Serotonin (5-hydroxytryptamine or 5-HT) modulates the activity of the central nervous system as well as processes throughout the body ranging from cardiovascular function to digestion, body temperature, endocrinology and reproduction1. Discovered in the late 1940s as a signalling molecule, serotonin increases vasoconstriction after blood clotting, that is, serum-tone2. In the brain, the raphe nuclei synthesize serotonin from tryptophan, and distribute serotonin via long projections that reach nearly every major brain region. Serotonin is released into the synaptic cleft between neurons, where it diffuses to activate serotonin receptors, a group of G-protein-coupled receptors and ligand-gated ion channels that participate in both excitatory and inhibitory neurotransmission and modulate the release of many neurotransmitters and hormones. Thus, serotonergic signalling influences neurological processes including sleep, mood, cognition, pain, hunger and aggression behaviours. The discovery that serotonin reuptake into nerve terminals is inhibited by the tricyclic antidepressant imipramine in a manner similar to norepinephrine (also known as noradrenaline) reuptake provided an initial clue that transport occurs by a related reuptake system3–5. Prozac was introduced as one of the first selective serotonin reuptake inhibitors (SSRIs) for the treatment of depression and, subsequently, the serotonin transporter gene (SERT, also known as SLC6A4) was cloned and proven to be the target of SSRIs6,7.

SERT is a member of the neurotransmitter sodium symporter (NSS) family of transporters, which also includes the dopamine (DAT) and norepinephrine (NET) transporters. NSSs are responsible for the sodium- and chloride-dependent reuptake of neurotransmitters, thus terminating signalling of the biogenic amines8,9. The unbinding of inhibitors can be further modulated by serotonin10 and antidepressants11 acting at an allosteric site. Several neurological conditions are associated with NSS dysregulation, including depression, anxiety disorder, attention-deficit hyperactivity disorder, epilepsy and Parkinson’s disease8,9,12. Pharmacological modulation of NSS function through the use of therapeutic drugs such as tricyclic antidepressants and SSRIs has been used to treat many psychiatric disorders13. Illicit drugs such as cocaine and methamphetamines block neurotransmitter reuptake and are commonly abused psychostimulants, diminishing the well-being of users and constituting a tremendous socioeconomic burden.

Knowledge of NSS structure has been guided, in part, by experiments on the bacterial orthologue LeuT, as well as by studies of the Drosophila DAT (dDAT). This previous work has shown that the NSS family of transporters contain an inverted-topological repeat of transmembrane helices (TM) 1–5 and TM6–TM10, a ‘central’ or primary binding site for substrate and ions approximately halfway across the membrane-spanning region of the transporters14–18 and, in the outward-open conformation, a large extracellular vestibule. Recently, structures of the invertebrate dDAT have provided insight into NSS pharmacology19–21. Nevertheless, these studies fall short of defining the structural determinants responsible for the markedly diverse pharmacological profiles of NSSs, the allosteric mechanism of human SERT, and important characteristics of human transporters. Here we present structures of the human serotonin transporter in complex with two of the most widely prescribed antidepressants: (S)-citalopram and paroxetine. Structures of SERT illuminate the molecular features of SSRI inhibition and allosteric regulation, as well as structural elements not reported in transporters previously studied.

Thermostable SERT–Fab complex

Wild-type human SERT22 is unstable in detergent micelles and refractory to crystallization. We thus screened a panel of SERT mutants for enhanced thermostability using a high-throughput ligand binding assay23, and by fluorescence-detection size exclusion chromatography24. Two thermostabilizing mutations, Ile291Ala and Thr439Ser, were introduced into SERT, yielding the ts2 construct, stable in short-chain detergents. Using transporter protein isolated from baculovirus-transduced mammalian cells25, together with a recombinant anti-SERT Fab, we obtained small crystals of a ts2–Fab–paroxetine complex that diffracted X-rays to 4.5 Å resolution. To improve crystal order, we included a third thermostabilizing mutation (Tyr110Ala), yielding the ts3 construct, which further improved stability and produced crystals of the Fab complex with either (S)-citalopram or paroxetine that diffracted X-rays to 3.15 Å resolution (Extended Data Tables 1 and 2). Whereas the wild-type transporter exhibits serotonin transport with a Michaelis constant ($K_m$) of 1.9±0.3 μM (mean±s.e.m.) and a maximal velocity ($V_{max}$) of 23±1 pmol min$^{-1}$, similar to reported values8, ts2 has a $K_m$
value of $4.5 \pm 0.6 \mu M$ and $V_{\text{max}}$ value of $21 \pm 5$ pmol min$^{-1}$ (Fig. 1a). No detectable transport activity was found for ts3.

**Architecture of human SERT**

The structure of human SERT bound to (S)-citalopram or paroxetine exhibits an outward-open conformation with the antidepressant drug bound to the central site, halfway across the membrane and wedged into a cavity made up of residues from TM1, TM3, TM6, TM8 and TM10 (Fig. 1b, c). A second (S)-citalopram molecule was found in the allosteric site, within the extracellular vestibule of the (S)-citalopram ocystal structure, approximately 13 Å from the central site. Akin to dDAT and LeuT, SERT has 12 transmembrane-spanning helices with TM1–TM5 and TM6–TM10 related by a pseudo-two-fold axis$^{14,16,20,21}$ (Extended Data Fig. 1). The ts2 and ts3 transporters superimpose well (Extended Data Table 3), demonstrating that the additional mutation of the ts3 construct does not substantially perturb the functionally active ts2 transporter structure (Extended Data Fig. 2a). TM1 and TM6 adopt short regions of non-helical conformation as they skirt the central ligand site and contribute residues that bind inhibitors as well as coordinate Na$^+$ and Cl$^-$ ions. The conformations of TM1 and TM6 are incompatible with the formation of an occluded state, suggesting that the antidepressant molecules have locked the transporter in an outward-open conformation, similar to the inhibitor-bound outward-open conformations of dDAT and Leu$^{T14,16,19,21,26}$ (Extended Data Table 3).

