N, Van Gaal LF, Maggioni AP, Torp-Pedersen C, Sharma AM, Shepherd GM, Rode RA, et al. (2010) Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. *N Engl J Med* **363:**905–917.
Jardetzky O (1966) Simple allosteric model for membrane pumps. *Nature* **211:**969–970.
Javitch JA (1998) Probing structure of neurotransmitter transporters by substituted-cysteine accessibility method. *Methods Enzymol* **296:**331–346.
Jayanthi LD, Annamalai B, Samuvel DJ, Gether U, and Ramamoorthy S (2006) Phosphorylation of the norepinephrine transporter at threonine 258 and serine 259 is linked to protein kinase C-mediated transporter internalization. *J Biol Chem* **281:**23326–23340.
Jayanthi LD, Samuvel DJ, and Ramamoorthy S (2004) Regulated internalization and phosphorylation of the native norepinephrine transporter in response to phorbol esters. Evidence for localization in lipid rafts and lipid raft-mediated internalization. *J Biol Chem* **279:**19315–19326.
Jayanthi LD, Samuvel DJ, Blakely RD, and Ramamoorthy S (2005) Evidence for biphasic effects of protein kinase C on serotonin transporter function, endocytosis, and phosphorylation. *Mol Pharmacol* **67:**2077–2087.
Jeannotte AM and Sidhu A (2007) Regulation of the norepinephrine transporter by α-synuclein-mediated interactions with microtubules. *Eur J Neurosci* **26:**1509–1520.
Jennings KA, Loder MK, Sheward WJ, Pei Q, Deacon RM, Benson MA, Olverman HJ, Hastie ND, Harmar AJ, Shen S, et al. (2006) Increased expression of the 5-HT transporter confers a low-anxiety phenotype linked to decreased 5-HT transmission. *J Neurosci* **26:**8955–8964.
Jensen K, Chiu CS, Sokolova I, Lester HA, and Mody I (2003) GABA transporter-1 (GAT1) deficient mice: differential tonic activation of $GABA_A$ versus $GABA_B$ receptors in the hippocampus. *J Neurophysiol* **90:**2690–2701.
Jess U, El Far O, Kirsch J, and Betz H (2002) Interaction of the C-terminal region of the rat serotonin transporter with MacMARCKS modulates 5-HT uptake regulation by protein kinase C. *Biochem Biophys Res Commun* **294:**272–279.
Jin C, Navarro HA, and Carroll FI (2008a) Development of 3-phenyltropane analogues with high affinity for the dopamine and serotonin transporters and low affinity for the norepinephrine transporter. *J Med Chem* **51:**8048–8056.
Jin C, Navarro HA, Page K, and Carroll FI (2008b) Synthesis and monoamine transporter binding properties of 2β-[3′-(substituted benzyl)isoxazol-5-yl]- and

2β-[3′-methyl-4′-(substituted phenyl)isoxazol-5-yl]-3β-(substituted phenyl)tropanes. *Bioorg Med Chem* **16:**6682–6688.
Johnson LA, Furman CA, Zhang M, Guptaroy B, and Gnegy ME (2005a) Rapid delivery of the dopamine transporter to the plasmalemmal membrane upon amphetamine stimulation. *Neuropharmacology* **49:**750–758.
Johnson LA, Guptaroy B, Lund D, Shamban S, and Gnegy ME (2005b) Regulation of amphetamine-stimulated dopamine efflux by protein kinase Cβ. *J Biol Chem* **280:**10914–10919.
Johnston GA, Krogsgaard-Larsen P, and Stephanson A (1975) Betel nut constituents as inhibitors of γ-aminobutyric acid uptake. *Nature* **258:**627–628.
Jones SR, Gainetdinov RR, Jaber M, Giros B, Wightman RM, and Caron MG (1998) Profound neuronal plasticity in response to inactivation of the dopamine transporter. *Proc Natl Acad Sci USA* **95:**4029–4034.
Jorenby D (2002) Clinical efficacy of bupropion in the management of smoking cessation. *Drugs* **62:**25–35.
Jørgensen AM, Tagmose L, Jørgensen AM, Bøgesø KP, and Peters GH (2007a) Molecular dynamics simulations of Na^+/Cl^--dependent neurotransmitter transporters in a membrane-aqueous system. *ChemMedChem* **2:**827–840.
Jørgensen AM, Tagmose L, Jørgensen AM, Topiol S, Sabio M, Gundertofte K, Bøgesø KP, and Peters GH (2007b) Homology modeling of the serotonin transporter: insights into the primary escitalopram-binding site. *ChemMedChem* **2:**815–826.
Jørgensen S, Nielsen EØ, Peters D, and Dyhring T (2008) Validation of a fluorescence-based high-throughput assay for the measurement of neurotransmitter transporter uptake activity. *J Neurosci Methods* **169:**168–176.
Ju P, Aubrey KR, and Vandenberg RJ (2004) Zn^{2+} inhibits glycine transport by glycine transporter subtype 1b. *J Biol Chem* **279:**22983–22991.
Jursky F, Tamura S, Tamura A, Mandiyan S, Nelson H, and Nelson N (1994) Structure, function and brain localization of neurotransmitter transporters. *J Exp Biol* **196:**283–295.
Just H, Sitte HH, Schmid JA, Freissmuth M, and Kudlacek O (2004) Identification of an additional interaction domain in transmembrane domains 11 and 12 that supports oligomer formation in the human serotonin transporter. *J Biol Chem* **279:**6650–6657.
Kahlig KM, Javitch JA, and Galli A (2004) Amphetamine regulation of dopamine transport. Combined measurements of transporter currents and transporter imaging support the endocytosis of an active carrier. *J Biol Chem* **279:**8966–8975.
Kahlig KM, Lute BJ, Wei Y, Loland CJ, Gether U, Javitch JA, and Galli A (2006) Regulation of dopamine transporter trafficking by intracellular amphetamine. *Mol Pharmacol* **70:**542–548.
Kaiser C and Setler PE (1981) Antipsychotic agents, in *Burger's Medicinal Chemistry* (Wolff ME ed) pp 859–980, John Wiley & Sons, New York.
Kanner BI (2006) Structure and function of sodium-coupled GABA and glutamate transporters. *J Membr Biol* **213:**89–100.
Kanner BI (2008) Structural biology: it's not all in the family. *Nature* **454:**593–594.
Kanner BI, Bendahan A, Pantanowitz S, and Su H (1994) The number of amino acid residues in hydrophilic loops connecting transmembrane domains of the GABA transporter GAT-1 is critical for its function. *FEBS Lett* **356:**191–194.
Kanner BI and Zomot E (2008) Sodium-coupled neurotransmitter transporters. *Chem Rev* **108:**1654–1668.
Karakossian MH, Spencer SR, Gomez AQ, Padilla OR, Sacher A, Loo DD, Nelson N, and Eskandari S (2005) Novel properties of a mouse γ-aminobutyric acid transporter (GAT4). *J Membr Biol* **203:**65–82.
Kardos J, Palló A, Bencsura A and Simon A (2010) Assessing structure, function and druggability of major inhibitory neurotransmitter γ-aminobutyrate symporter subtypes. *Curr Med Chem* **17:**2203–2213.
Karlin A and Akabas MH (1998) Substituted-cysteine accessibility method. *Methods Enzymol* **293:**123–145.
Katz JL, Kopajtic TA, Agoston GE, and Newman AH (2004) Effects of *N*-substituted analogs of benztropine: diminished cocaine-like effects in dopamine transporter ligands. *J Pharmacol Exp Ther* **309:**650–660.
Kaufmann KW, Dawson ES, Henry LK, Field JR, Blakely RD, and Meiler J (2009) Structural determinants of species-selective substrate recognition in human and *Drosophila* serotonin transporters revealed through computational docking studies. *Proteins* **74:**630–642.
Kavanaugh MP, Arriza JL, North RA, and Amara SG (1992) Electrogenic uptake of γ-aminobutyric acid by a cloned transporter expressed in *Xenopus* oocytes. *J Biol Chem* **267:**22007–22009.
Keller NR, Diedrich A, Appalsamy M, Tuntrakool S, Lonce S, Finney C, Caron MG, and Robertson D (2004a) Norepinephrine transporter-deficient mice exhibit excessive tachycardia and elevated blood pressure with wakefulness and activity. *Circulation* **110:**1191–1196.
Keller PC 2nd, Stephan M, Glomska H, and Rudnick G (2004b) Cysteine-scanning mutagenesis of the fifth external loop of serotonin transporter. *Biochemistry* **43:**8510–8516.
Kent JM (2000) SNaRIs, NaSSAs, and NaRIs: new agents for the treatment of depression. *Lancet* **355:**911–918.
Keshet GI, Bendahan A, Su H, Mager S, Lester HA, and Kanner BI (1995) Glutamate-101 is critical for the function of the sodium and chloride-coupled GABA transporter GAT-1. *FEBS Lett* **371:**39–42.
Keyes SR and Rudnick G (1982) Coupling of transmembrane proton gradients to platelet serotonin transport. *J Biol Chem* **257:**1172–1176.
Keynan S and Kanner BI (1988) γ-Aminobutyric acid transport in reconstituted preparations from rat brain: coupled sodium and chloride fluxes. *Biochemistry* **27:**12–17.
Khoshbouei H, Sen N, Guptaroy B, Johnson L, Lund D, Gnegy ME, Galli A, and Javitch JA (2004) N-terminal phosphorylation of the dopamine transporter is required for amphetamine-induced efflux. *PLoS Biol* **2:**E78.
Kilic F, Murphy DL, and Rudnick G (2003) A human serotonin transporter mutation causes constitutive activation of transport activity. *Mol Pharmacol* **64:**440–446.
Kilic F and Rudnick G (2000) Oligomerization of serotonin transporter and its functional consequences. *Proc Natl Acad Sci USA* **97:**3106–3111.
Kilty JE, Lorang D, and Amara SG (1991) Cloning and expression of a cocaine-sensitive rat dopamine transporter. *Science* **254:**578–579.
Kim E and Sheng M (2004) PDZ domain proteins of synapses. *Nat Rev Neurosci* **5:**771–781.
Kim KM, Kingsmore SF, Han H, Yang-Feng TL, Godinot N, Seldin MF, Caron MG, and Giros B (1994) Cloning of the human glycine transporter type 1: molecular and pharmacological characterization of novel isoform variants and chromosomal localization of the gene in the human and mouse genomes. *Mol Pharmacol* **45:**608–617.
Kinney GG, Sur C, Burno M, Mallorga PJ, Williams JB, Figueroa DJ, Wittmann M, Lemaire W, and Conn PJ (2003) The glycine transporter type 1 inhibitor *N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]sarcosine potentiates NMDA receptor-mediated responses in vivo and produces an antipsychotic profile in rodent behavior. *J Neurosci* **23:**7586–7591.
Kitayama S and Dohi T (2003) Norepinephrine transporter splice variants and their interaction with substrates and blockers. *Eur J Pharmacol* **479:**65–70.
Kitayama S, Shimada S, Xu H, Markham L, Donovan DM, and Uhl GR (1992) Dopamine transporter site-directed mutations differentially alter substrate transport and cocaine binding. *Proc Natl Acad Sci USA* **89:**7782–7785.
Kiyohara C and Yoshimasu K (2010) Association between major depressive disorder and a functional polymorphism of the 5-hydroxytryptamine (serotonin) transporter gene: a meta-analysis. *Psychiatr Genet* **20:**49–58.
Kniazeff J, Loland CJ, Goldberg N, Quick M, Das S, Sitte HH, Javitch JA, and Gether U (2005) Intramolecular cross-linking in a bacterial homolog of mammalian SLC6 neurotransmitter transporters suggests an evolutionary conserved role of transmembrane segments 7 and 8. *Neuropharmacology* **49:**715–723.
