Supplemental Data

Serotonin transporter occupancy in rats exposed to serotonin reuptake inhibitors *in utero* or via breast milk

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Materials and Methods

Partition Coefficient

Partition coefficients were measured in a 10 mL volume containing 5 mL of octanol and 5 mL PBS (pH 7.4). For citalopram and paroxetine measurements, [^{3}H]citalopram or [^{3}H]paroxetine were used to spike the 10 mL solution. The solution was vortexed and 10 µL of the aqueous PBS phase was added to scintillation fluid and counted on a liquid scintillation counter. Other compounds were measured by adding 10 µg of compound to 10 ml of the octanol:PBS solution as above.. The solution was vortexed for 1 hour and a small portion of the octanol phase was diluted directly with the mobile phase for HPLC-UV quantification as previously described. Data is the mean of three separate experiments, each prepared and extracted on different days.

Serotonin Transporter Western Blot

For western blotting, the cortex was dissected on dry ice. Frozen cortices (~50 mg) were homogenized in 1 mL of homogenization buffer containing 50 mM Tris (pH 7.2), 1 mM EDTA, 6 mM $MgCl_2$, 10% sucrose, and 1:1,000 protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO) with a PowerGen 125 Homogenizer (Fisher Scientific, Pittsburgh, PA) on ice. Homogenate was spun at 3,500 x *g* for 5 min at 4°C. The supernatant was spun at 16,000 x *g* for 45 min at 4°C. The pellet was resuspended in homogenization buffer and stored at -20°C. Protein concentrations were determined with the BCA assay (Pierce Biotechnology, Rockford, IL).

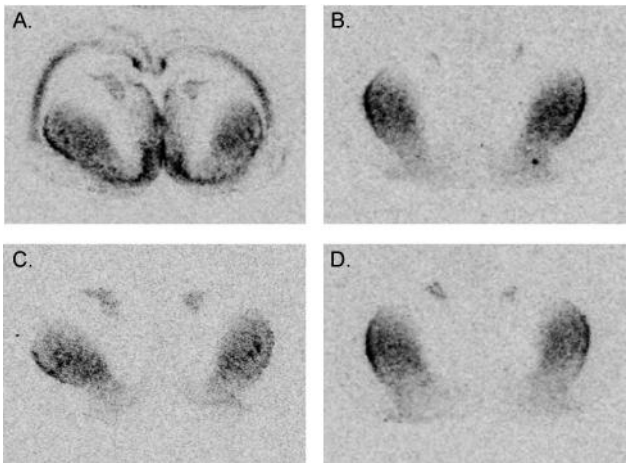
Cortical extracts (10 µg) and transfected HEK extracts (0.2 µg) were loaded onto a 3-8% tris-acetate gel (Invitrogen, Carlsbad, CA) and separated by gel electrophoresis. The gel was blotted onto a PVDF membrane (Invitrogen, Carlsbad, CA) and blocked in 7.5% skim milk-TBST nutating at 4°C overnight. Primary antibody for SERT was a generous gift from Dr. Randy Blakely (Qui et al. 1995). Blots were incubated for 2 hrs with primary antibody for SERT CT-2 (1:5,000). Blots were incubated with goat anti-rabbit IgG-poly HRP (Pierce Biotechnology, Rockford, IL) and visualized with the SuperSignal West Dura Extended Duration Chemiluminescent Kit (Pierce Biotechnology, Rockford, IL). Blots were exposed to ECL Hyperfilm (GE Healthcare Biosciences, Pittsburgh, PA) and analyzed with AIS 6.0 Imaging Software (Imaging Research, St. Catharines, Canada).

Table S1. Partition coefficients of antidepressants and their metabolites.

Compound	$logD_{7.4}$	Octanol:PBS 7.4
O-desmethylvenlafaxine	0.39	2.5
Venlafaxine	0.68	4.7
Citalopram	1.11	13
Escitalopram	1.11	13
Fluvoxamine	1.80	64
Paroxetine	1.84	69
Norfluoxetine	2.10	128
Fluoxetine	2.27	186
Desmethylsertraline	2.87	737
Sertaline	2.92	840