The extracellular surface of SERT is largely composed of extracellular loop (EL) 2, EL4 and EL6, with EL2 ‘combed-over’ the extracellular surface and providing 3,376 Å$^2$ of solvent-accessible surface area. A conserved disulfide bridge is formed between Cys200 and Cys209 in EL2 (ref. 27). EL2 is predicted to contain two N-linked glycosylation sites, Asn208 and Asn217 (ref. 28), and electron density for a N-acetylglucosamine moiety was found linked to Asn208; weak density was also found near Asn217. Similar to dDAT, the intracellular surface of the transporter is capped by intracellular loop (IL) 1, IL5 and the carboxy-terminal helix. Unlike LeuT, yet reminiscent of dDAT, TM12 has a pronounced kink halfway across the membrane. There is a cholesterol hemisuccinate (CHS) molecule bound near TM12a.

The crystal lattice packing between two SERT molecules occurs at the kink in TM12, which also overlaps with a two-fold axis of crystallographic symmetry (Extended Data Fig. 2c), thus generating an apparent SERT ‘dimer’. Experiments suggest that SERT is an oligomer molecules at the central and allosteric site are shown as sticks in dark green and cyan, respectively. Sodium ions are shown as spheres in salmon. Cholesteryl hemisuccinate (CHS) and N-acetylglucosamine (NAG) are shown as sticks. c, View of SERT from the extracellular side of the membrane.
The amine groups of (S)-citalopram and paroxetine occupy subsite A and interact with the carboxylate of the conserved Asp98 (ref. 34) at a distance of 4.1 and 3.1 Å (Fig. 2f, g), perhaps explaining, in part, why paroxetine has a higher affinity for SERT in comparison to (S)-citalopram. Tyr95 localizes 4.2 and 5.3 Å beneath the amine groups of (S)-citalopram and paroxetine, forming a cation--π interaction crucial for citalopram and mazindol potency35. Tyr95 may also form a hydrogen bond with the oxygen of (S)-citalopram. Ser336 partners in an interaction network with ligands and ions by participating in Na\(^+\) and Cl\(^-\) coordination, ions that are essential for ligand binding and substrate transport36–38.

Subsite B is particularly important for high-affinity antidepressant action as evidenced by mutations that influence citalopram binding32,39. Tyr176 engages in hydrophobic interactions with the fluorophenyl and benzodioxol groups of (S)-citalopram and paroxetine while also hydrogen bonding with Asp98. Ile172 and Phe341 define a non-polar ridge that cradles the hydrophobic groups of the drugs, and inhibitor binding is weakened upon mutation of these residues32,40. Phe341 in SERT, which is equivalent to Phe325 in dDAT, has swung ‘downward’ by nearly 40° and forms an aromatic interaction with the ‘face’ of the cyanothalamine of (S)-citalopram and with the ‘edge’ of the fluorophenyl group of paroxetine (Extended Data Fig. 3a, b). Ser439, Leu443, Ala169 and Ala173 define a cavity that is more hydrophobic in SERT in comparison to the equivalent cavity in dDAT, and into which the fluoro and oxiexciplex groups of (S)-citalopram and paroxetine are inserted (Extended Data Fig. 3c).

The fluorophenyl group of (S)-citalopram is positioned 1.5 Å deeper into this space compared with the benzodioxol of paroxetine (Extended Data Fig. 3b).

The fluorophenyl group of paroxetine stacks parallel to the ring of Phe335 in subsite C. By contrast, for (S)-citalopram, the cyanothalamine forms an edge-to-face aromatic interaction. Phe335 defines the extracellular gate, and TM1 and TM6 are markedly different when comparing SERT to dDAT bound to a substrate analogue35 (Extended Data Table 3), showing that (S)-citalopram and paroxetine ‘prop’ TM6a in an outward-open conformation. Val501 and Thr497 form a mixed non-polar-polar surface into which the fluoro and cyano groups of paroxetine and (S)-citalopram are found. In the case of (S)-citalopram, the cyano group is inserted 2.1 Å further into subsite C, and the hydroxyl group of Thr497 is positioned 1.7 Å away from its position in the paroxetine state (Extended Data Fig. 3b). In accord with the SERT-citalopram X-ray structure, a modest increase in citalopram affinity is observed for the Thr497Ala mutant39, which would allow additional space for the cyano group.

**Ion-binding sites**

Na\(^+\) and Cl\(^-\) ions, which are essential for substrate transport and SSRI binding41, could be identified with electron densities >3σ in F\(_o\) – F\(_c\) ‘omit’ maps (Extended Data Fig. 4), at positions similar to those found in dDAT (Extended Data Table 4). The Na\(^+\) site is made up of residues contributed from TM1, TM6 and TM7 and the ion is coordinated by Ala96, Asn101, Ser336 and Asn368. Whereas Na\(^+\) is coordinated, in part, by a water molecule in dDAT, which in turn is hydrogen-bonded to Asp46, in SERT there is not sufficient density to place water at a similar position. The chloride ion is coordinated by Tyr121, Gln332, Ser336 and Ser337 from TM2, TM6 and TM7 with a mean coordination distance of 3.1 Å. Strong density for Na\(^2+\) could be seen in the (S)-citalopram structure with the ion coordinated by Gly94, Val97, Leu434, Asp437 and Ser438 from TM1 and TM8. Placing ions in the omit densities led to a loss in F\(_o\) – F\(_c\) density and the B-values of the ions match the values of surrounding residues. The mean coordination distance (2.4 Å) corresponds to known coordinate distances for sodium32. Only weak density for Na\(^2+\) could be seen in the paroxetine structure, while in the (S)-citalopram complex the density for Cl\(^-\) was weak, perhaps reflecting the overall weaker density in these regions rather than a difference in occupancy.

