Prenatal Stress Induces Increased Striatal Dopamine Transporter Binding in Adult Nonhuman Primates

Supplemental Information

Methodological Considerations and Sensitivity Analyses

The following describes in greater detail the methodological considerations and sensitivity analyses that are presented in the Discussion.

Tracer Mass

There was 61% greater tracer mass concentration observed in the reference region for the subjects not exposed to prenatal stress because of unintentional differences in specific activity of injected [^{18}F]FECNT (Table 1, $M_{ref,}$ p = 0.095). This had the potential to artificially decrease [^{18}F]FECNT binding in those subjects due to occupancy of dopamine transporter (DAT) in the target regions by non-radioactive FECNT. There are no reported measures of striatal DAT occupancy as a function of injected FECNT mass. However, given the reported in vivo dissociation constant, K_d, of [^{11}C]methylphenidate for DAT in rat striatum (1), we estimate K_d = 79 pmol/mL for FECNT in rhesus striatum, and this would imply that the stress groups would exhibit only 0.5% greater [^{18}F]FECNT binding due to lower occupancy by non-radioactive tracer (see below). Nevertheless, we performed an analysis of covariance (ANCOVA) with tracer mass in the reference region, M_{ref}, included as a covariate. The fractional differences in binding for stress vs no stress decreased by approximately 3% when adjusted for the effect of tracer mass: striatum 12% (p = 0.055), putamen 14% (p = 0.040), and head of caudate 9% (p = 0.127) (Table S2). The regression against tracer

mass resulting from this ANCOVA corresponds to an in vivo dissociation constant of K_d = 11 pmol/mL (see below).

The following estimation suggests that lower FECNT mass concentration observed in the reference region in the stress groups would yield striatal occupancy that is only negligibly lower than in the non-stress groups compared to the observed difference in binding: In our hands, the binding potential of [^{11}C]methylphenidate in rhesus striatum, BP_{ND}(MP), is approximately 24% that of [^{18}F]FECNT (*n* = 3, unpublished data). Given $BP_{ND} = f_{ND} \times B_{avail} / K_d$ (2), and neglecting differences in the free fraction of ligand in the nondisplaceable tissue compartment, f_{ND}, it follows that K_d(FECNT) = BP_{ND}(MP) / BP_{ND}(FECNT) x K_d(MP) = 0.24 x K_d(MP). The observed in vivo dissociation constant of [^{11}C]methylphenidate in rat striatum is 331 +/- 63 pmol/mL (1). Assuming the in vivo dissociation constant in rhesus is the same as rat yields K_d(FECNT) = 0.24 x 331 pmol/mL = 79 pmol/mL. By the Michaelis-Menten relation, the fraction of bound target sites is $B/B_{max} = F/(K_d + F)$. Taking the observed reference region mass concentration, M_{ref}, as the free concentration, F, yields

Stress (M_{ref} = 0.66 pmol/mL): $B/B_{max} = 0.66/(79 + 0.66) = 0.8\%$

Non-stress (M_{ref} = 1.06 pmol/mL): $B/B_{max} = 1.06/(79 + 1.06) = 1.3\%$,

i.e., 0.5% higher radioactive tracer binding would be expected in the stress subjects due to lower cold tracer mass occupancy, as compared to the 15% elevation observed.

We can also estimate K_d from our present data as follows. The linear regression of binding vs M_{ref} from the ANCOVA described in the text yields BP = -0.714 x M_{ref} + 8.18, so the change in binding per pmol/mL change in M_{ref} is DBP/BP = -0.714/8.18 = -8.7%. This may be due to a change in occupancy, $D(B/B_{max})$, of 8.7% per 1 pmol/mL of FECNT. For small F, the Michaelis-Menten relation becomes $B/B_{max} = F/(K_d + F) = F/K_d$, and therefore $K_d = DF / D(B/B_{max}) =$ 1 pmol/mL / 8.7% = 11.5 pmol/mL. This would imply

Stress (M_{ref} = 0.66 pmol/mL): $B/B_{max} = 0.66/(11.5 + 0.66) = 5.4\%$

Non-stress (M_{ref} = 1.06 pmol/mL): $B/B_{max} = 1.06/(11.5 + 1.06) = 8.4\%$,

i.e., 3.0% higher radioactive tracer binding would be expected in the stress subjects due to lower cold tracer mass occupancy, which corresponds to the corrected results of the ANCOVA.

