C957T-mediated Variation in Ligand Affinity Affects the Association between ^{11}C-raclopride Binding Potential and Cognition

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Abstract

■ The dopamine (DA) system plays an important role in cognition. Accordingly, normal variation in DA genes has been found to predict individual differences in cognitive performance. However, little is known of the impact of genetic differences on the link between empirical indicators of the DA system and cognition in humans. The present work used PET with ^{11}C-raclopride to assess DA D2-receptor binding potential (BP) and links to episodic memory, working memory, and perceptual speed in 179 healthy adults aged 64–68 years. Previously, the T-allele of a DA D2-receptor single-nucleotide polymorphism, *C957T*, was associated with increased apparent affinity of ^{11}C-raclopride, giving rise to higher BP values despite similar receptor density values between allelic groups. Consequently, we hypothesized that ^{11}C-raclopride BP measures inflated by affinity rather than D2-receptor density in T-allele carriers would not be predictive of DA integrity and therefore prevent finding an association between ^{11}C-raclopride BP and cognitive performance. In accordance with previous findings, we show that ^{11}C-raclopride BP was increased in T-homozygotes. Importantly, ^{11}C-raclopride BP was only associated with cognitive performance in groups with low or average ligand affinity (C-allele carriers of *C957T*, $n = 124$), but not in the high-affinity group (T-homozygotes, $n = 55$). The strongest ^{11}C-raclopride BP–cognition associations and the highest level of performance were found in C-homozygotes. These findings show that genetic differences modulate the link between BP and cognition and thus have important implications for the interpretation of DA assessments with PET and ^{11}C-raclopride in multiple disciplines ranging from cognitive neuroscience to psychiatry and neurology. ■

INTRODUCTION

Reduced dopamine (DA) function is a feature of the normal aging process as well as part of the pathophysiology of several psychiatric and neurological disorders, including schizophrenia, attention-deficit hyperactivity disorder, depression, and parkinsonian diseases (Cools & D'Esposito, 2011; Howes & Kapur, 2009; Dunlop & Nemeroff, 2007; Cools, 2006; Solanto, 2002; Arnsten, 1997; Goldman-Rakic, 1997). These states are all characterized by reduced cognitive performance, hence suggesting a causal link between DA and cognition (Bäckman, Nyberg, Lindenberger, Li, & Farde, 2006) and placing the DA system as a target of investigation in several disciplines.

DA receptor availability can be assessed with PET by estimating the binding potential (BP) for a radioligand. BP is a combined measure of receptor density (B_{max}) and apparent affinity of the ligand to the receptor (the inverse of the dissociation constant, K_D; Mintun, Raichle, Kilbourn, Wooten, & Welch, 1984).

$$BP = B_{max} \cdot 1/K_D$$

The practice of using BP as the primary outcome measure in PET studies, rather than determining receptor density and ligand apparent affinity separately, relates to the laborious nature of the latter task, as it typically involves a minimum of two PET sessions per individual (Holden, Jivan, Ruth, & Doudet, 2002).

^{11}C-raclopride is a commonly used PET ligand in studies assessing DA D2-receptor (D2DR) availability. It has been used to demonstrate neurochemical alterations of the D2DR system and associations to behavioral correlates in aged individuals and in groups with psychiatric and neurological disorders (Rajji et al., 2017; Nyberg et al., 2016; Sawamoto et al., 2008; Lou et al., 2004; Volkow et al., 1998). It is a reversible-binding ligand sensitive to competition from endogenous DA. Research has demonstrated that ^{11}C-raclopride BP changes from drug-induced manipulation of DA levels, with increased BP values observed after DA depletion and vice versa

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after DA augmentation (Laruelle, 2000). Variations in BP upon changes in DA levels likely result from changed number of available binding sites (B_{max}) but, particularly, changed ligand apparent affinity to D2DRs (K_D; Doudet & Holden, 2003; Carson, Channing, Der, Herscovitch, & Eckelman, 2002; Ginovart, Farde, Halldin, & Swahn, 1997, 1999). In interindividual comparisons, negative associations have been found between estimations of DA synthesis with ^{11}C-DOPA and ^{11}C-raclopride BP, where the highest ^{11}C-raclopride BP values were observed in young individuals with the lowest DA levels (Ito et al., 2011). The same pattern was found in patients with Parkinson's disease using ^{11}C-raclopride (Ishibashi, Ishii, Oda, Mizusawa, & Ishiwata, 2010; Kaasinen et al., 2000), but not when using a high-affinity D2DR ligand less sensitive to DA competition (Ishibashi et al., 2010). Hence, the sensitivity of ^{11}C-raclopride BP values to DA tone may obscure interpretations of receptor measures in interindividual comparisons as well.

^{11}C-raclopride BP measurements in healthy human populations have revealed substantial interindividual variability in both younger and older age groups (Nevalainen et al., 2015; Pohjalainen, Rinne, Någren, Lehikoinen, et al., 1998; Farde, Hall, Pauli, & Halldin, 1995), which has been suggested to result from differences in D2DR density (Farde et al., 1995). However, interindividual variability in ^{11}C-raclopride apparent affinity has been demonstrated as well (Kuwabara et al., 2012; Hirvonen, Laakso, et al., 2009; Hietala, Någren, Lehikoinen, Ruotsalainen, & Syvälahti, 1999; Pohjalainen, Rinne, Någren, Syvälahti, & Hietala, 1998). Genetic variation can alter functions of proteins regulating DA metabolism and uptake. One single-nucleotide polymorphism (SNP) located in the D2DR gene, *C957T*, was associated with allelic group differences in K_D values (i.e., apparent affinity) in an ^{11}C-raclopride PET study (Hirvonen, Laakso, et al., 2009). Functional effects from *C957T* were reported for the T-allele, which included reduced messenger RNA stability and translation and altered DA-induced upregulation of D2DR expression (Duan et al., 2003). Hence, the observed allelic group differences in ^{11}C-raclopride apparent affinity may originate from DA system dissimilarities, such as variations in DA tone, and were large enough to yield differences in BP values between allelic groups despite similar values for receptor density (Hirvonen, Laakso, et al., 2009; Hirvonen et al., 2004).

