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History of cigarette smoking is associated with higher limbic $GABA_A$ receptor availability

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ABSTRACT

Cigarette smoking presents a significant worldwide healthcare challenge. Preclinical, genetic association and clinical trials studies provide considerable evidence for the involvement of the human γ-aminobutyric acid (GABA) system in the neurobiology of nicotine addiction. However there are few human GABA neurochemical imaging studies of nicotine addiction. We investigated limbic $GABA_A$ receptor availability in volunteers with a history of cigarette smoking using [^{11}C]Ro15 4513 positron emission tomography (PET). Eight [^{11}C]Ro15 4513 PET scans from volunteers with a history of cigarette smoking were compared to twelve scans from volunteers who were non-smokers. Total, α1 and α5 $GABA_A$ receptor subtype [^{11}C]Ro15 4513 V_T values were quantified using spectral analysis of limbic regions implicated in nicotine addiction. Spectral analysis allows quantification of the overall [^{11}C]Ro15 4513 spectral frequency as well as α1 and α5 $GABA_A$ receptor subtype specific spectral frequency components. Volunteers with a history of cigarette smoking showed significantly higher total [^{11}C]Ro15 4513 V_T values in the presubgenual cingulate and parahippocampal gyrus, and at a trend level in the insula, nucleus accumbens and subgenual cingulate. In six abstinent previous smokers ('ex-smokers'), total [^{11}C]Ro15 4513 binding was significantly higher in all limbic regions studied, with higher α5 availability in the amygdala, anterior cingulate, nucleus accumbens and presubgenual cingulate. These results suggest that limbic $GABA_A$ receptor availability is higher in volunteers with a history of cigarette smoking which may reflect either higher expression of $GABA_A$ receptors or lower endogenous GABA levels. The findings in ex-smokers suggest that higher $GABA_A$ receptor availability continues with abstinence indicating that this may be a trait marker for nicotine addiction or that alterations in GABA function associated with cigarette smoking persist.

Introduction

Tobacco cigarette smoking is a major worldwide healthcare challenge. Cigarette smokers are at much greater risk of developing serious health problems such as cancer, cardiovascular and respiratory diseases. Cigarette smoking causes five million deaths annually and of the over one billion cigarette smokers in the world, half will die from smoking related diseases (Hatsukami et al., 2008). Cigarette smoking is also associated with a higher frequency of mental health symptoms, such as depression and anxiety, as well as being much more prevalent in severe mental disorders such as schizophrenia (Boden et al., 2010; de Leon and Diaz, 2005). Although smoking cessation medications are increasingly available in the developed world, relapse rates are high which emphasises the highly addictive nature of tobacco use. A better understanding of the neurochemistry of cigarette smoking and nicotine addiction is therefore critical in shaping public health approaches towards cigarette smoking and in developing new smoking cessation treatments.

Both animal and human studies indicate the involvement of the GABA system in the neurobiology of nicotine addiction. GABA neurones express nicotinic acetylcholine receptors and exert inhibitory control over the limbic dopaminergic system which is implicated in the rewarding and mood elevating properties of nicotine (Brody et al., 2004; Brody et al., 2006; Brody et al., 2009; Montgomery et al., 2007). In animal studies, pharmacologically induced increases in GABA transmission are associated with reductions in the self-administration of nicotine (Markou et al., 2004), nicotine related behaviours (Bevins et al., 2001) and nicotine-induced dopamine release in the nucleus accumbens (Dewey et al., 1999). These findings are reflected in human studies where administration of agents which enhance GABA transmission reduces both the rewarding effects of nicotine and the symptoms of

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nicotine withdrawal (Herman et al., 2012; Sofuoglu et al., 2005). A number of human genetic studies have also identified associations between nicotine dependence and genes encoding for both $GABA_A$ and $GABA_B$ receptors (Agrawal et al., 2009; Li et al., 2009; Lou et al., 2007; Philibert et al., 2009).

