SUPPLEMENTAL DATA:

ANIMAL PREPARATION

Animals were fasted for up to 12 hours prior to imaging. On the imaging day, the animal was anesthetized with intramuscular ketamine (10 mg/kg) and glycopyrrolate (0.1-0.2 mg/kg) at ~2 hours prior to radiotracer injection, transferred to the positron emission tomography (PET) camera, and immediately intubated with an endotracheal tube for continued anesthesia with 1.5-2.5% isoflurane administered through a re-breathing circuit. An intravenous (i.v.) line was established in a cephalic and/or saphenous vein for injection of fluids for hydration and radiotracer injection. For arterial blood samples collection, a line was established in a femoral or radial artery. Body temperature was maintained by heated water blanket and monitored by rectal thermometer. Vital signs, including end tidal CO_2, SpO_2, heart rate, respiration rate and blood pressure, were monitored continuously and recorded at least every 15 minutes during the study.

BLOOD SAMPLES PROCESSING AND ANALYSIS

Radioactivity in whole blood and plasma was assessed using a well-type γ-counter using a 400-1400 keV window (Perkin Elmer Wallac 2480, USA). Plasma samples were processed by acetonitrile denaturation and analyzed by HPLC on a Luna C18(2) column with a mobile phase of MeCN/H_2O-Et_3N (0.8%) 55/45 at a flow rate of 4 mL/min to estimate the parent fraction. The free protein binding fraction was determined using ultrafiltration units (Centrifree® 30K, Millipore).

ESTIMATION OF ^{18}F-MNI698 ID_{50} AND UPPER MASS DOSE LIMITS

The methodology used to estimate the mass doses resulting in 50% receptor occupancy (ID_{50}) and to calculate the upper mass dose limits that will result in 5% or 10% receptor occupancy (D_5 or D_{10}, respectively) was based on previously published literature (*1*). Briefly, once the BP_{ND}s measured with the scan using a high mass dose are plotted against the corresponding regional BP_{ND}s of the scan using a low mass dose, the individual ID_{50} can then be calculated using the slope (α) of the regression line in the occupancy plot, where α<1 represents the negative effect of higher mass dose on BP_{ND} measurements (Eq. 1). Then, the upper mass dose limits can be estimated by rearranging the conventional occupancy (O) equation (Eq. 2).

$$ID_{50} = \frac{\alpha D_{High} - D_{Low}}{1-\alpha}$$ (Supplemental Eq. 1)

$$O = \frac{D}{D + ID_{50}}$$ (Supplemental Eq. 2)

where D = mass dose.

OCCUPANCY ESTIMATES - CORRECTION FOR SELF-OCCUPANCY

The correction for self-occupancy applied in this study was based on previously published work (*2*). In brief:

In the absence of neurotransmitter (N=0) and with true radiotracer (i.e. no mass effect, T=0), the receptor occupancy (O_c) for a certain drug dose can be calculated using:

$$O_c = 1 - \frac{D}{D + ID_{50}^D} = 1 - \frac{1}{1 + \frac{D}{ID_{50}^D}} = \frac{\frac{D}{ID_{50}^D}}{1 + \frac{D}{ID_{50}^D}} = \frac{\delta_m}{1 + \delta_m}$$

(Supplemental Eq. 1)

where O_c is the measured occupancy in the absence of neurotransmitter and with true radiotracer, D is the drug dose, ID_{50}^D is the drug dose resulting in 50% receptor occupancy and $\delta_m = \frac{D}{ID_{50}^D}$.

When N>0 and T>0 then O_m is:

$$O_m = 1 - \frac{1}{1 + \frac{T}{ID_{50}^T} + \frac{D}{ID_{50}^D} + \frac{N}{ID_{50}^N}} = 1 - \frac{1}{1 + \tau_T + \delta_m + \tau_N}$$

(Supplemental Eq. 2)

where O_m is the measured occupancy, T is the radiotracer mass dose, N is the neurotransmitter mass dose, ID_{50}^T is the tracer dose resulting in 50% receptor occupancy, ID_{50}^N is the neurotransmitter dose resulting in 50% receptor occupancy, $\tau_T = \frac{T}{ID_{50}^T}$ and $\tau_N = \frac{N}{ID_{50}^N}$.