Sex and Tracer Mass

After adjusting for both sex and tracer mass, binding in the striatum remained significantly related to both sensory magnitude (b = 0.200, SE = 0.090, $F_{(1,32)} = 4.91$, $p = 0.034$) and habituation (b = -1.997, SE = 0.816, $F_{(1,32)} = 5.99$, $p = 0.020$) (see Figures S4 and S5). When adjusted for both sex and tracer mass, the fractional differences in binding for stress vs no stress were further decreased: striatum 7.7% ($p = 0.23$), putamen 10.1% ($p = 0.16$), and head of caudate 6.1% ($p = 0.33$) but remained in the direction of higher binding in the Stress condition. There is a large literature on selecting confounders for statistical analyses. Given that individually the sex and tracer mass

adjustments did not alter the stress effect, fitting the model with both potential confounders may not be justified (3).

Tracer Metabolite

An inactive metabolite of [^{18}F]FECNT may cross the blood brain barrier, distribute evenly in the brain, and thus reduce the apparent binding potential of [^{18}F]FECNT determined by the reference tissue method used here (4; 5). The radioactivity observed in the cerebellar reference region scaled to injected dose/body weight differed by only 3% between the prenatally stressed and non-stressed groups (Table 1, A_{ref}), which suggests that any confound due to differences in radiotracer metabolism would be small compared to the observed effects. Nevertheless, to look for the effect of such a metabolite, binding potentials were determined using the slope of the Logan plots for the period 60-120 minutes post-injection and compared to those for 90-150 minutes as used in the main analysis. To the extent that the relative concentration of metabolite in the brain is lower at earlier times, the binding potential calculated using the 60-120 minute period is expected to be higher and closer to the true value. BP_{ND} in striatum was somewhat higher (+2.6% +/- 4.0%, mean +/- SD, n = 38) for 60-120 min compared to 90-150 min. A 2 (Stress) x 2 (Alcohol) ANOVA of BP_{ND} determined from the early data yielded a stress effect similar to that reported in Table 1, i.e. striatum 12% (p = 0.033), putamen 14% (p = 0.030), and head of caudate 11% (p = 0.049). Binding potentials calculated using earlier time points are expected to be less reliable as the [^{18}F]FECNT is not as well equilibrated (Figure S3), and this increased variance may contribute to the reduced significance.

Table S1. Potential effect of sex on main effect of stress.

	Main Effect of Stress			Effect of Sex	
ROI	Difference	*F*	*p*	Smallest *p* for sex	Model term
Striatum	12%	3.96	0.056	0.12	alc x sex
Putamen	15%	4.79	0.037	0.088	alc x sex
Caudate	9%	2.17	0.151	0.078	sex x stress
Head	10%	2.84	0.102	0.11	stress x sex
Body	1%	0.02	0.89	0.01	sex main
Tail	2%	0.11	0.75	0.065	sex x stress
Acb	7%	1.66	0.207	0.027	sex x stress
SN/VTA	3%	0.39	0.54	0.036	alc x sex

Analysis of variance including sex as a factor. Difference = (stress / no stress) -1, 2 (Stress) x 2 (Alcohol) x 2 (Sex), $F_{(1,30)}$, *p*: 2-tailed.

Acb, nucleus accumbens; Alc, alcohol; ROI, region of interest; SN, substantia nigra; VTA, ventral tegmental area.