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**Figure 2 | Antidepressant binding and recognition.** a. Graph of [\(^3\)H](R/S)-citalopram saturation binding to wild-type (black, circles), ts2 (blue, squares) and ts3 (red, triangles) transporters, showing the average (Fig. 2e). It is noteworthy that the chemically equivalent fluorophenyl groups of (S)-citalopram and paroxetine are positioned in different subsites.
Extracellular and intracellular gates

The SERT–SSRI complexes adopt an outward-open conformation that exposes the cone-shaped extracellular vestibule to aqueous solution, providing a pathway for substrates, inhibitors and ions to reach the central binding site, approximately halfway across the membrane bilayer. As in LeuT, the extracellular vestibule contains residues that form the extracellular gate, and is lined by TM1b and TM6a, as well as by extracellular regions of TM3, TM8, TM10 and TM11, together with EL6 and the ‘tip’ of EL4. The mixed polar and non-polar character of the extracellular vestibule provides low affinity binding sites for small molecules, similar to LeuT, and in SERT we find electron density attributed to a second (S)-citalopram molecule in the (S)-citalopram cocrystral structure and a maltose detergent head group in the paroxetine complex within the extracellular vestibule\textsuperscript{4,43–45} (Fig. 3a, b).

At the base of the vestibule is the extracellular gate, and near the cytoplasmic face of SERT is the intracellular gate (Extended Data Fig. 5a, b). In SERT, Tyr176 and Phe335 define the lower portion of the extracellular gate and are separated by a distance of 10 Å, thus providing open access to the extracellular vestibule. In comparison to the extracellular gate of dDAT, the equivalent region in SERT exhibits notable structural changes: Tyr176 and Asp98 are separated by 4.0 Å and TM10 is closer to TM1b, bringing Glu494 and Arg104 within 4.8 Å, and thus the central site can only be accessed through the extracellular vestibule. The intracellular gate of SERT is closed, similar to the outward-facing conformations of dDAT and LeuT, thus precluding direct access from the central ligand binding site to the intracellular solution (Fig. 3 and Extended Data Fig. 5b).

Allosteric site

To determine whether the off-rate of inhibitor from the central site is modulated by a ligand binding to an allosteric site in the ts3 construct, we measured the dissociation of [\textsuperscript{3}H](R/S)-citalopram from the central site in the presence of saturating concentrations of cold (S)-citalopram. As shown in previous studies, micromolar concentrations of (S)-citalopram, serotonin and other ligands slow dissociation from the central site\textsuperscript{9,11}. For ts3, 100 μM (S)-citalopram decreased the first-order rate of [\textsuperscript{3}H](R/S)-citalopram dissociation by nearly tenfold compared to buffer alone (0.0032 ± 0.0007 versus 0.025 ± 0.002 min\textsuperscript{−1}) (Fig. 4a), with the wild-type and ts2 transporters exhibiting similar effects (wild-type: 0.004 ± 0.001 versus 0.035 ± 0.004 min\textsuperscript{−1}; ts2: 0.0028 ± 0.001 versus 0.08 ± 0.03 min\textsuperscript{−1}), thus showing that allosteric modulation of ligand unbinding is intact in the ts2 and t3 constructs.

The allosteric binding site of (S)-citalopram is defined by residues in TM1b, TM6a, TM10 and TM11, and in EL4 and EL6 (Fig. 4b and Extended Data Fig. 5c) with prominent electron density (>5σ in $F_o-F_c$ omit maps) present in this region for crystals soaked with (S)-citalopram. Interestingly, mutagenesis of residues proximal to the allosteric site has been reported to severely alter allosteric potency\textsuperscript{46} yet the physiological role of this site is not well established\textsuperscript{47}. Residues of the extracellular gate, Glu494 and Arg104, are located 4.1 and 4.8 Å away from the aminopropyl group, while Asp328 is 6.8 Å away. Arg104 is also located 3.6 Å from the cyanothiaphenyl plane and probably participates in a cation–π interaction, while the cyano group of the phthalate ring is 3.1 Å from the side-chain amide of Gln332. Ala331 forms a non-polar groove into which the ring system of (S)-citalopram is butressed. Phe556 is 3.5 Å from the fluorophenyl group and participates in aromatic interactions while a proline repeat (Pro560–Pro561) in EL6 demarcates the upper portion of the allosteric site, 6.6 Å from the fluorophenyl entity.

To confirm the identity of the ligand bound to the allosteric site, we soaked crystals with Br-citalopram. A strong anomalous signal (>5σ) corresponding to bromine was detected in anomalous difference electron density maps, confirming the position and pose of citalopram in the Br-citalopram (blue)–(S)-citalopram-bound (pink) structures. Maltose is in orange sticks. Superposition was performed over all Cα atoms of the transporter.

In the paroxetine complex, we found electron density for a putative maltose entity, presumably derived from a detergent molecule (Extended Data Fig. 5d) occupying a position in the extracellular vestibule that partially overlaps with (S)-citalopram bound in the allosteric site (Fig. 4d). Upon analysis of the allosteric site of the (S)-citalopram and paroxetine complexes, we note considerable plasticity, presumably owing to the nature of the bound molecule. Relative
to the (S)-citalopram-bound allosteric site, in the paroxetine structure Phe556 moves ‘downwards’ towards TM6a, to a position underneath the maltose. In addition, Arg104 moves 2 Å further into the allosteric site, while EL6 also moves 1.3 Å towards TM10, with the largest change occurring at Pro561. The malleability of the allosteric site opens the possibility that, depending on the shape and size of the allosteric ligand, occupancy of the allosteric site might not necessarily abrogate transport activity. Indeed, it is conceivable that there could be a spectrum of small molecules that range from inhibiting to enhancing transport activity.

