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Effect of Cigarette Smoking on a Marker for Neuroinflammation: A [^{11}C]DAA1106 Positron Emission Tomography Study

Arthur L Brody*[,1,2], Robert Hubert[3], Ryutaro Enoki[3], Lizette Y Garcia[3], Michael S Mamoun[3], Kyoji Okita[3,4], Edythe D London[4], Erika L Nurmi[4], Lauren C Seaman[4] and Mark A Mandelkern[5]

[1]Department of Psychiatry, University of California at San Diego, San Diego, CA, USA; [2]Department of Psychiatry, VA San Diego Healthcare System, San Diego, CA, USA; [3]Department of Research, VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA; [4]Department of Psychiatry, University of California at Los Angeles, Los Angeles, CA, USA; [5]Department of Radiology, VA Greater Los Angeles Healthcare System, Los Angeles, CA, USA

In the brain, microglia continuously scan the surrounding extracellular space in order to respond to damage or infection by becoming activated and participating in neuroinflammation. When activated, microglia increase the expression of translocator protein (TSPO) 18 kDa, thereby making the TSPO expression a marker for neuroinflammation. We used the radiotracer [^{11}C]DAA1106 (a ligand for TSPO) and positron emission tomography (PET) to determine the effect of smoking on availability of this marker for neuroinflammation. Forty-five participants (30 smokers and 15 non-smokers) completed the study and had usable data. Participants underwent a dynamic PET scanning session with bolus injection of [^{11}C]DAA1106 (with smokers in the satiated state) and blood draws during PET scanning to determine TSPO affinity genotype and plasma nicotine levels. Whole-brain standardized uptake values (SUVs) were determined, and analysis of variance was performed, with group (smoker *vs* non-smoker) and genotype as factors, thereby controlling for genotype. Smokers and non-smokers differed in whole-brain SUVs ($P=0.006$) owing to smokers having 16.8% lower values than non-smokers. The groups did not differ in injected radiotracer dose or body weight, which were used to calculate SUV. An inverse association was found between whole-brain SUV and reported cigarettes per day ($P<0.05$), but no significant relationship was found for plasma nicotine. Thus, smokers have less [^{11}C]DAA1106 binding globally than non-smokers, indicating less microglial activation. Study findings are consistent with much prior research demonstrating that smokers have impaired inflammatory functioning compared with non-smokers and that constituents of tobacco smoke other than nicotine affect inflammatory processes.
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INTRODUCTION

Inflammation is a critical component of normal tissue repair and is fundamental to the body's defense against infection (Goncalves *et al*, 2011). In the brain, microglia continuously scan the surrounding extracellular space (Nayak *et al*, 2014) in order to respond swiftly to damage or infection by becoming activated and participating in neuroinflammation (Anthony and Pitossi, 2012). In this context, activated microglia participate in functions such as clearance of apoptotic cells and extracellular pathogens, removal of degenerating neurons and extracellular proteins, and cytokine/chemokine production (Anthony and Pitossi, 2012). When activated, microglial cellular morphology changes and the expression of the translocator protein (TSPO) 18 kDa is increased, thereby making the expression of TSPO a marker for neuroinflammation.

The radioligand *N*-(2,5-dimethoxybenzyl)-N-(5-fluoro-2-phenoxyphenyl) acetamide labeled with carbon-11 (abbreviated as [^{11}C]DAA1106) has emerged as a reliable second-generation radiotracer for labeling TSPO (Maeda *et al*, 2004; Okubo *et al*, 2004; Zhang *et al*, 2003) with high affinity (Chaki *et al*, 1999; Chauveau *et al*, 2008; Venneti *et al*, 2007a, 2008) for positron emission tomography (PET) scanning *in vivo*. Because [^{11}C]DAA1106 and other newer radiotracers have higher affinity for TSPO than previously used radiotracers (eg, [^{11}C]PK11195), they are more useful for quantifying PET data by having the sensitivity to account for genetic TSPO predispositions (discussed in more detail below) (Owen *et al*, 2011) and smaller changes in neuroinflammation (Venneti *et al*, 2008). TSPO was originally called the 'peripheral benzodiazepine receptor' (Zhang *et al*, 2003) because it was identified by benzodiazepine binding but was renamed to acknowledge its many potential functions and

*Correspondence: Dr AL Brody, Department of Psychiatry, University of California at San Diego, VA San Diego Healthcare System, 3350 La Jolla Village Drive (116A), San Diego, CA 92161, USA, Tel: +858-552-8585, E-mail: abrody@ucsd.edu
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location in the central nervous system (as well as in the periphery) (Papadopoulos *et al*, 2006). Specific binding of DAA1106 correlates with the presence of activated microglia identified by immunohistochemistry *in situ* (Venneti *et al*, 2008) and immunohistochemistry combined with autoradiography in brain tissue (Venneti *et al*, 2007a).