Kniazeff J, Shi L, Loland CJ, Javitch JA, Weinstein H, and Gether U (2008) An intracellular interaction network regulates conformational transitions in the dopamine transporter. *J Biol Chem* **283:**17691–17701.
Koldsø H, Severinsen K, Tran TT, Celik L, Jensen HH, Wiborg O, Schiøtt B, and Sinning S (2010) The two enantiomers of citalopram bind to the human serotonin transporter in reversed orientations. *J Am Chem Soc* **132:**1311–1322.
Kragler A, Höfner G, and Wanner KT (2005) Novel parent structures for inhibitors of the murine GABA transporters mGAT3 and mGAT4. *Eur J Pharmacol* **519:**43–47.
Krieter PA, Gohdes M, Musick TJ, Duncanson FP, and Bridson WE (2008) Pharmacokinetics, disposition, and metabolism of bicifadine in humans. *Drug Metab Dispos* **36:**252–259.
Krishnamurthy H, Piscitelli CL, and Gouaux E (2009) Unlocking the molecular secrets of sodium-coupled transporters. *Nature* **459:**347–355.
Kristensen AS, Larsen MB, Johnsen LB, and Wiborg O (2004) Mutational scanning of the human serotonin transporter reveals fast translocating serotonin transporter mutants. *Eur J Neurosci* **19:**1513–1523.
Krogsgaard-Larsen P, Falch E, Larsson OM, and Schousboe A (1987) GABA uptake inhibitors: relevance to antiepileptic drug research. *Epilepsy Res* **1:**77–93.
Krogsgaard-Larsen P, Frølund B, and Frydenvang K (2000) GABA uptake inhibitors. Design, molecular pharmacology and therapeutic aspects. *Curr Pharm Des* **6:**1193–1209.
Krogsgaard-Larsen P and Johnston GA (1975) Inhibition of GABA uptake in rat brain slices by nipectoic acid, various isoxazoles and related compounds. *J Neurochem* **25:**797–802.
Krogsgaard-Larsen P, Johnston GA, Curtis DR, Game CJ, and McCulloch RM (1975) Structure and biological-activity of a series of conformationally restricted analogues of GABA. *J Neurochem* **25:**803–809.
Kuhn R (1958) The treatment of depressive states with G-22355 (imipramine hydrochloride). *Am J Psychiatry* **115:**459–464.
Kurian MA, Zhen J, Cheng SY, Li Y, Mordekar SR, Jardine P, Morgan NV, Meyer E, Tee L, Pasha S, et al. (2009) Homozygous loss-of-function mutations in the gene encoding the dopamine transporter are associated with infantile parkinsonism-dystonia. *J Clin Invest* **119:**1595–1603.
Kvist T, Christiansen B, Jensen AA, and Bräuner-Osborne H (2009) The four human γ-aminobutyric acid (GABA) transporters: pharmacological characterization and validation of a highly efficient screening assay. *Comb Chem High Throughput Screen* **12:**241–249.
Laakso A and Hietala J (2000) PET studies of brain monoamine transporters. *Curr Pharm Des* **6:**1611–1623.
Labbate LA, Croft HA, and Oleshansky MA (2003) Antidepressant-related erictile dysfunction: management via avoidance, switching antidepressants, antidotes, and adaptation. *J Clin Psychiatry* **64:**11–19.
LaRoche SM and Helmers SL (2004) The new antiepileptic drugs: scientific review. *JAMA* **291:**605–614.
Larsen MB, Elfving B, and Wiborg O (2004) The chicken serotonin transporter discriminates between serotonin-selective reuptake inhibitors. A species-scanning mutagenesis study. *J Biol Chem* **279:**42147–42156.
Lasky-Su JA, Faraone SV, Glatt SJ, and Tsuang MT (2005) Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. *Am J Med Genet B Neuropsychiatr Genet* **133B:**110–115.
Law RM, Stafford A, and Quick MW (2000) Functional regulation of γ-aminobutyric acid transporters by direct tyrosine phosphorylation. *J Biol Chem* **275:**23986–23991.
Lechner SM (2006) Glutamate-based therapeutic approaches: inhibitors of glycine transport. *Curr Opin Pharmacol* **6:**75–81.
Lee FJ, Liu F, Pristupa ZB, and Niznik HB (2001) Direct binding and functional coupling of α-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. *FASEB J* **15:**916–926.
Lee FJ, Pei L, and Liu F (2009) Disruption of the dopamine transporter-dopamine D2 receptor interaction in schizophrenia. *Synapse* **63:**710–712.
Lee FJ, Pei L, Moszczynska A, Vukusic B, Fletcher PJ, and Liu F (2007) Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D_2 receptor. *EMBO J* **26:**2127–2136.
Lee KH, Kim MY, Kim DH, and Lee YS (2004) Syntaxin 1A and receptor for

activated C kinase interact with the N-terminal region of human dopamine transporter. *Neurochem Res* **29:**1405–1409.
Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, and Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274:**1527–1531.
Lester HA, Cao Y, and Mager S (1996) Listening to neurotransmitter transporters. *Neuron* **17:**807–810.
Lew R, Vaughan R, Simantov R, Wilson A, and Kuhar MJ (1991) Dopamine transporters in the nucleus accumbens and the striatum have different apparent molecular weights. *Synapse* **8:**152–153.
Li LB, Chen N, Ramamoorthy S, Chi L, Cui XN, Wang LC, and Reith ME (2004) The role of N-glycosylation in function and surface trafficking of the human dopamine transporter. *J Biol Chem* **279:**21012–21020.
Liang Y, Shaw AM, Boules M, Briody S, Robinson J, Oliveros A, Blazar E, Williams K, Zhang Y, Carlier PR, et al. (2008) Antidepressant-like pharmacological profile of a novel triple reuptake inhibitor, (1*S*,2*S*)-3-(methylamino)-2-(naphthalen-2-yl)-1-phenylpropan-1-ol (PRC200-SS). *J Pharmacol Exp Ther* **327:**573–583.
Lin PY (2007) Meta-analysis of the association of serotonin transporter gene polymorphism with obsessive-compulsive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* **31:**683–689.
Lin Z and Uhl GR (2002) Dopamine transporter mutants with cocaine resistance and normal dopamine uptake provide targets for cocaine antagonism. *Mol Pharmacol* **61:**885–891.
Lin Z, Wang W, Kopajtic T, Revay RS, and Uhl GR (1999) Dopamine transporter: transmembrane phenylalanine mutations can selectively influence dopamine uptake and cocaine analog recognition. *Mol Pharmacol* **56:**434–447.
Lin Z, Zhang PW, Zhu X, Melgari JM, Huff R, Spieldoch RL, and Uhl GR (2003) Phosphatidylinositol 3-kinase, protein kinase C, and MEK1/2 kinase regulation of dopamine transporters (DAT) require N-terminal DAT phosphoacceptor sites. *J Biol Chem* **278:**20162–20170.
Little KY, Elmer LW, Zhong H, Scheys JO, and Zhang L (2002) Cocaine induction of dopamine transporter trafficking to the plasma membrane. *Mol Pharmacol* **61:**436–445.
Little KY, Kirkman JA, Carroll FI, Breese GR, and Duncan GE (1993) [^{125}I]RTI-55 binding to cocaine-sensitive dopaminergic and serotonergic uptake sites in the human brain. *J Neurochem* **61:**1996–2006.
Liu GX, Cai GQ, Cai YQ, Sheng ZJ, Jiang J, Mei Z, Wang ZG, Guo L, and Fei J (2007) Reduced anxiety and depression-like behaviors in mice lacking GABA transporter subtype 1. *Neuropsychopharmacology* **32:**1531–1539.
Liu QR, López-Corcuera B, Mandiyan S, Nelson H, and Nelson N (2003) Molecular characterization of four pharmacologically distinct α-aminobutyric acid transporters in mouse brain. *J Biol Chem* **268:**2106–2112.
Liu QR, López-Corcuera B, Mandiyan S, Nelson H, and Nelson N (1993) Cloning and expression of a spinal cord-specific and brain-specific glycine transporter with novel structural features. *J Biol Chem* **268:**22802–22808.
Liu QR, López-Corcuera B, Nelson H, Mandiyan S, and Nelson N (1992a) Cloning and expression of a cDNA encoding the transporter of taurine and β-alanine in mouse brain. *Proc Natl Acad Sci USA* **89:**12145–12149.
Liu QR, Nelson H, Mandiyan S, López-Corcuera B, and Nelson N (1992b) Cloning and expression of a glycine transporter from mouse brain. *FEBS Lett* **305:**110–114.
Liu X, Mitrovic AD, and Vandenberg RJ (2009) Glycine transporter 1 associates with cholesterol-rich membrane raft microdomains. *Biochem Biophys Res Commun* **384:**530–534.
Loder MK and Melikian HE (2003) The dopamine transporter constitutively internalizes and recycles in a protein kinase C-regulated manner in stably transfected PC12 cell lines. *J Biol Chem* **278:**22168–22174.
Loland CJ, Desai RI, Zou MF, Cao J, Grundt P, Gerstbrein K, Sitte HH, Newman AH, Katz JL, and Gether U (2008) Relationship between conformational changes in the dopamine transporter and cocaine-like subjective effects of uptake inhibitors. *Mol Pharmacol* **73:**813–823.
Loland CJ, Grånäs C, Javitch JA, and Gether U (2004) Identification of intracellular residues in the dopamine transporter critical for regulation of transporter conformation and cocaine binding. *J Biol Chem* **279:**3228–3238.
Loland CJ, Norgaard-Nielsen K, and Gether U (2003) Probing dopamine transporter structure and function by Zn2+-site engineering. *Eur J Pharmacol* **479:**187–197.
Loland CJ, Norregaard L, and Gether U (1999) Defining proximity relationships in the tertiary structure of the dopamine transporter. Identification of a conserved glutamic acid as a third coordinate in the endogenous Zn^{2+}-binding site. *J Biol Chem* **274:**36928–36934.
Loland CJ, Norregaard L, Litman T, and Gether U (2002) Generation of an activating Zn^{2+} switch in the dopamine transporter: mutation of an intracellular tyrosine constitutively alters the conformational equilibrium of the transport cycle. *Proc Natl Acad Sci USA* **99:**1683–1688.
Lolkema JS and Slotboom DJ (2008) The major amino acid transporter superfamily has a similar core structure as Na^+-galactose and Na^+-leucine transporters. *Mol Membr Biol* **25:**567–570.
Loo DD, Eskandari S, Boorer KJ, Sarkar HK, and Wright EM (2000) Role of Cl^- in electrogenic Na^+-coupled cotransporters GAT1 and SGLT1. *J Biol Chem* **275:**37414–37422.
Lopez-Corcuera B, Liu QR, Mandiyan S, Nelson H, and Nelson N (1992) Expression of a mouse brain cDNA encoding novel γ-aminobutyric acid transporter. *J Biol Chem* **267:**17491–17493.
López-Corcuera B, Martínez-Maza R, Núñez E, Roux M, Supplisson S, and Aragón C (1998) Differential properties of two stably expressed brain-specific glycine transporters. *J Neurochem* **71:**2211–2219.
López-Corcuera B, Núñez E, Martínez-Maza R, Geerlings A, and Aragón C (2001) Substrate-induced conformational changes of extracellular loop 1 in the glycine transporter GLYT2. *J Biol Chem* **276:**43463–43470.
Lowe JA 3rd, Drozda SE, Fisher K, Strick C, Lebel L, Schmidt C, Hiller D, and Zandi KS (2003) [^{3}H]-(*R*)-NPTS, a radioligand for the type 1 glycine transporter. *Bioorg Med Chem Lett* **13:**1291–1292.
MacAulay N, Bendahan A, Loland CJ, Zeuthen T, Kanner BI, and Gether U (2001) Engineered Zn^{2+} switches in the γ-aminobutyric acid (GABA) transporter-1. Differential effects on GABA uptake and currents. *J Biol Chem* **276:**40476–40485.