Comparison of the allosteric site of SERT with the equivalent region of dDAT shows how the SERT site is distinct from that of dDAT, even though SERT and dDAT are highly similar in structure within their cores around the central ligand binding site (TM1–TM8; Extended Data Table 3). Indeed, there are marked differences between SERT and dDAT for TM9–TM12 and the extracellular loops (Fig. 5a and Extended Data Table 3). EL2, centrally positioned within the extracellular domain, is longer in SERT than in dDAT (Fig. 5a and Extended Data Table 3). EL2, centrally positioned within the extracellular domain, is longer in SERT than in dDAT (Fig. 5a and Extended Data Table 3). EL2, centrally positioned within the extracellular domain, is longer in SERT than in dDAT (Fig. 5a and Extended Data Table 3).

The conformation of TM9–TM12 also defines the allosteric site (Fig. 5d). Comparisons between dDAT and SERT illustrate that in SERT TM9 is shifted towards TM12, perhaps coordinated by contacts via EL5 and TM10, the latter of which contains a short stretch of α-helix near Glu494, a key residue of the extracellular gate. In SERT, TM11 extends further into the putative membrane environment in comparison to dDAT, thus providing a larger cavity for allosteric ligands, while TM12a splays inward to buttress TM10 and TM11. Finally, interaction of cholesterol, which is known to modulate transport and ligand binding to together with other lipid molecules, may reinforce the conformation of TM12. Indeed, in SERT a CHS molecule stacks against Trp573 in a groove formed by Leu577, Ile576 and Ala580 and the extracellular portion of TM12a (Extended Data Fig. 6b), along with a presumed alkyl chain of a detergent molecule bound in a cavity composed of residues from TM10 and TM12a (Extended Data Fig. 6c).

**Intracellular surface and C-terminal hinge**

IL5 and the intracellular half of TM11 are highly similar to dDAT, while IL4 is partially unwound due to the insertion of Trp561 (Fig. 5d). The C terminus of SERT mimics dDAT with a similar hinge and helix region (Fig. 5e). Glu615 is thought to form a salt bridge with Arg152 in IL1 (ref. 49), but no side-chain density is present, which makes assignment of C-terminal register not possible. We propose that the disorder of the C terminus is due to dynamic properties, perhaps related to its importance in trafficking.

**Conclusion**

The SERT–SSRI complexes capture the transporter in an inhibitor-bound, outward-open conformation, illustrating how the bulky ligands lodge in the central binding site, preventing substrate binding and transporter isomerization to occluded and inward-open conformations. Extensive interactions throughout the central binding site explain, in large part, the selectivity of SSRIs. The allosteric site is poised ‘above’ the central site, within the ‘walls’ of the extracellular vestibule, directly obstructing ligand egress from the central site, thus explaining how allosteric ligands slow the off-rate of inhibitors bound to the central site (Fig. 6). Taken together, the structures of the human serotonin transporter shed fresh insight into antidepressant recognition and the molecular basis for allosteric modulation of inhibitor binding and of transporter activity, thus providing a platform to design small molecules targeting the central and allosteric binding sites.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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PROTEIN CONSTRUCTS
The cDNA encoding the human wild-type SERT\textsuperscript{22} was cloned into the BacMam vector\textsuperscript{22} with a C-terminal GFP tag. For crystallization studies, the ts3 variant contained thermostabilizing mutations Y110A, I291A, T439S and K527M, and was fused to a C-terminal GFP followed by twin Strep\textsuperscript{[TrpSerHisProGlnPheGlu]ys} (Gly GlyGlySer)\textsuperscript{23} and His\textsuperscript{10} purification tags. The His\textsuperscript{10} tag was used in thermostability studies to capture SERT on copper scintillation beads\textsuperscript{24} while the twin Strep tag was used for large-scale purification. Thrombin cleavage sites (LeuValProArgGlySer) were introduced in the N and C terminus after residues Gin76 and Thr168. The ts2 construct is identical to ts3 except that ts2 does not have the Y110A mutation. To raise the 886 antibody, residues 73–616 of wild-type SERT were cloned into BacMam with a C-terminal StrepII tag (TrpSerHisProGlnPheGlu)ys and without GFP.

ANTI-HUMAN SERT ANTIBODY DISCOVERY AND FAB EXPRESSION
The 886 monoclonal antibody against SERT was raised by D. Cawley. StrepII-tagged SERT was purified by Strep Tactin affinity chromatography as described subsequently in DDM with 1 μM paroxetine. Liposomes containing asolectin: cholesterol:lipid A:brain polar lipid (60:17:3:20) were prepared in TBS (20 mM Tris, pH 8, 100 mM NaCl) at a concentration of 40 mg ml\textsuperscript{-1} by extrusion through 200-nm filters. Liposomes were saturated with 5 mM d-decyl-β-d-maltoside (DDM) and purified SERT was added to the detergent-lipid mixture. DDM was removed by three successive additions of 80 mg ml\textsuperscript{-1} biobeads. For the first two additions, the biobeads were incubated for 2 h; the final incubation was overnight. Paroxetine (10 μM) was added to the proteoliposomes after reconstitution. SERT-knockout mice were purchased from the Jackson Laboratory (mouse strain: 008355) and immunized with ~30 μg of recomposed SERT. Hybridoma cell lines were generated as described\textsuperscript{25} and screened by fluorescence-detection size-exclusion chromatography (FSEC)\textsuperscript{26} and western blotting to select antibodies which recognize tertiary epitopes. The 886 monoclonal antibody was purified from hybridoma supernatants using 4-mercapto-ethanol-lye resin. Fab was purified from papain digested monoclonal antibody by cation exchange chromatography and was stored in 20 mM Tris, pH 8, 150 mM NaCl and 10% glycerol.