PET studies using [^{11}C]DAA1106 and similar radiotracers have examined a range of conditions thought to be associated with neuroinflammation. This method was used recently to demonstrate increases in radiotracer binding in patients with Alzheimer's disease (Fan *et al*, 2015; Kreisl *et al*, 2013; Suridjan *et al*, 2015; Varrone *et al*, 2015; Yasuno *et al*, 2008, 2012), Lewy body dementia (Surendranathan *et al*, 2015), amyotrophic lateral sclerosis (Zurcher *et al*, 2015), stroke (Lartey *et al*, 2014), and non-smokers with major depression (Setiawan *et al*, 2015), but not Parkinson's disease (Koshimori *et al*, 2015) or normal aging (Suridjan *et al*, 2014). Increases in this marker have also been demonstrated in animal models of brain injury (Sandiego *et al*, 2015; Venneti *et al*, 2007b; Wang *et al*, 2014; Yu *et al*, 2010) and stroke (Walberer *et al*, 2014), along with subsequent normalization with time after a brain insult (Ory *et al*, 2015; Walberer *et al*, 2014; Wang *et al*, 2014). In contrast, a decrease in the marker for neuroinflammation was found with administration of propofol anesthesia (Hines *et al*, 2013).

Over the past 30+ years, a large body of research has addressed the effects of cigarette smoking on inflammation in the body (Goncalves *et al*, 2011; Towler, 2000). A driving force behind this research is the known impairment of wound healing by smoking. Comprehensive literature reviews have recommended preoperative and postoperative abstinence periods of $\geqslant 4$ weeks in smokers undergoing surgical procedures (Pluvy *et al*, 2015; Rinker, 2013). Though the mechanism by which smoking impairs wound healing has not been fully elucidated, cigarette smoke contains >250 toxins, many of which are known to affect healing (Rinker, 2013), and studies of laboratory animals exposed to cigarette smoke have demonstrated significant alterations (both decreases and increases) in markers of neuroinflammation (Khanna *et al*, 2013). Reviews of this literature indicate that the inflammatory healing response is attenuated in smokers by reduced inflammatory cell chemotactic responsiveness, diminished migratory function, and increased oxidative stress (Reuther and Brennan, 2014; Sorensen, 2012).

In the absence of studies directly examining the effect of human cigarette smoking on neuroinflammation *in vivo*, we used PET scanning to determine whether cigarette smokers have altered binding of [^{11}C]DAA1106, a marker for neuroinflammation, compared with non-smokers. We hypothesized that non-smoker *vs* smoker effects would occur globally throughout the brain, as prior research by our group (Brody *et al*, 2006a, 2009a, 2011, 2013) and others (Cosgrove *et al*, 2009; Staley *et al*, 2006) demonstrates widespread effects of smoking when studying systems (eg, the nicotinic cholinergic system) that are widely distributed. We also sought to examine the effect of menthol, as menthol cigarette smoking is common (~1/3 of US smokers) (SAMHSA, 2009) and menthol smokers have more difficulty quitting in standard treatment programs (Gandhi *et al*, 2009; Okuyemi *et al*, 2007; Pletcher *et al*, 2006), elevated serum nicotine/cotinine/exhaled carbon monoxide (CO) levels (in some (Williams *et al*, 2007), but not all (Abobo *et al*, 2012; Muscat *et al*, 2009), studies), and more severe upregulation of brain nicotinic acetylcholine receptors (Brody *et al*, 2013) when compared with non-menthol cigarette smokers. Therefore, we also hypothesized that effects of smoking on [^{11}C]DAA1106 binding would be greater in menthol than in non-menthol smokers.

MATERIALS AND METHODS

Forty-five participants (30 smokers and 15 non-smokers) completed the study and had usable data. These participants underwent telephone and in-person screening, a bolus [^{11}C]DAA1106 PET scanning session, blood draws during PET to determine TSPO affinity genotype and plasma nicotine (and metabolite) levels, and a structural magnetic resonance imaging (MRI) scan, as described below. An additional 6 participants underwent PET scanning but were excluded due to genotype ($n=4$, see below) or technical PET scanning issues ($n=2$) (Figure 1).