Macdonald RL and Olsen RW (1994) $GABA_A$ receptor channels. *Ann Rev Neurosci* **17:**569–602.
Machado RD, Koehler R, Glissmeyer E, Veal C, Suntharalingam J, Kim M, Carlquist J, Town M, Elliott CG, Hoeper M, et al. (2006) Genetic association of the serotonin transporter in pulmonary arterial hypertension. *Am J Respir Crit Care Med* **173:**793–797.
Madsen KK, Clausen RP, Larsson OM, Krogsgaard-Larsen P, Schousboe A, and White HS (2009) Synaptic and extrasynaptic GABA transporters as targets for anti-epileptic drugs. *J Neurochem* **109:**139–144.
Madsen KK, White HS, and Schousboe A (2010) Neuronal and non-neuronal GABA transporters as targets for antiepileptic drugs. *Pharmacol Ther* **125:**394–401.
Mager S, Kleinberger-Doron N, Keshet GI, Davidson N, Kanner BI, and Lester HA (1996) Ion binding and permeation at the GABA transporter GAT1. *J Neurosci* **16:**5405–5414.
Mager S, Naeve J, Quick M, Labarca C, Davidson N, and Lester HA (1993) Steady states, charge movements, and rates for a cloned GABA transporter expressed in *Xenopus* oocytes. *Neuron* **10:**177–188.
Magnani F, Tate CG, Wynne S, Williams C, and Haase J (2004) Partitioning of the serotonin transporter into lipid microdomains modulates transport of serotonin. *J Biol Chem* **279:**38770–38778.
Mallorga PJ, Williams JB, Jacobson M, Marques R, Chaudhary A, Conn PJ, Pettibone DJ, and Sur C (2003) Pharmacology and expression analysis of glycine transporter GlyT1 with [^{3}H]-(*N*-[3-(4′-fluorophenyl)-3-(4′-phenylphenoxy)propyl]) sarcosine. *Neuropharmacology* **45:**585–593.
Marazziti D, Mandillo S, Di Pietro C, Golini E, Matteoni R, and Tocchini-Valentini GP (2007) GPR37 associates with the dopamine transporter to modulate dopamine uptake and behavioral responses to dopaminergic drugs. *Proc Natl Acad Sci USA* **104:**9846–9851.
Marcusson J and Tiger G (1988) [^{3}H]Imipramine binding of protein nature in human platelets: inhibition by 5-hydroxytryptamine and 5-hydroxytryptamine uptake inhibitors. *J Neurochem* **50:**1032–1036.
Martina M, Gorfinkel Y, Halman S, Lowe JA, Periyalwar P, Schmidt CJ, and Bergeron R (2004) Glycine transporter type 1 blockade changes NMDA receptor-mediated responses and LTP in hippocampal CA1 pyramidal cells by altering extracellular glycine levels. *J Physiol* **557:**489–500.
Martínez-Maza R, Poyatos I, López-Corcuera B, N úñez E, Giménez C, Zafra F, and Aragón C (2001) The role of N-glycosylation in transport to the plasma membrane and sorting of the neuronal glycine transporter GLYT2. *J Biol Chem* **276:**2168–2173.
Massari S, Vanoni C, Longhi R, Rosa P, and Pietrini G (2004) Protein kinase C-mediated phosphorylation of the BGT1 epithelial γ-aminobutyric acid transporter regulates its association with LIN7 PDZ proteins: a post-translational mechanism regulating transporter surface density. *J Biol Chem* **280:**7388–7397.
Massari S, Vanoni C, Longhi R, Rosa P, and Pietrini G (2005) Protein kinase C-mediated phosphorylation of the BGT1 epithelial γ-aminobutyric acid transporter regulates its association with LIN7 PDZ proteins: a post-translational mechanism regulating transporter surface density. *J Biol Chem* **280:**7388–7397.
Matskevitch I, Wagner CA, Stegen C, Bröer S, Noll B, Risler T, Kwon HM, Handler JS, Waldegger S, Busch AE, et al. (1999) Functional characterization of the betaine/γ-aminobutyric acid transporter BGT-1 expressed in *Xenopus* oocytes. *J Biol Chem* **274:**16709–16716.
Matthies HJ, Han Q, Shields A, Wright J, Moore JL, Winder DG, Galli A, and Blakely RD (2009) Subcellular localization of the antidepressant-sensitive norepinephrine transporter. *BMC Neurosci* **10:**65.
Matthies HJ, Moore JL, Saunders C, Matthies DS, Lapierre LA, Goldenring JR, Blakely RD, and Galli A (2010) Rab11 supports amphetamine-stimulated norepinephrine transporter trafficking. *J Neurosci* **30:**7863–7877.
Mazei-Robison MS and Blakely RD (2005) Expression studies of naturally occurring human dopamine transporter variants identifies a novel state of transporter inactivation associated with Val382Ala. *Neuropharmacology* **49:**737–749.
Mazei-Robison MS, Bowton E, Holy M, Schmudermaier M, Freissmuth M, Sitte HH, Galli A, and Blakely RD (2008) Anomalous dopamine release associated with a human dopamine transporter coding variant. *J Neurosci* **28:**7040–7046.
Mazei-Robison MS, Couch RS, Shelton RC, Stein MA, and Blakely RD (2005) Sequence variation in the human dopamine transporter gene in children with attention deficit hyperactivity disorder. *Neuropharmacology* **49:**724–736.
McConathy J, Owens MJ, Kilts CD, Malveaux EJ, Camp VM, Votaw JR, Nemeroff CB, and Goodman MM (2004) Synthesis and biological evaluation of [^{11}C]talopram and [^{11}C]talsupram: candidate PET ligands for the norepinephrine transporter. *Nucl Med Biol* **31:**705–718.
McHugh EM, Zhu W, Milgram S, and Mager S (2004) The GABA transporter GAT1 and the MAGUK protein Pals1: interaction, uptake modulation, and coexpression in the brain. *Mol Cell Neurosci* **26:**406–417.
McIntosh JM, Corpuz GO, Layer RT, Garrett JE, Wagstaff JD, Bulaj G, Vyazovkina A, Yoshikami D, Cruz LJ, and Olivera BM (2000) Isolation and characterization of a novel conus peptide with apparent antinociceptive activity. *J Biol Chem* **275:**32391–32397.
Mead AN, Rocha BA, Donovan DM, and Katz JL (2002) Intravenous cocaine induced-activity and behavioural sensitization in norepinephrine-, but not dopamine-transporter knockout mice. *Eur J Neurosci* **16:**514–520.
Meiergerd SM, McElvain JS, and Schenk JO (1994) Effects of cocaine and repeated cocaine followed by withdrawal. Alterations of dopaminergic transporter turnover with no changes in kinetics of substrate recognition. *Biochem Pharmacol* **47:**1627–1634.
Meinild AK, Loo DD, Skovstrup S, Gether U, and MacAulay N (2009) Elucidating

conformational changes in the γ-aminobutyric acid transporter-1. *J Biol Chem* **284:**16226–16235.
Meldrum BS and Rogawski MA (2007) Molecular targets for antiepileptic drug development. *Neurotherapeutics* **4:**18–61.
Melikian HE and Buckley KM (1999) Membrane trafficking regulates the activity of the human dopamine transporter. *J Neurosci* **19:**7699–7710.
Melikian HE, McDonald JK, Gu H, Rudnick G, Moore KR, and Blakely RD (1994) Human norepinephrine transporter. Biosynthetic studies using a site-directed polyclonal antibody. *J Biol Chem* **269:**12290–12297.
Melikian HE, Ramamoorthy S, Tate CG, and Blakely RD (1996) Inability to N-glycosylate the human norepinephrine transporter reduces protein stability, surface trafficking, and transport activity but not ligand recognition. *Mol Pharmacol* **50:**266–276.
Melloni P, Carniel G, Dellatorre A, Bonsignori A, Buonamici M, Pozzi O, Ricciardi S, and Rossi AC (1984) Potential antidepressant agents: α-aryloxy-benzyl derivatives of ethanolamine and morpholine. *Eur J Med Chem* **19:**235–242.
Meyer JH (2007) Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J Psychiatry Neurosci* **32:**86–102.
Meyer JH, Houle S, Sagrati S, Carella A, Hussey DF, Ginovart N, Goulding V, Kennedy J, and Wilson AA (2004) Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. *Arch Gen Psychiatry* **61:**1271–1279.
Mezler M, Hornberger W, Mueller R, Schmidt M, Amberg MS, Amberg W, Amberg MS, Braje W, Ochse M, Schoemaker H, et al. (2008) Inhibitors of GlyT1 affect glycine transport via discrete binding sites. *Mol Pharmacol* **74:**1705–1715.
Micó JA, Ardid D, Berrocoso E, and Eschalier A (2006) Antidepressants and pain. *Trends Pharmacol Sci* **27:**348–354.
Millan MJ (2006) Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol Ther* **110:**135–370.
Millan MJ (2009) Dual- and triple-acting agents for treating core and co-morbid symptoms of major depression: novel concepts, new drugs. *Neurotherapeutics* **6:**53–77.
Miller KJ and Hoffman BJ (1994) Adenosine A_3 receptors regulate serotonin transport via nitric oxide and cGMP. *J Biol Chem* **269:**27351–27356.
Miranda M and Sorkin A (2007) Regulation of receptors and transporters by ubiquitination: new insights into surprisingly similar mechanisms. *Mol Interv* **7:**157–167.
Miranda M, Sorkina T, Grammatopoulos TN, Zawada WM, and Sorkin A (2004) Multiple molecular determinants in the carboxyl terminus regulate dopamine transporter export from endoplasmic reticulum. *J Biol Chem* **279:**30760–30770.
Miranda M, Wu CC, Sorkina T, Korstjens DR, and Sorkin A (2005) Enhanced ubiquitylation and accelerated degradation of the dopamine transporter mediated by protein kinase C. *J Biol Chem* **280:**35617–35624.
Mitchell P (1957) A general theory of membrane transport from studies of bacteria. *Nature* **180:**134–136.
Mitchell P (1990) Osmochemistry of solute translocation. *Res Microbiol* **141:**286–289.
Mitchell SM, Lee E, Garcia ML, and Stephan MM (2004) Structure and function of extracellular loop 4 of the serotonin transporter as revealed by cysteine-scanning mutagenesis. *J Biol Chem* **279:**24089–24099.
Moltzen EK and Bang-Andersen B (2006) Serotonin reuptake inhibitors: the corner stone in treatment of depression for half a century. *Curr Top Med Chem* **6:**1801–1823.
Morioka N, Abdin JM, Morita K, Kitayama T, Nakata Y, and Dohi T (2008) The regulation of glycine transporter GLYT1 is mainly mediated by protein kinase Cα in C6 glioma cells. *Neurochem Int* **53:**248–254.
Morón JA, Zakharova I, Ferrer JV, Merrill GA, Hope B, Lafer EM, Lin ZC, Wang JB, Javitch JA, Galli A, et al. (2003) Mitogen-activated protein kinase regulates dopamine transporter surface expression and dopamine transport capacity. *J Neurosci* **23:**8480–8488.
Mortensen OV, Kristensen AS, and Wiborg O (2001) Species-scanning mutagenesis of the serotonin transporter reveals residues essential in selective, high-affinity recognition of antidepressants. *J Neurochem* **79:**237–247.
Mortensen OV, Kristensen AS, Rudnick G, and Wiborg O (1999) Molecular cloning, expression and characterization of a bovine serotonin transporter. *Mol Brain Res* **71:**120–126.
Mortensen OV, Larsen MB, Prasad BM, and Amara SG (2008) Genetic complementation screen identifies a mitogen-activated protein kinase phosphatase, MKP3, as a regulator of dopamine transporter trafficking. *Mol Biol Cell* **19:**2818–2829.
