What were they thinking?
Cognitive states may influence $^{[11]}$C]raclopride binding potential in the striatum

Karmen K. Yoder$^{a,*}$, David A. Kareken$^b$, Evan D. Morris$^{a,c}$

$^a$Department of Radiology, Indiana University School of Medicine, Indianapolis, IN, United States
$^b$Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, United States
$^c$Department of Biomedical Engineering, Indiana University-Purdue University at Indianapolis, Indianapolis, IN, United States

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Abstract

$^{[11]}$C]Raclopride ($^{[11]}$C]RAC) is a selective dopamine D$_2$/D$_3$ antagonist that is commonly used in positron emission tomography (PET) studies to assess both basal levels of receptor availability and changes in availability caused by alterations in striatal dopamine concentration. When designing $^{[11]}$C]RAC studies, it is important to understand what variables may affect the results. Here, we examined differences in baseline striatal $^{[11]}$C]RAC binding potential (BP$_{ND}$) under two different “rest” conditions. Thirteen subjects received $^{[11]}$C]RAC scans. Eight subjects were aware prior to initiation of scanning that they would receive a “baseline” scan, and that no additional procedures would take place during the scan (“certain rest” group, CER). Five subjects were informed that they might or might not receive an IV alcohol infusion during the scan (“uncertain rest” group, UNC). This group was informed five min after scan start that they would not receive alcohol. Voxel-wise analyses of binding potential (BP$_{ND}$) images generated for both “rest” conditions indicated that receptor availability was higher in UNC than in CER. This result was confirmed by a region-of-interest analysis, which indicated that the average BP$_{ND}$ in right and left putamen was statistically higher in UNC. There were no differences in groups with respect to age or raclopride mass dose that could account for the difference in D$_2$/D$_3$ receptor availability. Our findings suggest that even slight differences in cognitive states between groups can have an effect on BP$_{ND}$, presumably mediated by changes in endogenous dopamine concentration.

Keywords: Positron emission tomography; Raclopride; Binding potential; Dopamine; Baseline; Reward

$^{[11]}$C]Raclopride ($^{[11]}$C]RAC) is a competitive D$_2$/D$_3$ antagonist (K$_D$ $\sim$1–10 nM [6,9,10,12]) that is sensitive to changes in endogenous dopamine (DA) levels [32,41]. Neuroimagers have taken advantage of the kinetic properties of $^{[11]}$C]RAC to study changes in striatal DA concentration ([DA]) using positron emission tomography [11,16,20,21] (for extensive review, see [16]). Typically, this involves a design requiring two PET scans: one at a “baseline” or “resting” state, and one during which a pharmacological challenge is administered or a cognitive or a motor task is performed (it should be noted that single-scan paradigms have been developed [1,26]). If the index of receptor availability, the binding potential (BP$_{ND}$; in brief, binding potential calculated as bound tracer concentration relative to nondisplaceable tracer concentration [14]), in the experimental condition is different from the baseline BP$_{ND}$, then the observed changes in BP$_{ND}$ are attributed to changes in endogenous [DA] [7,8,32,41]. Decreases in BP$_{ND}$ relative to baseline indicate increases in [DA], and increases in BP$_{ND}$ relative to the baseline BP$_{ND}$ indicate decreases in [DA].

It is assumed that the BP$_{ND}$ obtained during the “baseline” or “resting” condition is representative of a subject’s striatal D$_2$/D$_3$ receptor state under “normal” conditions. However, it is conceivable that subtle differences in cognitive state may result in changes in [DA], leading to measurable changes in BP$_{ND}$. If baseline BP$_{ND}$ is “artificially” altered consistently in one direction by induction of a specific cognitive state, it could affect the reliability of PET measures of stimulus-induced changes in striatal [DA]. To assess whether cognitive states of subjects could affect BP$_{ND}$, we tested for differences in resting...
BPND in normal subjects who were given different instructions with differing expectancies about what would occur during the [11C]RAC scans.

All procedures were carried out in accordance with the Declaration of Helsinki, and were approved by the Indiana University Institutional Review Board. The study was carefully explained to the subjects and informed consent was obtained prior to initiation of the study. Subjects were 13 healthy, non-smoking volunteers without histories of significant neurological disturbances or psychiatric diagnoses. None of the subjects were taking medications with central nervous system effects. Subjects received a urine drug screen on the day of scanning and all tested negative for amphetamines, barbiturates, benzodiazepines, cannabis, cocaine, and opiates.

