Test–Retest Variability of [^{11}C]Raclopride-Binding Potential in Nontreatment-Seeking Alcoholics

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ABSTRACT Knowledge of the reproducibility of striatal [^{11}C]raclopride (RAC) binding is important for studies that use RAC PET paradigms to estimate changes in striatal dopamine (DA) during pharmacological and cognitive challenges. To our knowledge, no baseline test–retest data exist for nontreatment-seeking alcoholics (NTS). We determined the test–retest reproducibility of baseline RAC binding potential (BP_{ND}) in 12 male NTS subjects. Subjects were scanned twice with single-bolus RAC PET on separate days. Striatal RAC BP (BP_{ND}) for left and right dorsal caudate, dorsal putamen, and ventral striatum was estimated using the Multilinear Reference Tissue Method (MRTM) and Logan Graphical Analysis (LGA) with a reference region. Test–retest variability (TRV), % change in BP_{ND} between scan days, and the intraclass correlation coefficient (ICC) were used as metrics of reproducibility. For MRTM, TRV for striatal RAC binding in NTS subjects was ±6.5% and ±7.1% for LGA. Average striatal ICCs were 0.94 for both methods ($P < 0.0001$). Striatal BP_{ND} values were similar to those reported previously for detoxified alcoholics. The results demonstrate that baseline striatal RAC binding is highly reproducible in NTS subjects, with a low variance similar to that reported for healthy control subjects. **Synapse 00:000–000, 2010.**

INTRODUCTION

[^{11}C]Raclopride (RAC) is a D_2/D_3 antagonist that is routinely used in positron emission tomography studies to estimate striatal D_2/D_3 receptor availability. This radioligand is sensitive to competition by endogenous striatal dopamine (DA) (Dewar et al., 1989; Seeman et al., 1989; Young et al., 1991) and is commonly used in paradigms that assess relative changes in DA concentration in response to pharmacological or cognitive paradigms [for review, see Egerton et al. (2009) and Laruelle (2000)]. Accurate and reproducible measurement of "baseline" D_2/D_3 availability is therefore crucial for a proper interpretation of such studies (Yoder et al., 2008). Moreover, knowledge of the within-subject variance of a population aids in power analyses and guides study design for paradigms in which the effect size of the dopaminergic challenge may be relatively small. The reliability of baseline striatal RAC measurements with single bolus protocols has been demonstrated for healthy control subjects in several small (Hietala et al., 1999; Volkow et al., 1993) and moderately sized studies (Hirvonen et al., 2003; Schlosser et al., 1998). Although both the methods for determining binding availability and the

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TABLE I. Subject demographic and drinking characteristics

n	Race	Ethnicity	Age (years)	Education (years)	D/Dday	D/wk	D/mon
12	3 C 9 AA	12 NHL	39.3 ± 10.0 (23–54)	12.1 ± 0.79 (11–14)	10.3 ± 3.11 (5.51–15.7)	46.9 ± 23.8 (21.0–95.2)	201 ±102 (90–238)

Top row is mean ± s.d.; bottom row is data range. C, Caucasian; AA, African-American; NHL, not Hispanic or Latino; D/Dday, drinks per drinking day; D/wk, drinks per week; D/mon, drinks per month.

metrics used to gauge reproducibility vary across studies, estimates typically range between ~±5–10%.

However, it cannot be assumed that populations with psychiatric and/or neurological disorders will exhibit the same stability of RAC binding as healthy controls. Regardless of the method used to assess RAC binding, D_2/D_3 availability is invariably a function of both number of receptors and the presence of endogenous DA. We recently observed that, even in control subjects, variance in "resting state" D_2/D_3 availability can be accounted for by tracking other variables (Yoder et al., 2008). For example, apparent differences in baseline cognitive states caused large sample variations in striatal D_2/D_3 availability—up to almost 20% between baseline conditions. This is an important consideration when there is a distinct possibility that striatal DA may frequently change as a consequence of subjective states such as expectation or craving (Brody et al., 2004, 2006; Volkow et al., 2006; Wong et al., 2006; Yoder et al., 2008). Our laboratory is currently studying several dopaminergic challenge paradigms in nontreatment-seeking (NTS) alcoholics. This subject group presents a unique challenge, as the majority of such individuals are chronic smokers (Meyerhoff et al., 2006; Room, 2004). If subjects in nicotine withdrawal crave a cigarette, this could conceivably alter the dopaminergic state (Brody et al., 2004, 2006; Volkow et al., 2006; Wong et al., 2006). Here, we sought to determine the test–retest reproducibility of baseline RAC binding availability in NTS alcoholics in whom nicotine levels were controlled across scans.