Moszczynska A, Saleh J, Zhang H, Vukusic B, Lee FJ, and Liu F (2007) Parkin disrupts the α-synuclein/dopamine transporter interaction: consequences toward dopamine-induced toxicity. *J Mol Neurosci* **32:**217–227.
Müller HK, Wiborg O, and Haase J (2006) Subcellular redistribution of the serotonin transporter by secretory carrier membrane protein 2. *J Biol Chem* **281:**28901–28909.
Munafò MR, Durrant C, Lewis G, and Flint J (2009) Gene X environment interactions at the serotonin transporter locus. *Biol Psychiatry* **65:**211–219.
Murphy DL and Lesch KP (2008) Targeting the murine serotonin transporter: insights into human neurobiology. *Nat Rev Neurosci* **9:**85–96.
Muth EA, Haskins JT, Moyer JA, Husbands GE, Nielsen ST, and Sigg EB (1986) Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivative. *Biochem Pharmacol* **35:**4493–4497.
Nelson N (1998) The family of Na^+/Cl^- neurotransmitter transporters. *J Neurochem* **71:**1785–1803.
Nemeroff CB and Owens MJ (2002) Treatment of mood disorders. *Nat Neurosci* **5:**1068–1070.
Neubauer HA, Hansen CG, and Wiborg O (2006) Dissection of an allosteric mechanism on the serotonin transporter: a cross-species study. *Mol Pharmacol* **69:**1242–1250.
Newman AH, Kline RH, Allen AC, Izenwasser S, George C, and Katz JL (1995) Novel 4′-substituted and 4′,4″-disubstituted 3-α-(diphenylmethoxy)tropane analogs as potent and selective dopamine uptake inhibitors. *J Med Chem* **38:**3933–3940.
Newman AH and Kulkarni S (2002) Probes for the dopamine transporter: new leads toward a cocaine-abuse therapeutic–a focus on analogues of benztropine and rimcazole. *Med Res Rev* **22:**429–464.
Nguyen TT and Amara SG (1996) N-linked oligosaccharides are required for cell surface expression of the norepinephrine transporter but do not influence substrate or inhibitor recognition. *J Neurochem* **67:**645–655.
Ni YG, Chen JG, Androutsellis-Theotokis A, Huang CJ, Moczydlowski E, and Rudnick G (2001) A lithium-induced conformational change in serotonin transporter alters cocaine binding, ion conductance, and reactivity of Cys-109. *J Biol Chem* **276:**30942–30947.
Nielsen CK, Lewis RJ, Alewood D, Drinkwater R, Palant E, Patterson M, Yaksh TL, McCumber D, and Smith MT (2005) Anti-allodynic efficacy of the chi-conopeptide, Xen2174, in rats with neuropathic pain. *Pain* **118:**112–124.
Nielsen EB, Suzdak PD, Andersen KE, Knutsen LJ, Sonnewald U, and Braestrup C (1991) Characterization of tiagabine (NO-328), a new potent and selective GABA uptake inhibitor. *Eur J Pharmacol* **196:**257–266.
Nirenberg MJ, Vaughan RA, Uhl GR, Kuhar MJ, and Pickel VM (1996) The dopamine transporter is localized to dendritic and axonal plasma membranes of nigrostriatal dopaminergic neurons. *J Neurosci* **16:**436–447.
Norgaard-Nielsen K and Gether U (2006) Zn^{2+} modulation of neurotransmitter transporters. *Handb Exp Pharmacol* **(175):**1–22.
Norregaard L, Frederiksen D, Nielsen EO, and Gether U (1998) Delineation of an endogenous zinc-binding site in the human dopamine transporter. *EMBO Journal* **17:**4266–4273.
Norregaard L and Gether U (2001) The monoamine neurotransmitter transporters: structure, conformational changes and molecular gating. *Curr Opin Drug Discov Devel* **4:**591–601.
Norregaard L, Loland CJ, and Gether U (2003) Evidence for distinct sodium-, dopamine-, and cocaine-dependent conformational changes in transmembrane segments 7 and 8 of the dopamine transporter. *J Biol Chem* **278:**30587–30596.
Norregaard L, Visiers I, Loland CJ, Ballesteros J, Weinstein H, and Gether U (2000) Structural probing of a microdomain in the dopamine transporter by engineering of artificial Zn^{2+} binding sites. *Biochemistry* **39:**15836–15846.
Noskov SY (2008) Molecular mechanism of substrate specificity in the bacterial neutral amino acid transporter LeuT. *Proteins* **73:**851–863.
Noskov SY and Roux B (2008) Control of ion selectivity in LeuT: two Na^+ binding sites with two different mechanisms. *J Mol Biol* **377:**804–818.
Nourry C, Grant SG, and Borg JP (2003) PDZ domain proteins: plug and play! *Sci STKE* **2003:**RE7.
Núñez E, Pérez-Siles G, Rodenstein L, Alonso-Torres P, Zafra F, Jiménez E, Aragón C, and López-Corcuera B (2009) Subcellular localization of the neuronal glycine transporter GLYT2 in brainstem. *Traffic* **10:**829–843.
Nurnberg HG, Hensley PL, Gelenberg AJ, Fava M, Lauriello J, and Paine S (2003) Treatment of antidepressant-associated sexual dysfunction with sildenafil: a randomized controlled trial. *JAMA* **289:**56–64.
O'Riordan C, Phillips OM, and Williams DC (1990) Two affinity states for [^{3}H]imipramine binding to the human platelet 5-hydroxytryptamine carrier: an explanation for the allosteric interaction between 5-hydroxytryptamine and imipramine. *J Neurochem* **54:**1275–1280.
Ohno K, Koroll M, El Far O, Scholze P, Gomeza J, and Betz H (2004) The neuronal glycine transporter 2 interacts with the PDZ domain protein syntenin-1. *Mol Cell Neurosci* **26:**518–529.
Olivares L, Aragón C, Giménez C, and Zafra F (1995) The role of N-glycosylation in the targeting and activity of the GLYT1 glycine transporter. *J Biol Chem* **270:**9437–9442.
Osawa I, Saito N, Koga T, and Tanaka C (1994) Phorbol ester-induced inhibition of GABA uptake by synaptosomes and by *Xenopus* oocytes expressing GABA transporter (GAT1). *Neurosci Res* **19:**287–293.
Owens MJ, Knight DL, and Nemeroff CB (2001) Second-generation SSRIs: human monoamine transporter binding profile of escitalopram and *R*-fluoxetine. *Biol Psychiatry* **50:**345–350.
Oz M, Libby T, Kivell B, Jaligam V, Ramamoorthy S, and Shippenberg TS (2010) Real-time, spatially resolved analysis of serotonin transporter activity and regulation using the fluorescent substrate, ASP^+. *J Neurochem* **114:**1019–1029.
Ozaki N, Goldman D, Kaye WH, Plotnicov K, Greenberg BD, Lappalainen J, Rudnick G, and Murphy DL (2003) Serotonin transporter missense mutation associated with a complex neuropsychiatric phenotype. *Mol Psychiatry* **8:**933–936.
Ozaslan D, Wang S, Ahmed BA, Kocabas AM, McCastlain JC, Bene A, and Kilic F (2003) Glycosyl modification facilitates homo- and hetero-oligomerization of the serotonin transporter. A specific role for sialic acid residues. *J Biol Chem* **278:**43991–44000.
Pacholczyk T, Blakely RD, and Amara SG (1991) Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter. *Nature* **350:**350–354.
Paczkowski FA, Bryan-Lluka LJ, Pörzgen P, Brüss M, and Bönisch H (1999) Comparison of the pharmacological properties of cloned rat, human, and bovine norepinephrine transporters. *J Pharmacol Exp Ther* **290:**761–767.
Paczkowski FA, Sharpe IA, Dutertre S, and Lewis RJ (2007) χ-Conotoxin and tricyclic antidepressant interactions at the norepinephrine transporter define a new transporter model. *J Biol Chem* **282:**17837–17844.
Pantanowitz S, Bendahan A, and Kanner BI (1993) Only one of the charged amino acids located in the transmembrane α-helices of the γ-aminobutyric acid transporter (subtype A) is essential for its activity. *J Biol Chem* **268:**3222–3225.
Paradela A and Albar JP (2008) Advances in the analysis of protein phosphorylation. *J Proteome Res* **7:**1809–1818.
Patel A, Uhl G, and Kuhar MJ (1993) Species differences in dopamine transporters: postmortem changes and glycosylation differences. *J Neurochem* **61:**496–500.
Patel AP, Cerruti C, Vaughan RA, and Kuhar MJ (1994) Developmentally regulated glycosylation of dopamine transporter. *Brain Res Dev Brain Res* **83:**53–58.

Penado KM, Rudnick G, and Stephan MM (1998) Critical amino acid residues in transmembrane span 7 of the serotonin transporter identified by random mutagenesis. *J Biol Chem* **273:**28098–28106.
Perry KW, Falcone JF, Fell MJ, Ryder JW, Yu H, Love PL, Katner J, Gordon KD, Wade MR, Man T, et al. (2008a) Neurochemical and behavioral profiling of the selective GlyT1 inhibitors ALX5407 and LY2365109 indicate a preferential action in caudal vs. cortical brain areas. *Neuropharmacology* **55:**743–754.
Petroff OA, Rothman DL, Behar KL, and Mattson RH (1996) Low brain GABA level is associated with poor seizure control. *Ann Neurol* **40:**908–911.
Pickel VM and Chan J (1999) Ultrastructural localization of the serotonin transporter in limbic and motor compartments of the nucleus accumbens. *J Neurosci* **19:**7356–7366.
Pike LJ (2006) Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. *J Lipid Res* **47:**1597–1598.
Pinard E, Alanine A, Alberati D, Bender M, Borroni E, Bourdeaux P, Brom V, Burner S, Fischer H, Hainzl D, et al. (2010) Selective GlyT1 inhibitors: discovery of [4-(3-fluoro-5-trifluoromethylpyridin-2-yl)piperazin-1-yl][5-methanesulfonyl-2-((*S*)-2,2,2-trifluoro-1-methylethoxy)phenyl]methanone (RG1678), a promising novel medicine to treat schizophrenia. *J Med Chem* **53:**4603–4614.
Pinard E, Alberati D, Borroni E, Fischer H, Hainzl D, Jolidon S, Moreau JL, Narquizian R, Nettekoven M, Norcross R, et al. (2010) Glycine transporter 1 inhibitors for the treatment of schizophrenia, in *Frontiers in Medicinal Chemistry*, Gesellschaft Deutscher Chemiker, Münster, Germany.
Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, and Sperk G (2000) $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. *Neuroscience* **101:**815–850.
Plenge P and Mellerup ET (1985) Antidepressive drugs can change the affinity of [^{3}H]imipramine and [^{3}H]paroxetine binding to platelet and neuronal membranes. *Eur J Pharmacol* **119:**1–8.
Plenge P, Mellerup ET, and Laursen H (1991) Affinity modulation of [^{3}H]imipramine, [^{3}H]paroxetine and [^{3}H]citalopram binding to the 5-HT transporter from brain and platelets. *Eur J Pharmacol* **206:**243–250.
Ponce J, Biton B, Benavides J, Avenet P, and Aragon C (2000) Transmembrane domain III plays an important role in ion binding and permeation in the glycine transporter GLYT2. *J Biol Chem* **275:**13856–13862.
Povlock SL and Amara SG (1996) The structure and function of norepinephrine, dopamine and serotonin transporters, in *Neurotransmitter Transporters: Structure, Function, and Regulation* (Reith ME ed) pp 1–29, Humana Press, Totowa, NJ.
Prasad BM and Amara SG (2001) The dopamine transporter in mesencephalic cultures is refractory to physiological changes in membrane voltage. *J Neurosci* **21:**7561–7567.