The sequences of the 886 Fab light and heavy chain genes were determined by standard techniques. The genes of the 886 Fab were cloned into a bacitracin insect cell expression vector, including a GP67 signal peptide. A thrombin cleavage site (LeuValProArgGlySer) was introduced in the N and C termini containing GFP and purification tags were removed by thrombin digestion and N-linked sugars were truncated using EndoH. SERT was mixed into the proteoliposomes after reconstitution. SERT-knockout mice were purchased from the Jackson Laboratory (mouse strain: 008355) and immunized with ~30 μg of recomposed SERT. Hybridoma cell lines were generated as described\textsuperscript{25} and screened by fluorescence-detection size-exclusion chromatography (FSEC)\textsuperscript{26} and western blotting to select antibodies which recognize tertiary epitopes. The 886 monoclonal antibody was purified from hybridoma supernatants using 4-mercapto-ethanol-lye resin. Fab was purified from papain digested monoclonal antibody by cation exchange chromatography and was stored in 20 mM Tris, pH 8, 150 mM NaCl and 10% glycerol.

Transporter expression and purification.

The human SERT constructs were expressed as C-terminal GFP fusions using baculovirus-mediated transduction of mammalian HEK293S GnTI\textsuperscript{−} cells, as previously described\textsuperscript{25,52}. Cells were subsequently solubilized in 50 mM Tris, pH 8, 150 mM NaCl containing 20 mM DDM, 2.5 mM CHS, 0.5 mM dithiothreitol (DTT) in the presence of 1 μM inhibitor (paroxetine, (S)-citalopram, or Br-citalopram). The lysate was passed over 10 ml of Strep Tactin resin, washed with 18 column volumes of 1 mM DDM, 0.2 mM CHS, 5% glycerol, 25 μM lipid (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine, and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol) at a molar ratio of 1:1:1, and 1 μM ligand in TBS. SERT was eluted in the same buffer containing 5 mM desthiobiotin. SERT was eluted in the same buffer containing 5 mM desthiobiotin. The purified SERT-8B6 complex was concentrated to the proteoliposomes after reconstitution. SERT-knockout mice were purchased from the Jackson Laboratory (mouse strain: 008355) and immunized with ~30 μg of recomposed SERT. Hybridoma cell lines were generated as described\textsuperscript{25} and screened by fluorescence-detection size-exclusion chromatography (FSEC)\textsuperscript{26} and western blotting to select antibodies which recognize tertiary epitopes. The 886 monoclonal antibody was purified from hybridoma supernatants using 4-mercapto-ethanol-lye resin. Fab was purified from papain digested monoclonal antibody by cation exchange chromatography and was stored in 20 mM Tris, pH 8, 150 mM NaCl and 10% glycerol.

the sequences of the 886 Fab light and heavy chain genes were determined by standard techniques. The genes of the 886 Fab were cloned into a bacitracin insect cell expression vector, including a GP67 signal peptide. A thrombin cleavage site and 8His tags were fused to the C terminus of residues 1–235 of the heavy chain. The 886 Fab was purified from S9 supernatant of cells containing the antibody by a cation exchange chromatography process. The sequences of the 886 Fab light and heavy chain genes were determined by standard techniques. The genes of the 886 Fab were cloned into a bacitracin insect cell expression vector, including a GP67 signal peptide. A thrombin cleavage site and 8His tags were fused to the C terminus of residues 1–235 of the heavy chain. The 886 Fab was purified from S9 supernatant of cells containing the antibody by a cation exchange chromatography process.

Ligand binding and uptake assays.

Ligand binding experiments were carried out by adding HEK293 membranes containing SERT to a final concentration of 2 nM in 1 ml of TBS with either [3H]paroxetine 0.01–10 nM or [3H](R/S)-citalopram 0.01–20 nM. Reactions were rotated at room temperature for 4 h followed by filtering through a glass microfibre filter prewet with 0.4% polyethylenimine in TBS. Membranes were washed three times with 4 ml of TBS followed by liquid scintillation counting. Data was fit to a single-site binding curve accounting for ligand depletion. For dissociation experiments, 20 mM SERT in membranes was mixed with 40 nM [3H](R/S)-citalopram in 10 μl; samples were diluted to 1 ml in TBS with 100 μM (S)-citalopram, or without ligand, followed by the detection of the Fab fragment by ELISA. For uptake assays, ~1 × 10\textsuperscript{11} HEK293 cells in 96-well CytoTec plates were transfected with 0.2 μg of plasmid with PolyJet. After 24–36 h, cells were washed with 25 mM HEPES-Tris, pH 7.0, 130 mM NaCl, 5.4 mM KCl, 1.2 mM CaCl\textsubscript{2}, 1.2 mM MgSO\textsubscript{4}, 1 mM ascorbic acid and 5 mM glucose. For a control, 10 μM paroxetine was added. [4C]-Hydroxytryptamine at concentrations of 0.02–400 nM was added and uptake was followed using a MicroBeta scintillation counter. Data were fit to a Michaelis–Menten equation.