Participants were veterans who were recruited through Internet (eg, Craigslist) advertisements and posted flyers. Inclusion criteria were: (1) healthy adult (18–65 years) cigarette smokers (10–40 cigarettes per day) who met DSM-IV criteria (First *et al*, 1995) for Nicotine Dependence or non-smokers (<100 cigarettes lifetime and none within the past year), (2) smoking primarily ($>80\%$) either menthol or non-menthol cigarettes (for the smoker group), (3) ability to read, write, and give voluntary informed consent, and (4) an exhaled CO $\geqslant$ or <8 ppm (and urine cotinine $\geqslant$ or <200 ng/ml) during the study screening visit to support smoking or non-smoking status, respectively. Exclusion criteria were: (1) any Axis I diagnosis (including mood, anxiety, psychotic, and substance abuse disorders) within the past year, (2) any current medication or history of a medical condition that might affect the central nervous system at the time of scanning (eg, current treatment with a psychotropic medication or history of severe head trauma with loss of consciousness, epilepsy, or other neurological diseases), (3) regular use ($>1\times$/week) of anti-inflammatory medication, such as steroidal or non-steroidal anti-inflammatory medications (eg, corticosteroids, ibuprofen, naproxen, aspirin, or celecoxib (Celebrex)), (4) unstable cardiovascular disease, severe liver disease, or renal insufficiency, which might make tolerating study procedures difficult, or (5) pregnancy. Occasional drug/alcohol use not meeting criteria for abuse or dependence was not exclusionary, but participants were instructed to abstain from drug/alcohol use for at least 48 h prior to PET scanning.

For the telephone screening, a thorough smoking history, including age of first cigarette, maximum smoking habit, menthol or non-menthol cigarette use, length and dates of abstinence periods, previous treatments used, and current smoking habit, was obtained. A brief medical, psychiatric, and substance use history was also obtained during the telephone screening. During a subsequent in-person visit, eligibility criteria were confirmed and general demographics, smoking history, and symptom ratings were obtained with screening questions from the SCID for DSM-IV (First *et al*, 1995), the Smoker's Profile Form (Brody *et al*, 2006a), the Fagerström Test for Nicotine Dependence (FTND)

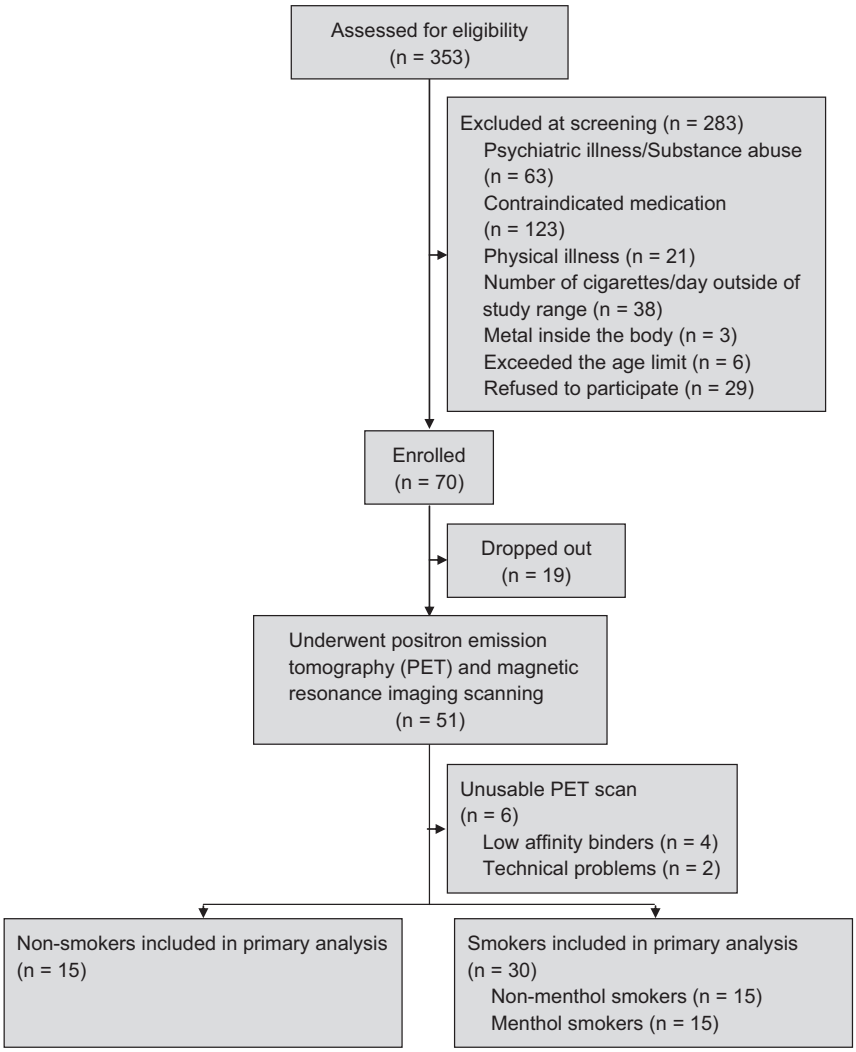

Figure 1 Flow diagram showing the number of potential and actual participants at each step of the study, including reasons for potential participants being screened out of participation.