Prasad HC, Steiner JA, Sutcliffe JS, and Blakely RD (2009) Enhanced activity of human serotonin transporter variants associated with autism. *Philos Trans R Soc Lond B Biol Sci* **364:**163–173.
Prasad HC, Zhu CB, McCauley JL, Samuvel DJ, Ramamoorthy S, Shelton RC, Hewlett WA, Sutcliffe JS, and Blakely RD (2005) Human serotonin transporter variants display altered sensitivity to protein kinase G and p38 mitogen-activated protein kinase. *Proc Natl Acad Sci USA* **102:**11545–11550.
Pratuangdejkul J, Schneider B, Jaudon P, Rosilio V, Baudoin E, Loric S, Conti M, Launay JM, and Manivet P (2005) Definition of an uptake pharmacophore of the serotonin transporter through 3D-QSAR analysis. *Curr Med Chem* **12:**2393–2410.
Pratuangdejkul J, Schneider B, Launay JM, Kellermann O, and Manivet P (2008) Computational approaches for the study of serotonin and its membrane transporter SERT: implications for drug design in neurological sciences. *Curr Med Chem* **15:**3214–3227.
Qian Y, Melikian HE, Rye DB, Levey AI, and Blakely RD (1995) Identification and characterization of antidepressant-sensitive serotonin transporter proteins using site-specific antibodies. *J Neurosci* **15:**1261–1274.
Quick M, Winther AM, Shi L, Nissen P, Weinstein H, and Javitch JA (2009) Binding of an octylglucoside detergent molecule in the second substrate (S2) site of LeuT establishes an inhibitor-bound conformation. *Proc Natl Acad Sci USA* **106:**5563–5568.
Quick M, Yano H, Goldberg NR, Duan L, Beuming T, Shi L, Weinstein H, and Javitch JA (2006) State-dependent conformations of the translocation pathway in the tyrosine transporter Tyt1, a novel neurotransmitter:sodium symporter from Fusobacterium nucleatum. *J Biol Chem* **281:**26444–26454.
Quick MW (2002) Role of syntaxin 1A on serotonin transporter expression in developing thalamocortical neurons. *Int J Dev Neurosci* **20:**219–224.
Quick MW (2003) Regulating the conducting states of a mammalian serotonin transporter. *Neuron* **40:**537–549.
Quick MW, Hu J, Wang D, and Zhang HY (2004) Regulation of a γ-aminobutyric acid transporter by reciprocal tyrosine and serine phosphorylation. *J Biol Chem* **279:**15961–15967.
Ramamoorthy S (2002) Regulation of monoamine transporters: regulated phosphorylation, dephosphorylation, and trafficking, in *Neurotransmitter Transporters: Structure, Function, and Regulation, 2nd ed* (Reith ME ed) pp 1–23, Humana Press, Totowa, NJ.
Ramamoorthy S, Bauman AL, Moore KR, Han H, Yang-Feng T, Chang AS, Ganapathy V, and Blakely RD (1993) Antidepressant-sensitive and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci USA* **90:**2542–2546.
Ramamoorthy S and Blakely RD (1999) Phosphorylation and sequestration of serotonin transporters differentially modulated by psychostimulants. *Science* **285:**763–766.
Ramamoorthy S, Giovanetti E, Qian Y, and Blakely RD (1998a) Phosphorylation and regulation of antidepressant-sensitive serotonin transporters. *J Biol Chem* **273:**2458–2466.
Ramamoorthy S, Melikian HE, Qian Y, and Blakely RD (1998b) Biosynthesis, N-glycosylation, and surface trafficking of biogenic amine transporter proteins. *Methods Enzymol* **296:**347–370.
Ramamoorthy S, Samuvel DJ, Buck ER, Rudnick G, and Jayanthi LD (2007) Phosphorylation of threonine residue 276 is required for acute regulation of serotonin transporter by cyclic GMP. *J Biol Chem* **282:**11639–11647.
Ramos EJ, Meguid MM, Campos AC, and Coelho JC (2005) Neuropeptide Y, α-melanocyte-stimulating hormone, and monoamines in food intake regulation. *Nutrition* **21:**269–279.
Rasmussen SG and Gether U (2005) Purification and fluorescent labeling of the human serotonin transporter. *Biochemistry* **44:**3494–3505.
Rasmussen TN, Plenge P, Bay T, Egebjerg J, and Gether U (2009) A single nucleotide polymorphism in the human serotonin transporter introduces a new site for N-linked glycosylation. *Neuropharmacology* **57:**287–294.
Ravna AW (2006) Three-dimensional models of neurotransmitter transporters and their interactions with cocaine and *S*-citalopram. *World J Biol Psychiatry* **7:**99–109.
Ravna AW and Edvardsen O (2001) A putative three-dimensional arrangement of the human serotonin transporter transmembrane helices: a tool to aid experimental studies. *J Mol Graph Model* **20:**133–144.
Ravna AW, Jaronczyk M, and Sylte I (2006) A homology model of SERT based on the $LeuT_{Aa}$ template. *Bioorg Med Chem Lett* **16:**5594–5597.
Ravna AW, Sylte I, and Dahl SG (2003) Molecular mechanism of citalopram and cocaine interactions with neurotransmitter transporters. *J Pharmacol Exp Ther* **307:**34–41.
Reddy PS and Corley RB (1998) Assembly, sorting, and exit of oligomeric proteins from the endoplasmic reticulum. *Bioessays* **20:**546–554.
Rees MI, Harvey K, Pearce BR, Chung SK, Duguid IC, Thomas P, Beatty S, Graham GE, Armstrong L, Shiang R, et al. (2006) Mutations in the gene encoding GlyT2 (SLC6A5) define a presynaptic component of human startle disease. *Nat Genet* **38:**801–806.
Reiterer V, Maier S, Sitte HH, Kriz A, Rüegg MA, Hauri HP, Freissmuth M, and Farhan H (2008) Sec24- and ARFGAP1-dependent trafficking of GABA transporter-1 is a prerequisite for correct axonal targeting. *J Neurosci* **28:**12453–12464.
Reith ME, Berfield JL, Wang LC, Ferrer JV, and Javitch JA (2001) The uptake inhibitors cocaine and benztropine differentially alter the conformation of the human dopamine transporter. *J Biol Chem* **276:**29012–29018.
Ressl S, Terwisscha van Scheltinga AC, Vonrhein C, Ott V, and Ziegler C (2009) Molecular basis of transport and regulation in the Na^+/betaine symporter BetP. *Nature* **458:**47–52.
Rice ME and Cragg SJ (2008) Dopamine spillover after quantal release: rethinking dopamine transmission in the nigrostriatal pathway. *Brain Res Rev* **58:**303–313.
Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, Griem A, Kovacs M, Ott J, and Merikangas KR (2009) Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA* **301:**2462–2471.
Rosenberg A and Kanner BI (2008) The substrates of the γ-aminobutyric acid transporter GAT-1 induce structural rearrangements around the interface of transmembrane domains 1 and 6. *J Biol Chem* **283:**14376–14383.
Roth BL, Sheffler DJ, and Kroeze WK (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov* **3:**353–359.
Rothman DL, Petroff OA, Behar KL, and Mattson RH (1993) Localized 1H NMR measurements of gamma-aminobutyric acid in human brain in vivo. *Proc Natl Acad Sci USA* 90:5662–5666.
Rothman RB and Baumann MH (2006) Therapeutic potential of monoamine transporter substrates. *Curr Top Med Chem* **6:**1845–1859.
Rothman RB, Blough BE, and Baumann MH (2006) Dual dopamine-5-HT releasers: potential treatment agents for cocaine addiction. *Trends Pharmacol Sci* **27:**612–618.
Roux MJ and Supplisson S (2000) Neuronal and glial glycine transporters have different stoichiometries. *Neuron* **25:**373–383.
Roy R, Hohng S, and Ha T (2008) A practical guide to single-molecule FRET. *Nat Methods* **5:**507–516.
Rudnick G (2006) Serotonin transporters–structure and function. *J Membr Biol* **213:**101–110.
Rudnick G (2007) What is an antidepressant binding site doing in a bacterial transporter? *ACS Chem Biol* **2:**606–609.
Rudnick G and Clark J (1993) From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. *Biochim Biophys Acta* **1144:**249–263.
Ryckmans T, Balançon L, Berton O, Genicot C, Lamberty Y, Lallemand B, Pasau P, Pirlot N, Quéré L, and Talaga P (2002a) First dual NK_1 antagonists-serotonin reuptake inhibitors: synthesis and SAR of a new class of potential antidepressants. *Bioorg Med Chem Lett* **12:**261–264.
Ryckmans T, Berton O, Grimée R, Kogej T, Lamberty Y, Pasau P, Talaga P, and Genicot C (2002b) Dual NK_1 antagonists–serotonin reuptake inhibitors as potential antidepressants. Part 2: SAR and activity of benzyloxyphenethyl piperazine derivatives. *Bioorg Med Chem Lett* **12:**3195–3198.
Sacher A, Nelson N, Ogi JT, Wright EM, Loo DD, and Eskandari S (2002) Presteady-state and steady-state kinetics and turnover rate of the mouse γ-aminobutyric acid transporter (mGAT3). *J Membr Biol* **190:**57–73.
Sakai N, Sasaki K, Nakashita M, Honda S, Ikegaki N, and Saito N (1997) Modulation of serotonin transporter activity by a protein kinase C activator and an inhibitor of type 1 and 2A serine/threonine phosphatases. *J Neurochem* **68:**2618–2624.
Salahpour A, Ramsey AJ, Medvedev IO, Kile B, Sotnikova TD, Holmstrand E, Ghisi V, Nicholls PJ, Wong L, Murphy K, et al. (2008) Increased amphetamine-induced hyperactivity and reward in mice overexpressing the dopamine transporter. *Proc Natl Acad Sci USA* **105:**4405–4410.
Samuvel DJ, Jayanthi LD, Bhat NR, and Ramamoorthy S (2005) A role for p38 mitogen-activated protein kinase in the regulation of the serotonin transporter: evidence for distinct cellular mechanisms involved in transporter surface expression. *J Neurosci* **25:**29–41.
Sato K, Adams R, Betz H, and Schloss P (1995a) Modulation of a recombinant glycine

transporter (GLYT1b) by activation of protein kinase C. *J Neurochem* **65:**1967–1973.
Sato K, Betz H, and Schloss P (1995b) The recombinant GABA transporter GAT1 is downregulated upon activation of protein kinase C. *FEBS Lett* **375:**99–102.
Sato Y, Zhang YW, Androutsellis-Theotokis A, and Rudnick G (2004) Analysis of transmembrane domain 2 of rat serotonin transporter by cysteine scanning mutagenesis. *J Biol Chem* **279:**22926–22933.
Saunders C, Ferrer JV, Shi L, Chen J, Merrill G, Lamb ME, Leeb-Lundberg LM, Carvelli L, Javitch JA, and Galli A (2000) Amphetamine-induced loss of human dopamine transporter activity: an internalization-dependent and cocaine-sensitive mechanism. *Proc Natl Acad Sci USA* **97:**6850–6855.
Schmid JA, Scholze P, Kudlacek O, Freissmuth M, Singer EA, and Sitte HH (2001) Oligomerization of the human serotonin transporter and of the rat GABA transporter 1 visualized by fluorescence resonance energy transfer microscopy in living cells. *J Biol Chem* **276:**3805–3810.
Schmitt KC and Reith ME (2010) Regulation of the dopamine transporter: aspects relevant to psychostimulant drugs of abuse. *Ann NY Acad Sci* **1187:**316–340.
Scholze P, Freissmuth M, and Sitte HH (2002) Mutations within an intramembrane leucine heptad repeat disrupt oligomer formation of the rat GABA transporter 1. *J Biol Chem* **277:**43682–43690.
