Supplementary Information

Ligand selectivity among the dopamine and the serotonin transporter specified by the forward binding reaction

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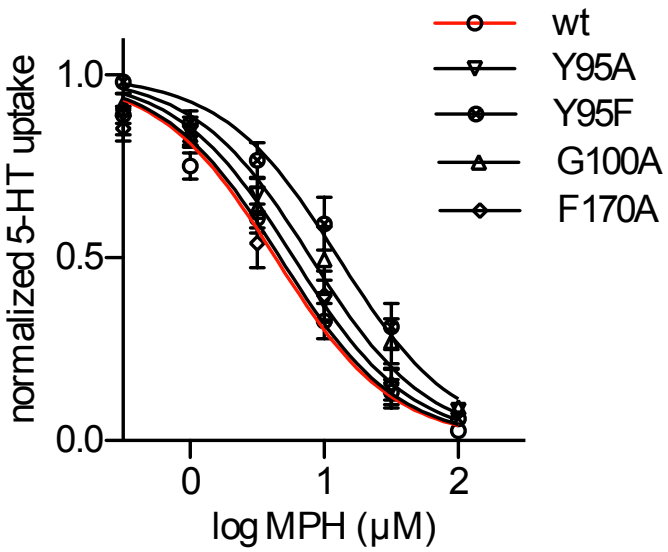
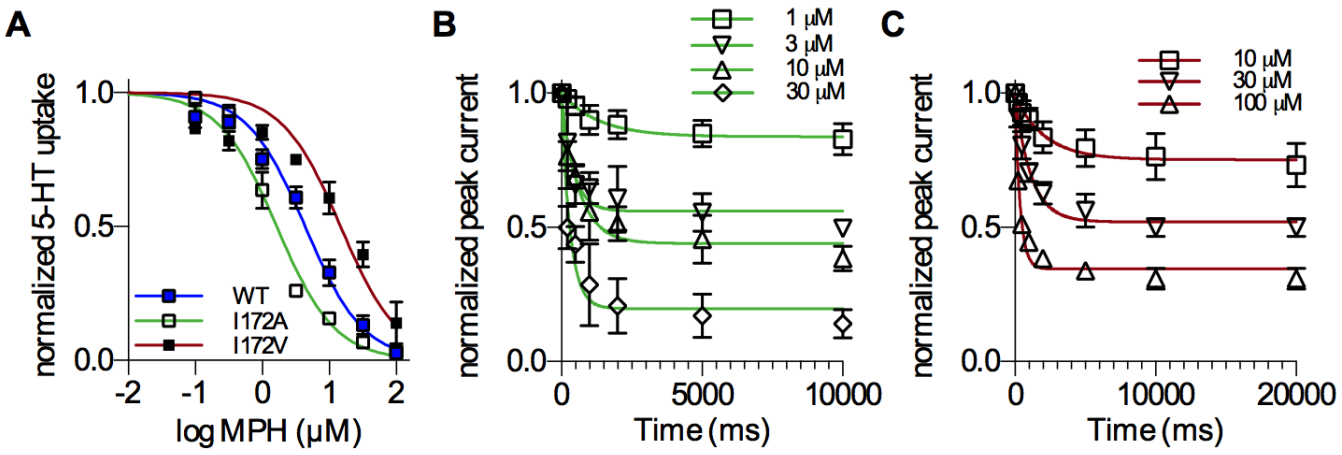
Supplementary Text

Differences in the association rate of compounds may be a general feature that contributes to the discrimination between closely related binding sites. If this was the case, we hypothesized that it could be possible to accelerate binding of the inhibitor by mutagenesis of the binding site or the access pathway of the ligand. Accordingly, we searched for mutations in SERT that rendered the protein more or less susceptible to inhibition by methylphenidate (MPH).

SERT and MPH are ideally suitable for these experiments because MPH binds SERT slower than DAT (Fig. 3, see main text). Hence, it may be possible to accelerate MPH binding to SERT by site-directed mutagenesis. We mutated several residues in the substrate binding site of SERT that differ between DAT and SERT. Of the positions (Supplementary Fig. 1 and 2A), which we mutated, we identified one - I^{172} – that, when mutated to alanine, lowered, and when mutated to valine, increased the IC_{50} of MPH in an uptake inhibition assay (1.65 [1.37-1.98] *vs*. 4.35 [3.62-5.23] *vs*. 14.72 [11.41-18.97] µM, respectively; Supplementary Fig. 2A). The same rank order in affinity was observed, when occupancy by MPH was measured by recording its effect on the peak current (Fig. 5A). Thus, this pair of mutants provided an opportunity to examine whether we could ascribe mutation-induced affinity changes to changes in k_{on} and k_{off}. The observations were unequivocal: MPH bound more rapidly to SERT-I^{172}A (k_{on} = 8.66 [6.68-10.06] *10^4 $M^{-1}s^{-1}$- Fig. 5B and Supplementary Fig. 2B) than to SERT-I^{172}V (k_{on} = 2.42 [2.12-2.72] *10^4 $M^{-1}s^{-1}$- Supplementary Fig. 5B and Supplementary Fig. 2C), whereas direct and indirect determination of k_{off} revealed no difference in dissociation rates (0.55 [0.38-0.73] s^{-1} *vs*. 0.43 [0.28-0.58] s^{-1}- Fig. 5C).



Supplementary Fig. 1. Uptake inhibition by methylphenidate (MPH) assessed in HEK-293 cells expressing wild-type SERT and several binding site mutants thereof. In SERT ^{Y95F}, SERT ^{G100A} and SERT ^{F170A}, the respective residues were mutated to the equivalent residues in DAT. The IC_{50} values for uptake inhibition for the mutants were: 12.83[9.54-17.24] µM, 8.00[6.01-10.66] µM and 4.72[3.65-6.10] µM, respectively. None of these mutants showed an increase in affinity to MPH – *i.e.* the IC_{50} for wild-type SERT was 4.35[3.62-5.23] µM. The SERT ^{F95A} mutant displayed a moderate loss in affinity for MPH -5.95[4.67-7.60] µM- compared to the DAT-like SERT ^{Y95F} mutant.



Supplementary Fig. 2. Inhibition of (A) [^{3}H]5-HT uptake in wild-type SERT (blue), SERT-I^{172}A (green) and SERT-I^{172}V (red), expressed in HEK-293 cells. Data in (A) are means ± S.D of three independent experiments conducted in triplicate and from 5 experiments, respectively. (B and C) The association rate constant for MPH binding to SERT-I^{172}A (B) and SERT-I^{172}V (C) was determined from the time-dependent block of the peak current as outlined in Fig. 1C (main text). The k_{app}-values from these curves were plotted against [MPH] to estimate the values of k_{on} and k_{off} from the slope and the y-intercept of the regression lines (see Fig. 5 B main text). All peak currents were normalized to the amplitude of the peak current in the absence of MPH. The data are means ± S.D from 4 to 8 independent measurements.