MATERIALS AND METHODS

Subjects

All procedures were approved by the Indiana University Institutional Review Board. Informed consent for participation for this study was obtained only after confirmation that breath alcohol concentration (BrAC) was 0 mg%. Twelve male NTS alcoholics participated. Subjects were right-handed cigarette smokers with a family history of alcoholism, defined as having at least two or more primary or secondary relatives identified by the Family History Assessment Module (Rice et al., 1995) that had at least three "yes" answers on the Individual Assessment Module. NTS subjects met DSM-IV criteria for alcohol dependence as determined by the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994; Hesselbrock et al., 1999; NIAAA, 2003). Subjects had neither received treatment for alcohol use disorders within the past year nor were they actively seeking treatment. Demographic and drinking characteristics are presented in Table I.

Study procedures

Subjects underwent identical procedures on 2 separate days. A typical study-day timeline is presented in Figure 1. Briefly, subjects presented to the Indiana Clinical Research Center at ~8 a.m. BrAC measurements were taken to ensure BrAC = 0 mg%. An IV catheter was placed in an antecubital vein. Subjects were given a full breakfast. The Clinical Withdrawal Assessment for Alcohol, Revised (CIWA-Ar; Sullivan et al., 1989), Alcohol Urge Questionnaire (AUQ; Bohn et al., 1995), and Cigarette Withdrawal Scale (CWS; Etter, 2005) were given periodically throughout each study day. The AUQ and CWS are both self-report Likert rating scales. The AUQ is eight items, with a seven-point scale for each item (score range = 7–56). Nicotine craving was measured with the second dimension on the CWS, which specifically captures the individual's current subjective state of cigarette craving. There are four questions on this dimension, each with a five-point scale; possible scores for cigarette craving range from 4 to 20. Ratings were taken upon arrival for the study (time 1) and before and after the resting (baseline) scan (times 2 and 3). The CIWA-Ar was administered by study personnel; a score of <8 was required for study participation.

To control for both dosage and timing of last exposure to alcohol, all subjects received an IV alcohol infusion to a target of 60 mg% using the Alcohol Clamp Technique (O'Connor et al., 1998; Ramchandani et al., 1999). During the infusion, subjects were in a reclined position on a hospital bed. Target BrAC was achieved over a "ramp" period of 15 min and then "clamped" at target for 30 min.

To avoid the potential confound of nicotine withdrawal or cigarette craving on repeated measurements of baseline D_2 receptor availability (Brody et al., 2004, 2006; Volkow et al., 2006; Wong et al., 2006), transdermal nicotine patches were placed on the subjects shortly after arrival. Patch dose was based on self-reported number of cigarettes smoked per day.

Scanning procedures

A magnetization prepared rapid gradient echo (MP-RAGE) magnetic resonance image (MRI) was acquired

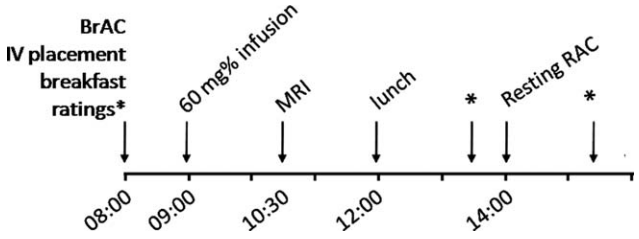

Fig. 1. General outline of study protocol. Typically, the magnetic resonance image (MRI) was acquired on day 1. BrAC, breath alcohol concentration; RAC, [^{11}C]raclopride positron emission tomography scan. Ratings were taken upon arrival and before and after the resting (baseline) RAC scan.

on all subjects (Siemens 3T Trio) for anatomic coregistration of PET data (see "Image Processing Procedures").

Subjects received two baseline RAC scans in the early afternoon on 2 separate days. Time of injection was typically between 14:00 and 15:00. BrAC was 0 mg% before scanning. RAC synthesis was completed as described previously (Fei et al., 2004). RAC PET scans were acquired on a Siemens EXACT HR+ (3D mode; septa retracted). Before each PET scan, a 10-min transmission scan using three internal rod sources was acquired for attenuation correction. RAC PET scans were initiated with the IV infusion of 14.0 ± 1.46 mCi [^{11}C]RAC (mass dose: 0.14 ± 0.07 nmol/kg) over 1.5 min. Dynamic acquisition occurred for 50 min.

Image processing procedures

Image processing is similar to that described previously (Yoder et al., 2007, 2008, 2009). MRI DICOM and RAC PET images were converted to Neuroimaging Informatics Technology Initiative format (http://nifti.nimh.nih.gov/) using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/). For each subject, all dynamic PET data were coregistered to an early-time mean image to facilitate motion correction. The early mean PET image was coregistered to the MRI scan using the normalized mutal information algorithm in SPM5, with the transformation matrix from this coregistration subsequently applied to the motion-corrected dynamic PET data. Each subject's MRI was spatially normalized to Montreal Neurological Institute space. The transformation matrix obtained from the spatial normalization step was then applied to the motion-corrected, MRI-registered PET data from each subject.