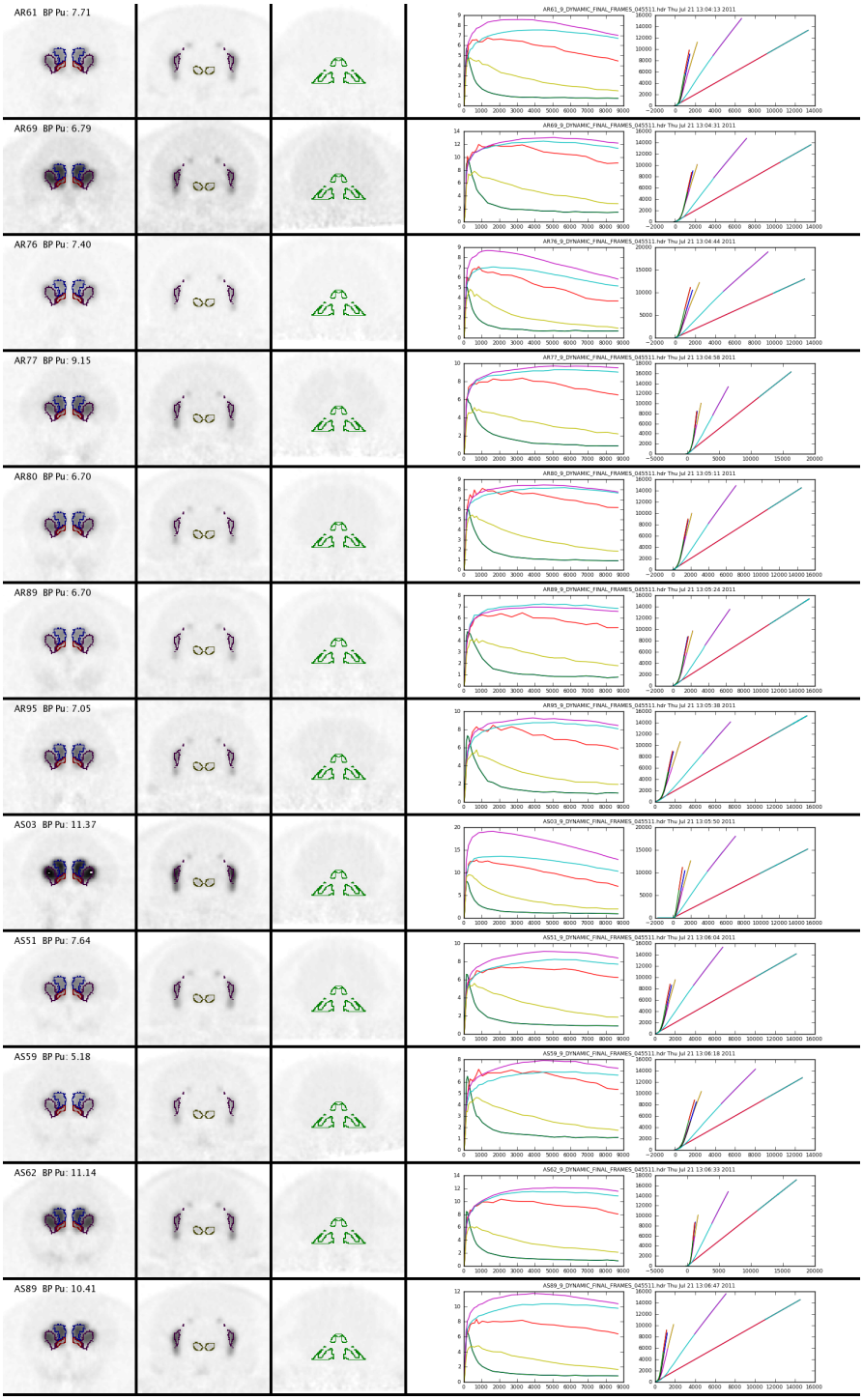
Table S2. Main effect of stress adjusted for potential effect of non-radioactive tracer mass.

	Main Effect of Stress		
ROI	Difference	*F*	*p*
Striatum	12%	3.97	0.055
Putamen	14%	4.57	0.040
Caudate	9%	2.45	0.127
Head	10%	3.08	0.089
Body	5%	0.41	0.526
Tail	2%	0.06	0.813
Acb	5%	1.12	0.297
SN/VTA	-1%	0.04	0.848

Analysis of covariance performed with reference region tracer mass (M_{ref}) as a covariate. Difference = (stress / no stress) -1, 2 (Stress) x 2 (Alcohol) ANCOVA, $F_{(1,33)}$, *p*: 2-tailed.

Acb, nucleus accumbens; ROI, region of interest; SN, substantia nigra; VTA, ventral tegmental area.