Subjects had previously participated in one of two [11C]RAC PET protocols, each of which assessed the effects of a different dose of IV alcohol infusion on striatal [DA][39,40]. These protocols were designed to assess changes in [DA], and thus consisted of two scans, a “baseline” scan, and a scan during which alcohol was administered. The present work is a retrospective analysis of differences in the baseline scans between the two protocols (i.e., only one PET scan from each subject was included in the present comparison).

Each group had one female subject, and one left-handed subject. Ethnicity of all subjects was non-hispanic/latino. Mean ± S.D. age were 25.0 ± 5.76 (CER; see below) and 25.2 ± 3.19 (UNC).

[11C]RAC scans were performed as previously described [39,40] on an EXACT HR + scanner (CTI, Knoxville, TN) Upon arrival on the study day (typically, 2–3h before the baseline scan), subjects were informed of one of two ways about scanning procedures. CER subjects (n = 8) were told that they would receive a baseline scan and that during this scan, no other procedures would be performed while they were in the scanning environment. UNC subjects (n = 5) were informed that they might or might not receive an IV alcohol infusion during the scan, and that the type of scan would be revealed to them immediately after the scan started. UNC subjects were informed 5 min after the start of RAC injection (see below) by study personnel that “This is a No-Alcohol scan.” Both groups were instructed to otherwise lie quietly in the scanner.

Scans were initiated with the IV injection of 14.5 ± 3.09 mCi (CER) or 14.6 ± 8.21 mCi (UNC) of [11C]RAC, and dynamic data were acquired for 60 min. Specific activity at time of injection was 1.12 ± 0.67 Ci/μmol (CER) and 0.42 ± 0.22 Ci/μmol (UNC). Subject weights were 76.5 ± 14.4 kg (CER) and 68.6 ± 12.8 kg (UNC). Injected raclopride mass doses were 0.24 ± 0.15 nmol/kg (CER) and 0.65 ± 0.39 nmol/kg (UNC). Subjects also received a heavily T1-weighted magnetic resonance image (MR; 3D spoiled gradient echo recalled) on a 1.5T GE Echospeed LX scanner (GE Medical Systems, Waukesha, WI).

Image processing procedures using Statistical Parametric Mapping 2 software (SPM2) (http://www.fil.ion.ucl.ac.uk/spm/) have been described in detail elsewhere [39]. For each scan, a summed image was created from the first ten minutes of dynamic [11C]RAC data using the Realign function in SPM2. These summed images contained a mixture of blood flow and specific striatal D2/D3 binding, permitting accurate registration of all time frames to a single image. The summed image was co-registered to the individual subject’s MR scan using the SPM2. Motion correction was achieved by co-registering individual PET frames to the co-registered, summed PET image. Each subject’s MR was normalized into Montreal Neurological Institute (MNI) stereotactic space using SPM2’s default normalization parameters. The transformation matrix obtained from this normalization step was applied to the motion-corrected, co-registered PET data from each subject, placing all dynamic PET data in MNI stereotactic space.

The binding potential of [11C]RAC (BPND, [14]) is an index of how many DA D2/D3 receptors are available for binding. Parametric BPND images were generated from the spatially normalized dynamic PET data as described previously [39], using a multilinear reformulation of the Logan reference region graphical analysis [13,17]. The parametric whole brain BPND images were smoothed with an 8 mm kernel [5,27,42].

We restricted the search area during the voxel-wise paired t-test analysis, as (1) our sole focus was the striatum, and (2) the striatum has the highest density of D2/D3 receptors in the brain, and is the only brain structure with high enough signal-to-noise ratio to support quantitation of D2/D3 receptor availability using [11C]RAC. Ligands with higher signal-to-noise ratios in extrastriatal areas are required to quantify D2/D3 receptor availability outside of the striatum [4,22,23]. A bilateral striatal binary mask smoothed with a 10 mm kernel [39] was applied to the whole brain BPND images to create striatal BPND images that were used for all analyses reported herein. Unidirectional, voxel-wise independent t-tests between the CER and UNC striatal BPND images were conducted in SPM2 as follows: CER BPND > UNC BPND, and UNC BPND > CER BPND. The statistical threshold for the SPM results was p < 0.005 (uncorrected).

ROIs were created for the left caudate, right caudate, left putamen, and right putamen from the publically available MarsBaR Automated Anatomic Labeling Region-of-Interest library (http://marsbar.sourceforge.net/). The anatomic regions are described in Tzourio-Mazoyer et al. [33]. ROIs were smoothed with a 10 mm kernel. Mean BPND values for each ROI were extracted from the smoothed parametric images using the MarsBaR toolbox for SPM2 (http://marsbar.sourceforge.net/). Two-tailed Student’s t-tests were used to test for differences in BPND between groups. Statistical significance was set at p < 0.05.