Region of interest analysis

All regions of interest (ROIs) were drawn on an average normalized MRI from all subjects, using MRIcron (http://www.sph.s.c..edu/comd/rorden/mricron/). Striatal ROIs consisted of the left and right ventral striatum (VST), dorsal caudate (DCA), and dorsal putamen (DPU) and were drawn according to anatomic landmarks described previously (Mawlawi et al., 2001). The DCA ROI contains the pre- and postcommissural caudate as described in Martinez (2003); the DPU contains the pre- and postcommissural putamen. For the reference region (tissue that contains little to no D_2/D_3 receptor density), an ROI was created that contained all cerebellar gray matter except for the vermis. Time-activity curves for each ROI were generated from the dynamic RAC data using the MarsBaR toolbox for SPM5 (http://marsbar.sourceforge.net/). For each striatal ROI, D_2/D_3 receptor availability was indexed with BP_{ND}, the binding potential (BP) of RAC calculated as bound tracer concentration relative to nondisplaceable tracer concentration (Innis et al., 2007). Estimations of BP_{ND} were conducted using the Multilinear Reference Tissue Model (MRTM; Ichise et al., 2003) and Logan reference region graphical analysis (LGA, Logan et al., 1996). All Logan plots were linear.

Metrics of test–retest reproducibility of baseline RAC binding

The relative reproducibility of striatal BP_{ND} between days 1 and 2 was examined with three calculations, test–retest variability (TRV), percent change in BP_{ND} (ΔBP_{ND}), and the intraclass correlation coefficient (ICC; one-way random effects model) as implemented in the PASW statistical package (McGraw and Wong, 1996; Shrout and Fleiss, 1979). TRV was calculated as: $|BP_{day1} - BP_{day2}|/[(BP_{day1} + BP_{day2})/2]$ (Hirvonen et al., 2003; Mawlawi et al., 2001). ΔBP between days 1 and 2 was calculated as: $-[(BP_{day1} - BP_{day2})/BP_{day1}] \times 100$.

Parametric images for SPM analysis

BP_{ND} was estimated at each voxel throughout the brain using the multilinear reference tissue method with a common reference region efflux rate to facilitate robust performance on noisy voxel data (MRTM2) (Ichise et al., 2003). The resulting parametric BP_{ND} images were smoothed with an 8-mm Gaussian kernel (Costes et al., 2005; Picard et al., 2006; Ziolko et al., 2006). The search area for the voxelwise paired *t*-tests was restricted to the striatum as (1) our sole focus was the striatum and (2) the striatum has the highest density of D_2/D_3 receptors in the brain and is the only brain structure with high-enough signal-to-noise ratio to support quantification of D_2/D_3 receptor availability with RAC. A bilateral striatal binary mask was created on an average image of all subjects' raw parametric images by excluding any BP_{ND} values less than one. Striatal ΔBP images were generated (i.e., ΔBP at each voxel within the striatal mask), and one-sample *t*-tests

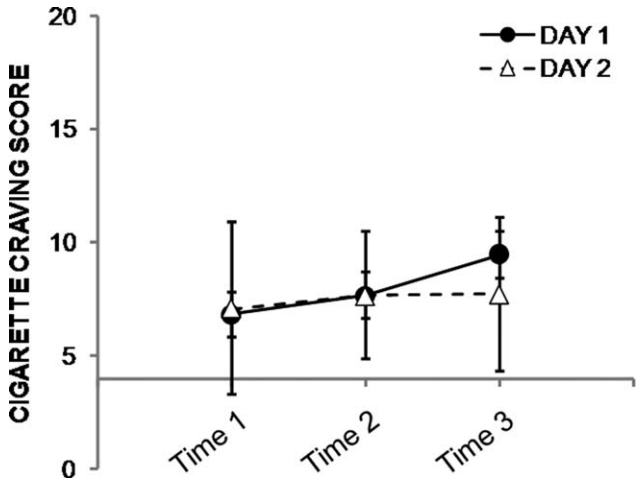

Fig. 2. Mean ± s.d. cigarette craving ratings from day 1 (filled circles) and day 2 (open triangles). The *x*-axis crosses the *y*-axis at the value of four to denote that four is the lowest possible score on the index; for reference, 20 is the highest possible score. Craving ratings did not vary within subjects across either day or time point (see text for details).