Given this background, this study tested the hypothesis that ^{11}C-raclopride BP values elevated via increased apparent affinity in *C957T* T-allele carriers may not represent DA system integrity and therefore not predict cognitive performance. Analyses were carried out in a large cohort of healthy older adults (n = 179; 64–68 years old) from the Cognition, Brain, and Aging (COBRA) study (Nevalainen et al., 2015). The sample was stratified according to the D2DR *C957T* polymorphism, as previous work has shown ^{11}C-raclopride apparent affinity differences for this SNP in the direction of C/C < C/T < T/T (Hirvonen, Laakso, et al., 2009). Participants underwent ^{11}C-raclopride PET, MRI, and tests of episodic memory, working memory, and perceptual speed. More specifically, we tested whether ^{11}C-raclopride BP_{ND}–cognition associations were different in groups with low-to-average (C-carriers) versus high (T-homozygotes) affinity. Because of the previously reported gradual increase in ^{11}C-raclopride apparent affinity, BP_{ND}–cognition associations and cognitive performance were further compared among C/C, and C/T, and T/T allelic groups. BP is abbreviated BP_{ND} in this work and denotes the ratio of specific binding over nondisplaceable binding in a receptor-free reference area (Innis et al., 2007).

METHODS

Sample

This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Informed consent was obtained from participants before any testing. A detailed description of the COBRA sample and test battery has been published previously (Nevalainen et al., 2015). The original sample consisted of healthy older individuals (age = 64–68 years, n = 181; 100 men) randomly selected from the population registry of Umeå in northern Sweden. Exclusion criteria were factors that affect brain and cognitive functions, such as cognitive impairment, brain pathology, mental and physical disability, and certain medications (e.g., treatment for diabetes and cancer), and MRI-inhibiting factors (e.g., metal implants). Objective measures included a Mini-Mental State Examination (required: 27/30) and radiological evaluation of MR images.

During the first test session, participants underwent half of the cognitive test battery and an MRI session. Two days later, they performed the second part of the cognitive test battery and a ^{11}C-raclopride PET session. Lifestyle data, a medical anamnesis, and blood samples were also collected.

In the present work, two participants were excluded because of observations of atrophy in the left temporal lobe and lack of genetic data, respectively. The remaining sample of 179 persons was subdivided into allelic variants of *C957T* (C/C: n = 32; C/T: n = 92; T/T: n = 55). The distribution was in Hardy–Weinberg equilibrium ($\chi^2 < 1, p > .1$).

Genotyping

Deoxyribonucleic acid (DNA) extraction and genotyping services were performed by LGC genomics using their in-house products (Hoddesdon). In brief, DNA was extracted from the buffy-coat fraction of blood samples using the Kleargene XL nucleic-acid extraction kits, and genotyping was performed with KASP genotyping assays. When conducting the genotyping analysis of *C957T* (rs6277), the DNA template was mixed with a KASP

master mix (containing KASP Taq polymerase, deoxynucleoside triphosphates, buffers, salts, and two fluorescently labeled reporter cassettes) and an SNP-specific KASP assay mix (containing two allele-specific forward primers and one common reverse primer). Two allele-specific forward primers that differed at one base in the 3′-end (5′-CAT GGT CTC CAC AGC ACT CC T/C-3′) and one common reverse primer (5′-TCT CRG GTT TGG CGG GGC TGT-3′) were constructed. After polymerase chain reaction sessions, allelic variants were determined via detection of either fluorophore for homozygous alleles or both fluorophores for heterozygous alleles.

Cognitive Assessment

Each ability (episodic memory, working memory, and perceptual speed) was tested using three separate tasks with verbal, numerical, and figural stimuli, respectively. Episodic memory was assessed with word recall, number–word recall, and object-position recall; working memory was tested with letter-string updating, numerical 3-back, and spatial updating; and perceptual speed was evaluated with letter comparison, number comparison, and figure comparison. For each separate test, scores were summarized across the total number of blocks or trials and standardized to form composites (*T* score: mean = 50, *SD* = 10). The three composites were then averaged to create one composite score for episodic memory, working memory, and perceptual speed, respectively. Missing values (<1.2% for all variables) were replaced by the average of the available observed scores.

Imaging Procedures and Analyses

Structural MRI

All MRI scans were performed on a 3-T scanner (Discovery MR 750; General Electric). A 3-D fast spoiled gradient-echo sequence was used to obtain high-resolution anatomical T1-weighted images. Imaging parameters were 176 sagittal slices, with a slice thickness of 1 mm, repetition time = 8.2 msec, echo time = 3.2 msec, flip angle = 12°, and field of view = 25 × 25 cm.

Volumetric analyses. Subcortical brain structures were delineated with the Freesurfer 5.3 software (surfer.nmr.mgh.harvard.edu; Fischl et al., 2002), and cortical parcellation was achieved according to the Desikan–Killiany atlas (Desikan et al., 2006). When necessary, Voxel Edit mode in Freeview was used to correct striatal volumes manually. The number of voxels within delineated structures was used to calculate gray matter volumes.