Figure S1, continued on next page



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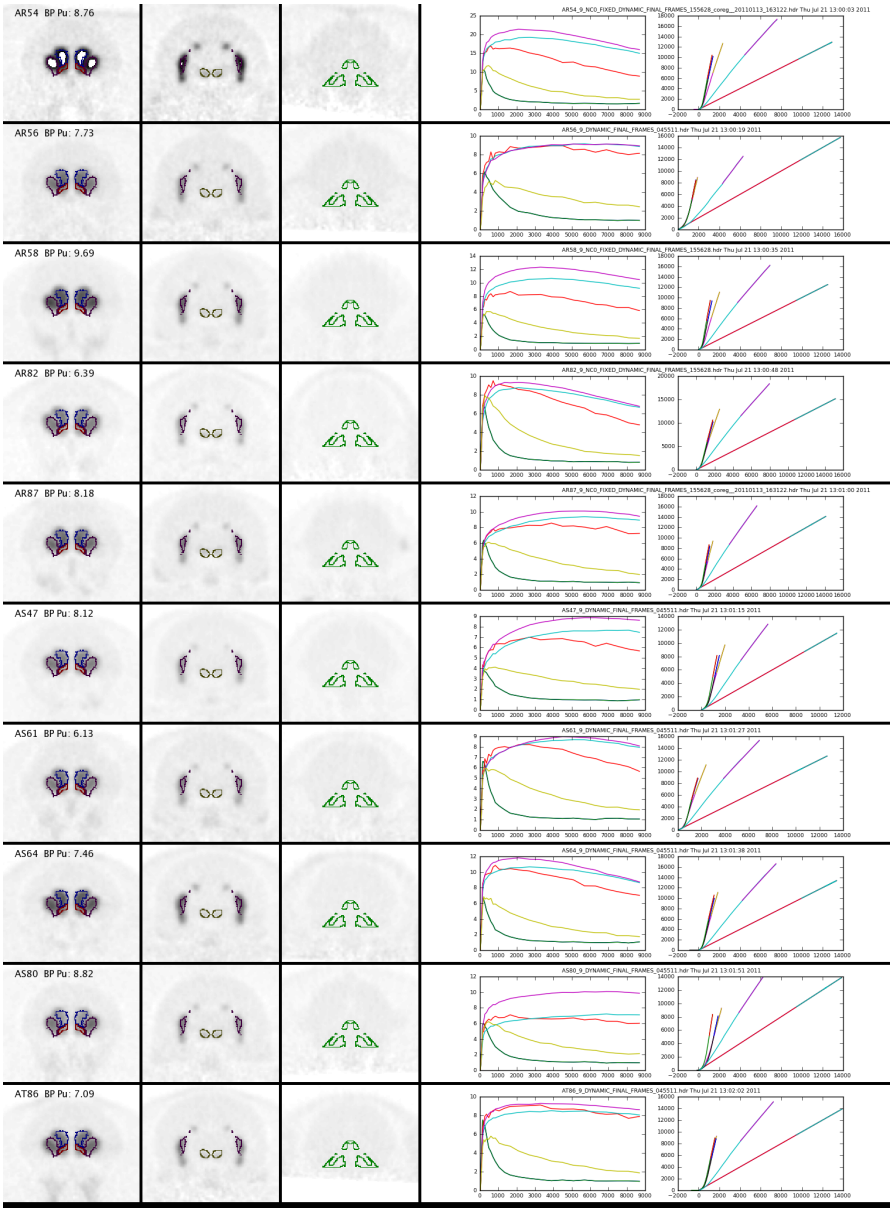