Scholze P, Zwach J, Kattinger A, Pifl C, Singer EA, and Sitte HH (2000) Transporter-mediated release: a superfusion study on human embryonic kidney cells stably expressing the human serotonin transporter. *J Pharmacol Exp Ther* **293:**870–878.
Schou M, Halldin C, Sóvágó J, Pike VW, Gulyás B, Mozley PD, Johnson DP, Hall H, Innis RB, and Farde L (2003) Specific *in vivo* binding to the norepinephrine transporter demonstrated with the PET radioligand, (*S,S*)-[^{11}C]MeNER. *Nucl Med Biol* **30:**707–714.
Schou M, Halldin C, Sóvágó J, Pike VW, Hall H, Gulyás B, Mozley PD, Dobson D, Shchukin E, Innis RB, et al. (2004) PET evaluation of novel radiofluorinated reboxetine analogs as norepinephrine transporter probes in the monkey brain. *Synapse* **53:**57–67.
Schousboe A (2000) Pharmacological and functional characterization of astrocytic GABA transport: a short review. *Neurochem Res* **25:**1241–1244.
Schousboe A, Sarup A, Larsson OM, and White HS (2004) GABA transporters as drug targets for modulation of GABAergic activity. *Biochem Pharmacol* **68:**1557–1563.
Schreiber TB, Mäusbacher N, Breitkopf SB, Grundner-Culemann K, and Daub H (2008) Quantitative phosphoproteomics–an emerging key technology in signal-transduction research. *Proteomics* **8:**4416–4432.
Schröder W, Tzschentke TM, Terlinden R, De Vry J, Jahnel U, Christoph T, and Tallarida RJ (2011) Synergistic interaction between the two mechanisms of action of tapentadol in analgesia. *J Pharmacol Exp Ther* **337:**312–320.
Schroeter S, Apparsundaram S, Wiley RG, Miner LH, Sesack SR, and Blakely RD (2000) Immunolocalization of the cocaine- and antidepressant-sensitive L-norepinephrine transporter. *J Comp Neurol* **420:**211–232.
Schulze S, Köster S, Geldmacher U, Terwisscha van Scheltinga AC, and Kühlbrandt W (2010) Structural basis of Na^+-independent and cooperative substrate/product antiport in CaiT. *Nature* **467:**233–236.
Schwartz JW, Blakely RD, and DeFelice LJ (2003) Binding and transport in norepinephrine transporters. Real-time, spatially resolved analysis in single cells using a fluorescent substrate. *J Biol Chem* **278:**9768–9777.
Schwartz JW, Novarino G, Piston DW, and DeFelice LJ (2005) Substrate binding stoichiometry and kinetics of the norepinephrine transporter. *J Biol Chem* **280:**19177–19184.
Segonzac A, Raisman R, Tateishi T, Schoemaker H, Hicks PE, and Langer SZ (1985) Tryptamine, a substrate for the serotonin transporter in human platelets, modifies the dissociation kinetics of [^{3}H]imipramine binding: possible allosteric interaction. *J Neurochem* **44:**349–356.
Seiden LS, Sabol KE, and Ricaurte GA (1993) Amphetamine: effects on catecholamine systems and behavior. *Annu Rev Pharmacol Toxicol* **33:**639–677.
Semyanov A, Walker MC, and Kullmann DM (2003) GABA uptake regulates cortical excitability via cell type-specific tonic inhibition. *Nat Neurosci* **6:**484–490.
Semyanov A, Walker MC, Kullmann DM, and Silver RA (2004) Tonically active $GABA_A$ receptors: modulating gain and maintaining the tone. *Trends Neurosci* **27:**262–269.
Sen N, Shi L, Beuming T, Weinstein H, and Javitch JA (2005) A pincer-like configuration of TM2 in the human dopamine transporter is responsible for indirect effects on cocaine binding. *Neuropharmacology* **49:**780–790.
Serretti A, Kato M, De Ronchi D, and Kinoshita T (2007) Meta-analysis of serotonin transporter gene promoter polymorphism (5-HTTLPR) association with selective serotonin reuptake inhibitor efficacy in depressed patients. *Mol Psychiatry* **12:**247–257.
Sesack SR, Hawrylak VA, Matus C, Guido MA, and Levey AI (1998) Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci* **18:**2697–2708.
Sette M, Briley MS, and Langer SZ (1983) Complex inhibition of [^{3}H]imipramine binding by serotonin and nontricyclic serotonin uptake blockers. *J Neurochem* **40:**622–628.
Severinsen K, Sinning S, Müller HK, and Wiborg O (2008) Characterisation of the zebrafish serotonin transporter functionally links TM10 to the ligand binding site. *J Neurochem* **105:**1794–1805.
Shaffer PL, Goehring A, Shankaranarayanan A, and Gouaux E (2009) Structure and mechanism of a Na^+-independent amino acid transporter. *Science* **325:**1010–1014.
Shaikh SA and Tajkhorshid E (2010) Modeling and dynamics of the inward-facing state of a Na^+/Cl^--dependent neurotransmitter transporter homologue. *PLoS Comput Biol* **6:**e1000905.
Shannon JR, Flattem NL, Jordan J, Jacob G, Black BK, Biaggioni I, Blakely RD, and Robertson D (2000) Orthostatic intolerance and tachycardia associated with norepinephrine-transporter deficiency. *N Engl J Med* **342:**541–549.
Sharp SI, McQuillin A, and Gurling HM (2009) Genetics of attention-deficit hyperactivity disorder (ADHD). *Neuropharmacology* **57:**590–600.
Sharpe IA, Gehrmann J, Loughnan ML, Thomas L, Adams DA, Atkins A, Palant E, Craik DJ, Adams DJ, Alewood PF, et al. (2001) Two new classes of conopeptides inhibit the α1-adrenoceptor and noradrenaline transporter. *Nat Neurosci* **4:**902–907.
Sharpe IA, Palant E, Schroeder CI, Kaye DM, Adams DJ, Alewood PF, and Lewis RJ (2003) Inhibition of the norepinephrine transporter by the venom peptide χ-MrIA. Site of action, Na^+ dependence, and structure-activity relationship. *J Biol Chem* **278:**40317–40323.
Shelton RC (2006) Olanzapine/fluoxetine combination for bipolar depression. *Expert Rev Neurother* **6:**33–39.
Shi L, Quick M, Zhao Y, Weinstein H, and Javitch JA (2008) The mechanism of a neurotransmitter:sodium symporter–inward release of Na^+ and substrate is triggered by substrate in a second binding site. *Mol Cell* **30:**667–677.
Shimada S, Kitayama S, Lin CL, Patel A, Nanthakumar E, Gregor P, Kuhar M, and Uhl G (1991) Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. *Science* **254:**576–578.
Shimamura T, Weyand S, Beckstein O, Rutherford NG, Hadden JM, Sharples D, Sansom MS, Iwata S, Henderson PJ, and Cameron AD (2010) Molecular basis of alternating access membrane transport by the sodium-hydantoin transporter Mhp1. *Science* **328:**470–473.
Sieghart W (1995) Structure and pharmacology of γ-aminobutyric $acid_A$ receptor subtypes. *Pharmacol Rev* **47:**181–234.
Simons K and Toomre D (2000) Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* **1:**31–39.
Singh SK (2008) LeuT: a prokaryotic stepping stone on the way to a eukaryotic neurotransmitter transporter structure. *Channels (Austin)* **2:**380–389.
Singh SK, Piscitelli CL, Yamashita A, and Gouaux E (2008) A competitive inhibitor traps LeuT in an open-to-out conformation. *Science* **322:**1655–1661.
Singh SK, Yamashita A, and Gouaux E (2007) Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. *Nature* **448:**952–956.
Sinning S, Musgaard M, Jensen M, Severinsen K, Celik L, Koldsø H, Meyer T, Bols M, Jensen HH, Schiøtt B, et al. (2010) Binding and orientation of tricyclic antidepressants within the central substrate site of the human serotonin transporter. *J Biol Chem* **285:**8363–8374.
Sitte HH, Farhan H, and Javitch JA (2004) Sodium-dependent neurotransmitter transporters: oligomerization as a determinant of transporter function and trafficking. *Mol Interv* **4:**38–47.
Sitte HH, Hiptmair B, Zwach J, Pifl C, Singer EA, and Scholze P (2001) Quantitative analysis of inward and outward transport rates in cells stably expressing the cloned human serotonin transporter: inconsistencies with the hypothesis of facilitated exchange diffusion. *Mol Pharmacol* **59:**1129–1137.
Sitte HH, Scholze P, Schloss P, Pifl C, and Singer EA (2000) Characterization of carrier-mediated efflux in human embryonic kidney 293 cells stably expressing the rat serotonin transporter: a superfusion study. *J Neurochem* **74:**1317–1324.
Sivilotti L and Nistri A (1991) GABA receptor mechanisms in the central nervous system. *Prog Neurobiol* **36:**35–92.
Skolnick P, Krieter P, Tizzano J, Basile A, Popik P, Czobor P, and Lippa A (2006) Preclinical and clinical pharmacology of DOV 216,303, a "triple" reuptake inhibitor. *CNS Drug Rev* **12:**123–134.
Skolnick P, Popik P, Janowsky A, Beer B, and Lippa AS (2003) Antidepressant-like actions of DOV 21,947: a "triple" reuptake inhibitor. *Eur J Pharmacol* **461:**99–104.
Skovstrup S, Taboureau O, Bräuner-Osborne H, and Jørgensen FS (2010) Homology modelling of the GABA transporter and analysis of tiagabine binding. *ChemMedChem* **5:**986–1000.
Smicun Y, Campbell SD, Chen MA, Gu H, and Rudnick G (1999) The role of external loop regions in serotonin transport. Loop scanning mutagenesis of the serotonin transporter external domain. *J Biol Chem* **274:**36058–36064.
Smith G, Ruhland T, Mikkelsen G, Andersen K, Christoffersen CT, Alifrangis LH, Mørk A, Wren SP, Harris N, Wyman BM, et al. (2004) The synthesis and SAR of 2-arylsulfanyl-phenyl piperazinyl acetic acids as glyT-1 inhibitors. *Bioorg Med Chem Lett* **14:**4027–4030.
Smith KE, Borden LA, Hartig PR, Branchek T, and Weinshank RL (1992) Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. *Neuron* **8:**927–935.
Sorkina T, Doolen S, Galperin E, Zahniser NR, and Sorkin A (2003) Oligomerization of dopamine transporters visualized in living cells by fluorescence resonance energy transfer microscopy. *J Biol Chem* **278:**28274–28283.
Sorkina T, Hoover BR, Zahniser NR, and Sorkin A (2005) Constitutive and protein kinase C-induced internalization of the dopamine transporter is mediated by a clathrin-dependent mechanism. *Traffic* **6:**157–170.
Sorkina T, Miranda M, Dionne KR, Hoover BR, Zahniser NR, and Sorkin A (2006) RNA interference screen reveals an essential role of Nedd4–2 in dopamine transporter ubiquitination and endocytosis. *J Neurosci* **26:**8195–8205.
Sorkina T, Richards TL, Rao A, Zahniser NR, and Sorkin A (2009) Negative regulation of dopamine transporter endocytosis by membrane-proximal N-terminal residues. *J Neurosci* **29:**1361–1374.
Sotnikova TD, Beaulieu JM, Gainetdinov RR, and Caron MG (2006) Molecular biology, pharmacology and functional role of the plasma membrane dopamine transporter. *CNS Neurol Disord Drug Targets* **5:**45–56.
Spielewoy C, Biala G, Roubert C, Hamon M, Betancur C, and Giros B (2001) Hypolocomotor effects of acute and daily D-amphetamine in mice lacking the dopamine transporter. *Psychopharmacology (Berl)* **159:**2–9.