The inhibitory effects of GABA within the brain are mediated in part through $GABA_A$ receptors. These are comprised of at least six different subunits and both the localisation and function of $GABA_A$ receptors vary between subtypes. For example, the α1 receptor subtype is predominantly localised in cortical regions and is involved in sedation and sleep, whereas the α5 receptor subtype is particularly highly expressed in limbic regions and is involved in learning and memory (Lingford-Hughes et al., 2002; Tan et al., 2011). A number of functional neuroimaging studies have also highlighted the involvement of limbic regions in nicotine addiction (Brody et al., 2002; Franklin et al., 2007; Hong et al., 2010; Janes et al., 2010b; Naqvi et al., 2007; Rose et al., 2007; Smolka et al., 2006; Stein et al., 1998; Yalachkov et al., 2009).

We have previously shown that limbic $GABA_A$ receptor availability in the living human brain can be measured using [^{11}C]Ro15 4513 positron emission tomography (PET) (Lingford-Hughes et al., 2002), which binds to the benzodiazepine site of the $GABA_A$ receptor, and that lower limbic [^{11}C]Ro15 4513 binding is associated with alcohol dependence (Lingford-Hughes et al., 2012). Although [^{11}C]Ro15-4513 is more selective for the α5 receptor subtype, there is also measurable in vivo binding for the α1 receptor subtype (Hadingham et al., 1993) and we have recently shown that [^{11}C]Ro15 4513 $GABA_A$ α1 receptor subtype binding can be quantified in PET studies, by partitioning fast and slow ligand kinetics (Myers et al., 2012). This provides an opportunity, for the first time, to assess $GABA_A$ α1 and α5 receptor subtype availability in volunteers with a history of cigarette smoking.

In this study, we investigated total, α1 and α5 subtype $GABA_A$ receptor availability in volunteers with and without a history of cigarette smoking using a database of [^{11}C]Ro15 4513 PET scans. We sought to test the hypothesis that volunteers with a history of cigarette smoking would show higher limbic $GABA_A$ receptor availability compared to non-smokers as a consequence of higher receptor expression compensating for lower GABA function in nicotine addiction.

Methods and materials

Participants

Using a [^{11}C]Ro15 4513 PET database, eight scans from male volunteers with a history of cigarette smoking (mean age 48.4 years, SD: 6.8) were compared to twelve [^{11}C]Ro15 4513 scans from male non-smoking volunteers (mean age 46.5 years, SD: 7.9). Volunteers with a history of cigarette smoking were required to either be current cigarette smokers at the time of imaging or have previously smoked a minimum of 5 cigarettes per day for at least a six month period. Non-smokers were included in the study if they reported smoking less than 100 cigarettes over their lifetime. All volunteers had previously undergone [^{11}C]Ro15 4513 PET imaging at the MRC Clinical Sciences Centre, Hammersmith Hospital, London, UK.

Lifetime cigarette smoking history was assessed using participant self-reports of their tobacco use from a semi-structured screening interview prior to imaging and also using reports of tobacco use completed as part of the cannabis experiences questionnaire (CEQ3) (Barkus et al., 2006) (95% of volunteers) which additionally assesses cannabis use, alcohol use and use of other recreational drugs. Finally, where there was ambiguity from screening or CEQ3 cigarette smoking history responses, smoking histories were re-confirmed through telephone interview or email responses. All volunteers had been previously assessed by a psychiatrist to exclude current or previous significant mental health disorders and alcohol or recreational drug dependency as defined by DSM-IV, serious physical illness, past neurological disorders or previous use of psychotropic medications. All volunteers had previously given written informed consent to undergo [^{11}C]Ro15 4513 PET imaging, which was approved both by the Hammersmith Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee, UK.