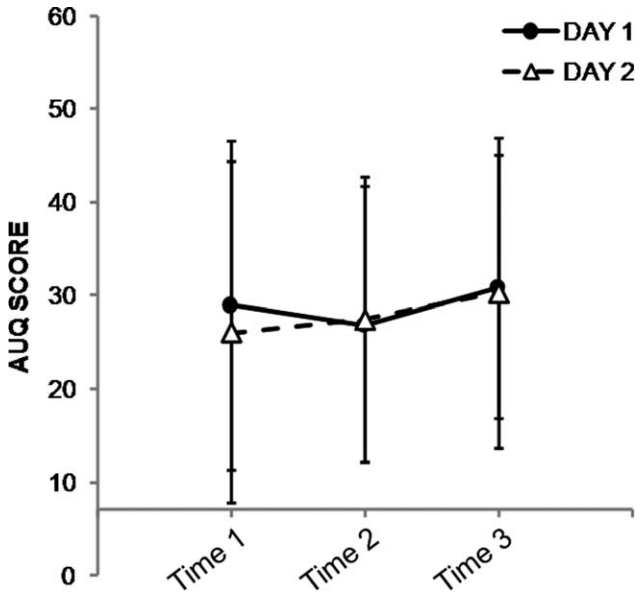

Fig. 3. Mean ± s.d. alcohol craving score from the Alcohol Urge Questionnaire (AUQ) from day 1 (filled circles) and day 2 (open triangles). The *x*-axis crosses the *y*-axis at the value of seven to denote that seven is the lowest possible score on the index; for reference, 56 is the highest possible score. Craving ratings did not vary within subjects across either day or time point (see text for details).

were used in SPM5 to test the null hypothesis that $\Delta BP = 0$. Specifically, model contrasts were $\Delta BP > 0$ and $\Delta BP < 0$. The statistical threshold was set at $P < 0.05$, two-tailed (i.e., $P < 0.025$ for each contrast).

Other statistical tests

Independent *t*-tests were used to test for differences in injected radioactivity and injected mass dose between scan days. To examine the stability of cigarette craving (CWS dimension two score), alcohol craving (AUQ), and alcohol withdrawal (CIWA-Ar), repeated-measures ANOVA was used to test for effects of scan day, time point, and day × time point. Paired *t*-tests were used to determine if striatal BP_{ND} values were significantly different between scan days. Spearman's correlation coefficient was used to assess whether number of days between scans was associated with the absolute value of ΔBP_{ND} (days-between-scan data were non-normally distributed). Pearson's correlation coefficient was used to (1) determine how similar estimates of BP_{ND} for the ROI data were between MRTM and LGA and (2) compare the relative performance of MRTM and LGA with respect to TRV.

Statistical tests were conducted with Microsoft Excel 2007 and PASW Statistics 17 and 18.

RESULTS

RAC scan parameters

Average number of days between scans was 8.83 ± 14.8 (range, 1–45 days). Injected radioactivity of RAC on days 1 and 2 was 13.7 ± 1.82 and 14.2 ± 0.98 mCi, respectively. Corresponding mass doses were 0.15 ± 0.07 and 0.13 ± 0.06 nmol/kg. Injected radioactivity and mass doses were not significantly different between scan days.

Subject data

The demographic and drinking characteristics of the subjects are shown in Table I. Although every effort was made to screen out potential polysubstance users at the screening visit, four subjects had positive urine drug screens on one or both scan days. One subject tested positive for opiates on both scan days (self-report was of a few days' use of painkillers for a neck injury); one tested positive for cocaine on both scan days (subject denied "intentional" use of illicit drugs), one tested positive for cannabis use on both scan days, and another tested positive for cannabis use on the first scan day only (the latter two subjects endorsed infrequent use of marijuana). None of these subjects were apparent outliers in the dataset in any respect, and all four subjects had test–retest variability of striatal RAC binding equal to or below that of the sample average (see below; <6.5%).

Exact timing of patch placement was not available for two subjects on day 1. Across the remaining data points ($n = 22$), the interval between patch placement and resting scan was 5.9 ± 0.6 h. Ratings for CWS, AUQ, and CIWA-Ar were unavailable for one subject at day 1, time 3, and for another subject on day 2, time 3. Results from the repeated-measure ANOVAs indicated that all three ratings were stable within

subjects (i.e., there were no effects of day, timepoint, or interactions of day × timepoint). Cigarette and alcohol-craving scores are presented in Figures 2 and 3. Subjects reported very few acute alcohol withdrawal symptoms (Fig. 4). The range of CIWA-Ar scores was 0–3; of 70 measurements, a score of two was given five times; the score of three was observed on three occasions.