Two-tailed Student’s t-tests were used to test for group differences in subject age and total mass dose. Statistical significance was set at p < 0.05.

There were no differences between groups with respect to age or weight. The specific activity was significantly higher in the CER group (p < 0.05). Total raclopride mass dose per body weight was significantly lower in CER (p < 0.02).

For the contrast, CER BPND > UNC BPND, no significant voxels were detected, even when the statistical threshold was lowered to p < 0.05. However, for the contrast UNC BPND > CER BPND, significant voxels were detected in the right and left putamen, indicating that the UNC had higher...
The age-related decline of striatal D2/D3 receptors is well-documented [3,19,28,34–36,38], which raised the possibility that age differences between CER and UNC could have accounted for the results. However, subjects in our studies were not statistically different in age by group. The tight distribution around the mean of 25 years of age also makes it very unlikely that age-related correlations with BPND would be detectable, as the rates of receptor loss are slow (e.g., 0.6% per year [3], 7.9% per decade [35]) and are even slower with proper correction for partial volume effects [19]). Therefore, we are confident that age of the subjects did not play a role in the differential BPND values.

In addition, mass effects were not responsible for the differences in striatal BPND between groups. A high mass dose per body weight given during tracer administration could cause underestimation of BPND. This happens when the amount of cold ligand administered is not truly a “trace” amount (i.e., “non-tracer” conditions). Although mass dose is traditionally a concern limited to the realm of small animal neuroligand imaging, it is theoretically possible for mass dose effects to occur in human studies. With [11C]RAC, non-tracer conditions cause two significant sources of confound: (1) Mass effect: Occupation of receptors by cold raclopride could become significant, reducing the number of receptors available for binding by the hot ligand, and causing underestimation of BPND. (2) Drug effect: Raclopride itself could induce DA release via antagonism of pre-synaptic D2 autoreceptors [2,30,31,37]. The DA release caused by cold raclopride could also contribute to reduction of [11C]RAC signal, and contribute to the underestimation of BPND. However, it is highly unlikely that our scans were conducted under non-tracer conditions, especially if the “safe” mass dose range determined in small animals may be extrapolated to human studies. The highest dose given was 1.1 nmol/kg, which is still under the cutoff for a mass dose effect in rats (1.5 nmol/kg, [15,24]). Furthermore, the data do not support evidence for a mass dose effect causing differences in BPND between groups: the mass doses given per subject were significantly different, but UNC had a significantly higher mass dose than CER (this was driven by two outliers in the UNC group at 1.09 and 1.06 nmol/kg; all other subjects – in both groups – were under 0.45 nmol/kg). This is inconsistent with the concept of a mass dose effect. For mass dose to have played a role in the differences in BPND, UNC (which had a higher BPND than CER) would have to have had a significantly lower mass dose compared to CER. Therefore, we can safely rule out mass dose as the cause of higher BPND in UNC.

A more plausible and theoretically meaningful explanation for the differences in BPND between CER and UNC could lie in the expectations the subjects had for the scan condition. While somewhat speculative in the absence of quantitative data on subjects’ expectations, it nevertheless seems reasonable to assume that subjects in CER had no ambivalence about the scan condition; they were told definitively they would be undergoing a resting scan. UNC, however, had several hours to contemplate the possibility of receiving alcohol, and perhaps cultivate an anticipation or hope of alcohol administration during the first scan (alcohol administration was highlighted during recruitment). It is also possible that when the UNC subjects were told they were not going to receive alcohol, this constituted a negative prediction error (e.g., an anticipated reward was not delivered), which can result in a decrease in the firing rates of dopamine neurons [18,25,29], and presumably a concomitant decrease in striatal [DA].
Our present retrospective analysis has limitations: most notably, small sample size and the lack of a quantitative assessment of subjective state regarding anticipation, expectation, and disappointment before, during, and after the “baseline” scan. Future studies are needed to assess the effects of various cognitive states on “resting” measures of binding potential for $[^{11}C]$raclopride and other tracers.

In summary, striatal D2/D3 receptor availability was significantly different in two subject groups provided with different instructions about what to expect during a “baseline” scan. Our results suggest that alterations in DA level induced by cognitive state may affect measurements of binding potential. When designing studies that assess changes in dopamine concentration via changes in $BP_{ND}$ relative to a baseline scan, investigators should carefully consider the conditions under which “baseline” scans are conducted.

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References


