Published in final edited form as:

Curr Opin Struct Biol. 2010 August ; 20(4): 415–422. doi:10.1016/j.sbi.2010.05.007.

Substrate and drug binding sites in LeuT

Ajeeta Nyola¹, Nathan K. Karpowich¹, Juan Zhen², Jennifer Marden¹, Maarten E. Reith², and Da-Neng Wang¹

¹ Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, and Department of Cell Biology, New York University School of Medicine, 540 First Avenue, New York, NY 10016, USA

² Departments of Psychiatry and of Pharmacology, New York University School of Medicine, 540 First Avenue, New York, NY 10016, USA

Summary of recent advances

LeuT is a member of the neurotransmitter/sodium symporter family, which includes the neuronal transporters for serotonin, norepinephrine and dopamine. The original crystal structure of LeuT shows a primary leucine binding site at the center of the protein. LeuT is inhibited by different classes of antidepressants that act as potent inhibitors of the serotonin transporter. The newly determined crystal structures of LeuT-antidepressant complexes provide opportunities to probe drug binding in the serotonin transporter, of which the exact position remains controversial. Structure of a LeuT-tryptophan complex shows an overlapping binding site with the primary substrate site. A secondary substrate binding site was recently identified, where the binding of a leucine triggers the cytoplasmic release of the primary substrate. This two binding site model presents opportunities for a better understanding of drug binding and the mechanism of inhibition for mammalian transporters.

1. INTRODUCTION

The sodium-dependent neurotransmitter symporter (NSS) family of membrane proteins includes the neuronal transporters for the monoamines serotonin, norepinephrine and dopamine (SERT, NET and DAT, respectively) [1–3]. The subset of such transporters from the human genome also forms the solute carrier 6 (SLC6) family of transporters. Progress in genomic sequencing at the turn of the century has revealed that the NSS family includes transporter proteins of many bacteria, which often transport amino acids driven by sodium gradients [4]. The sequence identity between NSS proteins from mammals and bacteria is in the range of 20–30%, indicating a common ancestor and a shared protein fold. The determination of the crystal structure of the leucine transporter LeuT from *Aquifex aeolicus* [5] represents an important breakthrough in the mechanistic understanding of the NSS proteins (Figure 1). The structure consists of a twelve-helix bundle, with the helices TMs 1–5 and helices TMs 6–10 being related by inverted symmetry relative to the membrane (Figure 2a). This arrangement reveals that the substrate leucine, along with two sodium ions, binds at the center of the transporter between the two inverted domains (the S1 site). The substrate is shielded from the extramembrane space by both a cytoplasmic and an extracellular gate. The alternating opening

*Corresponding authors: maarten.reith@med.nyu.edu, +1 (212) 263-8267, wang@saturn.med.nyu.edu, +1 (212) 263-8634.

Conflict of interests

None

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and closing of these gates is believed to allow the translocation of the substrate across the membrane. Much of the mutagenesis, biochemical and transport activity data on SERT, NET and DAT can be understood within the structural framework of LeuT. Indeed, the substrate binding site is conserved enough to allow the engineering of a chloride binding site in LeuT based on the SERT and DAT data [6,7].

2. Secondary substrate binding site in NSS proteins

In the crystal structure of LeuT in its occluded state there is just one leucine bound [5]. Therefore, it was surprising when Shi and colleagues discovered a secondary leucine binding (S2) site in the periplasmic vestibule [8]. This is the same site where tricyclic antidepressant (TCA) molecules had previously been shown to bind (Figures 2a & 2b) [9,10], and molecular dynamics (MD) simulations had suggested passage of leucine through such a site [11]. LeuT purified in detergent solution was found to bind leucine at a ratio of 1:2 [8]. Importantly, binding experiments showed that the binding of a TCA molecule is competitive to substrate binding not at the S1 site but at the S2 site. Also surprisingly, strong evidence was provided by these investigators that the binding of substrate to S2 triggers the cytoplasmic release of the leucine molecule from the primary S1 binding site.

Intriguingly, this two-site model for transport recently (Figure 1) [8] received support from the crystallization studies of a related membrane transporter. Despite a lack of amino acid sequence identity, several families of membrane transporters have been shown to share the same LeuT-like fold [5], and presumably they operate using a similar transport mechanism. One such protein is the carnitine transporter (CaiT) from *E. coli*, a member of the betaine/choline/carnitine transporter (BCCT) family [12]. In addition to the similarities in the protein fold as well as the position of the primary substrate binding site, the crystal structure of CaiT revealed a secondary substrate binding site on the cytoplasmic side of the protein—an exporter—as opposed to the periplasmic secondary site found in the importer LeuT. Such symmetry, both in structure and in functionality, probably reflects a high degree of similarity for the common ancestor of both protein families [13].