Test–retest reproducibility of resting RAC signal

BP_{ND} values for both scan days, percent change of BP_{ND} between days (%Δ), the test–retest variability (TRV), and ICCs are presented in Table II (MRTM) and Table III (LGA). For both MRTM and Logan, the average striatal ICC values were very high. The observed striatal RAC BP_{ND} values are very similar to BP_F values reported previously for detoxified alcoholics in RAC single-bolus protocols with arterial sampling (Volkow et al., 1996; Volkow et al., 2002; Volkow et al., 2007), but slightly higher than BP_{ND} reported for detoxified alcoholics in a RAC bolus-infusion paradigm [see Table III from Martinez et al. (2005)]. There was no association between number of days between scans and the absolute value of ΔBP in any ROI (i.e., length of days between scans did not contribute to observed variability).

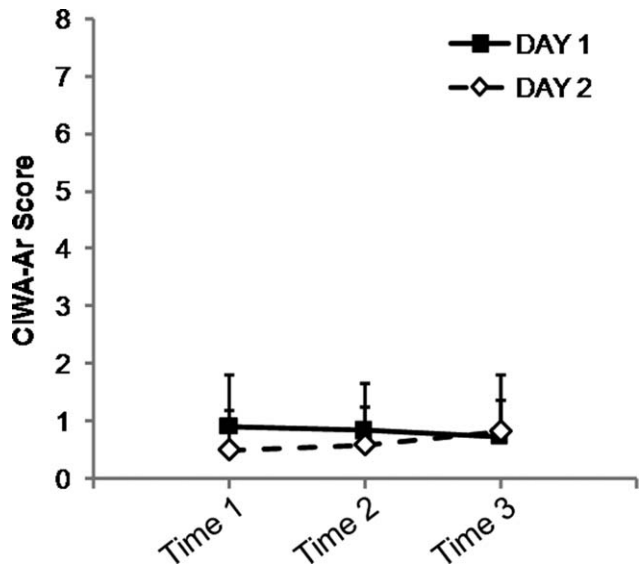

Fig. 4. Mean + s.d. CIWA-Ar ratings (for graphical clarity, only positive s.d. is shown) from day 1 (filled squares) and day 2 (open diamonds). CIWA-Ar score did not vary within subjects across either day or time point (see text for details). In this protocol, a score of ≥8 would require a subject to be withdrawn from the study to receive immediate medical attention for alcohol withdrawal symptoms.

Overall, there were no overt differences between MRTM and LGA performance. As expected, estimates of BP_{ND} from these methods are highly correlated (Fig. 5). MRTM and LGA had very similar values for the test–retest metrics (Tables II and III). Although MRTM and LGA are statistically identical with respect to observed TRV across regions, Figure 6 suggests that relative TRV is not entirely consistent between estimation schemes. However, the fact that MRTM and LGA produce statistically identical TRVs indicates that test–retest metrics should not be a deciding factor in selection of one method over another.

SPM analysis

In general, the SPM analysis agreed with the ROI analyses. BP_{ND} was slightly lower in the right VST on day 2 (Fig. 7). The SPM analysis also picked up lower BP_{ND} on day 2 in an area that overlapped the left posterior lateral putamen (data not shown). The majority of this cluster was outside the anatomical boundary of the putamen, and thus the cluster was considered to be an artifact.

DISCUSSION

This study is the first to report the test–retest reproducibility of resting (baseline) striatal RAC binding availability in NTS alcoholics under conditions designed to control for the potential confound of cigarette craving on endogenous DA. The results demonstrate excellent test–retest variability (~6.5–7.1%). Overall, the reproducibility in striatal BP_{ND} in NTS

TABLE II. Test–retest data from resting [^{11}C]raclopride (RAC) scans in 12 nontreatment-seeking alcoholics

	BP_{ND} estimated with MRTM				
Region	BP_{ND} Day 1	BP_{ND} Day 2	%Δ	%TRV	ICC*
L DCA	2.02 ± 0.41	2.01 ± 0.46	−0.46 ± 9.76	6.27 ± 6.63	0.93
R DCA	2.08 ± 0.40	2.02 ± 0.49	−3.80 ± 9.67	8.54 ± 6.86	0.93
L DPU	2.88 ± 0.33	2.89 ± 0.31	0.64 ± 6.5	5.56 ± 2.77	0.85
R DPU	2.81 ± 0.35	2.79 ± 0.34	−0.48 ± 5.31	4.23 ± 3.07	0.92
L VST	2.32 ± 0.36	2.26 ± 0.29	−1.87 ± 10.4	8.02 ± 5.93	0.79
R VST	2.29 ± 0.38	2.18 ± 0.34†	−4.65 ± 5.92	6.17 ± 4.84	0.89
STRIATUM‡	2.40 ± 0.49	2.36 ± 0.51	−1.77 ± 8.13	6.46 ± 5.29	0.94

See Methods section for details. BP_{ND}, RAC binding potential estimated with MRTM. %Δ, percent change in BP_{ND} from day 1; %TRV, test–rest variability; ICC, intraclass correlation coefficient; L, left; R, right; DCA, dorsal caudate; DPU, dorsal putamen; VST, ventral striatum. Data are mean ± s.d.
*All ICC were statistically significant, $P < 0.0001$, except for L VST ($P < 0.001$).
†BP_{ND} values for R VST were significantly different between scan days, $P < 0.05$.
‡Data are averaged across six striatal regions in 12 subjects ($n = 72$).