PET

A 55-min, 18-frame (9 × 120 sec + 3 × 180 sec + 3 × 260 sec + 3 × 300 sec) dynamic PET scan (Discovery PET/CT 690; General Electric) was acquired during resting-state conditions after an intravenous bolus injection of 250-MBq ^{11}C-raclopride. A CT scan (20 mA, 120 kV, 0.8 sec/revolution) for attenuation correction purposes preceded ligand injection. Attenuation- and decay-corrected images (47 slices, field of view = 25 cm, transaxial images of 256 × 256 pixels, voxel size = 0.977 × 0.977 × 3.27 mm^3) were reconstructed with the iterative algorithm VUE Point HD-SharpIR (General Electric; Bettinardi et al., 2011); six iterations, 24 subsets, 3.0-mm postfiltering), yielding an FWHM of 3.2 mm (Wallstén, Axelsson, Sundström, Riklund, & Larsson, 2013). Head movements were minimized with individually fitted thermoplastic masks attached to the bed surface.

PET image data were converted from DICOM to NIfTI format and corrected for head movements. The T1 images were segmented into gray- and white-matter maps, which were used for creating a sample-specific group template with DARTEL (Ashburner, 2007). The PET and T1 images were coregistered, normalized to Montreal Neurological Institute space with DARTEL-created transformation maps, and smoothed using an 8-mm Gaussian filter. All these steps were performed using the SPM software (SPM8). BP_{ND} was calculated using time–activity curves for each voxel with orthogonal regression reference Logan analysis (Logan et al., 1996). Logan regression was based on Frames 10–18 (18–55 min). BP was calculated as BP_{ND} = distribution volume ratio − 1. Cerebellar gray matter served as the reference area, because of its negligible D2DR expression (Camps, Cortés, Gueye, Probst, & Palacios, 1989; Farde, Hall, Ehrin, & Sedvall, 1986).

^{11}C-raclopride BP_{ND} was also determined in extrastriatal ROIs. For BP_{ND} in dorsolateral pFC (DLPFC), a mask from the MRIcron atlas (www.mccauslandcenter.sc.edu/mricro/mricron/index.html) was applied to the normalized brain template describe above. Median BP_{ND} was extracted for Brodmann's areas (BAs) 46 and 9 for each individual, for which average BP_{ND} was entered into the analyses. In determining ^{11}C-raclopride BP_{ND} in subcortical ROIs, individual T1-weighted images and PET emission scans were merged (i.e., operating in native space). ROIs consisting of Freesurfer-delineated structures were applied to calculate BP_{ND} as described above, from which the median BP_{ND} value was used in ROI-based analyses. BP_{ND} measures for striatal ROIs were excluded for six individuals, because of imperfect segmentation of MR images or problems with PET/MR coregistration, or for being statistical outliers according to the outlier labeling rule with 2.2 interquartile ranges (Hoaglin & Iglewicz, 1987).

Statistical Analyses

Between-group comparisons were made using one- or two-way ANOVAs, independent sample *t* tests, or chi-square tests. Descriptive data are presented as frequencies or with mean values and standard deviations. Correlations are reported with the Pearson correlation coefficient (*r*).

First, we checked whether allelic groups differed in striatal ^{11}C-raclopride BP_{ND}. Second, the effect of differences in ligand apparent affinity on ^{11}C-raclopride BP_{ND}–cognition correlations was assessed in ROI-based analyses. Correlations were compared between groups with low-to-average (*C957T* C-carriers, $n = 124$) versus high (T-homozygotes, $n = 55$) affinity using Fisher's z transformation (statistical threshold of $p < .05$, two-tailed). ROIs consisted of hippocampus, caudate, and DLPFC, with the latter represented by BAs 46 and 9 (Rajkowska & Goldman-Rakic, 1995; Brodmann, 1909). The region selection was based on previous work by us and others indicating that these areas and their DA constituents are central for cognitive functions including episodic memory, working memory, and perceptual speed (Nyberg, 2017; Nyberg et al., 2016; D'Esposito & Postle, 2015; Eriksson, Vogel, Lansner, Bergström, & Nyberg, 2015; Takahashi, Yamada, & Suhara, 2012; Bäckman et al., 2000). Additional ROIs consisted of select regions where no associations were expected. Gray matter volumes were controlled for to limit partial volume effects on ^{11}C-raclopride BP_{ND}.

Next, whole-brain analyses were performed to assess the regional specificity of the ROI-based analyses. Composite cognitive scores were regressed onto a set of

Table 1. Lifestyle, Brain, and Cognitive Descriptives across *C957T* Allelic Groups

	C/C	*C/T*	*T/T*
Number of participants	32	92	55
Men/women	15/17	54/38	29/26
Educational attainment (years)	13.2 ± 4.1	13.1 ± 3.4	13.7 ± 3.2
BMI	25.4 ± 3.4	26.4 ± 3.8	26.1 ± 3.2
Nicotine consumption (%)	21.9	17.4	14.5
^{11}C-raclopride BP_{ND}			
Putamen	3.30 ± 0.24	3.30 ± 0.29	3.39 ± 0.23
Caudate	2.15 ± 0.22	2.20 ± 0.27	2.27 ± 0.25*
Hippocampus	0.25 ± 0.05	0.27 ± 0.05	0.26 ± 0.04
Amygdala	0.40 ± 0.06	0.40 ± 0.06	0.40 ± 0.05
Globus pallidus	1.35 ± 0.18	1.36 ± 0.20	1.39 ± 0.17
DLPFC	0.15 ± 0.04	0.14 ± 0.04	0.14 ± 0.04
Occipital cortex	0.25 ± 0.05	0.24 ± 0.05	0.25 ± 0.05
Ligand injection			
Radioactivity dose (MBq)	264.6 ± 16.2	261.1 ± 18.7	266.7 ± 21.1
Raclopride (nM)	3.3 ± 5.5	2.3 ± 3.3	2.2 ± 1.7
Gray matter volume (cm^3)			
Putamen	4.3 ± 0.3	4.5 ± 0.6	4.4 ± 0.6
Caudate	3.6 ± 0.4	3.7 ± 0.6	3.6 ± 0.5
Hippocampus	3.8 ± 0.5	3.9 ± 0.4	3.9 ± 0.5
Amygdala	1.4 ± 0.1	1.4 ± 0.2	1.4 ± 0.2
Globus pallidus	1.4 ± 0.2	1.5 ± 0.2	1.5 ± 0.2
Cortical	436.5 ± 39.7	454.9 ± 53.0	447.9 ± 44.4
White matter volume (cm^3)	579.2 ± 61.0	615.3 ± 72.8	600.6 ± 73.6
Cognitive task performance			
Episodic memory	51.3 ± 9.0	49.9 ± 8.2	49.7 ± 6.6
Working memory	51.7 ± 7.6	50.3 ± 7.3	48.8 ± 7.6
Perceptual speed	51.1 ± 8.1	49.6 ± 8.5	49.9 ± 9.3