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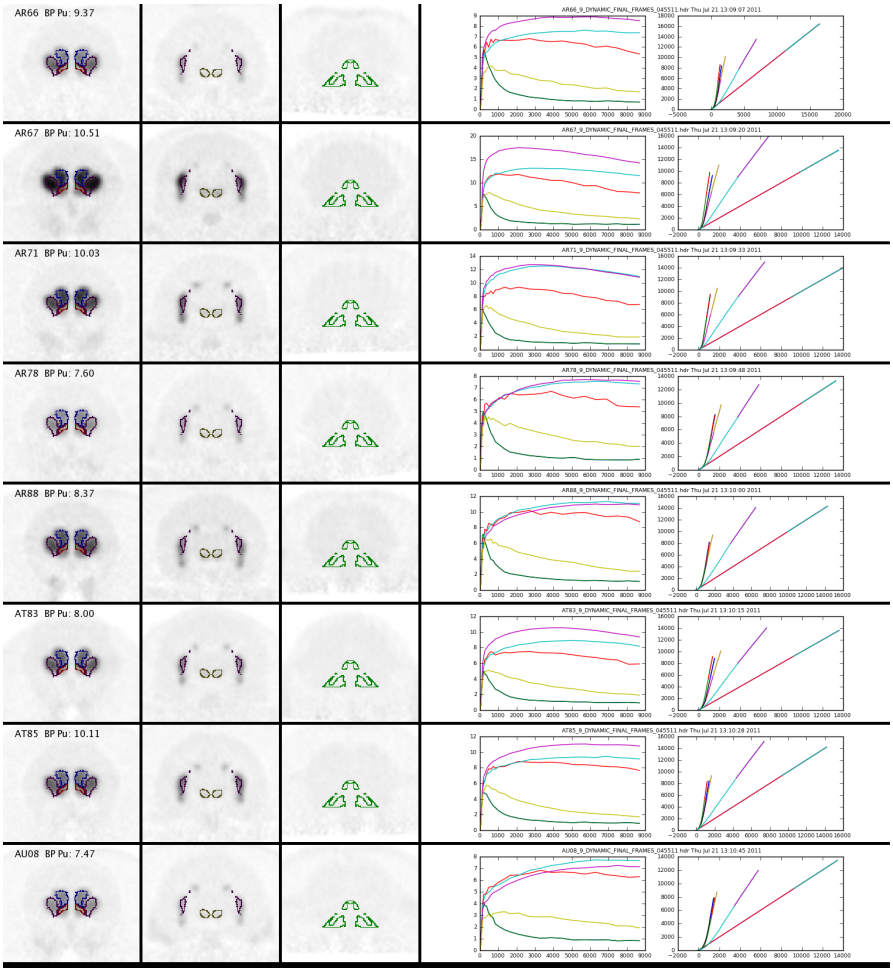