TABLE III. Test–retest data from resting [^{11}C]raclopride (RAC) scans in 12 nontreatment-seeking alcoholics

	BP_{ND} estimated with LGA				
Region	BP_{ND} Day 1	BP_{ND} Day 2	%Δ	%TRV	ICC*
L DCA	1.95 ± 0.41	1.96 ± 0.45	0.01 ± 8.69	6.06 ± 5.40	0.95
R DCA	2.03 ± 0.40	1.94 ± 0.50	−5.20 ± 11.8	10.9 ± 8.52	0.89
L DPU	2.84 ± 0.32	2.83 ± 0.31	−0.25 ± 5.95	4.82 ± 3.12	0.87
R DPU	2.75 ± 0.35	2.74 ± 0.35	−0.25 ± 5.31	4.43 ± 2.72	0.92
L VST	2.24 ± 0.35	2.18 ± 0.29	−1.73 ± 11.8	7.72 ± 7.60	0.78
R VST	2.18 ± 0.37	2.07 ± 0.33†	−4.50 ± 8.64	8.64 ± 4.88	0.84
STRIATUM‡	2.33 ± 0.50	2.29 ± 0.52	−1.98 ± 8.98	7.10 ± 6.02	0.94

See Methods section for details. BP_{ND}, RAC binding potential estimated with LGA; %Δ, percent change in BP_{ND} from day 1; %TRV, test–rest variability; ICC, intraclass correlation coefficient; L, left; R, right; DCA, dorsal caudate; DPU, dorsal putamen; VST, ventral striatum. Data are mean ± s.d.
*All ICC were statistically significant, $P < 0.0001$, except for L VST ($P < 0.001$).
†BP_{ND} values for R VST were different at trend-level significance, $P = 0.06$.
‡Data are averaged across six striatal regions in 12 subjects ($n = 72$).

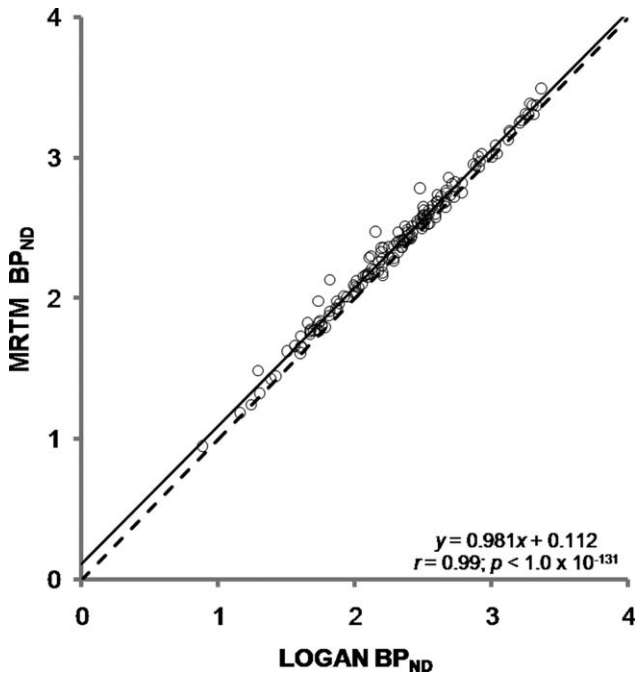

Fig. 5. BP_{ND} values from MRTM and LGA are highly correlated. The regression line (solid) almost overlaps with the line of identity (dashed). Pearson's correlation coefficient and the significance value are denoted within the graph.