Presently, one can only speculate as to whether a secondary substrate-binding site exists for mammalian members of the NSS family. The lack of high quality purified protein preparations makes the sophisticated experiments for selective binding and competition, as has been done for LeuT [8], next to impossible. Indirect support comes from our recent study on the interaction between bivalent ligands and DAT [14,15]. Bivalent ligands - compounds incorporating two receptor-interacting moieties linked by a flexible chain - often exhibit profoundly enhanced binding affinity as compared with their monovalent components, implying concurrent binding to multiple sites on the target protein. Molecules possessing two substrate-like phenylalkylamine moieties linked by a progressively longer aliphatic spacer were found to be more potent DAT inhibitors [14]. One compound bearing two dopamine-like pharmacophoric 'heads' separated by an 8-carbon linker achieved an 82-fold gain in inhibition of cocaine-analog binding compared with dopamine itself; bivalent 6-carbon linker combinations of dopamine-, amphetamine- and β -phenethylamine-like heads all resulted in considerable gains in affinity. Docking into a DAT homology model suggested simultaneous occupancy of two discrete substrate-binding domains. Similar results were obtained by Nielson and colleagues, who used bivalent phenyl tropanes—part of the cocaine molecule—to probe binding to DAT, SERT and NET [15]. When tethered together with an ester linker of 10 atoms, bivalent phenyl tropanes showed gains of affinity of up to 45-fold. These observations point to a secondary binding site in DAT that has affinity for both dopamine-like substrates and phenyl tropane-like inhibitors; the distance of approximately 13 Å between the two heads of the bivalent ligands in both the Schmitt et al. [14] and Nielsen et al. [15] studies fit the distance of 11–13 Å between the S1 and S2 sites in the LeuT structure model [8].

3. LeuT-TCA/SSRI structures

Serotonin selective reuptake inhibitors (SSRIs, e.g. fluoxetine and sertraline) and tricyclic antidepressants (TCAs) (Figures 3a & 3e) are the most widely prescribed medications for clinical depression. Compounds from both classes bind to SERT and inhibit serotonin reuptake, in most studies, in a competitive manner (for example see [16]). Surprisingly, these compounds also inhibit the transport activity of LeuT although with lower potency than the human NSS proteins. Crystal structures of LeuT in complex individually with TCAs and SSRIs have been published recently (Figure 2) [9,10,17]. In LeuT, all of the co-crystallized antidepressant compounds were found to bind at the S2 substrate site, separated from the S1 leucine-binding site by the transporter's extracellular gate. In contrast to the binding of a second substrate at this S2 site, bound TCA and SSRI molecules prevent conformational changes and block subsequent substrate transport by directly locking the gate.

LeuT cocrystal structures with desipramine [9,10], imipramine and chlorimipramine [10] show that TCAs bind in the extracellular vestibule S2 site, about 11 Å above the substrate and the two sodium ions located at the S1 site (Figure 2d and Table 1). In all three of the complex structures, the tricyclic rings are superimposable and the dipole moments of the drug molecules align in the same direction. The drug molecule is held in place by the EL4 hairpin loop on the extracellular side and by a salt bridge. The third ring of the drug forms cation- π interactions with gate residues Arg30 and Phe253, stabilizing the occluded conformation. This in turn prevents gate opening on the cytoplasmic side, supported by the measured reduction of the leucine dissociation off-rate [10].

Similarly, in cocrystal structures of LeuT with three SSRIs (sertraline, R-fluoxetine and S-fluoxetine), the drug molecules all bind to LeuT at the S2 site and in a very similar manner (Figure 2 and Table 1). The SSRI binding site shares a number of residues with the S1 substrate binding site. For example, Leu25 is simultaneously within a 4 Å distance to both the bound sertraline and the S1 leucine (Figure 2a). The electronic dipole moment in each drug points in approximately the same direction, from TM10 to TM1. Not only are the phenyl and phenoxy groups of the three SSRIs roughly superimposable, but in each compound, the halogen moieties were found to insert into the same halogen binding pocket (HBP) of LeuT (Figure 2d). It is of note that all of the shared key features of SSRI molecules are important for binding to LeuT.

4. Antidepressant binding in SERT

Whether the antidepressant binding site found in LeuT is conserved in the human NSS protein is intriguing and controversial. TCAs and SSRIs bind and inhibit NSS proteins from *Drosophila* and *C. elegans* as tightly as they do to the mammalian proteins, prompting Roubert and colleagues to propose in 2001 that "... in the evolution process the first amine transporters already had the ability to strongly bind TCA in a binding pocket formed by disseminated residues." [18]. Can one extend the hypotheses further back in evolution to include the bacterial amino acid transporters in the NSS family?