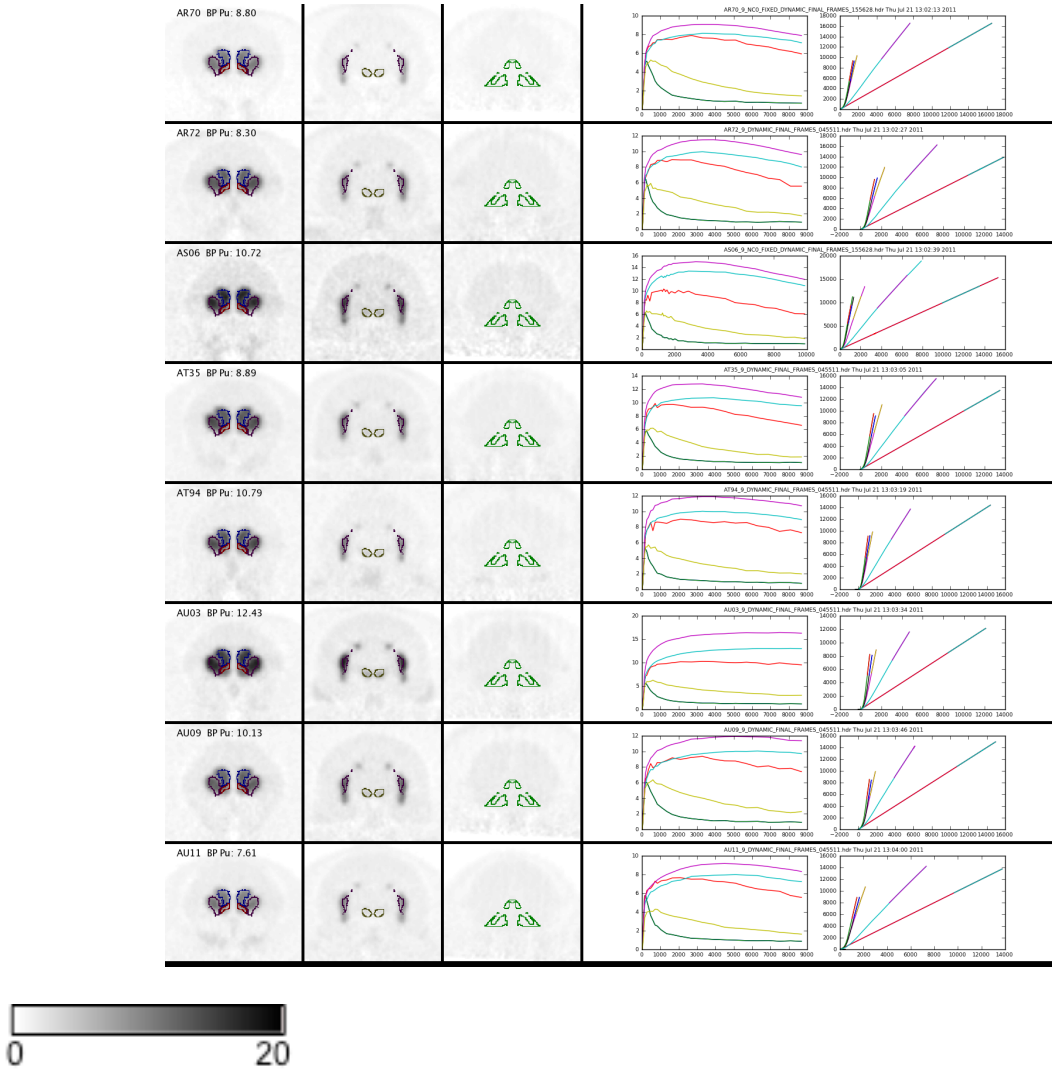

Figure S1. Images, time activity curves (TACs), and Logan plots for each subject. Data are shown ordered by in utero treatment group: Control (*n* = 12), Alcohol (*n* = 10), Stress (*n* = 8) and Alcohol + Stress (*n* = 8). Coronal slices of 0-150 min summed images are shown at anterior commissure +5, -9, and -36 mm. Regions of interest and corresponding TACs are shown for putamen (Pu magenta), head of caudate (CdHd blue), nucleus accumbens (Acb red), substantia nigra / ventral tegmental area (SN/VTA yellow), and cerebellar reference region (Cb green). Images and TACs are scaled to injected dose/body weight and linear image scale runs from 0 to 20 (g/mL). Logan plots are shown with overlay of linear fit to points from 90-150 min: Cb (red, slope = 1), SN/VTA (cyan), Acb (magenta), CdHd (black), and Pu (green). Value of [^{18}F]FECNT binding in putamen is given at top of coronal view. Time axis of TACs runs from 0 to 9000 s, i.e. 0 to 150 min. Logan plot axes are Starget/target vs Sreference/target.