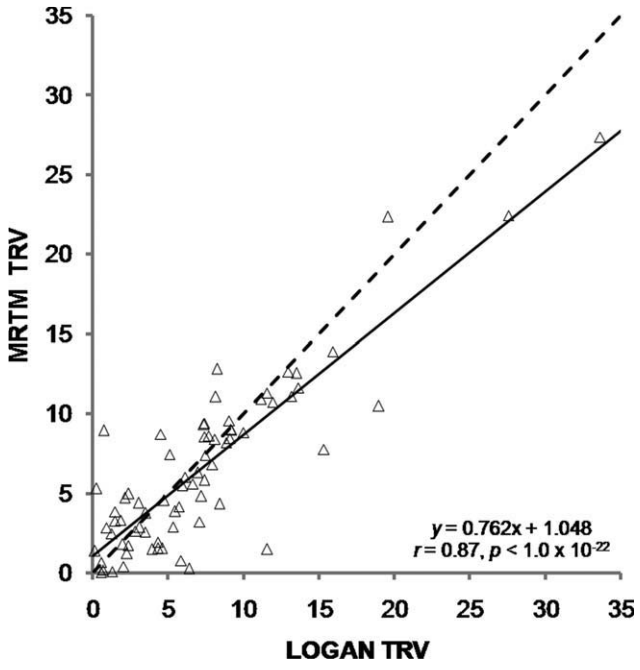

Fig. 6. Test–retest variability (TRV) from MRTM plotted against TRV from LGA. The regression line (solid) deviates from the line of identity (dashed), but Pearson's correlation coefficient is still highly statistically significant (inset).

alcoholics is commensurate with that reported for single-bolus RAC studies in healthy control subjects (Hietala et al., 1999; Hirvonen et al., 2003; Schlosser et al., 1998; Volkow et al., 1993).

Controlling for cognitive and physiological factors that may influence "baseline" RAC binding is critical for PET studies in which dopaminergic responses to pharmacological or cognitive challenges are being examined (Egerton et al., 2009; Yoder et al., 2008). In populations such as alcoholics, in whom smoking rates are high, it will be important to stabilize nicotine withdrawal/cigarette craving, which is likely to affect endogenous striatal DA (Brody et al., 2006; Brody et al., 2004). In this study, we found that use of nicotine patches was effective in controlling cigarette craving during the course of the study in NTS subjects. Given that (a) the test–retest reproducibility of striatal RAC binding availability in NTS was very similar to that of control subjects, and (b) the NTS striatal BP_{ND} values are comparable to those from detoxified alcoholics, transdermal nicotine delivery does not seem to have any adverse affect on obtaining a stable striatal BP_{ND} and thus seems to be a reasonable approach for controlling cigarette craving during DA challenge paradigms.

The ROI data revealed two unexpected findings. First, in this NTS sample, the rank order of BP_{ND} across striatal regions is unusual. Typically, the DPU has the highest D_2/D_3 availability in the striatum, followed by the DCA, and finally, the VST. This is true for control samples and even in detoxified alcoholics (Martinez et al., 2005). In our sample, BP_{ND} was

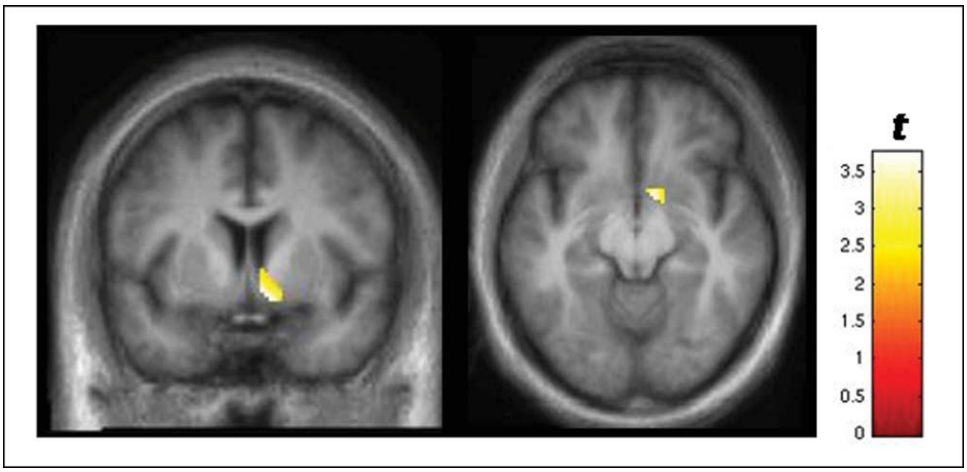

Fig. 7. Results from SPM analysis illustrating voxels in the right VST where BP_{ND} was lower on day 2 relative to day 1. Display threshold: $P < 0.025$. See text for details. The average $\%\Delta BP_{ND}$ of the voxels in this cluster was $-11.7\% \pm 10.3\%$. These results are consistent with the data in Table II.