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Extended Data Figure 1  | Construct design and secondary structure. Thrombin digestion sites were introduced within the N- and C-terminal regions before Glu76 and after Thr618. Mutations which were introduced to increase thermostability (Tyr110Ala, Ile291Ala and Thr439Ser) are indicated (red star). Surface exposed cysteine residues were mutated to alanine (Cys554 and Cys580) and indicated by a blue star. Residues that have no electron density are boxed in green. Secondary structure was analysed using DSSP (http://swift.cmbi.ru.nl/gv/dssp/) and displayed using ENDScript (http://endscript.ibcp.fr/). Secondary structure elements are shown using the following symbols: \( \alpha \)-helix (\( \alpha \)), \( \beta \)-strand (\( \beta \)), \( \pi \)-helix (\( \pi \)), \( 3_{10} \) helix (\( \eta \)), \( \alpha \)-turn (TTT letters), \( \alpha \)-turn (TT letters). Locations of carbohydrate (red, *\( ^* \)) and disulfide bonding cysteine (green digits) residues are also shown. A–C in italic means the residue has a crystallographic contact with a residue in chain A–C. The *\( ^\# \) symbol identifies a contact between two residues along the crystallographic two-fold axis of symmetry. Contacts between transporter residues and small molecules in the range of 3.2–5.0 \( \AA \) are also indicated (black, *\( ^\# \)). Hydropathy is calculated according to Kyte and Doolittle59 and shown with pink as hydrophobic (\( H > 1.5 \)), cyan as hydrophilic (\( H < 1.5 \)), and grey as intermediate. The secondary structure of the dopamine transporter (4M48) is shown for comparison.
Extended Data Figure 2  | Comparison of the ts3 and ts2 structures, crystal packing and antibody structure. a, Superposition of the ts2 (blue) and ts3 (grey) transporters, each in complex with paroxetine using all atoms (Extended Data Table 3). Paroxetine (pink sticks) and thermostabilizing mutations (yellow spheres). b, Position of amino acid changes due to single nucleotide polymorphisms and mutants associated with psychiatric disorders (yellow). Paroxetine is shown in pink. c, SERT is shown in green, Fab heavy chain (orange), light chain (blue). SERT molecules pack into the crystal lattice with SERT–SERT interface occurring along the kink of TM12 helices related by the crystallographic two-fold axis (blue box). d, Rotation by 90° reveals further lattice contacts. e, The binding site of the 8B6 Fab is made up of interactions of residues from EL2 and EL4 (sticks). f, Comparison of the high resolution Fab structure (grey) with SERT-bound Fab (Extended Data Table 3). The largest structural changes occur in the complementary determining regions (CDRs).
Extended Data Figure 3 | Comparison of ligand binding in SERT and in DAT. 
a, Comparison of SERT bound to paroxetine with dDAT (4M48) bound to nortriptyline (yellow); superposition based on TM1–TM12. SERT is shown in blue and DAT in grey. 
b, Alignment of paroxetine (blue) and (S)-citalopram (pink) structures using all atoms in superposition (Extended Data Table 3). Residues interacting with the antidepressant molecules are shown as sticks. Paroxetine (pink) and (S)-citalopram (green) are shown as sticks. c, Insertion of benzodioxol and fluorophenyl groups of paroxetine and (S)-citalopram into a cavity in subsite B made up of Leu443, Ala169, Ala173 and Ser439. Note that Ser439 is equivalent to Thr439 in wild-type SERT. Equivalent residues in dDAT are shown in grey.
Extended Data Figure 4 | Ion-binding sites. a, Overall view of the Na\(^+\) and Cl\(^-\) ion binding sites in the paroxetine bound transporter. Na\(^+\) (salmon) and Cl\(^-\) (green) are shown as spheres. Paroxetine is shown as pink sticks. b, Overall view of the (S)-citalopram bound transporter showing the Na\(^+\) binding site; (S)-citalopram (green sticks). c, Residues coordinating Na\(^+\) and Cl\(^-\). Ion \(F_0 - F_c\) omit densities are shown at 2\(\sigma\) and 3\(\sigma\) for Na\(^+\) and Cl\(^-\). d, Residues coordinating Na\(^+\). \(F_0 - F_c\) omit density is shown at 4\(\sigma\). A water molecule is shown as a yellow sphere. Coordination distances are given in Extended Data Table 4.
Extended Data Figure 5 | Extracellular and intracellular gates and the allosteric site of paroxetine and partially occupied (S)-citalopram.

a, The extracellular gate of the SERT–(S)-citalopram complex is shown, with (S)-citalopram bound to the central site. The width of the gate is depicted by the distances between Tyr176 and Phe335 (10.3 Å, CD1–CE2), Asp98 and Tyr176 (4.0 Å, OD2–OH), Glu494 and Arg104 (4.9 Å, OE1–NH1) dDAT (grey) is shown for comparison. b, Comparison of the intracellular gate of SERT (pink) versus DAT (4M48, grey). Superpositions were made by alignment of TM1–TM12 of SERT with dDAT. c, The allosteric site containing fully occupied (S)-citalopram (pink) was superposed with the partially occupied structure (olive). The $F_o - F_c$ omit density (blue mesh) of the partially occupied structure is shown at 2σ. (S)-citalopram is shown in green sticks. A 12-carbon chain (magenta) was modelled into this density but could instead represent a partially occupied (S)-citalopram. The structure with partial (S)-citalopram occupancy at the allosteric site was derived from crystals grown in the presence of 10μM ligand. Crystals with a higher occupancy at the allosteric site were soaked in a solution containing 5 mM (S)-citalopram before crystal cryo protection. d, The paroxetine-bound transporter contains a maltose detergent headgroup (orange) bound to the allosteric site. $F_o - F_c$ maltose omit density at 3σ.