BMI = body mass index. *$p < .05$ for differences between C- and T-homozygotes.

whole-brain BP_{ND} maps, generating a statistical brain map of the linear relation. Voxels in which linear associations were found between ^{11}C-raclopride BP_{ND} and cognitive performance were identified, noting the p value threshold at which they were found (ranging between $p < .01$ and $p < 1 \cdot 10^{-5}$ uncorrected), cluster size (minimum = 10 voxels), and r. An approach of stepwise logarithmic (10-based) increases in the p value threshold, while continuously noting at which threshold new clusters emerged, was performed to fully characterize the whole-brain pattern of BP–cognition associations (i.e., not used for statistical inference). Findings for the whole sample were contrasted with those for *C957T* C-carriers and T-homozygotes.

Finally, ^{11}C-raclopride BP_{ND}–cognition associations were compared among the three allelic groups to assess how the previously demonstrated gradual increase in apparent affinity (C/C < C/T < T/T; Hirvonen, Laakso, et al., 2009) may affect the associations. To complement the correlational analyses, interaction effects between factors Allelic group (C/C, C/T, T/T) and ^{11}C-raclopride BP_{ND} measures (low/high) were tested for cognitive performance (with two-way ANOVAs; statistical threshold of $p < .05$). Low and high BP_{ND} measures were defined as less than or equal to or greater than the median BP_{ND}, respectively, for the total sample within an ROI.

RESULTS

^{11}C-raclopride BP_{ND}–cognition Associations Are Found Exclusively for *C957T* C-allele Carriers

Cognitive and BP_{ND} data were normally distributed within allelic groups (skewness and kurtosis ranged between −1.0 to 1.2 and −0.96 to 1.46 for C/C, −0.39 to 0.48 and −0.56 to 0.43 for C/T, and −0.74 to 0.61 and −0.47 to 0.42 for T/T).

In agreement with previous findings (Hirvonen, Laakso, et al., 2009), T-homozygotes had higher striatal ^{11}C-raclopride BP_{ND} values than C-homozygotes ($t(83) = 2.17$, $p = .03$, for caudate; $t(83) = 1.77$, $p = .08$, for putamen; Table 1). No between-group differences were found in various lifestyle variables, nor when it comes to performance in the main cognitive tasks assessed in COBRA (episodic memory, working memory, and perceptual speed; Table 1).

ROI-based analyses demonstrated significant associations between episodic memory and ^{11}C-raclopride BP_{ND} in hippocampus, caudate, and DLPFC in C-carriers, but not in T-homozygotes (Table 2). The correlations in C-carriers were statistically different from those in T-homozygotes for BP_{ND} in hippocampus ($z = 2.52$, $p < .05$) and DLPFC ($z = 2.72$, $p < .01$) but did not reach significance for caudate ($z = 0.19$, $p > .05$). No links were found for any of the other regions tested, regardless of genotype, and there were no associations in corresponding analyses of working memory and speed.

The regional specificity of the ROI-based findings was supported by the whole-brain analysis, which yielded

Table 2. ^{11}C-raclopride BP_{ND}–Cognition Associations in C-carriers ($n = 124$), T-homozygotes ($n = 55$), and the Whole Sample ($n = 179$)

	Episodic Memory	*Working Memory*	*Perceptual Speed*
Hippocampus			
C-carriers	**.27****#	.07	.01
T/T	−.14	.13	.01
Whole sample	**.19***	.09	.01
Caudate			
C-carriers	**.20***	−.11	−.07
T/T	.17	.07	−.08
Whole sample	**.19***	−.07	−.07
DLPFC			
C-carriers	**.30*****##	−.03	−.08
T/T	−.14	−.16	.21
Whole sample	**.18***	−.07	.01
Putamen			
C-carriers	.15	−.06	−.01
T/T	−.04	−.07	−.15
Whole sample	.10	−.07	−.04
Occipital cortex			
C-carriers	.06	.04	−.12
T/T	−.19	−.02	.06
Whole sample	−.01	.02	−.06
Amygdala			
C-carriers	.14	−.01	.10
T/T	−.01	.23	−.03
Whole sample	.10	.06	.06
Globus pallidus			
C-carriers	.10	−.18	−.09
T/T	−.09	−.04	.19
Whole sample	.06	−.14	.01

Values in **bold** indicate significant correlations at *$p < .05$, **$p < .01$, or ***$p < .001$. Comparisons of correlations were carried out between C-carries and T-homozygotes (#$p < .05$ and ##$p < .01$).