(Fagerstrom, 1978; Heatherton *et al*, 1991) (to assess severity of Nicotine Dependence), Shiffman–Jarvik Withdrawal Scale (SJWS) (Shiffman and Jarvik, 1976) (to measure craving and withdrawal), and Spielberger State Trait Anxiety Index (STAI) (Spielberger, 1983) and Beck Depression Inventory (BDI) (Beck *et al*, 1996) (to confirm the absence of potentially confounding psychiatric symptoms). A brief medical review of systems and chart review were also performed by a study physician (ALB or MSM), along with an exhaled CO measurement (Micro+ Smokerlyzer Breath CO Monitor; Bedfont Scientific, UK), urine cotinine screen (The Accutest NicAlert; Jant Pharmacal, Encino, CA), breathalyzer (AlcoMatePro), urine toxicology screen (Test Country I-Cup Urine Toxicology Kit), and urine pregnancy test (Test Country Cassette Urine Pregnancy Test) to verify inclusion/exclusion criteria.

Participants meeting inclusion/exclusion criteria who wished to participate underwent a [^{11}C]DAA1106 PET scanning session 1 week later, using a procedure similar to the one developed in previous studies (Ikoma *et al*, 2007; Takano *et al*, 2010; Yasuno *et al*, 2008, 2012). At 1400 hours on the day of PET scanning, participants arrived at the VA Greater Los Angeles Healthcare System PET Center and underwent a brief clinical interview, breathalyzer, and urine cotinine, toxicology, and pregnancy screens, in order to verify continued meeting of inclusion/exclusion criteria (including confirmation of reports of drug abstinence at the time of scanning). From 1430 to 1445 hours, smokers smoked to satiety (2–3 cigarettes, favorite brand) in an outdoor area adjacent to the PET center. From 1445 to 1500 hours, participants were positioned on the PET scanner and a venous line was placed. At 1500 hours, participants received a bolus injection of 377 (±62) MBq of [^{11}C] DAA1106 and underwent dynamic PET scanning of the brain for the next 90 min. PET scans were obtained using the Philips Gemini TruFlight PET Scanner (Koninklijke Philips Electronics N.V., Eindhoven, The Netherlands). [^{11}C] DAA1106 was prepared by an established method (Wang *et al*, 2012). An investigational new drug (IND) approval from the Food and Drug Administration (IND 122041) was obtained to use the radiotracer [^{11}C]DAA1106 for the study described here.

A 5-ml blood sample was drawn prior to the initiation of PET scanning for genotyping of each individual's TSPO affinity subtype (high [C/C], medium [C/T], or low [T/T]), because these affinity subtypes have been shown to affect radiotracer binding for all currently used radiotracers determining TSPO availability (Owen *et al*, 2011; Owen *et al*, 2012; Yoder *et al*, 2013). For this sample, venous blood was drawn via a port in the catheter placed for radiotracer injection. Genomic DNA was extracted from whole blood using the QiaAmp DNA Blood Mini Kits (Qiagen, Valencia, CA) by study collaborators (EN and LS) and TSPO single-nucleotide polymorphism (rs6971) genotyping using the TaqMan Allelic Discrimination (Thermo Fisher Scientific, Canoga Park, CA) platform was performed in duplicate, according to the manufacturer's specified protocol. Quality control was ensured by perfect concordance of replicate samples, expected minor allele frequencies, and adherence to Hardy–Weinberg equilibrium. Only scans from participants with the high- or medium-affinity genotypes (known to be >90% of North Americans; Mizrahi *et al*, 2012) were included in study analyses in order to avoid a potential confound. The exclusion of low-affinity binders from data analysis is standard practice in recent research in this field (Hafizi *et al*, 2016; Hannestad *et al*, 2013; Koshimori *et al*, 2015; Zurcher *et al*, 2015).

In addition, blood samples were drawn 10 and 60 min after the initiation of PET scanning for determination of plasma nicotine/cotinine levels. Afternoon plasma cotinine has been shown to be a good measure of nicotine exposure for the past 24 h (Benowitz and Jacob, 1994). Samples were centrifuged to obtain plasma, packed on dry ice, and shipped to the Clinical Pharmacology Laboratory at the University of California, San Francisco for assay by gas chromatography by Peyton Jacob and colleagues.