Indeed, we have interpreted initial results from our work on LeuT and its corresponding human monoamine transporters as pointing to the S2 site being a tricyclic antidepressant binding site in both the bacterial LeuT and human SERT [9]. Subsequently, more extensive site-directed mutagenesis was carried out related to SSRI binding [17]. At the HBP, mutations of Ile179 or Ile108 in human SERT caused dramatic reductions in the protein's affinity to bind sertraline, with increases in IC₅₀ ranging from 180 to 1080 fold. Mutating Ile179 reduced the affinity of the other two SSRIs that were studied in the LeuT crystal structures, R- and S-fluoxetine, by 7 to 20 fold. Losses of SSRI affinity were also found upon mutations in two other areas, on the extracellular side and on the cytoplasmic side of the S2 site [17]. Compared with the changes observed in this study for fluoxetine interacting with the S2 site, SERT mutations related to

S1 have generated effects of similar [19] or greater [20–22] magnitude. Mutations at the HBP in the S2 site of SERT in the form of Gly100Ala and Thr178Ala produced a modest reduction in affinity for citalopram [23]. Similarly, Lys448 in the GABA transporter rGAT1, another member of the NSS family of transporters, corresponds to Asp401 in the S2 site of LeuT; the Lys448Asp or Lys448Glu mutants were found to be more sensitive to desipramine [24].

Some other recent studies, however, support the notion that antidepressant molecules instead bind at the S1 site in SERT. Mutation of Ser438 in the S1 site of SERT into threonine caused a loss of affinity of 20 to 300 fold for three TCAs (imipramine, clomipramine and amitriptyline) [25]. The loss of affinity to S-citalopram by the same mutation was as high as 2000 fold. Part of this effect can be compensated for by reciprocal removal of a methyl group from the inhibitor [26]. In computer docking into the SERT homology model, TCA molecules could be docked into the S1 site by induced fit, while allowing for protein flexibility in the docking calculations; docking into the S2 site pointed to a diffuse site with no particular drug orientation [22]. In addition, extensive mutagenesis data supported a role for S1 residues Asp98, Ala173, Phe335 and Thr439 in drug binding. These results, together with the previously identified Tyr95 and Ile172 residues believed to be involved in antidepressant binding [20], favor the S1 site as the substrate binding site. However, the cause of the Ile172Met mutation to the observed loss of antidepressant binding affinity has recently been attributed to be indirect via conformational changes [21,26].

In contemplating the location of drug binding sites in human SERT, the first consideration should be that the bacterial and human transporter could be sufficiently divergent as to impart different drug binding properties as a function of the drug studied. Another consideration is that there could be more than one binding pocket for a given drug, for example a particular antidepressant. Sinning and colleagues [22] suggest that the S2 site is the allosteric site previously proposed for citalopram [27]. However, a detailed mutagenesis study of residues impacting allosteric interactions with citalopram did not point to a cluster of S2 residues [26, 28]. In interpreting mutagenesis studies, one always needs to consider indirect conformational changes distal from the site of interest. Furthermore, in contemplating drug binding sites, it is important to recall that different drugs—cocaine-like phenyltropanes, TCAs and structurally heterologous SSRI antidepressants—often show dramatically different effects to the same mutation [17,20–22,25]. This calls for the separation of results for individual drugs, even among SSRIs, when interpreting mutagenesis and binding experiments. Another consideration is that the close spatial proximity between the S1 and S2 sites makes the total separation of the roles between the two sites difficult to ascertain. The two sites even share a number of common residues (Figure 2a). Therefore, even if the inhibitor and the substrate do not compete for space, they can still compete for those common residues. This issue is further complicated by the flexible nature of substrate binding sites in transporter proteins, which operate by an induced-fit mechanism [29]. It should thus be considered that some drugs could bind to both S1 and S2 sites, with differing impact on substrate translocation. Ultimately crystal structures of human SERT, or related mammalian transporters, with different compounds will provide the decisive information.

5. SERT specificity for SSRIs

SSRIs bind SERT with high specificity relative to NET and DAT (Figure 3) [30,31]. Both the position and type of substitution found on an aromatic moiety of the SSRI molecule are important determinants for SERT specificity [32,33]. All SSRIs have a halogen substitution at the 3rd (*meta*) or 4th (*para*) position of the phenyl or phenoxy ring [32,34–38], which, together with the amine group at the other end of the molecule, yields a dipole moment (Figure 3d). It is this halogen substitution and this characteristic dipole moment that appears to be largely responsible for the drugs' specificity to SERT over NET [39,40]. Similar halogen substitutions

also exist in serotonin-norepinephrine reuptake inhibitors [41,42] as well as in serotonin-norepinephrine-dopamine triple reuptake inhibitors [43–46] but not in norepinephrine selective reuptake inhibitors (NRIs) [32,37]. Strikingly, in fluoxetine-related antidepressants, [35,36] substitutions with CH₃- or OCH₃- at the 2nd (*ortho*) position in the phenoxy ring yield NRIs like tomoxetine and nisoxetine (Figures 2b & 2c).