Table 3. Results from Whole-brain Analysis of ^{11}C-raclopride BP_{ND}–Episodic Memory Associations in the Whole Sample (n = 179) and *C957T* C-carriers (n = 124)

Threshold (p)	*Region*	*Peak (x, y, z)*	*Cluster Size*	*r*
Whole sample				
<.001	Hippocampus	−18, −10, −18	23	.28
	pFC	−42, 12, 36	147	.32
<.01	Putamen	−28, 8, 4	273	.22
	Putamen/caudate	18, 16, 4	13	.20
C-carriers				
<.00001	Hippocampus	−18, −12, −18	51	.42
<.0001	Hippocampus	−18, −12, −18	352	.42
	pFC	−42, 10, 36	26	.37
	Putamen	−28, 8, 4	134	.30
	Putamen/caudate	20, 16, 4	21	.26

stronger relationships between episodic memory and ^{11}C-raclopride BP_{ND} in clusters located in hippocampus, pFC, and striatum in C-carriers compared with the whole sample (Table 3 and Figure 1A and B). In C-carriers, ^{11}C-raclopride BP_{ND}–episodic memory associations were observed in a hippocampal cluster already at $p < .00001$. Relaxing the threshold to $p < .0001$, the hippocampal cluster increased considerably in size without losing strength of the overall correlation, and prefrontal and striatal clusters emerged as well. As in the ROI-based analyses, no associations were found between ^{11}C-raclopride BP_{ND} and episodic memory in T-homozygotes, even at a very liberal threshold ($p < .01$; Figure 1C). Again, no associations or group differences in associations were found with measures of working memory or perceptual speed.

The Highest BP_{ND}–cognition Associations Are Seen for *C957T* C-homozygotes

To assess whether correlations become gradually stronger when apparent affinity varies from low to high (C/C < C/T < T/T; Hirvonen, Laakso, et al., 2009), BP_{ND}–cognition associations were compared among the three allelic groups. The strongest ^{11}C-raclopride BP_{ND}–cognition associations were found for C-homozygotes and, in particular, when considering episodic memory and BP_{ND} in hippocampus, caudate, and DLPFC (Table 4 and Figure 2). Notably, hippocampal ^{11}C-raclopride BP_{ND} in C-homozygotes was positively associated with performance in all three cognitive domains. Hippocampal ^{11}C-raclopride BP_{ND}–episodic memory correlations differed significantly between C- and T-homozygotes ($z = 2.49$, $p < .05$) and between heterozygotes and T-homozygotes ($z = 2.21$, $p < .05$). Similarly, correlations to perceptual speed differed between C- and T-homozygotes ($z = 2.88$, $p < .01$) and between C-homozygotes and heterozygotes ($z = 3.64$, $p < .001$). A trend was also found when comparing C-homozygotes and heterozygotes for measures of working memory ($z = 1.92$, $p = .05$). Moreover, correlations between ^{11}C-raclopride BP_{ND} in DLPFC and episodic memory differed significantly between C- and T-homozygotes ($z = 3.40$, $p < .001$) and between C/C and C/T groups ($z = 2.23$, $p < .05$). Although the association concerning caudate BP_{ND} appeared stronger in C-homozygotes, it was not statistically different from those in the C/T and T-homozygote groups ($z = 1.06$ and $z = 0.94$, respectively; $ps > .05$).

Furthermore, intraindividual differences were present when comparing striatal–extrastriatal ^{11}C-raclopride BP_{ND} correlations among allelic groups. Specifically, caudate and hippocampal BP_{ND} values were most highly correlated in C-homozygotes ($r = .55$, $p < .01$), followed by C/T ($r = .37$, $p < .001$) and T-homozygotes ($r = .22$, $p > .1$).

Highest Cognitive Performance in *C957T* C-homozygotes with High ^{11}C-raclopride BP_{ND}

Two-way ANOVAs demonstrated that ^{11}C-raclopride BP_{ND} predicts cognitive performance as a function of *C957T* genotype. Interaction effects from Allelic Group × Hippocampal ^{11}C-raclopride BP_{ND} measures were found for episodic memory, $F(2, 173) = 4.13$, $p = .02$, and, at trend level, for working memory, $F(2, 173) = 2.34$, $p = .10$ (Figure 3A and B). A similar trend was found for episodic