One week after the PET scanning session, an MRI scan of the brain was obtained on a 3.0-T scanner (Signa; GE Medical Systems, Milwaukee, WI) in order to aid in localization of regions on the PET scans. The MRI had the following specifications: three-dimensional Fourier-transform spoiled-gradient-recalled acquisition with TR = 30 ms, TE = 7 ms, 30-degree angle, 2 acquisitions, and 256 × 192 view matrix. The acquired volume was reconstructed as roughly 90 contiguous 1.5-mm thick transaxial slices.

As in previous research by our group (Brody *et al*, 2002, 2004, 2006a, b, 2009a, b), MRI/PET co-registration was performed using the Statistical Parametric Mapping software (FIL Methods Group, UK), and automated volumes of interest (VOIs) were determined on MRI using FSL tools for structural MRI. These automated VOIs were transferred from each participant's MRI to his/her co-registered PET scan and visually inspected using PMOD (PMOD Technologies, Zurich, Switzerland). The primary VOI was whole brain (including gray and white matter) for reasons cited in the Introduction section. However, as automated volumes are easily attained and regional differences are possible, VOIs were also determined for the amygdala, caudate, hippocampus, nucleus accumbens, putamen, and thalamus, similar to VOIs obtained in prior research (Takano *et al*, 2010; Yasuno *et al*, 2012).

In order to obtain a quantitative measurement of VOI binding to TSPO in the brain, standardized uptake values (SUVs) were calculated using the standard definition of SUV = mean tissue activity concentration (Bq/ml)/(injected dose (Bq)/body weight (g)). Mean tissue activity concentration from 20 to 40 min postinjection was used, based on time activity curves demonstrating stable activity during this time period. SUV was used as the primary outcome measure because it avoids invasive arterial blood sampling and has been shown to strongly correlate with total volume of distribution (Vt) values (Toth *et al*, 2015; Walker *et al*, 2015), has good test–retest reproducibility (Toth *et al*, 2015), and has less intersubject variability than Vt (Walker *et al*, 2015) for a similar radiotracer.

For statistical analysis of data, an analysis of variance (ANOVA) was performed, with whole-brain SUV as the measure of interest and both group (smokers *vs* non-smoker) and TSPO genotype (mixed or high affinity) as between-subject factors (Suridjan *et al*, 2015; Varrone *et al*, 2015). To determine whether group differences were due to differences in particular brain regions, a multivariate ANOVA (MANOVA), using the smaller automated VOIs, was performed with the same structure as the preceding ANOVA, followed by univariate ANOVAs for the individual VOIs. To quantify between-group differences, percentage of difference was calculated as: $100 \times (SUV_{non\text{-}smokers} - SUV_{smokers})/SUV_{non\text{-}smokers}$. Based on prior research reporting greater brain exposure to cigarette smoke in menthol than in non-menthol cigarette smokers, we also performed an ANOVA for whole-brain SUV with the same structure as the above test, using non-smoker *vs* menthol *vs* non-menthol cigarette preference as a between-subject factor. As an exploratory analysis, linear analyses were performed for the smoker group, with whole-brain SUV value as the dependent variable and independent variables related to smoking, controlling for TSPO genotype. Statistical tests were performed using the statistical software program SPSS/PASW version 24 (SPSS, Chicago, IL).

RESULTS

Study groups had no significant differences in age, sex, race/ethnicity, height, weight, depression/anxiety levels, or caffeine, alcohol, or marijuana use (Table 1). On average, the groups were middle-aged, mostly male, and had generally low levels of depression/anxiety and drug/alcohol use. No significant between-group differences were present for body weight or injected dose of radiotracer, which were used to calculate SUV.

PET data analysis comparing smokers and non-smokers revealed a significant effect of group for whole-brain SUV values (ANOVA, $F = 8.3$; $df = 1,41$; $P = 0.006$), due to smokers having mean 16.8% lower values than non-smokers (Table 2 and Figure 2). Consistent with this global finding, in the analysis of the smaller VOIs, a significant multivariate effect of group was found (MANOVA; $F = 2.8$, $df = 12,30$; $P = 0.01$), with all VOIs having a significant (or trend-level) between-group effect on univariate analysis (Table 2), owing to smokers having lower SUV values than non-smokers (range 14.6–19.7%) in all VOIs studied.