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Extended Data Figure 6 | Cholesteryl hemisuccinate and tetradecane binding sites. a, Overall view of the (S)-citalopram-bound structure showing CHS (red box) and tetradecane (C14, blue box). b, Zoomed view of the CHS binding site. Residues near CHS are shown as sticks. The $F_o - F_c$ omit density map is shown at $3\sigma$. c, Binding of tetradecane. The $F_o - F_c$ omit density map is contoured at $4\sigma$. d, Tetradecane was modelled on a two-fold axis of symmetry with partial occupancy as a single molecule. On the basis of the density, it is unclear if this molecule represents the alkyl chain of a lipid, detergent, or a molecule of PEG.
## Extended Data Table 1 | Data collection and refinement statistics

|                         | Paroxetine ts3  | Paroxetine ts2 † | (S)-citalopram ts3 ‡ | (S)-citalopram soaked ts3 § | 8B6 Fab || |
|-------------------------|-----------------|------------------|----------------------|-----------------------------|----------|||
| **Data collection**     |                 |                  |                      |                             |          |
| Space group             | APS24-IDE       | ALS 5.0.2        | ALS 5.0.2            | ALS 5.0.2                   | ALS 5.0.2 |
| Cell dimensions         | C222,           | C222,            | C222                 | C222                        | P43212   |
| **α, β, γ (°)**         | 129.2, 162.8, 140.4 | 129.8, 162.8, 140.1 | 129.7, 163.7, 140.6  | 129.9, 163.2, 140.5         | 81.6, 81.6, 142.2 |
| **Wavelength**          | 0.979           | 1.000            | 1.000                | 0.978                       | 1.000    |
| Resolution (Å)          | 53.17-3.14      | 40.50-4.53       | 53.33-3.15           | 47.68-3.24                  | 57.67-1.62 |
| l(Å)                    | (3.25-3.14)     | (4.69-4.53)      | (3.26-3.15)          | (3.36-3.24)                 | (1.68-1.62) |
| Completeness (%)        | 99.9 (100.0)    | 99.4 (95.6)      | 99.1 (98.9)          | 99.0 (92.0)                 | 97.7 (76.6) |
| Redundancy              | 17.7 (12.5)     | 7.1 (5.9)        | 5.5 (4.0)            | 10.5 (6.6)                  | 6.7 (2.9) |
| CC_{1/2} (%)            | 99.9 (13.8)     | 99.9 (24.5)      | 99.9 (14.0)          | 99.9 (11.0)                 | 99.9 (21.5) |
| **Refinement**          |                 |                  |                      |                             |          |
| Resolution (Å)          | 53.17-3.14      | 40.50-4.53       | 53.33-3.15           | 47.68-3.24                  | 57.67-1.62 |
| No. reflections         | 26151 (2585)    | 8837 (828)       | 25995 (2577)         | 23870 (2178)                | 59809 (4634) |
| R_{work}/R_{free}       | 23.8 (38.3) / 27.0 (41.9) | 28.0 (38.9) / 31.7 (37.1) | 24.0 (39.1) / 27.6 (40.0) | 23.5 (37.5) / 27.6 (38.0) | 19.8 (44.6) |
| No. atoms               | 7631            | 7544             | 7616                 | 7833                        | 3604     |
| Protein                 | 7526            | 7530             | 7515                 | 7503                        | 3302     |
| Ligand/ion              | 104             | 14               | 100                  | 129                         | N/A      |
| Water                   | 1               | 0                | 1                    | 1                           | 302      |
| **B-factors**           |                 |                  |                      |                             |          |
| Protein                 | 157.0           | 398.13           | 159.9                | 175.8                       | 40.1     |
| Ligand/ion              | 158.3           | 302.8            | 157.0                | 173.0                       | N/A      |
| Water                   | 133.5           | N/A              | 103.3                | 145.3                       | 43.0     |
| **R.m.s deviations**    |                 |                  |                      |                             |          |
| Bond lengths (Å)        | 0.008           | 0.004            | 0.008                | 0.015                       | 0.012    |
| Bond angles (°)         | 0.80            | 0.93             | 1.026                | 1.085                       | 1.15     |
| **Ramachandran plot**   |                 |                  |                      |                             |          |
| Favored (%)             | 95.7            | 96.1             | 95.6                 | 95.7                        | 98.1     |
| Allowed (%)             | 4.3             | 3.9              | 4.4                  | 4.3                         | 1.9      |
| Disallowed (%)          | 0               | 0                | 0                    | 0                           | 0        |

*Six crystals were merged for the paroxetine ts3 structure using microdiffraction assembly.
†A single crystal was used for the paroxetine ts2 structure.
‡A single crystal was used for the (S)-citalopram ts3 structure and processed by microdiffraction assembly.
§Three crystals were merged for the (S)-citalopram-soaked ts3 structure using microdiffraction assembly.
¶Highest resolution shell is shown in parentheses.
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Extended Data Table 2 | Anomalous data collection and refinement statistics

