Supplementary material

Table S1. *Molecular masses of peptides expected from CNBr cleavage of SERT mutants X3M, W103C, I179C and X3M2C.*

Peptide	Start	End	Mass
1	c-myc-1	135	14936
2	136	320	21202
3	321	370	5406
4	371	386	1767
5	387	417	3356
6	418	430	1504
7	431	528	10624
8	529	558	3563
9	559	583	2983
10	584	627	4928

Figure S1. *CNBr cleavage of SERT I179C, W103C and X3M2C.* HeLa cells expressing the indicated mutants were treated with the following concentrations of $CuPh_3$: 10 µM for I179C and X3M2C; 30 µM for W103C. The cells were treated as described in the legend to Figure 3. Lane 1 was a control with untransfected HeLa cells; samples in lane 2 were not treated; samples in lane 3 were treated with $CuPh_3$; samples in lane 4 were treated with $CuPh_3$ and then reduced with DTT prior to SDS-PAGE.

Origin of the ~30 kDa band in W103C. If this reaction represents cross-linking between Cys-103 and an endogenous SERT cysteine, it would require that the peptide containing that cysteine be large enough to account for the ~15 kDa increase in mass. No other single CNBr peptide is sufficiently large, although cleavage at Met-418 and Met-558 would generate a peptide of 15.6 kDa. Within this region are two cysteine residues (Cys-473 in TM 9 and Cys-540 in TM11) that roughly correspond to cysteines in GAT-1 (Cys 427 and Cys-498). However, this peptide contains methionines at 430 and 528, and it is not clear why they would not be cleaved by CNBr. Alternatively, both SERT and GAT-1 cross-linking experiments were performed in HeLa cells, and it is possible that the minor inactivation of W103C and consequent formation of a 30 kDa product represents cross-linking to a 15 kDa peptide derived from an endogenous protein in the HeLa cell plasma membrane.

Figure S2. *Inactivation of SERT W103C by MTSEA and effect of cocaine and ibogaine.* HeLa cells expressing W103C were treated for 10 min. with the indicated concentrations of MTSEA in the presence of 2.5 µM cocaine (squares) or 35 µM ibogaine (open circles) or neither (filled circles). 5-HT influx was measured at the indicated times after washing the cells free of MTSEA.