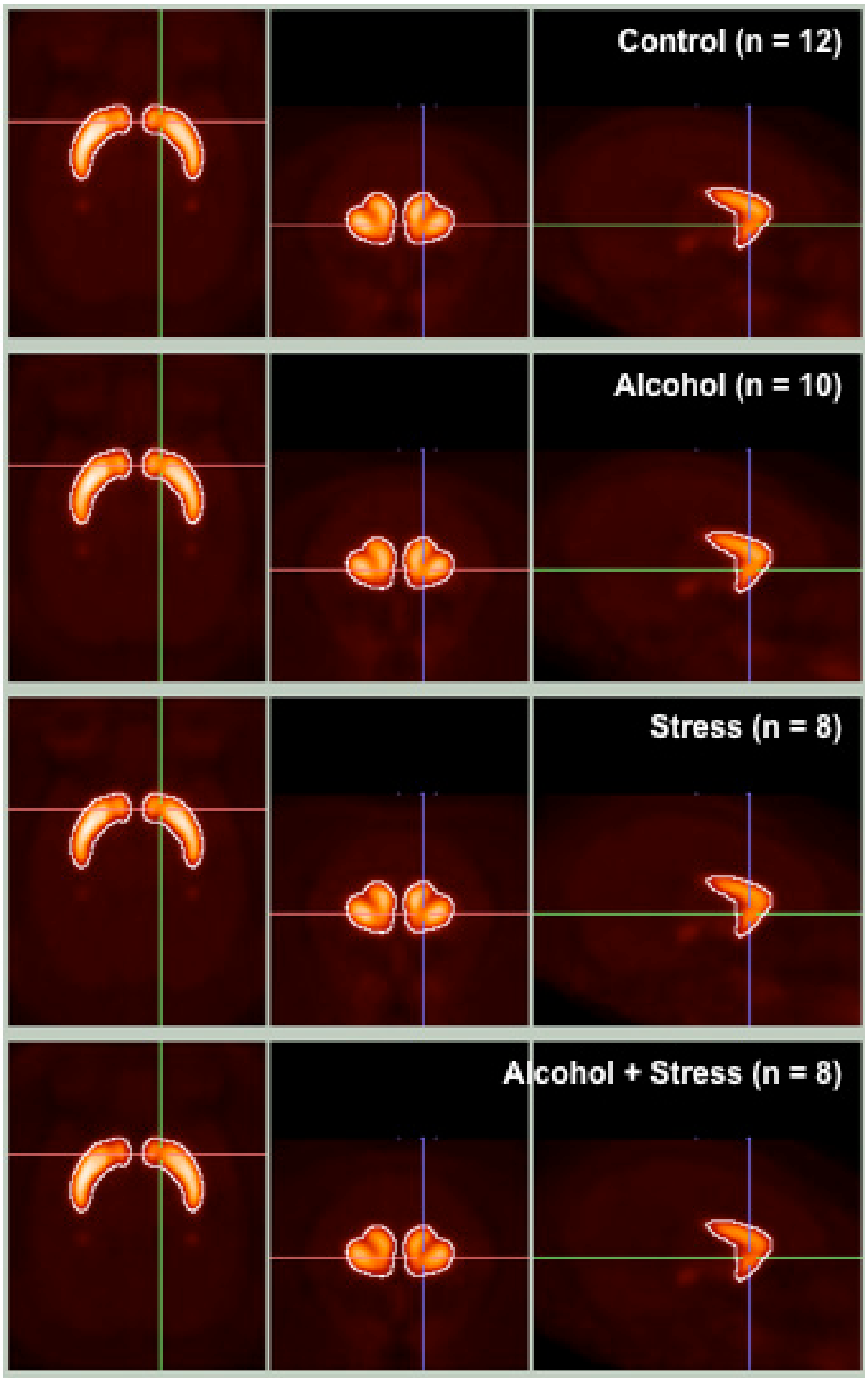

Figure S2. Alignment of groups. Mean whole brain-normalized 150 min images for each group. Contour shown in all four images is delineated on striatal radioactivity of Control group.

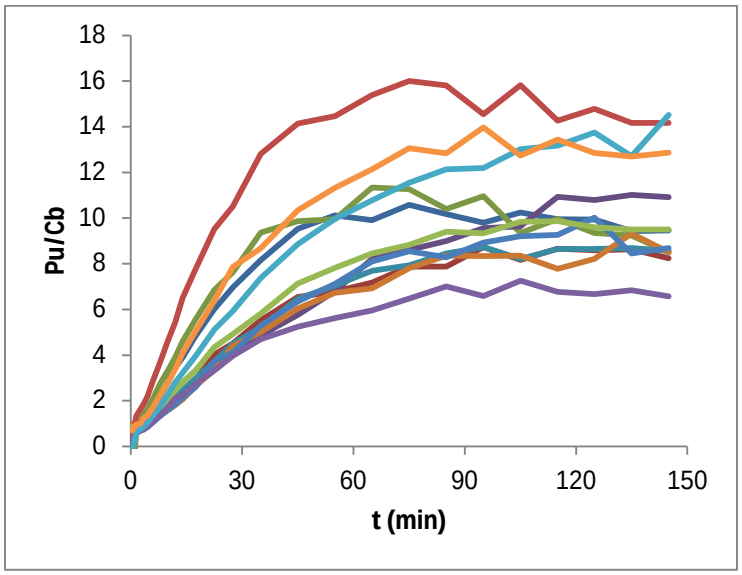

Figure S3. Equilibrium. Target to reference ratio stabilizes by 90 minutes. Putamen (Pu) in control subjects (*n* = 12).