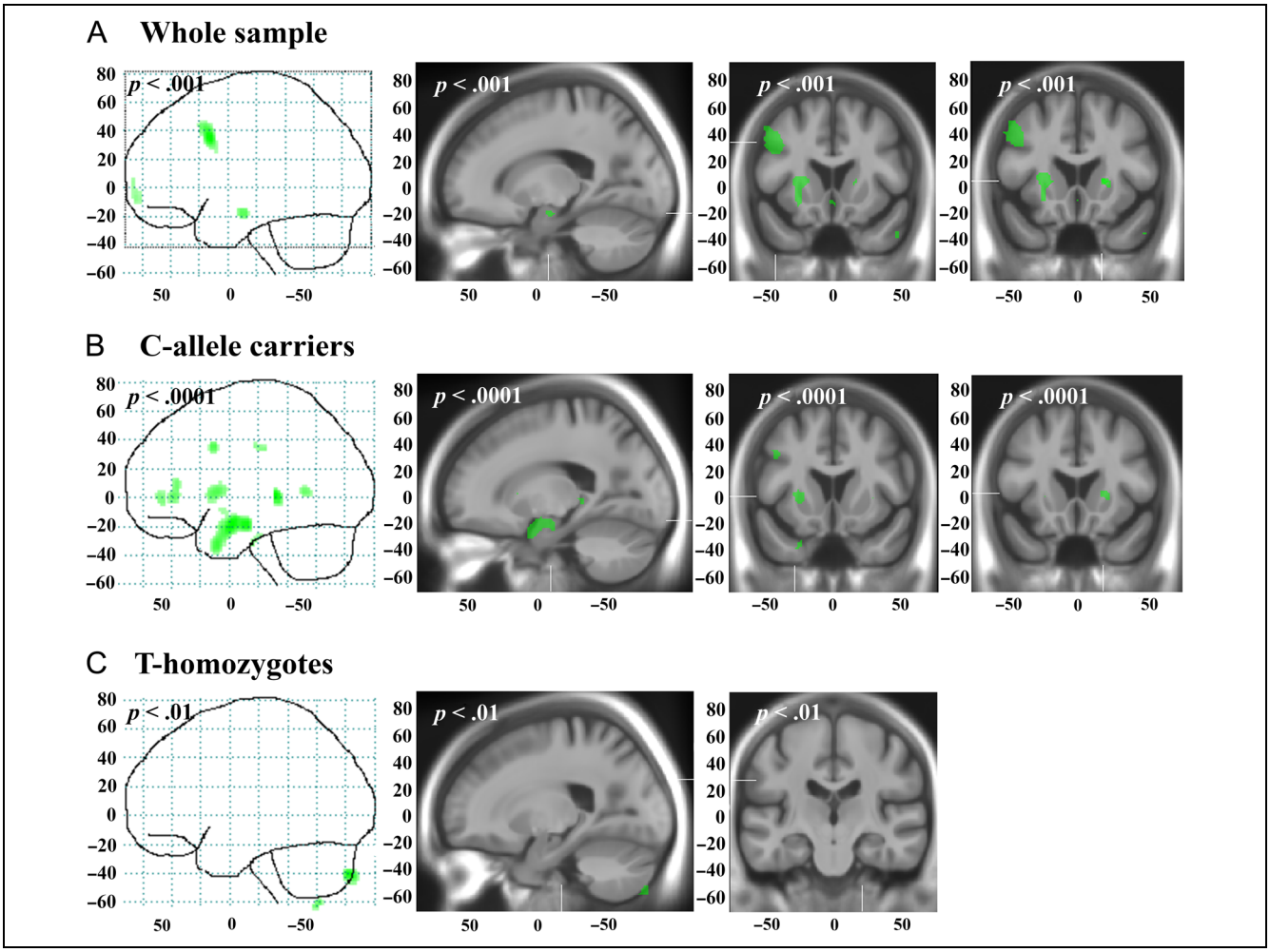

Figure 1. Whole-brain analyses of ^{11}C-raclopride BP_{ND}–episodic memory associations in the whole sample (A), *C957T* C-carriers (B), and T-homozygotes (C). ^{11}C-raclopride BP_{ND}–episodic memory associations are shown for hippocampal and striatal clusters. Stronger relationships emerged when removing T-homozygotes from the analysis, and no associations were found for T-homozygotes.

memory performance for Genotype × ^{11}C-raclopride BP_{ND} measures in DLPFC, $F(2, 170) = 2.62$, $p = .08$ (Figure 3C), but not for those in the caudate ($p > .10$; data not shown).

In line with the correlational findings, performance differences were only found between individuals with high versus low ^{11}C-raclopride BP_{ND} in C-carriers (hippocampus: $t(24) = 2.93$, $p = .01$ in C/C, and $t(90) = 2.25$, $p = .03$ in C/T for episodic memory; $t(30) = 1.9$, $p = .07$ for C/C for working memory; DLPFC: $t(29) = 2.09$, $p = .05$ in C/C), but not in T-homozygotes.

When comparing the Allelic Groups × ^{11}C-raclopride BP_{ND} groups, cognitive performance tended to peak in C-homozygotes with high ^{11}C-raclopride BP_{ND} measures and most evidently so for episodic memory performance when considering hippocampal BP_{ND}, $F(5, 173) = 3.20$, $p = .02$ (in C/C vs. C/T).

DISCUSSION

The frequent use of receptor availability, or BP, as the outcome measure in PET studies relates to its many advantages. It requires only one PET session per participant, is easily and reliably calculated, and has higher test–retest reliability than B_{max} and K_D assessed separately (Hietala et al., 1999; Logan et al., 1990, 1996). If the apparent affinity term for a radioligand can be assumed to be constant in interindividual comparisons, then the BP value likely reflects individual differences in DA system integrity via receptor density. However, when apparent affinity of a radioligand varies because of biological differences, BP values may not have the same meaning between individuals. In agreement with this hypothesis, we demonstrated increased striatal ^{11}C-raclopride BP_{ND} values and absent BP_{ND}–cognition associations for individuals with a genetic predisposition for high ^{11}C-raclopride apparent affinity (T-homozygotes of *C957T*; Hirvonen, Laakso, et al., 2009). Critically, ^{11}C-raclopride BP_{ND}–cognition correlations were only found in individuals with low-to-average affinity (C-carriers of the SNP *C957T*) and were strongest in the low-affinity group.

The present work demonstrated stronger ^{11}C-raclopride BP_{ND}–cognition associations when excluding a group in

Table 4. ^{11}C-raclopride BP_{ND}–Cognition Associations in C/C (n = 32), C/T (n = 92), and T/T (n = 55) Allelic Groups

	Episodic Memory	*Working Memory*	*Perceptual Speed*
Hippocampus			
C/C	**.41***#	**.38***	**.59*****##§§§
C/T	**.24***#	−.01	−.10
T/T	−.14	.13	.01
Caudate			
C/C	**.37***	.12	.24
C/T	.16	−.17	−.13
T/T	.17	.07	−.08
DLPFC			
C/C	**.57*****###§	−.06	−.03
C/T	.17	−.02	−.11
T/T	−.14	−.16	.21
Putamen			
C/C	.16	.10	.07
C/T	.13	−.12	−.03
T/T	−.04	−.07	−.15
Occipital cortex			
C/C	−.12	−.10	.06
C/T	.11	.08	−.19
T/T	−.19	−.02	.06
Amygdala			
C/C	.28	.10	.29
C/T	.07	−.04	.05
T/T	−.01	.23	−.03
Globus pallidus			
C/C	.07	−.07	.23
C/T	.11	−.21	−.19
T/T	−.09	−.04	.19

Values in **bold** indicate significant correlations at *$p < .05$ and ***$p < .001$. Comparisons of correlations were carried out between C/C–T/T and C/T–T/T groups (#$p < .05$, ##$p < .01$, ###$p > .001$) and between C/C and C/T groups (§$p < .05$, §§§$p < .001$).