|                          | Br-citalopram ts3 * | Br-citalopram soaked ts3 † |
|--------------------------|---------------------|-----------------------------|
| **Data collection**      | APS24-IDC           | ALS 5.0.2                   |
| **Space group**          | C222_1              | C222_1                      |
| **Cell dimensions**      |                     |                             |
| a, b, c (Å)              | 129.6, 164.0, 140.2 | 129.6, 163.4, 140.5         |
| α, β, γ (°)              | 90.0, 90.0, 90.0    | 90.0, 90.0, 90.0            |
| **Wavelength**           | 0.902               | 0.900                       |
| **Resolution (Å)**       | 101.7-3.40 (3.52-3.40) | 82.29-3.49 (3.61-3.46)     |
| **R_{free}**             | 6.3 (58.2)          | 8.8 (59.5)                  |
| **I/σI**                 | 17.60 (2.44)        | 17.76 (2.52)                |
| **Completeness (%)**     | 99.9 (97.0)         | 99.5 (95.3)                 |
| **Redundancy**           | 10.7 (7.0)          | 11.0 (6.7)                  |
| **CC_{1/2} (%)**         | 100.0 (23.2)        | 99.9 (12.5)                 |
| **Refinement**           |                     |                             |
| **Resolution (Å)**       | 101.7-3.40          | 82.29-3.49                  |
| **No. reflections**      | 20919 (2004)        | 19231 (1812)                |
| **R_{work}/ R_{free}**  | 26.6 (39.9) / 28.5  | 24.4 (38.5) / 28.6          |
|                         | (47.0)              | (36.3)                      |
| **No. atoms**            | 7610                | 7610                        |
| Protein                  | 7512                | 7497                        |
| Ligand/ion               | 98                  | 113                         |
| Water                    | 0                   | 0                           |
| **B-factors**            |                     |                             |
| Protein                  | 220.8               | 175.2                       |
| Ligand/ion               | 200.6               | 151.0                       |
| Water                    | N/A                 | N/A                         |
| **R.m.s deviations**     |                     |                             |
| Bond lengths (Å)         | 0.002               | 0.014                       |
| Bond angles (°)          | 0.665               | 0.737                       |
| **Ramachandran plot**    |                     |                             |
| Favored (%)              | 95.8                | 95.5                        |
| Allowed (%)              | 4.2                 | 4.5                         |
| Disallowed (%)           | 0                   | 0                           |

* A single crystal was used for the Br-citalopram ts3 structure and processed by microdiffraction assembly.
† A single crystal was used for the Br-citalopram-soaked ts3 structure and processed by microdiffraction assembly.
‡ Highest resolution shell is shown in parentheses.
§ 5% of reflections were used for calculation of R_{free}. 

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Extended Data Table 3  |  Superpositions of DAT, LeuT, ts2 SERT and ts3 (S)-citalopram conformational states versus ts3 SERT (paroxetine) and comparison of high-resolution Fab versus SERT-bound Fab (paroxetine)

| Structure | Rmsd (Å) / Ca # |
|-----------|-----------------|
| SERT TM1-12 vs. DAT (comp. inh: nortriptyline; 4M48) | 0.7 / 272 |
| SERT TM1-12 vs. DAT (sub. analog: 3,4-dichlorophenethylamine; 4XPA) | 0.8 / 283 |
| SERT TM1,6 vs. DAT (comp. inh: nortriptyline; 4M48) | 0.4 / 50 |
| SERT TM1,6 vs. DAT (sub. analog: 3,4-dichlorophenethylamine; 4XPA) | 0.6 / 52 |
| SERT TM1-8 vs. DAT (comp. inh: nortriptyline; 4M48) | 0.5 / 200 |
| SERT TM9-12 vs. DAT (comp. inh: nortriptyline; 4M48) | 1.2 / 137 |
| SERT EL2, EL4, EL6 vs. DAT (comp. inh: nortriptyline; 4M48) | 1.2 / 80 |
| SERT TM1-12 vs. LeuT (sub. free; 3TT1) | 1.3 / 260 |
| SERT TM1-12 vs. LeuT (comp. inh: tryptophan; 3F3A) | 1.4 / 249 |
| SERT TM1-12 vs. LeuT (sub. bound: leucine; 2A65) | 1.7 / 285 |
| SERT TM1-12 vs. LeuT (inward-open; 3TT3) | 2.9 / 282 |
| ts3 (S)-citalopram SERT vs. ts3 paroxetine SERT | 0.3 / 489 |
| ts2 SERT vs. ts3 SERT | 0.1 / 514 |
| High resolution Fab vs. SERT-bound Fab | 0.8 / 414 |

Superpositions were done by overlapping the Cα atoms of DAT, LeuT or SERT ts2 over Cα atoms of SERT ts3 (paroxetine) using PyMOL. A cutoff of 2.0 Å RMS was used throughout five cycles of superposition to reject outliers.
## Extended Data Table 4 | Ion-binding sites and coordination distances

| Site | Coordinating group | Location | Distance* (Å) |
|------|--------------------|----------|--------------|
| Na₁ † | Ala 96-CO-         | TM1a     | 2.3          |
|      | Asn 101 Oɛ1       | TM1b     | 2.3          |
|      | Ser 336 Oγ        | TM6a     | 2.5          |
|      | Ser 336-CO        | TM6a     | 2.4          |
|      | Asn 368 Oɛ1       | TM7      | 2.6          |
|      | mean distance     |          | 2.4          |
| Cl †  | Tyr 121 OH        | TM2      | 2.6          |
|       | Gln 332 Ne        | TM6a     | 3.1          |
|       | Ser 336 Oγ        | TM6a     | 3.6          |
|       | Ser 372 Oγ        | TM7      | 3.0          |
|       | mean distance     |          | 3.1          |
| Na₂ ‡ | Gly 94-CO-        | TM1a     | 2.5          |
|       | Val 97-CO-        | TM1b     | 2.4          |
|       | Leu 434-CO-       | TM8      | 2.3          |
|       | Asp 437 Oɛ1       | TM8      | 2.4          |
|       | Ser 438 Oγ        | TM8      | 2.5          |
|       | mean distance     |          | 2.4          |

*Interatomic distance between coordinating atom of protein chain to the bound ion(s).
†Determined from the ts3 paroxetine-bound structure.
‡Determined from the ts3 (S)-citalopram-bound structure.