Amino acids around the S2 site in SERT are largely homologous with those in LeuT, and within the HBP six of the seven residues are conserved between SERT, NET and DAT (Table 1). The only difference in primary sequence of the HBP between the three human proteins is found at Gly100 (SERT). When the equivalent residue in the HBP was mutated to a glycine, both NET and DAT showed modestly increased affinities to all three SSRIs tested, sertraline, R-fluoxetine and S-fluoxetine [17]. Indeed, both NET and DAT are the two very neurotransmitter transporters that SSRIs were developed to select against, and a single mutation can partially reverse the selectivity. The findings are consistent with the possibility that the specificity of the human SERT to this class of antidepressants is defined in large part by interaction of the drug's halogens with the protein's halogen-binding pocket. Obviously, given the size of the SSRI molecules, additional structural determinants in SERT must be implicated in its specificity for SSRIs.

6. LeuT-Trp structure

Tryptophan has recently been shown to be a competitive inhibitor of LeuT [47]. It binds at the primary leucine S1 site, trapping the protein in an outward-facing (C_o) conformation (Figure 4a). In the LeuT-tryptophan crystal structure [47], tryptophan bound at the S1 site interacts with the same residues as leucine, except it fails to form a hydrogen-bond with the hydroxyl group of the extracellular gate residue Tyr108. The structure shows that the gating residues Tyr108 and Phe253 are 3 Å farther apart in comparison to the leucine bound occluded (O_{cc}) state, leaving the S1 site accessible from the extracellular side. In addition, there is a second tryptophan molecule bound 4 Å on the extracellular side of the S1 site sandwiched between the Arg30 and Asp404 gating residues (Figure 4b). This secondary tryptophan molecule has been hypothesized to be the substrate-in-waiting, to be recruited as substrate after a desolvating passage through the Arg30 and Asp404 gate [47].

7. Cocaine binding to DAT

Cocaine's behavioral action is thought to be due primarily to inhibition of the reuptake of dopamine by DAT [48], in most studies found to be of a competitive nature [49]. Recently, molecular models for binding of dopamine, cocaine and the cocaine-analogue β-CFT to human DAT have been proposed on the basis of the LeuT structure, verified by extensive mutagenesis and chemical cross-linking experiments [50]. Although the binding sites for dopamine and cocaine were proposed to partially overlap, the two molecules appeared considerably different in their interaction with the gating residue Tyr156. In the DAT protein, Tyr156 appears to form a π-π stacking with the catechol ring of the dopamine and an H-bond with Asp79 via its hydroxyl group. When CFT is bound to the DAT, the interaction with Tyr156 is maintained but the hydrogen-bond with Asp79 gets disrupted leaving the non-polar pocket facing outward, open to the bulk solvent. MD simulation studies showed that disruption of this H-bond was accompanied by the permeation of water deep in the binding pocket [50].

8. Conclusions

The bacterial leucine transporter LeuT has proven to be an informative model for understanding the structure and mechanism of the neurotransmitter/sodium symporter family. Some of these properties may extend to mammalian members of this transporter family, but it is not yet clear how much overlap actually occurs in view of substantial detailed differences in bacterial and

mammalian sequences. Crystal structures of LeuT in complex with various inhibitors, TCAs, SSRIs and amino acids, have inspired new hypotheses on how antidepressants bind and inhibit mammalian SERT. These new hypotheses, for which some mutagenesis data lend support, are still controversial, with a large body of mutagenesis data pointing to interaction of antidepressant drugs, especially tricyclics and citalopram, with the primary S1 substrate site. Purified protein samples will eventually allow stoichiometry measurements of drug binding and site specific drug inhibition, as has been done for LeuT. Ultimately, the protein structure of mammalian SERT, and other mammalian members of the NSS transporter family, needs to be solved.

The identification of a secondary substrate-binding site in LeuT represents an important breakthrough in the understanding of the protein's transport mechanism. It also poses some key unanswered questions: How does a kinetic theory for such a two-binding-site protein differ from the original Michaelis–Menten kinetics for the single-binding-site protein, usually described by the alternating access model? How does a two-binding-site transporter display competitive inhibition? What experiments will be needed to dissect substrate/inhibitor interaction for each individual site? Could there be multiple stopover sites along the substrate permeation pathway with their primary role being to guide substrate passage? Answers to such questions will require the combination of a better theoretical frame of transport kinetics, crystal structures of mammalian NSS proteins in complex with drugs, and single molecule spectroscopic techniques that can define the complete transport cycle of the NSS protein.