which ligand apparent affinity has been shown to be elevated (*C957T* T-homozygotes, ≈30% of the total sample). Hence, individual differences in ligand apparent affinity may overshadow differences in BP between experimental groups. In C-carriers of *C957T*, ^{11}C raclopride BP_{ND}–episodic memory associations were found for hippocampus, pFC, and striatum. These areas, and their D2DRs, are important for episodic memory performance (Nyberg et al., 2016; Takahashi et al., 2012; Liggins, 2009; Bäckman et al., 2000; Tulving & Markowitsch, 1998). The lack of DLPFC ^{11}C raclopride BP_{ND}–working memory association may be due to a more crucial role of cortical D1DRs in working memory (Arnsten, Wang, & Paspalas, 2012; Takahashi et al., 2012). Although D2DR density is low in cortical areas, acceptable reliability has been shown for extrastriatal BP_{ND} measurements with ^{11}C-raclopride (Alakurtti et al., 2015). Importantly, no differences were observed between allelic groups for ligand concentration injected or radioactivity dose. Future directions may involve using a high-affinity D2DR ligand, such as ^{18}F-Fallypride, to further assess effects of *C957T* on extrastriatal D2DR BP_{ND}.

The highest ^{11}C-raclopride BP_{ND}–episodic memory correlations were observed in C-homozygotes. The same group also exhibited hippocampal BP_{ND} associations with working memory and perceptual speed, supporting hippocampal D2DRs as key players for a broad range of cognitive functions (Rocchetti et al., 2015; Liggins, 2009; Takahashi et al., 2007, 2008). The relatively low affinity observed for this group may reflect high extracellular DA levels, as ^{11}C-raclopride is sensitive to competition by endogenous DA (Laruelle, 2000). Consequently, the ^{11}C-raclopride BP_{ND} values in C-homozygotes may mirror DA system integrity and D2DR status accurately, thereby rendering stronger BP_{ND}–cognition associations. The opposite scenario would serve as an example of the lack of BP_{ND}–cognition associations in T-homozygotes, that is, reduced DA levels giving rise to elevated ^{11}C-raclopride affinity and resulting BP_{ND} values that are not representative of DA system status and do not relate to cognitive performance. No between-group differences were found when inspecting the Logan plots. Furthermore, BP values obtained from Logan analysis versus simplified reference tissue model were coherent and similar for all three groups (rs > .84 for putamen, caudate, and hippocampus; data not shown but available upon request from the first author). We therefore find it unlikely that the group differences arise from data modeling issues. Moreover, caudate–hippocampal BP_{ND} correlations were highest in C-homozygotes, hence individual differences in striatal BP may be reflected to a greater degree in extrastriatal areas in this subgroup. Striatal–hippocampal DA receptor coherence is indeed important for cognitive performance (Nyberg et al., 2016) and declines in aging (Rieckmann et al., 2011).

For definite quantification of the constituents of a BP value, receptor density and apparent affinity should be assessed separately. The resource-demanding nature of this task, requiring typically at least two PET sessions per participant, precludes such an approach for large-scale studies. Instead, we used a proxy to estimate apparent affinity, which relates to previous findings for the *C957T* SNP (Hirvonen, Laakso, et al., 2009). *C957T* is located in Exon 7 of the D2DR gene and has been

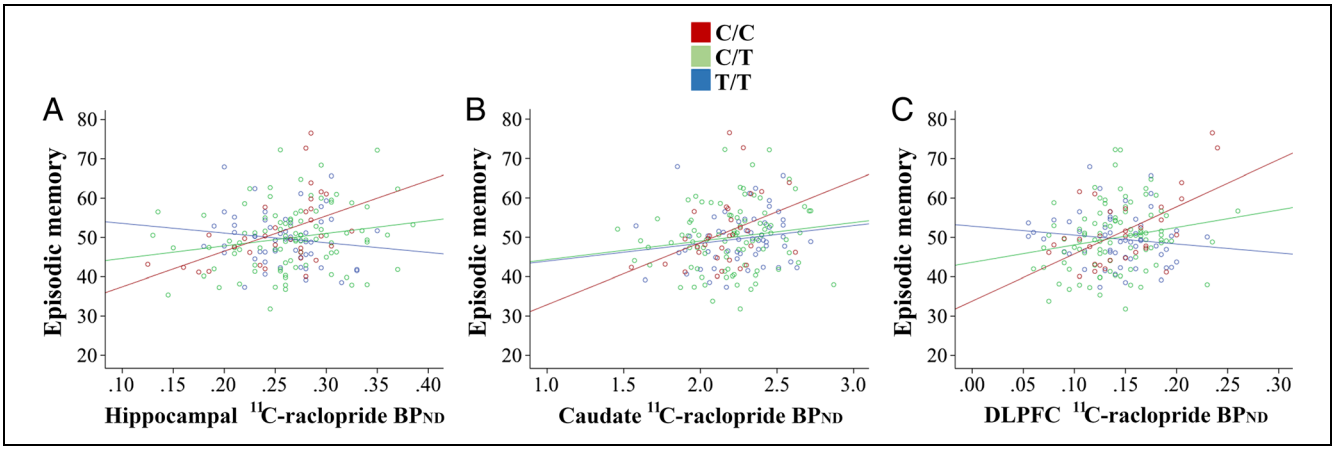

Figure 2. Associations of ^{11}C-raclopride BP_{ND} in hippocampus (A), caudate (B), and DLPFC (C) and episodic memory in *C957T* C/C (n = 32), C/T (n = 92), and T/T (n = 55) allelic groups.

shown to regulate D2DR availability (Smith et al., 2017; Hirvonen, Laakso, et al., 2009; Hirvonen, Lumme, et al., 2009; Duan et al., 2003; Grandy et al., 1989). Central to our work, this SNP has been associated with differences in ^{11}C raclopride apparent affinity. Notably, the between-group differences in striatal BP values reported by Hirvonen and colleagues (Hirvonen, Laakso, et al., 2009) were found in our sample as well. Recently, this finding was replicated when using another D2DR ligand, ^{18}F-fallypride (Smith et al., 2017); thus, increased striatal BP values in T-homozygotes are consistent between studies.