Steiner JA, Carneiro AM, and Blakely RD (2008) Going with the flow: trafficking-dependent and -independent regulation of serotonin transport. *Traffic* **9:**1393–1402.
Stephan MM, Chen MA, Penado KM, and Rudnick G (1997) An extracellular loop region of the serotonin transporter may be involved in the translocation mechanism. *Biochemistry* **36:**1322–1328.
Sucic S and Bryan-Lluka LJ (2002) The role of the conserved GXXXRXG motif in the expression and function of the human norepinephrine transporter. *Mol Brain Res* **108:**40–50.

Sudhof TC (2004) The synaptic vesicle cycle. *Annu Rev Neurosci* **27:**509–547.
Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, and Ewing A (1995) Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J Neurosci* **15:**4102–4108.
Sulzer D, Sonders MS, Poulsen NW, and Galli A (2005) Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol* **75:**406–433.
Sung U, Apparsundaram S, Galli A, Kahlig KM, Savchenko V, Schroeter S, Quick MW, and Blakely RD (2003) A regulated interaction of syntaxin 1A with the antidepressant-sensitive norepinephrine transporter establishes catecholamine clearance capacity. *J Neurosci* **23:**1697–1709.
Sung U, Jennings JL, Link AJ, and Blakely RD (2005) Proteomic analysis of human norepinephrine transporter complexes reveals associations with protein phosphatase 2A anchoring subunit and 14–3-3 proteins. *Biochem Biophys Res Commun* **333:**671–678.
Supplisson S and Bergman C (1997) Control of NMDA receptor activation by a glycine transporter co-expressed in *Xenopus* oocytes. *J Neurosci* **17:**4580–4590.
Supplisson S and Roux MJ (2002) Why glycine transporters have different stoichiometries. *FEBS Lett* **529:**93–101.
Sur C and Kinney GG (2004) The therapeutic potential of glycine transporter-1 inhibitors. *Expert Opin Investig Drugs* **13:**515–521.
Sutcliffe JS, Delahanty RJ, Prasad HC, McCauley JL, Han Q, Jiang L, Li C, Folstein SE, and Blakely RD (2005) Allelic heterogeneity at the serotonin transporter locus (SLC6A4) confers susceptibility to autism and rigid-compulsive behaviors. *Am J Hum Genet* **77:**265–279.
Talvenheimo J, Fishkes H, Nelson PJ, and Rudnick G (1983) The serotonin transporter-imipramine "receptor." *J Biol Chem* **258:**6115–6119.
Talvenheimo J, Nelson PJ, and Rudnick G (1979) Mechanism of imipramine inhibition of platelet 5-hydroxytryptamine transport. *J Biol Chem* **254:**4631–4635.
Tarkiainen J, Vercouille J, Emond P, Sandell J, Hiltunen J, Frangin Y, Guilloteau D, and Halldin C (2001) ^{11}C labelling of MADAM in two different positions: a highly selective PET radioligand for the serotonin transporter. *J Labelled Comp Radiopharm* **44:**1013–1023.
Tate CG (2001) Overexpression of mammalian integral membrane proteins for structural studies. *FEBS Lett* **504:**94–98.
Tate CG and Blakely RD (1994) The effect of N-linked glycosylation on activity of the Na^+- and Cl^--dependent serotonin transporter expressed using recombinant baculovirus in insect cells. *J Biol Chem* **269:**26303–26310.
Tate CG, Haase J, Baker C, Boorsma M, Magnani F, Vallis Y, and Williams DC (2003) Comparison of seven different heterologous protein expression systems for the production of the serotonin transporter. *Biochim Biophys Acta* **1610:**141–153.
Tatsumi M, Groshan K, Blakely RD, and Richelson E (1997) Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol* **340:**249–258.
Tavoulari S, Forrest LR, and Rudnick G (2009) Fluoxetine (Prozac) binding to serotonin transporter is moduclated by chloride and conformational changes. *J Neurosci* **29:**9635–9643.
Terui Y, Chu YW, Li JY, Ando T, Fukunaga T, Aoki T, and Toda Y (2008) New cyclic tetrapeptides from *Nonomuraea* sp. TA-0426 that inhibit glycine transporter type 1 (GlyT1). *Bioorg Med Chem Lett* **18:**6321–6323.
Thoeringer CK, Ripke S, Unschuld PG, Lucae S, Ising M, Bettecken T, Uhr M, Keck ME, Mueller-Myhsok B, Holsboer F, et al. (2009) The GABA transporter 1 (SLC6A1): a novel candidate gene for anxiety disorders. *J Neural Transm* **116:**649–657.
Thomas GH, Babbs AJ, Chatfield RE, Krülle TM, Widdowson PS, Provost D, and McCormack JG (2009) 5-HT_{1A} activation counteracts cardiovascular but not hypophagic effects of sibutramine in rats. *Obesity* **17:**467–473.
Thomsen C (2006) Glycine transporter inhibitors as novel antipsychotics. *Drug Discov Today Ther Strateg* **3:**539–545.
Thomsen C, Sørensen PO, and Egebjerg J (1997) 1-(3-(9H-carbazol-9-yl)-1-propyl)-4-(2-methoxyphenyl)-4-piperidinol, a novel subtype selective inhibitor of the mouse type II GABA-transporter. *Br J Pharmacol* **120:**983–985.
Torres GE and Amara SG (2007) Glutamate and monoamine transporters: new visions of form and function. *Curr Opin Neurobiol* **17:**304–312.
Torres GE, Carneiro A, Seamans K, Fiorentini C, Sweeney A, Yao WD, and Caron MG (2003a) Oligomerization and trafficking of the human dopamine transporter. Mutational analysis identifies critical domains important for the functional expression of the transporter. *J Biol Chem* **278:**2731–2739.
Torres GE, Gainetdinov RR, and Caron MG (2003b) Plasma membrane monoamine transporters: structure, regulation and function. *Nat Rev Neurosci* **4:**13–25.
Torres GE, Yao WD, Mohn AR, Quan H, Kim KM, Levey AI, Staudinger J, and Caron MG (2001) Functional interaction between monoamine plasma membrane transporters and the synaptic PDZ domain-containing protein PICK1. *Neuron* **30:**121–134.
Tran PV, Bymaster FP, McNamara RK, and Potter WZ (2003) Dual monoamine modulation for improved treatment of major depressive disorder. *J Clin Psychopharmacology* **23:**78–86.
Trinidad JC, Specht CG, Thalhammer A, Schoepfer R, and Burlingame AL (2006) Comprehensive identification of phosphorylation sites in postsynaptic density preparations. *Mol Cell Proteomics* **5:**914–922.
Tsai G and Coyle JT (2002) Glutamatergic mechanisms in schizophrenia. *Annu Rev Pharmacol Toxicol* **42:**165–179.
Tsai G, Ralph-Williams RJ, Martina M, Bergeron R, Berger-Sweeney J, Dunham KS, Jiang Z, Caine SB, and Coyle JT (2004) Gene knockout of glycine transporter 1: characterization of the behavioral phenotype. *Proc Natl Acad Sci USA* **101:**8485–8490.
Tsui-Pierchala BA, Encinas M, Milbrandt J, and Johnson EM Jr (2002) Lipid rafts in neuronal signaling and function. *Trends Neurosci* **25:**412–417.
Uchida J, Kiuchi Y, Ohno M, Yura A, and Oguchi K (1998) Ca^{2+}-dependent enhancement of [^{3}H]noradrenaline uptake in PC12 cells through calmodulin-dependent kinases. *Brain Res* **809:**155–164.
Uhl GR and Lin Z (2003) The top 20 dopamine transporter mutants: structure-function relationships and cocaine actions. *Eur J Pharmacol* **479:**71–82.
Vandenberg RJ, Shaddick K, and Ju P (2007) Molecular basis for substrate discrimination by glycine transporters. *J Biol Chem* **282:**14447–14453.
Vaughan RA (2004) Phosphorylation and regulation of psychostimulant-sensitive neurotransmitter transporters. *J Pharmacol Exp Ther* **310:**1–7.
Vaughan RA, Huff RA, Uhl GR, and Kuhar MJ (1997) Protein kinase C-mediated phosphorylation and functional regulation of dopamine transporters in striatal synaptosomes. *J Biol Chem* **272:**15541–15546.
Veenstra-Vanderweele J, Jessen TN, Thompson BJ, Carter M, Prasad HC, Steiner JA, Sutcliffe JS, and Blakely RD (2009) Modeling rare gene variation to gain insight into the oldest biomarker in autism: construction of the serotonin transporter Gly56Ala knock-in mouse. *J Neurodev Disord* **1:**158–171.
Volz TJ and Schenk JO (2005) A comprehensive atlas of the topography of functional groups of the dopamine transporter. *Synapse* **58:**72–94.
Waitekus AB and Kirkpatrick P (2004) Duloxetine hydrochloride. *Nat Rev Drug Discov* **3:**907–908.
Waldinger MD (2006) Emerging drugs for premature ejaculation. *Expert Opin Emerg Drugs* **11:**99–109.
Waldinger MD, Zwinderman AH, Schweitzer DH, and Olivier B (2004) Relevance of methodological design for the interpretation of efficacy of drug treatment of premature ejaculation: a systematic review and meta-analysis. *Int J Impot Res* **16:**369–381.
Wall SC, Gu H, and Rudnick G (1995) Biogenic amine flux mediated by cloned transporters stably expressed in cultured cell lines: amphetamine specificity for inhibition and efflux. *Mol Pharmacol* **47:**544–550.
Walline CC, Nichols DE, Carroll FI, and Barker EL (2008) Comparative molecular field analysis using selectivity fields reveals residues in the third transmembrane helix of the serotonin transporter associated with substrate and antagonist recognition. *J Pharmacol Exp Ther* **325:**791–800.
Wang D and Quick MW (2005) Trafficking of the plasma membrane γ-aminobutyric acid transporter GAT1. Size and rates of an acutely recycling pool. *J Biol Chem* **280:**18703–18709.
Wankerl M, Wüst S, and Otte C (2010) Current developments and controversies: does the serotonin transporter gene-linked polymorphic region (5-HTTLPR) modulate the association between stress and depression? *Curr Opin Psychiatry* **23:**582–587.
Watanabe A, Choe S, Chaptal V, Rosenberg JM, Wright EM, Grabe M, and Abramson J (2010) The mechanism of sodium and substrate release from the binding pocket of vSGLT. *Nature* **468:**988–991.
Wei Y, Williams JM, Dipace C, Sung U, Javitch JA, Galli A, and Saunders C (2007) Dopamine transporter activity mediates amphetamine-induced inhibition of Akt through a Ca^{2+}/calmodulin-dependent kinase II-dependent mechanism. *Mol Pharmacol* **71:**835–842.
Wein T and Wanner KT (2010) Generation of a 3D model for human GABA transporter hGAT-1 using molecular modeling and investigation of the binding of GABA. *J Mol Model* **16:**155–161.
Wells JA and McClendon CL (2007) Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature* **450:**1001–1009.
Wennogle LP and Meyerson LR (1983) Serotonin modulates the dissociation of [^{3}H]imipramine from human platelet recognition sites. *Eur J Pharmacol* **86:**303–307.
Wennogle LP and Meyerson LR (1985) Serotonin uptake inhibitors differentially modulate high-affinity imipramine dissociation in human platelet membranes. *Life Sci* **36:**1541–1550.
Wersinger C, Jeannotte A, and Sidhu A (2006a) Attenuation of the norepinephrine transporter activity and trafficking via interactions with α-synuclein. *Eur J Neurosci* **24:**3141–3152.
Wersinger C, Prou D, Vernier P, and Sidhu A (2003) Modulation of dopamine transporter function by α-synuclein is altered by impairment of cell adhesion and by induction of oxidative stress. *FASEB J* **17:**2151–2153.