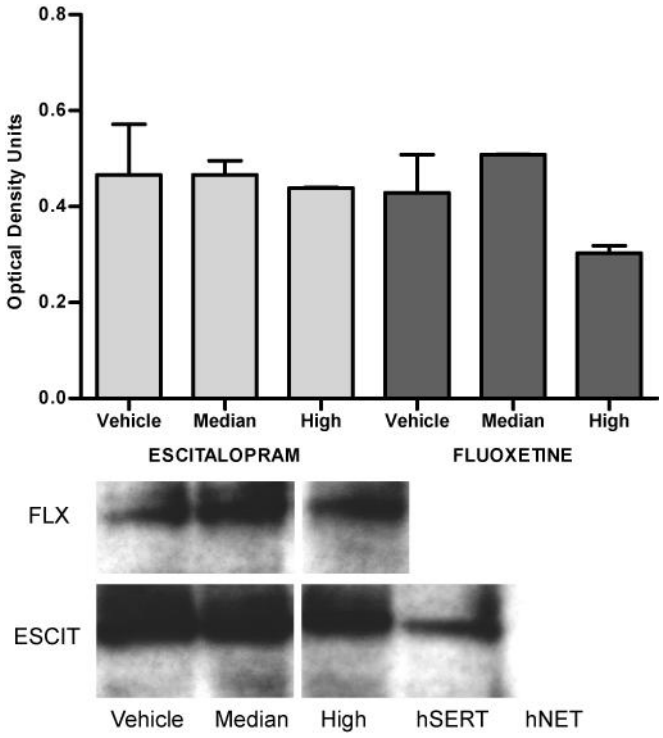
Compounds are arranged in order of increasing lipophilicity. Citalopram and escitalopram were measured by different detection methods but their partition coefficients were identical. This result validates the two different methods of compound detection since citalopram and escitalopram have identical partition coefficients.

Figure S1. Representative autoradiography of SERT binding.



Visualization of the SERT in E21 rats using [^{125}I]RTI-55 exposed *in utero* to paroxetine. 50 pM of [^{125}I]RTI-55 was used to assess SERT binding. (A) Total binding after *in utero* exposure to vehicle. (B) Binding after *in utero* exposure to the median dose of paroxetine (calculated as 98% SERT occupancy). Maternal serum concentration of paroxetine was 43 ± 28 ng/mL. (C) Binding after *in utero* exposure to the high dose of paroxetine (calculated as 98% SERT occupancy). Maternal serum concentration of paroxetine was 121 ±16 ng/mL. (D) Non-specific binding using 50 pM of [^{125}I]RTI-55 + 1 μM chloroimipramine.

Figure S2. Western blot for the serotonin transporter in E21 rats.



Cortex homogenate of E21 animals exposed *in utero* to vehicle, median, or high dose of escitalopram or fluoxetine. Representative western blots and quantification of 2 separate pups are shown. HEK293 cells transfected with hNET or hSERT were used as negative and positive controls, respectively. Data are mean ± SEM (n = 2).

Figure S3. Representative autoradiographs of *in utero* exposure to SRIs.