For the three-group comparison (non-smokers *vs* non-menthol cigarette smokers *vs* menthol cigarette smokers), the whole-brain SUV comparison was significant (ANOVA, $F = 6.1$; $df = 2,39$; $P = 0.005$), owing to a range of values from

Table 1 Baseline Demographics and Rating Scale Scores for the Non-Smoker and Smoker Groups

Variable	Non-smoker group ($n=15$)	Whole smoker group ($n=30$)	Non-menthol smoker subgroup ($n=15$)	Menthol smoker subgroup ($n=15$)
Age, years	47.6 (±13.8)	52.1 (±8.1)	49.9 (±8.4)	54.4 (±7.4)
Sex (% female)	26.7	20.0	20.0	20.0
Race/ethnicity (%)				
African American	26.7	46.7	33.3	60.0
Asian	26.7	10.0	6.7	13.3
Hispanic	26.7	13.3	13.3	13.3
White	20.0	30.0	46.7	13.3
Height (inches)	68.9 (±4.0)	68.2 (±4.0)	68.3 (±4.7)	68.2 (±3.5)
Weight (kg)	88.1 (±23.4)	84.0 (±16.2)	83.9 (±17.9)	84.1 (±14.9)
Cigarettes per day	0 (±0)	13.9 (±3.8)	13.5 (±3.8)	14.4 (±3.9)
Exhaled carbon monoxide (ppm)	1.6 (±0.6)	13.3 (±4.7)	13.0 (±4.2)	13.5 (±5.2)
Fagerström Test for Nicotine Dependence (FTND)	0 (±0)	4.0 (±2.3)	4.1 (±2.1)	3.9 (±2.4)
Beck Depression Inventory	1.0 (±1.3)	1.7 (±2.3)	1.3 (±1.8)	2.1 (±2.8)
State Trait Anxiety Inventory	58.5 (±15.0)	66.7 (±17.8)	68.3 (±18.1)	65.1 (±18.0)
Caffeine use (coffee cup equivalents/day)	1.1 (±1.3)	2.0 (±1.6)	2.0 (±1.7)	1.9 (±1.5)
Alcohol drinks per day	0.6 (±1.4)	1.0 (±2.1)	0.7 (±1.6)	1.3 (±2.5)
Marijuana cigarettes per week	0.0 (±0.0)	0.3 (±1.3)	0.5 (±1.8)	0.1 (±0.4)

All values are presented as means (± SD) or percentages. Using χ^2 tests for categorical variables and Student's *t*-tests for continuous variables, no between-group (or between-subgroup) tests were significant, other than differences in measures of smoking (cigarettes per day, exhaled carbon monoxide, and FTND scores) between the smoker groups/subgroups and the non-smoker group (all *P*-values < 0.0005).

Table 2 Standardized Uptake Values (SUVs) for the Whole Brain and Smaller Regions of Interest for Non-smokers and Smokers (and the Non-Menthol Smoker and Menthol Smoker Subgroups)

Brain region	SUV values—non-smokers ($n=15$)	SUV values—smokers ($n=30$)	SUV values—non-menthol smoker subgroup ($n=15$)	SUV values—menthol smoker subgroup ($n=15$)
Whole brain	0.20 (±0.03)	0.17 (±0.04)	0.18 (±0.04)	0.16 (±0.02)
Accumbens				
R	0.20 (±0.03)	0.17 (±0.04)	0.17 (±0.04)	0.16 (±0.03)
L	0.21 (±0.03)	0.17 (±0.04)	0.17 (±0.04)	0.16 (±0.03)
Amygdala				
R	0.18 (±0.03)	0.15 (±0.04)	0.16 (±0.04)	0.13 (±0.02)
L	0.17 (±0.03)	0.15 (±0.04)	0.16 (±0.04)	0.14 (±0.02)
Caudate				
R	0.18 (±0.04)	0.14 (±0.03)	0.15 (±0.03)	0.14 (±0.02)
L	0.18 (±0.03)	0.15 (±0.03)	0.15 (±0.03)	0.14 (±0.02)
Hippocampus				
R	0.19 (±0.03)	0.16 (±0.03)	0.17 (±0.04)	0.15 (±0.02)
L	0.19 (±0.03)	0.15 (±0.03)	0.16 (±0.04)	0.14 (±0.02)
Putamen				
R	0.23 (±0.03)	0.19 (±0.05)	0.21 (±0.06)	0.18 (±0.03)
L	0.23 (±0.03)	0.19 (±0.04)	0.20 (±0.05)	0.18 (±0.03)
Thalamus				
R	0.22 (±0.04)	0.19 (±0.04)	0.20 (±0.04)	0.18 (±0.03)
L	0.22 (±0.03)	0.18 (±0.04)	0.19 (±0.04)	0.17 (±0.03)

Abbreviations: L = left; R = right. All values are mean ± SD. All regions were analyzed using analysis of variance, with group (non-smoker *vs* smoker or non-smoker *vs* non-menthol smoker *vs* menthol smoker) and genotype as between-subject factors. All regions were significant for the non-smoker *vs* smoker comparison at the $P \leqslant 0.05$ level, except for the right and left accumbens, which approached significance (*P*-values = 0.08 and 0.06, respectively). Similarly, all regions were significant for the three group comparisons (non-smoker *vs* non-menthol smoker *vs* menthol smoker) at the $P \leqslant 0.05$ level. The automated regions listed here were generated using the FSL toolkit.