Now considering the case of a baseline study with no drug and a pre-blocking study with drug, and ignoring the neurotransmitter effects, O_m is:

$$O_m = \frac{O_{Drug} - O_{Baseline}}{1 - O_{Baseline}} = \frac{\left(1 - \frac{1}{1 + \tau_T^{Drug} + \delta_m^{Drug}}\right) - \left(1 - \frac{1}{1 + \tau_T^{Baseline}}\right)}{1 - \left(1 - \frac{1}{1 + \tau_T^{Baseline}}\right)}$$

$$= \frac{\left(\frac{1}{1 + \tau_T^{Baseline}}\right) - \left(\frac{1}{1 + \tau_T^{Drug} + \delta_m^{Drug}}\right)}{\left(\frac{1}{1 + \tau_T^{Baseline}}\right)}$$

$$= 1 - \frac{1 + \tau_T^{Baseline}}{1 + \tau_T^{Drug} + \delta_m^{Drug}}$$

$$= \frac{\tau_T^{Drug} - \tau_T^{Baseline} + \delta_m^{Drug}}{1 + \tau_T^{Drug} + \delta_m^{Drug}} \quad \text{(Eq. 3)}$$

In order to extract true drug occupancy in the absence of radiotracer mass and ignoring neurotransmitter effects (O_c) from the measured value (O_m), Eq. 1 is reversed:

$$\delta_m^{Drug} = \frac{O_c}{1 - O_c} \quad \text{(Eq. 4)}$$

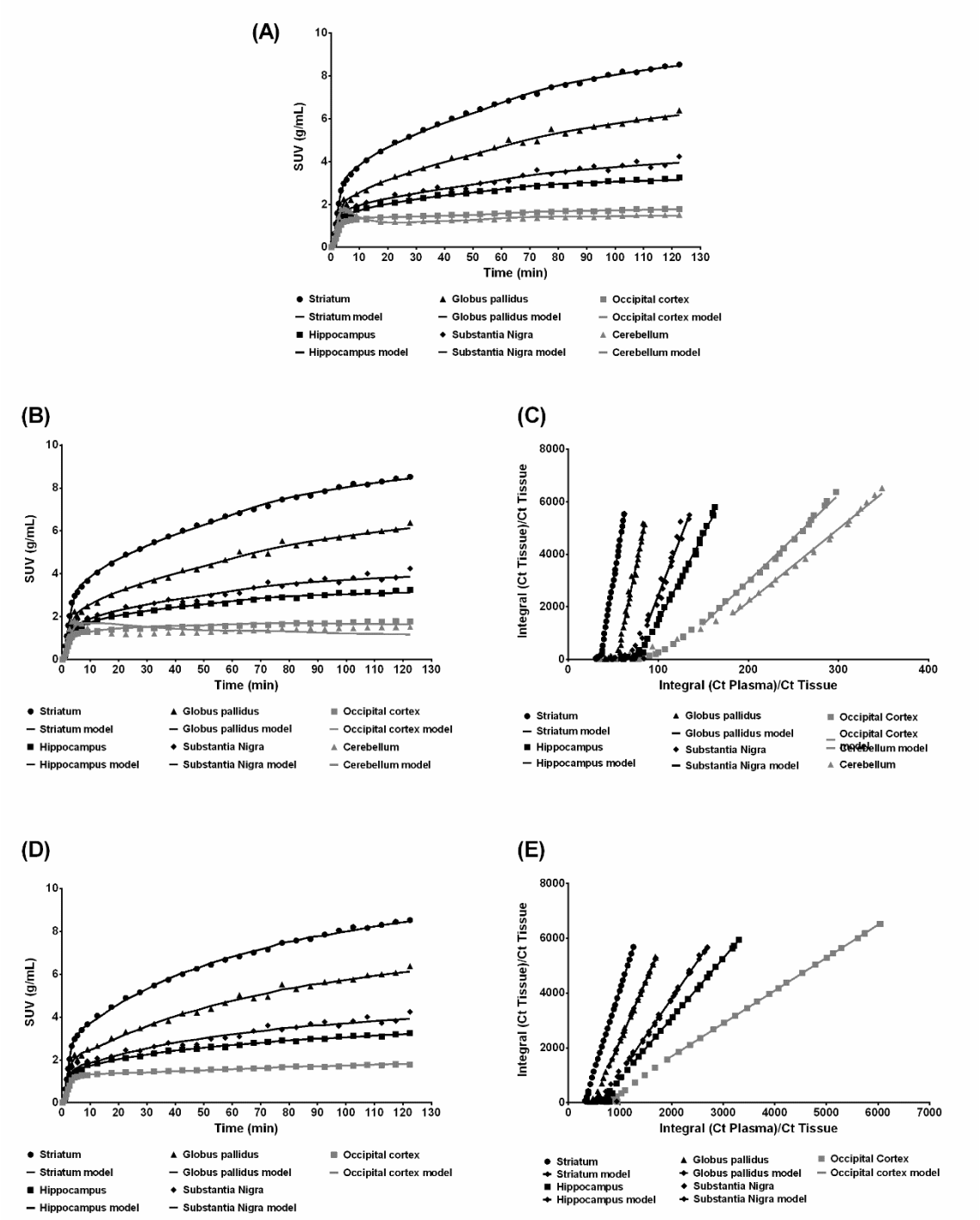
and plugged into Eq. 3:

$$O_m = \frac{\tau_T^{Drug} - \tau_T^{Baseline} + \frac{O_c}{1 - O_c}}{1 + \tau_T^{Drug} + \frac{O_c}{1 - O_c}} \quad \text{(Eq. 5)}$$

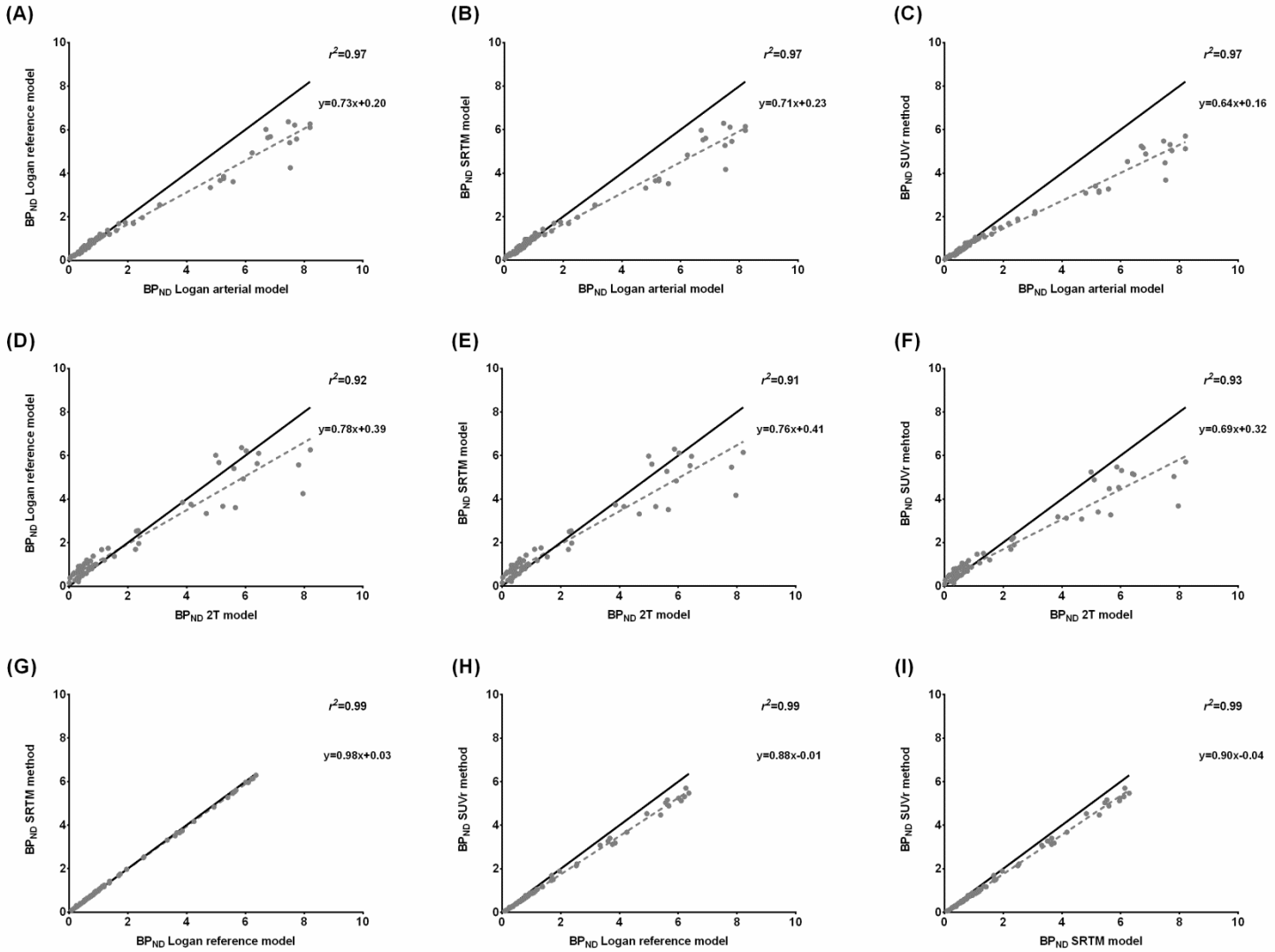
Further arrangement of Eq. 5 yields:

$$O_c = \frac{O_m\left(1 + \tau_{Drug}\right) + \tau_{Baseline} - \tau_{Drug}}{1 - \tau_{Drug} + \tau_{Baseline} + O_m \tau_{Drug}} \quad \text{(Eq. 6)}$$

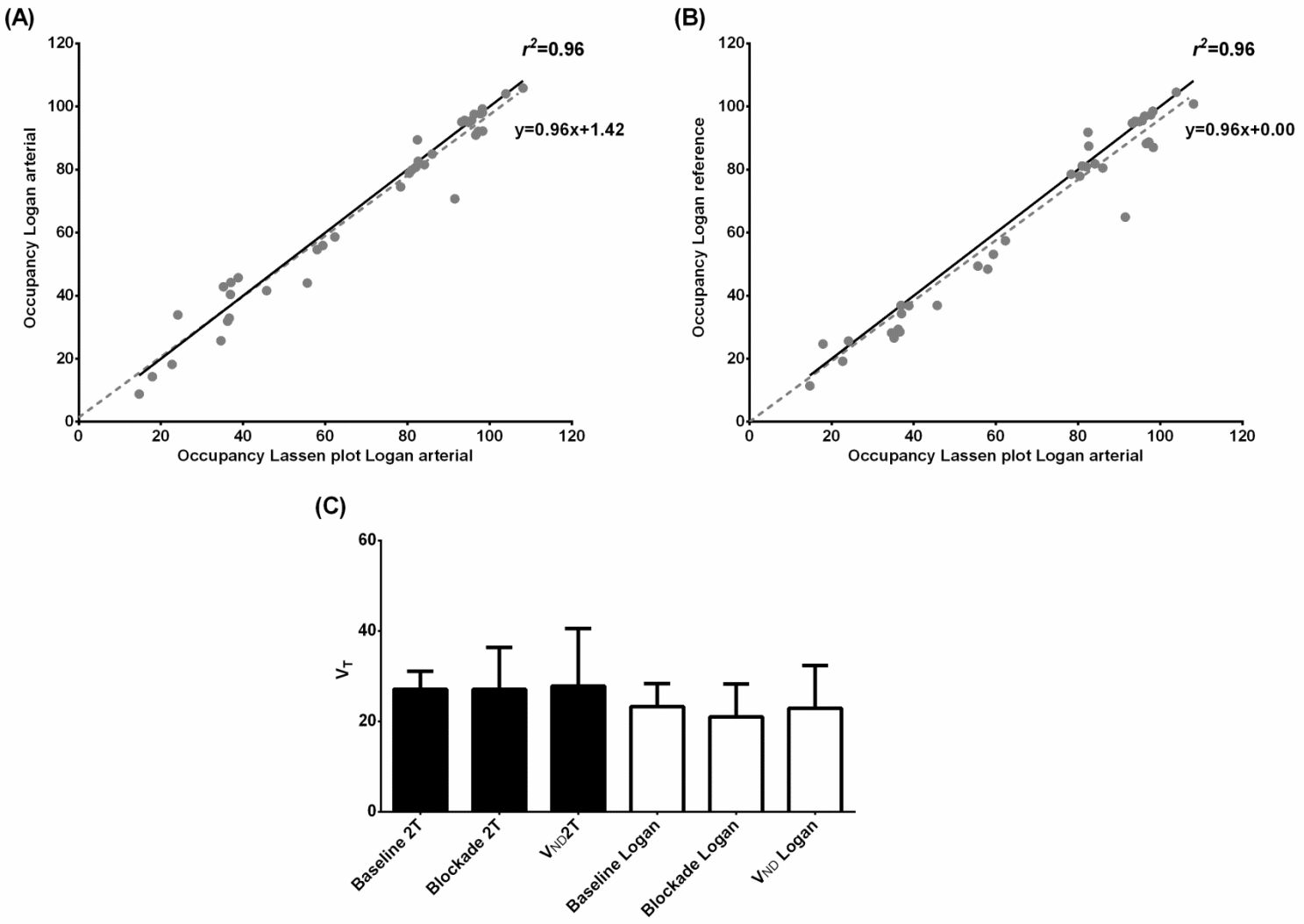
where O_c is the measured occupancy corrected for self-occupancy, O_m is the measured occupancy and $\tau = \frac{T}{ID_{50}}$, with T=mass dose concentration at baseline or at blockade conditions (drug study) and ID_{50} is the mass doses resulting in 50% receptor occupancy determined from the mass effect study.



SUPPLEMENTAL FIGURE 1. Representative time-activity curves in selected brain regions fitted with different kinetic models: 2T (A), 1T (B), Logan graphical plot with arterial input function (C), SRTM (D) and Logan non-invasive (E). For the non-invasive methods of analysis (SRTM and Logan non-invasive) the cerebellum was used as reference region.



SUPPLEMENTAL FIGURE 2. Comparative analysis of BP_{ND} values determined using multiple methods. (A) Logan invasive versus Logan non-invasive; (B) Logan invasive versus SRTM; (C) Logan invasive versus SUVr method; (D) 2T versus Logan non-invasive; (E) 2T versus SRTM; (F) 2T versus SUVr method; (G) Logan non-invasive versus SRTM; (H) Logan non-invasive versus SUVr method; and (I) SRTM versus SUVr method. Note the underestimation of BP_{ND} values when the non-invasive methods of quantification are used, in comparison with invasive methods (2T and Logan arterial). This is particularly evident in the regions with high density of $5HT_4$ receptors.



SUPPLEMENTAL FIGURE 3. Correlation between measured occupancy with the Lassen plot using Logan graphical analysis with arterial input function and the occupancy measured using Logan graphical analysis with arterial input function (A) and Logan non-invasive (B). Note the linear relationship between measurements and the proximity to the line of identity. Panel (C) shows the V_T values measured in the cerebellum at baseline and pre-blocking conditions, as well as derived V_{ND}s.

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