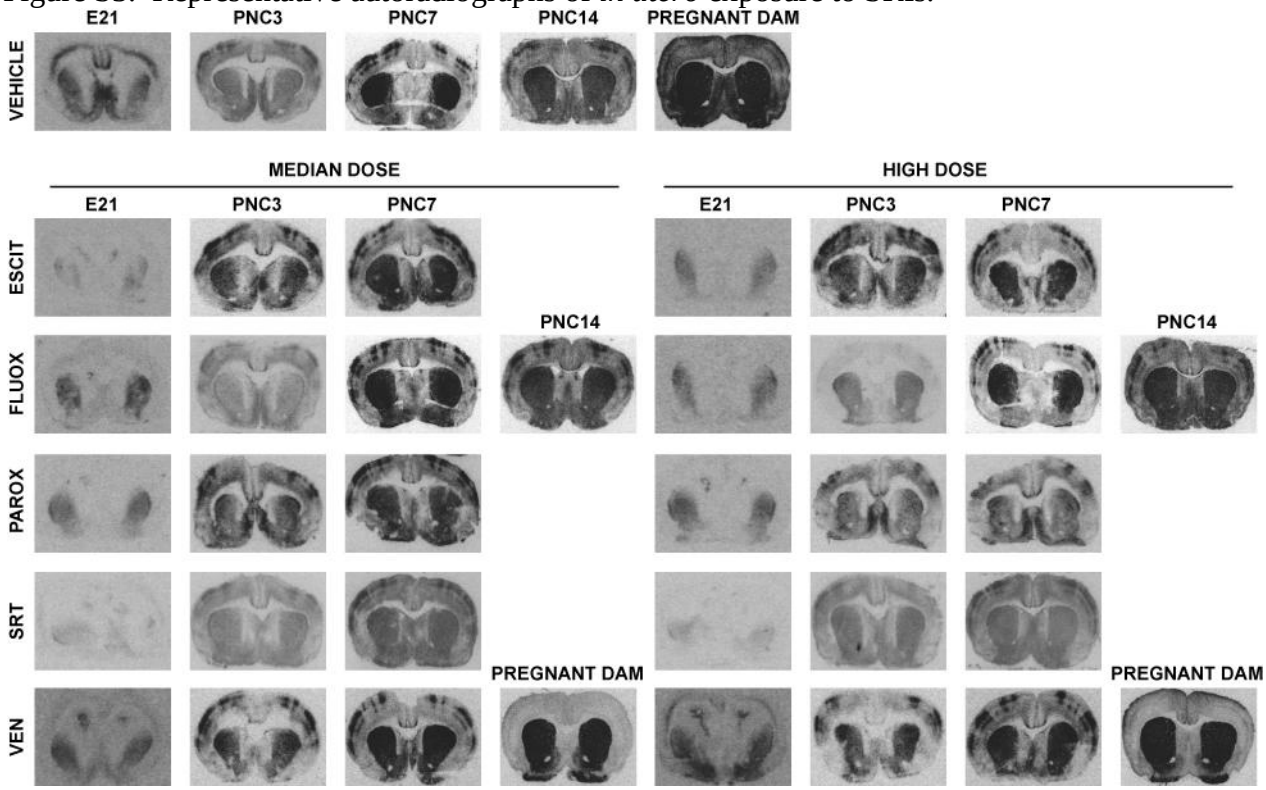
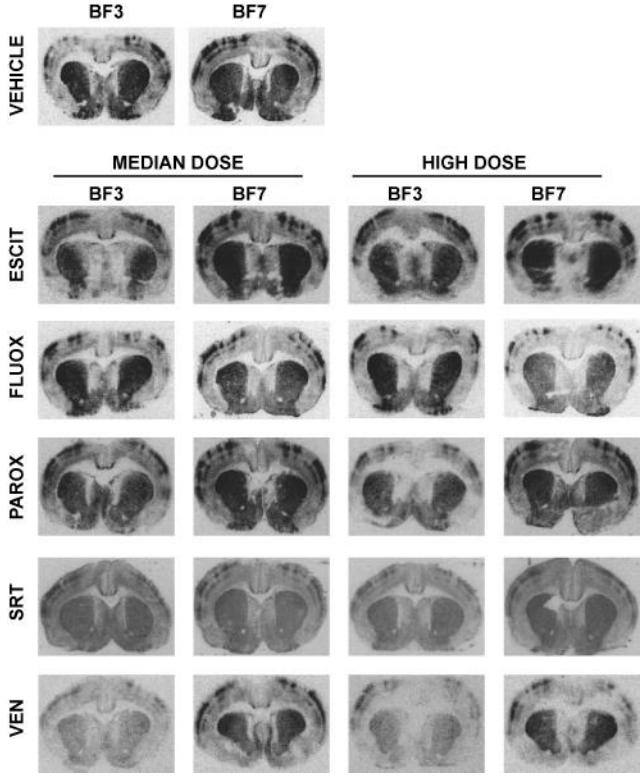


Figure S4. Representative autoradiographs of exposure to SRIs via breast milk.



Representative autoradiographs of escitalopram (ESCIT), fluoxetine (FLUOX), paroxetine (PAROX), sertraline (SRT), and venlafaxine (VEN) including representative pregnant dams exposed to VEN during pregnancy. Each treatment group had its own vehicle run in the same assay but one series is shown for reference. Total binding representative images are displayed for comparison. Dense patches of total binding in the somatosensory cortex are consistent with previous studies investigating SERT binding during the early postnatal period (D'Amato et al., 1987; Kelly et al., 2002).

References

D'Amato RJ, Blue ME, Largent BL, Lynch DR, Ledbetter DJ, Molliver ME, Snyder SH (1987) Ontogeny of the serotonergic projection to rat neocortex: transient expression of a dense innervation to primary sensory areas. *Proc Natl Acad Sci U S A* **84(12)**: 4322-4326.

Kelly PA, Ritchie IM, Quate L, McBean DE, Olverman HJ (2002) Function characterization of perinatal exposure to 3,4-methylenedioxymethamphetamine in rat brain. *Br J Pharmacol* **137**: 963-970.

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