Differences in ^{11}C-raclopride apparent affinity to D2DRs between *C957T* allelic groups may result from altered characteristics of D2DRs or, as previously suggested (Hirvonen, Laakso, et al., 2009), variations in extracellular DA levels. Although *C957T* is a silent mutation, it has functional implications for D2DR function. The T allele was associated with reduced D2DR protein-synthesis levels, significantly faster messenger RNA decay, and reduced DA-induced upregulation of D2DRs (Duan et al., 2003). Thus, the T allele gives rise to a less stable D2DR transcript and altered D2DR responses to DA levels. Given the role of D2DRs in presynaptic control of DA synthesis and reuptake, effects on DA levels are to be expected if this regulatory system is different in T-carriers. Further insight into how individual differences in extrastriatal DA levels affect the ^{11}C raclopride BP_{ND}–cognition association may be achieved by considering other DA gene polymorphisms previously linked to differences in extracellular DA levels and behavior, such as the 40-basepair variable number tandem repeat polymorphism located in the DA transporter gene (rs2836317; Brewer et al., 2015; Li et al., 2013; Vandenbergh et al., 1992).

Evidence for *C957T* as a functional DA polymorphism is also found at behavioral levels. *C957T* has been associated with reward-related behaviors such as overconsumption of alcohol, nicotine, and food (Davis et al., 2012; Swagell et al., 2012; Voisey et al., 2012) and with an increased risk for developing schizophrenia (Monakhov, Golimbet, Abramova, Kaleda, & Karpov, 2008; Lawford

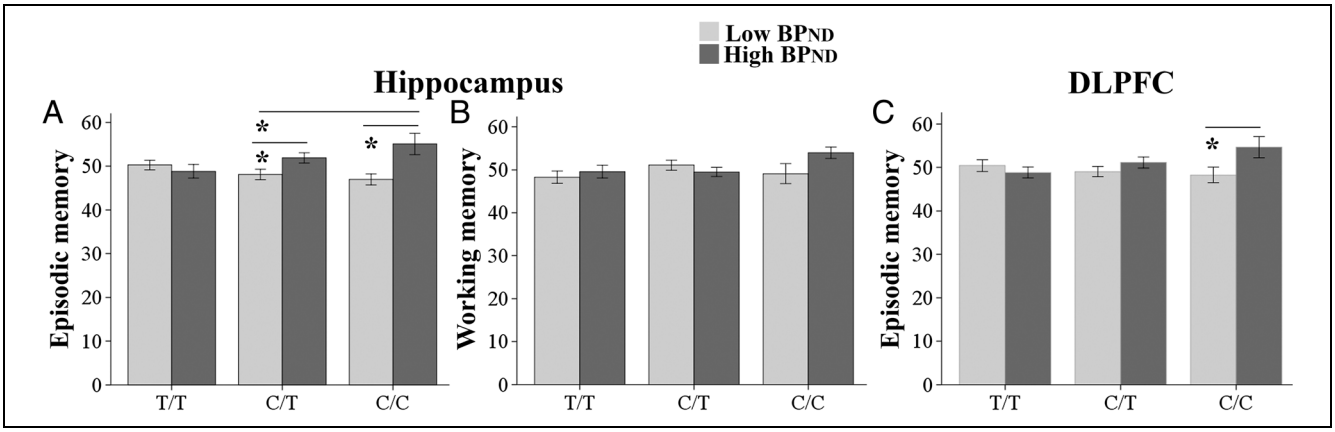

Figure 3. Interactions between *C957T* allelic group and ^{11}C-raclopride BP_{ND} status for cognitive performance. C-homozygotes with high hippocampal BP_{ND} exhibited the best episodic memory (A) and working memory (B) performance. Similarly, C-homozygotes with high BP_{ND} in DLPFC had the highest episodic memory performance (C). *p < .05.

et al., 2005). Moreover, T- but not C-homozygotes improved cognitive performance after supplementation of the DA precursor tyrosine (Colzato et al., 2016). Tyrosine conversion is inhibited by feedback mechanisms when DA levels are sufficient (Daubner, Le, & Wang, 2011; Weiner, Lee, Barnes, & Dreyer, 1977), suggesting that reduced DA levels in T-homozygotes underlie the group-specific performance improvements. The present work showed that C-homozygotes with high ^{11}C-raclopride BP_{ND} values had the highest cognitive performance, which is consistent with past research (Papenberg et al., 2014; Colzato, van den Wildenberg, & Hommel, 2013; Li et al., 2013). The lower mean ages of samples in previous studies indicate that the *C957T*-mediated allelic group differences in the DA system are present already at younger ages (Colzato et al., 2016; Hirvonen, Laakso, et al., 2009). Consequently, the results presented here may generalize across differences in sample age. However, as DA and cognitive decline occur throughout senescence (Bäckman et al., 2006) and the influence of genetics is magnified at older ages (Lindenberger et al., 2008), we cannot exclude that the effects from *C957T* reported here are exacerbated by aging, as previously suggested (Papenberg et al., 2014; Li et al., 2013).

The current findings emphasize the importance to consider how interindividual differences in genetic background may affect results derived from ^{11}C-raclopride assessments. Misleading interpretations of BP values may be detrimental to our understanding of the fate of the DA system and DA–behavior relations in healthy aging and in a variety of psychiatric and neurological disorders. This conclusion resonates well with the general plea to move toward a personalized and mechanistic account of psychopathology (Stephan et al., 2016; Insel & Cuthbert, 2015).

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