Acknowledgments

We thank current and former members of the Wang Lab and the Reith Lab for discussions. The authors' work was financially supported by the NIH (MH083840 to D.N.W. and M.E.A.R., GM075936 to D.N.W., DA019676 and DA013261 to M.E.A.R., and GM075026 to W.A. Hendrickson). N.K.K. thanks the American Heart Association and the NIH (F32HL091618) for postdoctoral fellowships.

References and annotations

1. Rudnick, G. Mechanisms of biogenic amine neurotransmitter transporters. In: Reith, MEA., editor. *Neurotransmitter Transporters: Structure, Function, and Regulation*. 2. Humana Press; 2002. p. 25-52.
2. Torres GE, Gainetdinov RR, Caron MG. Plasma membrane monoamine transporters: structure, regulation and function. *Nat Rev Neurosci* 2003;4:13–25. [PubMed: 12511858]
3. Kanner BI, Zomot E. Sodium-coupled neurotransmitter transporters. *Chem Rev* 2008;108:1654–1668. [PubMed: 18393466]
4. Androutsellis-Theotokis A, Goldberg NR, Ueda K, Beppu T, Beckman ML, Das S, Javitch JA, Rudnick G. Characterization of a functional bacterial homologue of sodium-dependent neurotransmitter transporters. *J Biol Chem* 2003;278:12703–12709. [PubMed: 12569103]
5. Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E. Crystal structure of a bacterial homologue of Na⁺/Cl⁻-dependent neurotransmitter transporters. *Nature* 2005;437:215–223. [PubMed: 16041361]
6. Forrest LR, Tavoulari A, Zhang YW, Rudnick G, Honig B. Identification of a chloride ion binding site in Na⁺/Cl⁻-dependent transporters. *Proc Natl Acad Sci U S A* 2007;104:12761–12766. [PubMed: 17652169]
7. Zomot E, Bendahan A, Quick M, Zhao Y, Javitch JA, Kanner BI. Mechanism of chloride interaction with neurotransmitter:sodium symporters. *Nature* 2007;449:726–730. [PubMed: 17704762]
- 8**. Shi L, Quick M, Zhao Y, Weinstein H, Javitch JA. The mechanism of a neurotransmitter:sodium symporter--inward release of Na⁺ and substrate is triggered by substrate in a second binding site. *Mol Cell* 2008;30:667–677. Binding and flux experiments, together with steered molecular dynamics simulations, identified a second substrate binding (S2) site located in the extracellular vestibule. The two binding sites can be occupied simultaneously. Binding of leucine substrate at the S2 site triggers intracellular release of substrate from the primary site. This two-site model of transport calls for new kinetics theories and provides opportunities for greater understanding of substrate- and drug-binding in NSS proteins. [PubMed: 18570870]