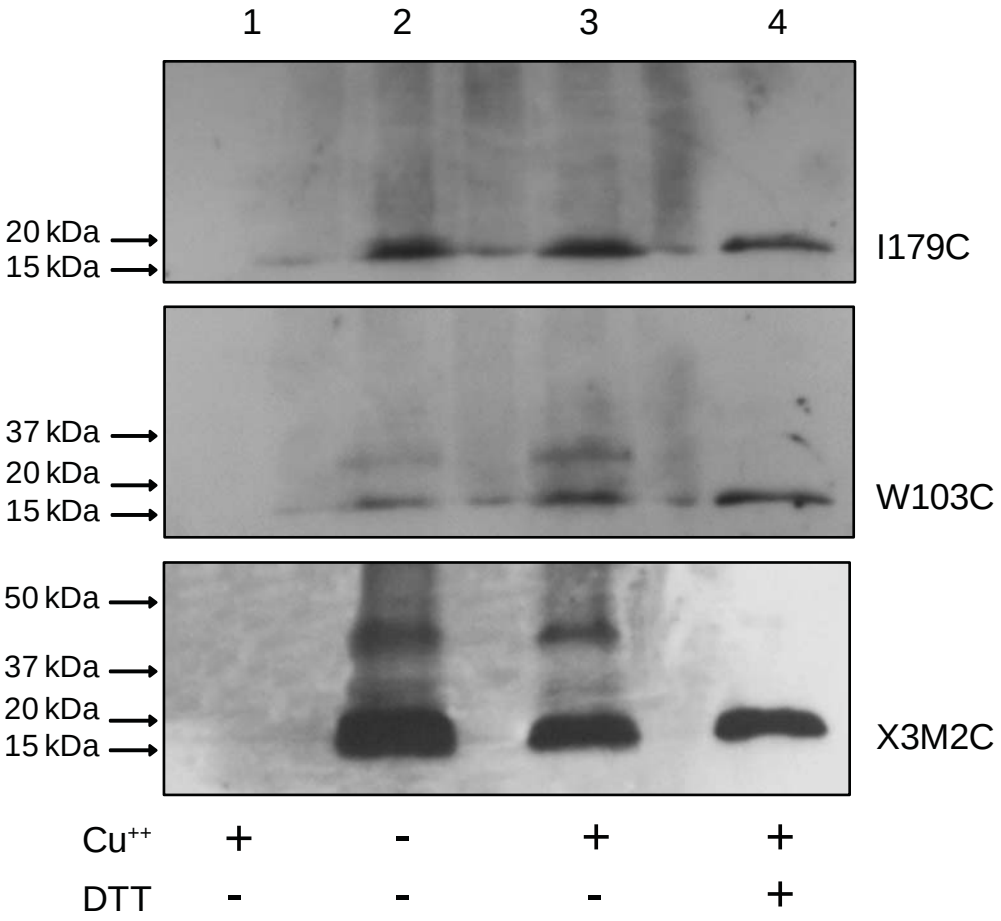

Figure S1

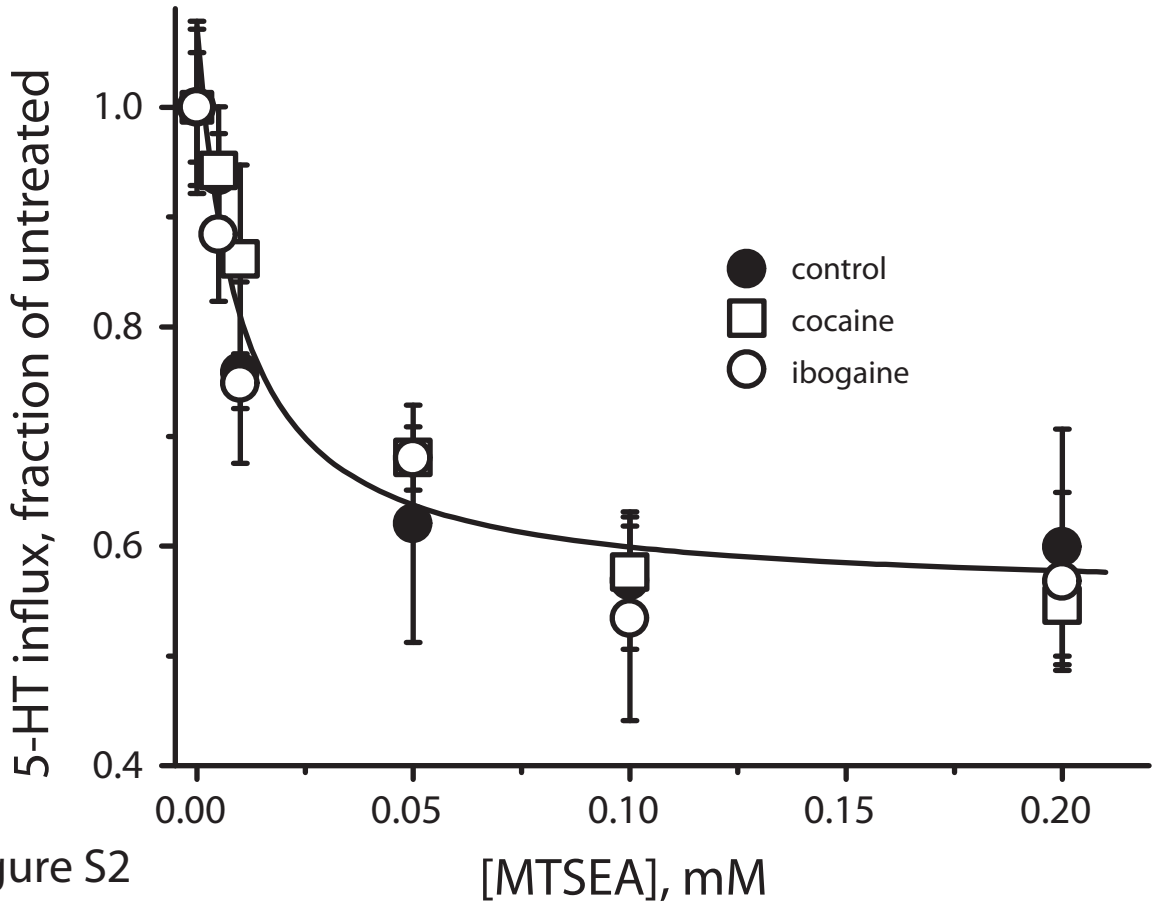

Figure S2