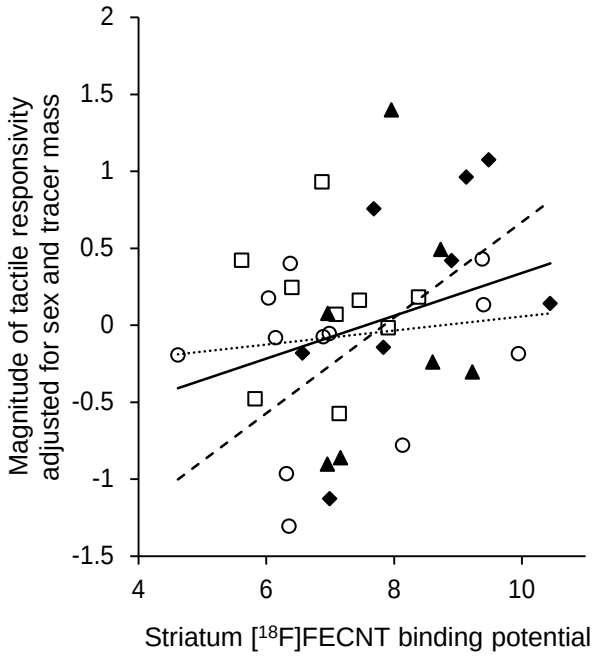

Figure S4. Magnitude of tactile responsivity adjusted for sex and tracer mass vs dopamine transporter availability in striatum as indexed by [^{18}F]FECNT binding. ¡ = control, p = alcohol, p = stress, u = alcohol + stress. Linear regressions: —— = overall (b = 0.200, SE = 0.090, $F_{(1,32)}$ = 4.91 p = 0.034), ··· = no stress, - - - = stress. There was no significant difference between the regressions for stress and no stress (p = 0.10), and neither was significant alone.

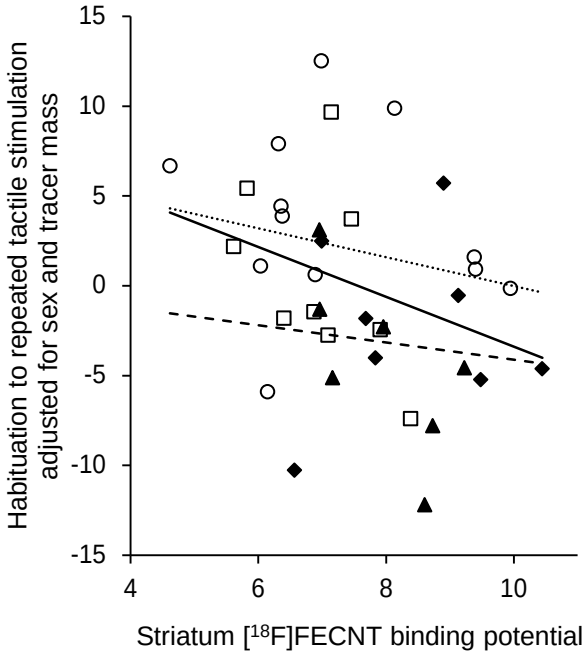

Figure S5. Habituation to repeated tactile stimulation adjusted for sex and tracer mass vs dopamine transporter availability in striatum as indexed by [^{18}F]FECNT binding. ¡ = control, p = alcohol, p = stress, u = alcohol + stress. Linear regressions: —— = overall (b = -1.997, SE = 0.816, $F_{(1,32)}$ = 5.99, p = 0.020), ··· = no stress, - - - = stress. There was no significant difference between the regressions for stress and no stress (p = 0.40), and neither was significant alone.

Supplemental References

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5. Price JC, Lopresti BJ, Meltzer CC, Smith GS, Mason NS, Huang Y, *et al.* (2001): Analyses of [(18)F]altanserin bolus injection PET data. II: consideration of radiolabeled metabolites in humans. *Synapse.* 41: 11–21.