9. Zhou Z, Zhen J, Karpowich NK, Goetz RM, Law CJ, Reith ME, Wang DN. LeuT-desipramine structure reveals how antidepressants block neurotransmitter reuptake. *Science* 2007;317:1390–1393. [PubMed: 17690258]
10. Singh SK, Yamashita A, Gouaux E. Antidepressant binding site in a bacterial homologue of neurotransmitter transporters. *Nature* 2007;448:952–956. [PubMed: 17687333]
11. Celik L, Schiott B, Tajkhorshid E. Substrate binding and formation of an occluded state in the leucine transporter. *Biophys J* 2008;94:1600–1612. [PubMed: 18024499]
- 12**. Tang L, Bai L, Wang WH, Jiang T. Crystal structure of the carnitine transporter and insights into the antiport mechanism. *Nat Struct Mol Biol* 2010;17:492–496. CaiT is an exporter of carnitine across the *E. coli* inner membrane. The CaiT crystal structure nonetheless revealed a LeuT fold, even though it belongs to another transporter family and does not share any sequence homology with the leucine transporter. In addition to the S1 site substrate, another substrate was found in a secondary S2 site located in the intracellular vestibule. [PubMed: 20357772]
13. Karpowich NK, Wang DN. Structural biology. Symmetric transporters for asymmetric transport. *Science* 2008;321:781–782. [PubMed: 18687947]
14. Schmitt KC, Mamidyalu S, Biswas S, Dutta AK, Reith ME. Bivalent phenethylamines as novel dopamine transporter inhibitors: evidence for multiple substrate-binding sites in a single transporter. *J Neurochem* 2010;112:1605–1618. [PubMed: 20067583]
15. Nielsen S, Pedersen CM, Hansen SG, Petersen MD, Sinning S, Wiborg O, Jensen HH, Bols M. An extended study of dimeric phenyl tropanes. *Bioorg Med Chem* 2009;17:4900–4909. [PubMed: 19560930]
16. Talvenheimo J, Nelson PJ, Rudnick G. Mechanism of imipramine inhibition of platelet 5-hydroxytryptamine transport. *J Biol Chem* 1979;254:4631–4635. [PubMed: 438209]
- 17**. Zhou Z, Zhen J, Karpowich NK, Law CJ, Reith ME, Wang DN. Antidepressants specificity of serotonin transporter suggested by three LeuT-SSRI complex structures. *Nat Struct Mol Biol* 2009;16:652–657. Cocrystal structures of LeuT with sertraline, R-fluoxetine or S-fluoxetine revealed that all three SSRI molecules bind at the S2 site in LeuT, with their halogens all binding to the same pocket. Mutation at this halogen-binding pocket (HBP) in human SERT markedly reduced the transporter's affinity for SSRIs. Conversely, when the only nonconserved HBP residue in both norepinephrine and dopamine transporters was mutated into that found in SERT, their affinities for all the three SSRIs increase uniformly. These results show that the specificity of SERT for SSRIs is dependent largely on interaction of the drug halogens with the protein's HBP. [PubMed: 19430461]
18. Roubert C, Cox PJ, Bruss M, Hamon M, Bonisch H, Giros B. Determination of residues in the norepinephrine transporter that are critical for tricyclic antidepressant affinity. *J Biol Chem* 2001;276:8254–8260. [PubMed: 11092898]
19. Barker EL, Blakely RD. 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* 1996;50:957–965. [PubMed: 8863842]
20. Henry LK, Field JR, Adkins EM, Parnas ML, Vaughan RA, Zou MF, Newman AH, Blakely RD. 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* 2006;281:2012–2023. [PubMed: 16272152]
21. Walline CC, Nichols DE, Carroll FI, Barker EL. 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* 2008;325:791–800. [PubMed: 18354055]
22. Sinning S, Musgaard M, Jensen M, Severinsen K, Celik L, Koldso H, Meyer T, Bols M, Jensen HH, Schiott B, et al. Binding and orientation of tricyclic antidepressants within the central substrate site of the human serotonin transporter. *J Biol Chem* 2010;285:8363–8374. [PubMed: 19948720]
23. Plenge P, Wiborg O. High- and low-affinity binding of S-citalopram to the human serotonin transporter mutated at 20 putatively important amino acid positions. *Neurosci Lett* 2005;383:203–208. [PubMed: 15955412]

24. Cherubino F, Miszner A, Renna MD, Sangaletti R, Giovannardi S, Bossi E. GABA transporter lysine 448: a key residue for tricyclic antidepressants interaction. *Cell Mol Life Sci* 2009;66:3797–3808. [PubMed: 19756379]
25. Andersen J, Taboureau O, Hansen KB, Olsen L, Egebjerg J, Stromgaard K, Kristensen AS. Location of the antidepressant binding site in the serotonin transporter: importance of Ser-438 in recognition of citalopram and tricyclic antidepressants. *J Biol Chem* 2009;284:10276–10284. [PubMed: 19213730]
26. Andersen J, Olsen L, Hansen KB, Taboureau O, Jorgensen FS, Jorgensen AM, Bang-Andersen B, Egebjerg J, Stromgaard K, Kristensen AS. Mutational mapping and modeling of the binding site for (S)-citalopram in the human serotonin transporter. *J Biol Chem* 2010;285:2051–2063. [PubMed: 19892699]
27. Plenge P, Møllerup ET. Antidepressive drugs can change the affinity of [3H]imipramine and [3H]paroxetine binding to platelet and neuronal membranes. *Eur J Pharmacol* 1985;119:1–8. [PubMed: 2935414]
28. Jorgensen AM, Topiol S. Driving forces for ligand migration in the leucine transporter. *Chem Biol Drug Des* 2008;72:265–272. [PubMed: 18844672]
29. Klingenberg M. Ligand-protein interaction in biomembrane carriers. The induced transition fit of transport catalysis. *Biochemistry* 2005;44:8563–8570. [PubMed: 15952762]
30. Tatsumi M, Groshan K, Blakely RD, Richelson E. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur J Pharmacol* 1997;340:249–258. [PubMed: 9537821]
31. Eshleman AJ, Carmolli M, Cumbay M, Martens CR, Neve KA, Janowsky A. Characteristics of drug interactions with recombinant biogenic amine transporters expressed in the same cell type. *J Pharmacol Exp Ther* 1999;289:877–885. [PubMed: 10215666]
32. Pinder RM, Wieringa JH. Third-generation antidepressants. *Med Res Rev* 1993;13:259–325. [PubMed: 8387126]
33. Roman DL, Walline CC, Rodriguez GJ, Barker EL. Interactions of antidepressants with the serotonin transporter: a contemporary molecular analysis. *Eur J Pharmacol* 2003;479:53–63. [PubMed: 14612137]
34. Welch WM, Kraska AR, Sarges R, Koe BK. Nontricyclic antidepressant agents derived from cis- and trans-1-amino-4-aryltetralins. *J Med Chem* 1984;27:1508–1515. [PubMed: 6492080]
35. Wong DT, Bymaster FP, Engleman EA. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. *Life Sci* 1995;57:411–441. [PubMed: 7623609]
36. Wong DT, Bymaster FP. Development of antidepressant drugs. Fluoxetine (Prozac) and other selective serotonin uptake inhibitors. *Adv Exp Med Biol* 1995;363:77–95. [PubMed: 7618533]
37. Iversen, L. Antidepressants. In: Abraham, DJ., editor. *Burger's Medical Chemistry and Drug Discovery*. 6. Vol. 6. Wiley; 2003. p. 483-524.
38. Eildal JN, Andersen J, Kristensen AS, Jorgensen AM, Bang-Andersen B, Jorgensen M, Stromgaard K. From the selective serotonin transporter inhibitor citalopram to the selective norepinephrine transporter inhibitor talopram: synthesis and structure-activity relationship studies. *J Med Chem* 2008;51:3045–3048. [PubMed: 18429609]
39. Rupp A, Kovar KA, Beuerle G, Ruf C, Folkers G. A new pharmacophoric model for 5-HT reuptake-inhibitors: differentiation of amphetamine analogues. *Pharm Acta Helv* 1994;68:235–244. [PubMed: 8208746]
40. Butler SG, Meegan MJ. Recent developments in the design of anti-depressive therapies: targeting the serotonin transporter. *Curr Med Chem* 2008;15:1737–1761. [PubMed: 18673223]
41. Heal DJ, Aspley S, Prow MR, Jackson HC, Martin KF, Cheetham SC. Sibutramine: a novel anti-obesity drug. A review of the pharmacological evidence to differentiate it from d-amphetamine and d-fenfluramine. *Int J Obes Relat Metab Disord* 1998;22 (Suppl 1):S18–28. [PubMed: 9758240]
42. Sanchez C, Hyttel J. Comparison of the effects of antidepressants and their metabolites on reuptake of biogenic amines and on receptor binding. *Cell Mol Neurobiol* 1999;19:467–489. [PubMed: 10379421]