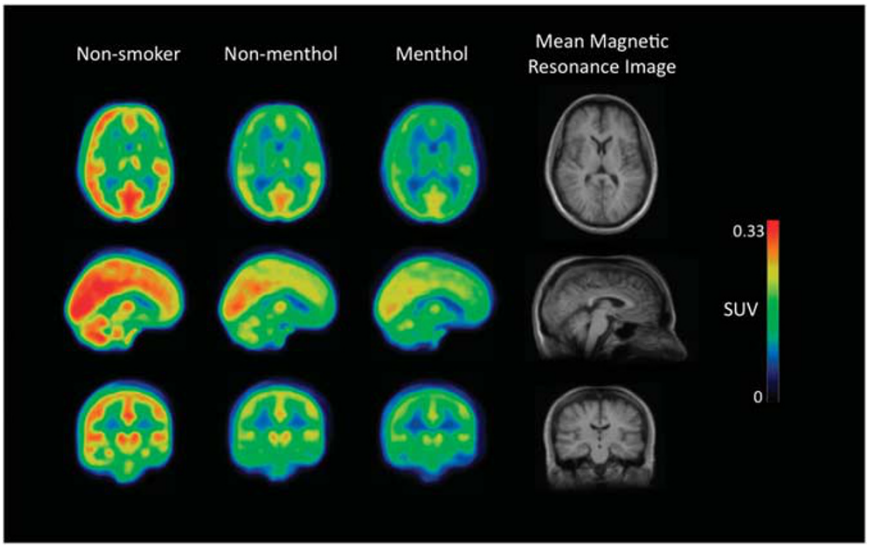

Figure 2 Mean positron emission tomography (PET) images from the study subgroups (non-smokers, non-menthol cigarette smokers, and menthol cigarette smokers) demonstrating higher [^{11}C]DAA1106 standardized uptake values (SUVs) for non-smokers than the two smoker subgroups. The first three columns consist of mean SUV PET images (transaxial, sagittal, and coronal from top to bottom) for the three study groups/subgroups ($n = 15$ each) and the far right column shows the group mean magnetic resonance image. For this figure representation of study results, the PET scans were spatially normalized into standard Montreal Neurological Institute (MNI) template space.

non-smokers (highest) to non-menthol cigarette smokers (middle) to menthol cigarette smokers (lowest) (Table 2). In the multivariate analysis of smaller VOIs, a significant effect of group was found (MANOVA; $F = 1.8$, $df = 24,56$; $P = 0.03$), with all VOIs having a significant between-group effect, owing to the range (from high to low) of SUV values from smokers to non-menthol smokers to menthol smokers (Table 2). In comparing only the non-menthol with the menthol cigarette smokers, the whole-brain SUV comparison did not reach significance (ANOVA; $F = 3.6$; $df = 1,26$; $P = 0.07$), and similar results were found for the smaller VOIs (ANOVAs; Ps = 0.03–0.21), possibly owing to the smaller samples used for comparing the non-menthol with the menthol cigarette smoker subgroups.

In the exploratory analysis of smoking-related variables, a significant relationship was found between cigarettes per day and whole-brain SUV ($F = 6.3$; $P = 0.02$), indicating that higher levels of reported smoking were associated with lower levels of TSPO availability. Similarly, a significant relationship between the stimulation subscale scores of the SJWS and whole-brain SUV was also found ($F = 5.6$; $P = 0.03$), indicating that higher levels of withdrawal stimulation were associated with lower levels of TSPO availability. No significant associations were found for FTND scores, CO levels, plasma nicotine/cotinine levels, or other subscales on the SJWS.

DISCUSSION

Cigarette smokers have less [^{11}C]DAA1106 binding than non-smokers throughout the brain, indicating less TSPO availability. Though several explanations for this finding are possible, a straightforward one is that smoking results in global impairment of microglial activation. This explanation is consistent with much prior research demonstrating that smokers have impaired inflammatory functioning in other parts of the body, which leads to compromised wound healing (Goncalves *et al*, 2011; Towler, 2000). Furthermore, the inverse correlation between [^{11}C]DAA1106 binding and participant reports of cigarette use per day indicates that the severity of impaired microglial activation may be related to the amount of current cigarette usage. Of note, the fact that study results were global (rather than regional) is also consistent with prior research demonstrating widespread effects of smoking on brain receptors (Brody *et al*, 2006a, 2009a, 2011, 2013; Cosgrove *et al*, 2009; Staley *et al*, 2006). These global effects of smoking are in line with known properties of cigarette smoke, namely, that it rapidly enters the body and brain due to high permeability through lung, vasculature, and brain cells (Henderson and Lester, 2015). Taken together, study results may demonstrate a significant widespread brain abnormality in smokers in the satiated state.