TABLE IV. BP_{ND} values for NTS subjects on days 1 and 2

	Day 1	Day 2
VST	2.30 ± 0.36	2.22 ± 0.31
preDCA	2.11 ± 0.43	2.07 ± 0.50
postCA	1.74 ± 0.30	1.74 ± 0.36

VST, ventral striatum; PostCA, postcommissural caudate (postCA); PreDCA, precommissural dorsal caudate. BPND was estimated with MRTM; values from left and right regions were not significantly different (paired *t*-test) and were averaged.

higher in the VST than in the DCA. To explore this further, we estimated BP_{ND} for pre- and postcommissural caudate (Martinez et al., 2003) to determine if either or both of these subdivisions contributed to the anomaly. The typical rank order of the caudate subdivisions and VST is preDCA > VST > postCA. The rank order in the NTS sample is of the ROIs here is VST > preDCA > postCA (Table IV). Visual comparisons of the caudate ROIs with each individual's spatially normalized MRI did not give any indication that a partial volume effect would account for this effect. At this time, it is not known whether this observation is unique to this sample, or whether NTS subjects have lower BP_{ND} in the precommissural DCA relative to controls (or even treatment-seeking alcoholic populations). To address this, we are currently conducting a study within a much larger sample of controls and NTS subjects.

The second unexpected effect was that, in the right VST, BP_{ND} was relatively lower on day 2 than on day 1. This effect was statistically significant for MRTM data and trend-level for LGA. For both ROI methods, the $\%\Delta BP_{ND}$ was about -4.5%, which is below the average TRV reported here for these subjects. These results were echoed in the SPM analysis. However, it should be noted that the statistical threshold for the SPM analysis was extremely low ($P < 0.025$, uncorrected). This apparent effect was greatly diminished at $P < 0.01$ and did not survive the more stringent threshold of $P < 0.005$ (data not shown). The cause of this putative effect in the right VST is not known at this time. This finding helps illustrate the importance of TRV metrics when interpreting data in which changes in BP_{ND} are modest (i.e., below test-retest thresholds).

A limitation of this study was the absence of test–retest values for striatal RAC binding in NTS subjects without nicotine patches, which would have assessed any variability in baseline striatal BP_{ND} attributable to nicotine withdrawal. However, the most important assumption in a typical dopaminergic RAC PET challenge paradigm is that the within-subject state of basal DA is stable. Thus, instead of directly studying the amount of variance that would occur in NTS subjects undergoing nicotine withdrawal, we elected to proactively control for the potential (and likely highly variable) influence of cigarette craving on endogenous striatal DA. An added benefit of this approach is that use of nicotine patches helps ensure subject comfort and study compliance.

There is evidence in both human and animal studies that acute withdrawal from alcohol dependence causes decrease in basal striatal DA release (Ebert et al., 2002; Weiss et al., 1996). Although NTS subjects did not report many symptoms associated with alcohol withdrawal, we cannot exclude the possibility that subtle physiological effects of alcohol withdrawal after the low-dose morning alcohol infusion contributed to the observed variance in test–retest reproducibility. Conversely, we also do not know how variable baseline D_2/D_3 availability would be in NTS subjects had we not explicitly controlled for last time and dose of alcohol. Future studies in NTS subjects will be

necessary to understand the effects of alcohol withdrawal on the DA system.

Finally, several reports have documented that detoxified alcohol-dependent subjects have relatively lower striatal D_2/D_3 availability compared to control subjects (Martinez et al., 2005; Volkow et al., 1996; Volkow et al., 2002). Although our BP_{ND} values from NTS subjects are similar to data for detoxified alcoholic populations, we cannot make statements about whether the NTS sample shares this deficit. An ongoing parallel study with smoking controls will soon allow us to directly test this hypothesis.

In this study, we controlled for two factors that presumably could cause variability in baseline measurements of RAC binding, specifically, nicotine craving/withdrawal and alcohol withdrawal. However, there are many reasons why the types of interventions utilized herein (nicotine patches and low-dose IV alcohol infusion) may not be desirable or even feasible. For example, subject safety is always the first priority. If a RAC scan paradigm is being used to test for dopaminergic effects of a drug, all potential pharmacological interactions between the drug and, for example, nicotine and/or alcohol need to be considered carefully. Some cognitive or motor paradigms may require that subjects be completely nicotine-free (even if this results in nicotine craving/withdrawal). Alternatively, depending on the experimental setting and population being tested, it simply may not be practical to administer alcohol, and/or nicotine patches to smokers. Thus, optimal controls for variability in DA tone may not always be possible. However, nonpharmacological methods that may assist in explaining variance in the data should be considered.

In summary, we report excellent test–retest stability of baseline striatal RAC binding availability in NTS alcoholics. Test–retest metrics were well within the range of what has been reported for healthy control subjects. Additionally, the BP_{ND} values in our NTS sample are similar to previous data in detoxified alcoholics. These data suggest that it is feasible to conduct dopaminergic challenge studies in this population with RAC PET.

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