43. Pearce RK, Smith LA, Jackson MJ, Banerji T, Scheel-Kruger J, Jenner P. The monoamine reuptake blocker brasofensine reverses akinesia without dyskinesia in MPTP-treated and levodopa-primed common marmosets. *Mov Disord* 2002;17:877–886. [PubMed: 12360536]
44. Lehr T, Staab A, Tillmann C, Trommeshauser D, Raschig A, Schaefer HG, Kloft C. Population pharmacokinetic modelling of NS2330 (tesofensine) and its major metabolite in patients with Alzheimer's disease. *Br J Clin Pharmacol* 2007;64:36–48. [PubMed: 17324246]
45. Rothman RB, Partilla JS, Baumann MH, Dersch CM, Carroll FI, Rice KC. Neurochemical neutralization of methamphetamine with high-affinity nonselective inhibitors of biogenic amine transporters: a pharmacological strategy for treating stimulant abuse. *Synapse* 2000;35:222–227. [PubMed: 10657029]
46. Skolnick P, Popik P, Janowsky A, Beer B, Lippa AS. Antidepressant-like actions of DOV 21,947: a "triple" reuptake inhibitor. *Eur J Pharmacol* 2003;461:99–104. [PubMed: 12586204]
- 47*. Singh SK, Piscitelli CL, Yamashita A, Gouaux E. A competitive inhibitor traps LeuT in an open-to-out conformation. *Science* 2008;322:1655–1660. Cocystal structure showed that a competitive inhibitor, tryptophan, traps LeuT in an outward-facing conformation. A second tryptophan is found between the extracellular gate residues arginine 30 and aspartic acid 404, preventing the gate from closing. [PubMed: 19074341]
48. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 1987;237:1219–1223. [PubMed: 2820058]
49. Wu Q, Reith ME, Kuhar MJ, Carroll FI, Garri PA. Preferential increases in nucleus accumbens dopamine after systemic cocaine administration are caused by unique characteristics of dopamine neurotransmission. *J Neurosci* 2001;21:6338–6347. [PubMed: 11487657]
- 50*. Beuming T, Kniazeff J, Bergmann ML, Shi L, Gracia L, Raniszewska K, Newman AH, Javitch JA, Weinstein H, Gether U, et al. The binding sites for cocaine and dopamine in the dopamine transporter overlap. *Nat Neurosci* 2008;11:780–789. Homology modeling suggests that the binding site for cocaine is deeply buried between transmembrane segments 1, 3, 6 and 8, and overlaps with the binding sites for the substrate dopamine. This binding mode was validated by detailed mutagenesis, followed either by cross-linking engineered cysteines or with an engineered Zn²⁺-binding site. These data demonstrate the molecular basis for the competitive inhibition of dopamine transport by cocaine. [PubMed: 18568020]
51. DeLano, WL. *The PyMOL User's Manual*. San Carlos, CA: DeLano Scientific; 2002.