The negative association between SUV values and cigarettes per day, but not plasma nicotine levels (or other measures of smoking behavior), may indicate that components of cigarette smoke other than nicotine are responsible for the low level of microglial activation found here. Laboratory studies support this theory, with several studies demonstrating that whole tobacco smoke administration results in greater alterations in inflammatory markers than nicotine alone (Arimilli *et al*, 2015; Tilp *et al*, 2016). However, given the evidence that nicotine indeed impairs (Kalra *et al*, 2004; Piao *et al*, 2009) or attenuates (Gao *et al*, 2014) some inflammatory processes, and the relatively small sample of smokers studied here in the correlational analysis, the exact relationship between nicotine and neuroinflammation in human smokers remains to be confirmed.

Although impairment of neuroinflammation by smoking is a straightforward explanation of the study results, other explanations are possible, given the complex effects of cigarette smoking on the brain. Cigarette smoke contains

thousands of constituents (Green and Rodgman, 1996), with hundreds having known toxic effects (Baker *et al*, 2004; Fowles and Dybing, 2003). It is possible that one or more of these constituents directly interfered with [^{11}C]DAA1106 binding to TSPO, which would have resulted in the difference in binding between smokers in the satiated state and non-smokers found here. Additionally, acute smoking is known to disrupt blood–brain barrier function (Sajja *et al*, 2016), which could have created differences in radiotracer binding for smokers and non-smokers for the PET time period of interest used here.

In addition to the overall difference between smokers and non-smokers, the menthol cigarette smoker subgroup had less [^{11}C]DAA1106 binding than the non-menthol cigarette smoker subgroup. This finding is consistent with prior research by our group (Brody *et al*, 2013) showing greater upregulation of nicotinic acetylcholine receptors throughout almost all brain regions in menthol than in non-menthol cigarette smokers. Also, research by others demonstrates that menthol cigarette smoking is associated with more severe biological abnormalities in some (Williams *et al*, 2007), but not all (Abobo *et al*, 2012; Muscat *et al*, 2009), studies that have examined this issue. Therefore, as in prior research, the present finding may be due to greater brain exposure to cigarette smoke (leading to greater impairment of microglial activation) in menthol cigarette smokers, a direct effect of menthol flavoring, or some other mechanism.

The primary limitation of this study was the absence of arterial blood sampling such that total distribution volume (Vt) was not ascertained. Vt may control for the potential confounds of between-subject differences in radiotracer metabolism and binding to vascular endothelium and plasma protein (Koshimori *et al*, 2015; Rizzo *et al*, 2014; Turkheimer *et al*, 2015). Although Vt is a common outcome measure in PET studies examining TSPO in conditions other than tobacco dependence (Colasanti *et al*, 2016; Haarman *et al*, 2016; Narendran *et al*, 2014), recent research demonstrates that the less invasive SUV measure tends to correlate well with Vt within individual PET studies (Toth *et al*, 2015; Yoder *et al*, 2015) and has high test–retest reliability (Nair *et al*, 2016; Toth *et al*, 2015). Other similar studies have used pseudo-reference regions for PET data analysis (Colasanti *et al*, 2016; Coughlin *et al*, 2014; Hamelin *et al*, 2016; Kreisl *et al*, 2016; Lyoo *et al*, 2015; Zurcher *et al*, 2015) to minimize potential confounds, but this method would not have been appropriate here due to the hypothesized and confirmed effect of smoking throughout the brain. Additional limitations included a modest sample size and the fact that smokers were scanned in the satiated state, such that we did not determine whether results were due to acute or chronic cigarette smoking. Future research could examine smokers in the abstinent state to determine the relationship between decreased [^{11}C]DAA1106 binding and recency of smoking.

In summary, cigarette smokers in the satiated state have decreased TSPO availability, which is related to participants' current smoking level (higher levels of smoking were associated with less TSPO availability). This effect appeared to be greater for menthol than for non-menthol cigarette smokers. Future research could examine the time course of recovery of TSPO availability upon smoking cessation and the interplay between smoking, neuroinflammation, and the progression of diseases thought to be mediated by neuroinflammation.

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