Wersinger C, Rusnak M, and Sidhu A (2006b) Modulation of the trafficking of the human serotonin transporter by human α-synuclein. *Eur J Neurosci* **24:**55–64.
Wersinger C and Sidhu A (2005) Disruption of the interaction of α-synuclein with microtubules enhances cell surface recruitment of the dopamine transporter. *Biochemistry* **44:**13612–13624.
Weyand S, Shimamura T, Yajima S, Suzuki S, Mirza O, Krusong K, Carpenter EP, Rutherford NG, Hadden JM, O'Reilly J, et al. (2008) Structure and molecular mechanism of a nucleobase-cation-symport-1 family transporter. *Science* **322:**709–713.
White HS, Sarup A, Bolvig T, Kristensen AS, Petersen G, Nelson N, Pickering DS, Larsson OM, Frølund B, Krogsgaard-Larsen P, et al. (2002) Correlation between anticonvulsant activity and inhibitory action on glial γ-aminobutyric acid uptake of the highly selective mouse γ-aminobutyric acid transporter 1 inhibitor 3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole and its N-alkylated analogs. *J Pharmacol Exp Ther* **302:**636–644.
White HS, Watson WP, Hansen SL, Slough S, Perregaard J, Sarup A, Bolvig T, Petersen G, Larsson OM, Clausen RP, et al. (2005) First demonstration of a functional role for central nervous system betaine/γ-aminobutyric acid transporter (mGAT2) based on synergistic anticonvulsant action among inhibitors of mGAT1 and mGAT2. *J Pharmacol Exp Ther* **312:**866–874.
White KJ, Kiser PD, Nichols DE, and Barker EL (2006) Engineered zinc-binding sites confirm proximity and orientation of transmembrane helices I and III in the human serotonin transporter. *Protein Science* **15:**2411–2422.
Whitworth TL and Quick MW (2001) Substrate-induced regulation of γ-aminobutyric acid transporter trafficking requires tyrosine phosphorylation. *J Biol Chem* **276:**42932–42937.
Whitworth TL, Herndon LC, and Quick MW (2002) Psychostimulants differentially regulate serotonin transporter expression in thalamocortical neurons. *J Neurosci* **22:**RC192.

Wilbrandt W and Rosenberg T (1961) The concept of carrier transport and its corollaries in pharmacology. *Pharmacol Rev* **13:**109–183.
Wiles AL, Pearlman RJ, Rosvall M, Aubrey KR, and Vandenberg RJ (2006) *N*-Arachidonyl glycine inhibits the glycine transporter, GLYT2a. *J Neurochem* **99:** 781–786.
Willers ED, Newman JH, Loyd JE, Robbins IM, Wheeler LA, Prince MA, Stanton KC, Cogan JA, Runo JR, Byrne D, et al. (2006) Serotonin transporter polymorphisms in familial and idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med* **173:**798–802.
Williams JM, Owens WA, Turner GH, Saunders C, Dipace C, Blakely RD, France CP, Gore JC, Daws LC, Avison MJ, et al. (2007) A Hypoinsulinemia regulates amphetamine-induced reverse transport of dopamine. *PLoS Biol* **5:**e274.
Wilson AA, Ginovart N, Schmidt M, Meyer JH, Threlkeld PG, and Houle S (2000) Novel radiotracers for imaging the serotonin transporter by positron emission tomography: synthesis, radiosynthesis, and in vitro and ex vivo evaluation of ^{11}C-labeled 2-(phenylthio)araalkylamines. *J Med Chem* **43:**3103–3110.
Wilson AA, Johnson DP, Mozley D, Hussey D, Ginovart N, Nobrega J, Garcia A, Meyer J, and Houle S (2003) Synthesis and *in vivo* evaluation of novel radiotracers for the *in vivo* imaging of the norepinephrine transporter. *Nucl Med Biol* **30:**85–92.
Wise RA (1996) Neurobiology of addiction. *Curr Opin Neurobiol* **6:**243–251.
Wong DT, Robertson DW, Bymaster FP, Krushinski JH, and Reid LR (1988) LY227942, an inhibitor of serotonin and norepinephrine uptake: biochemical pharmacology of a potential antidepressant drug. *Life Sci* **43:**2049–2057.
Wong DT, Threlkeld PG, Best KL, and Bymaster FP (1982) A new inhibitor of norepinephrine uptake devoid of affinity for receptors in rat brain. *J Pharmacol Exp Ther* **222:**61–65.
Wong EH, Nikam SS, and Shahid M (2008) Multi- and single-target agents for major psychiatric diseases: therapeutic opportunities and challenges. *Curr Opin Investig Drugs* **9:**28–36.
Xhaard H, Backström V, Denessiouk K, and Johnson MS (2008) Coordination of Na^+ by monoamine ligands in dopamine, norepinephrine, and serotonin transporters. *J Chem Inf Model* **48:**1423–1437.
Xu F, Gainetdinov RR, Wetsel WC, Jones SR, Bohn LM, Miller GW, Wang YM, and Caron MG (2000) Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nat Neurosci* **3:**465–471.
Xu YF, Cai YQ, Cai GQ, Jiang J, Sheng ZJ, Wang ZG, and Fei J (2008) Hypoalgesia in mice lacking GABA transporter subtype 1. *J Neurosci Res* **86:**465–470.
Yamashita A, Singh SK, Kawate T, Jin Y, and Gouaux E (2005) Crystal structure of a bacterial homologue of Na^+/Cl^--dependent neurotransmitter transporters. *Nature* **437:**215–223.
Yamauchi A, Uchida S, Kwon HM, Preston AS, Robey RB, Garcia-Perez A, Burg MB, and Handler JS (1992) Cloning of a Na^+- and Cl^--dependent betaine transporter that is regulated by hypertonicity. *J Biol Chem* **267:**649–652.
Yan J, Olsen JV, Park KS, Li W, Bildl W, Schulte U, Aldrich RW, Fakler B, and Trimmer JS (2008) Profiling the phospho-status of the BKCa channel α subunit in rat brain reveals unexpected patterns and complexity. *Mol Cell Proteomics* **7:**2188–2198.
Zafra F, Aragón C, Olivares L, Danbolt NC, Giménez C, and Storm-Mathisen J (1995a) Glycine transporters are differentially expressed among CNS cells. *J Neurosci* **15:**3952–3969.
Zafra F, Gomeza J, Olivares L, Aragón C, and Giménez C (1995b) Regional distribution and developmental variation of the glycine transporters GLYT1 and GLYT2 in the rat CNS. *Eur J Neurosci* **7:**1342–1352.
Zahniser NR and Doolen S (2001) Chronic and acute regulation of Na^+/Cl^- -dependent neurotransmitter transporters: drugs, substrates, presynaptic receptors, and signaling systems. *Pharmacol Ther* **92:**21–55.
Zapata A, Kivell B, Han Y, Javitch JA, Bolan EA, Kuraguntla D, Jaligam V, Oz M, Jayanthi LD, Samuvel DJ, et al. (2007) Regulation of dopamine transporter function and cell surface expression by D_3 dopamine receptors. *J Biol Chem* **282:**35842–35854.
Zarpellon A, Donella-Deana A, Folda A, Turetta L, Pavanetto M, and Deana R (2008) Serotonin (5-HT) transport in human platelets is modulated by Src-catalysed Tyr-phosphorylation of the plasma membrane transporter SERT. *Cell Physiol Biochem* **21:**87–94.
Zhang YW and Rudnick G (2005) Cysteine-scanning mutagenesis of serotonin transporter intracellular loop 2 suggests an α-helical conformation. *J Biol Chem* **280:** 30807–30813.
Zhang YW and Rudnick G (2006) The cytoplasmic substrate permeation pathway of serotonin transporter. *J Biol Chem* **281:**36213–36220.
Zhang YW, Gesmonde J, Ramamoorthy S, and Rudnick G (2007) Serotonin transporter phosphorylation by cGMP-dependent protein kinase is altered by a mutation associated with obsessive compulsive disorder. *J Neurosci* **27:**10878–10886.
Zhao Y, Quick M, Shi L, Mehler EL, Weinstein H, and Javitch JA (2010a) Substrate-dependent proton antiport in neurotransmitter:sodium symporters. *Nat Chem Biol* **6:**109–116.
Zhao Y, Terry D, Shi L, Weinstein H, Blanchard SC, and Javitch JA (2010b) Single-molecule dynamics of gating in a neurotransmitter transporter homologue. *Nature* **465:**188–193.
Zhou FC, Tao-Cheng JH, Segu L, Patel T, and Wang Y (1998) Serotonin transporters are located on the axons beyond the synaptic junctions: anatomical and functional evidence. *Brain Res* **805:**241–254.
Zhou J (2004) Norepinephrine transporter inhibitors and their therapeutic potential. *Drugs Future* **29:**1235–1244.
Zhou Y, Bennett ER, and Kanner BI (2004) The aqueous accessibility in the external half of transmembrane domain I of the GABA transporter GAT-1 is modulated by its ligands. *J Biol Chem* **279:**13800–13808.
Zhou Y, Zomot E, and Kanner BI (2006) Identification of a lithium interaction site in the γ-aminobutyric acid (GABA) transporter GAT-1. *J Biol Chem* **281:**22092–22099.
Zhou Z, Zhen J, Karpowich NK, Goetz RM, Law CJ, Reith ME, and Wang DN (2007) LeuT-desipramine structure reveals how antidepressants block neurotransmitter reuptake. *Science* **317:**1390–1393.
Zhou Z, Zhen J, Karpowich NK, Law CJ, Reith ME, and Wang DN (2009) Antidepressant specificity of serotonin transporter suggested by three LeuT-SSRI structures. *Nat Struct Mol Biol* **16:**652–657.
Zhu CB, Carneiro AM, Dostmann WR, Hewlett WA, and Blakely RD (2005) p38 MAPK activation elevates serotonin transport activity via a trafficking-independent, protein phosphatase 2A-dependent process. *J Biol Chem* **280:**15649–15658.
Zhu CB, Hewlett WA, Feoktistov I, Biaggioni I, and Blakely RD (2004a) Adenosine receptor, protein kinase G, and p38 mitogen-activated protein kinase-dependent up-regulation of serotonin transporters involves both transporter trafficking and activation. *Mol Pharmacol* **65:**1462–1474.
Zhu CB, Hewlett WA, Francis SH, Corbin JD, and Blakely RD (2004b) Stimulation of serotonin transport by the cyclic GMP phosphodiesterase-5 inhibitor sildenafil. *Eur J Pharmacol* **504:**1–6.
Zhu CB, Steiner JA, Munn JL, Daws LC, Hewlett WA, and Blakely RD (2007) Rapid stimulation of presynaptic serotonin transport by A_3 adenosine receptors. *J Pharmacol Exp Ther* **322:**332–340.
Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, and Hen R (2001) Hyperactivity and impaired response habituation in hyperdopaminergic mice. *Proc Natl Acad Sci USA* **98:**1982–1987.
Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, Setola V, Prisinzano TE, and Baumann MH (2009) Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *J Pharmacol Exp Ther* **329:**738–746.
Zomot E, Bendahan A, Quick M, Zhao Y, Javitch JA, and Kanner BI (2007) Mechanism of chloride interaction with neurotransmitter: sodium symporters. *Nature* **449:**726–729.
Zomot E and Kanner BI (2003) The interaction of the γ-aminobutyric acid transporter GAT-1 with the neurotransmitter is selectively impaired by sulfhydryl modification of a conformationally sensitive cysteine residue engineered into extracellular loop IV. *J Biol Chem* **278:**42950–42958.
Zomot E, Zhou Y, and Kanner BI (2005) Proximity of transmembrane domains 1 and 3 of the γ-aminobutyric acid transporter GAT-1 inferred from paired cysteine mutagenesis. *J Biol Chem* **280:**25512–25516.