Figure 1. Transport cycle of the leucine transporter LeuT. C_i: inward-facing conformation; C_o: outward-facing conformation; O_{cc}: occluded conformation.

Figure 2.

Interaction of LeuT with antidepressants. (a) Overall LeuT structure in its O_{cc} state, with leucine and two sodium ions bound at the S1 site and a sertraline bound at the S2 site (PDB ID: 3GWU). (b) Desipramine binding site in LeuT (PDB ID: 2QJU) [9]. (c) R-fluoxetine binding site in LeuT (PDB ID: 3GWV) [17]. (d) Superposition of the three LeuT-SSRI structures at the drug binding site. The figures were prepared using PyMol [51]. Sertraline molecule is colored yellow, R-fluoxetine orange, and S-fluoxetine green.

Figure 3.

Antidepressant and cocaine molecules and key determinants for specificity for SSRIs. **(a)** Molecular structures of serotonin selective reuptake inhibitors. **(b)** Molecular structures of norepinephrine selective reuptake inhibitors. **(c)** Illustration showing fluoxetine-derivative antidepressants. Substitutions at the 4th position in the phenoxy ring with F-, Cl-, CF₃-, CH₃- or OCH₃- produce SSRIs, whereas substitutions at the 2nd position with CH₃- or OCH₃- yield NRIs like tomoxetine and nisoxetine [35]. **(d)** Key determinants for SSRI's specificity for SERT [39,40]. The letter "X" denotes the electronegative halogen atom(s). The arrow represents the direction of the drug's dipole moment. **(e)** Molecular structures of TCAs. **(f)** Molecular structure of cocaine.

Figure 4. Interaction of LeuT with tryptophan [47]. (a) LeuT-Trp complex structure (PDB ID: 3F3A). (b) Two tryptophan molecules are bound to the LeuT.

Table 1

LeuT residues that interact with the drug molecule and their equivalents from human NSS proteins

Type of contact	LeuT*	SERT	NET	DAT
Desipramine				
van der Waals and cation- π	Arg ³⁰	Arg ¹⁰⁴	Arg ⁸¹	Arg ⁸⁵
van der Waals	Gln ³⁴	Ile ¹⁰⁸	Leu ⁸⁵	Leu ⁸⁹
van der Waals	Phe ²⁵³	Phe ³³⁵	Phe ³¹⁷	Phe ³²⁰
van der Waals	Ala ³¹⁹	Gly ⁴⁰²	Gly ³⁸³	Gly ³⁸⁶
van der Waals	Phe ³²⁰	Pro ⁴⁰³	Ala ³⁸⁴	Pro ³⁸⁷
van der Waals	Leu ⁴⁰⁰	Val ⁴⁸⁹	Leu ⁴⁶⁹	Phe ⁴⁷²
Salt-bridge	Asp ⁴⁰¹	Lys ⁴⁹⁰	Thr ⁴⁷⁰	Thr ⁴⁷³
Sertraline				
van der Waals	Leu ²⁹	Trp ¹⁰³	Trp ⁸⁰	Trp ⁸⁴
van der Waals	Arg ³⁰	Arg ¹⁰⁴	Arg ⁸¹	Arg ⁸⁵
van der Waals	Gln ³⁴	Ile ¹⁰⁸	Leu ⁸⁵	Leu ⁸⁹
van der Waals	Tyr ¹⁰⁸	Tyr ¹⁷⁶	Tyr ¹⁵²	Tyr ¹⁵⁶
van der Waals	Phe ²⁵³	Phe ³³⁵	Phe ³¹⁷	Phe ³²⁰
van der Waals	Ala ³¹⁹	Gly ⁴⁰²	Gly ³⁸³	Gly ³⁸⁶
van der Waals	Leu ⁴⁰⁰	Val ⁴⁸⁹	Leu ⁴⁶⁹	Phe ⁴⁷²
van der Waals	Asp ⁴⁰¹	Lys ⁴⁹⁰	Thr ⁴⁷⁰	Thr ⁴⁷³
Salt-bridge and van der Waals	Asp ⁴⁰⁴	Glu ⁴⁹³	Asp ⁴⁷³	Asp ⁴⁷⁶
van der Waals	Thr ⁴⁰⁹	Gly ⁴⁹⁸	Gly ⁴⁷⁸	Gly ⁴⁸¹

* Notes: Atomic coordinates are taken from crystal structures of LeuT in complex with desipramine (PDB ID: 2QJU) [9] and with sertraline (PDB ID: 3GWU) [17], respectively.