[^{11}C]Ro15 4513 PET imaging

All PET scans were acquired using an ECAT HR + 962 scanner (CTI/Siemens) with an axial field of view of 15.5 cm. A 10 min transmission scan was performed prior to each emission scan to measure tissue attenuation in two dimensional mode. Each volunteer received a fast intravenous bolus injection of about 493 MBq [^{11}C]Ro15 4513 through an intravenous cannula sited in the dominant antecubital fossa vein. Twenty four dynamic frames (1×30, 4×15, 4×60, 2×150, 10×300, 3×600 s) of data were acquired in 3D mode over 90 min and produced images containing 63 contiguous slices. Arterial blood sampling was used to produce a metabolite-corrected plasma input function as described previously (Lingford-Hughes et al., 2002).

MR imaging

All participants also underwent a structural T1 MRI scan for co-registration purposes. The MRI scans were performed using either a 1.5 T scanner (17 scans) (1.5 Eclipse system, Marconi Medical Systems, Cleveland, OH, USA; TR = 30 ms, TE = 3 ms, flip angle = 30°, NSA = 1, voxel dimensions $0.98\times1.6\times1.6$ mm^3), or a 3 T scanner (3 scans) (3 T Intera Philips Medical Systems; TR = 9.6 ms, TE = 4.6 ms, flip angle = 8°, NSA = 1, voxel dimensions $0.94\times0.94\times1.2$ mm^3).

Image analysis

All dynamic scans were corrected for head movement using frame by frame (FBF) realignment (Montgomery et al., 2006). This procedure was applied to all frames to generate a FBF-corrected dynamic image, which was then analysed using an automated region of interest (ROI) analysis. FBF corrected reconstructed [^{11}C]Ro15 4513 images were analysed using Analyze AVW version 8.1 (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN), Matlab 6, 6.5 and SPM5 (available via http://www.fil.ion.ucl.ac.uk/spm/). Metabolite-corrected arterial plasma input functions were calibrated against discrete blood samples using a well counter cross-calibrated to the scanner. Parent plasma input functions were generated by multiplying the calibrated arterial radioactivity by the plasma-over-blood ratio and the parent fraction in plasma using Clickfit v1.66 in-house software in Matlab 6.

[^{11}C]Ro15 4513 V_T (volume of distribution) values were generated using spectral analysis. Spectral analysis convolves the arterially derived metabolite-corrected plasma input function with a first order poly-exponential, without specifying the number of tissue compartments, to fit the time activity curve for any region, under the assumptions of system linearity and time invariance (Cunningham and Jones, 1993; Turkheimer et al., 1994). Voxel-by-voxel parametric V_T images were produced with spectral analysis using parent plasma input functions within RPM. A slow frequency boundary of 0.00063 s^{-1} was used, rather than the default boundary of 0.0008 s^{-1}, in order to maximise the reliability of binding quantification (Barros et al., 2010). Weighted summed activity images ("add images") were created of all 24 frames to provide the best possible anatomical PET data for co-registration or normalization to structural MR images.

Individual [^{11}C]Ro15 4513 PET add images were then co-registered to individual T1 structural MRI images and the T1 image segmented within SPM5. The deformation parameters from this segmentation were then used to normalize an 83-region maximum probability atlas (Gousias et al., 2008; Hammers et al., 2003) to individual PET space and converted into an "object map" in Analyze 8.1. Prior to regional sampling, the goodness of fit of each object map to the individual brain was checked by visual inspection. The following ROIs from

the atlas previously implicated in nicotine addiction were sampled: nucleus accumbens, insula, cingulate cortex (anterior, subgenual and presubgenual portions), amygdala, hippocampus and parahippocampal gyrus (Brody et al., 2002; Franklin et al., 2007; Hong et al., 2010; Janes et al., 2010b; Naqvi et al., 2007; Rose et al., 2007; Smolka et al., 2006; Stein et al., 1998; Yalachkov et al., 2009). The superior parietal gyrus was used a comparator region as it is anatomically relatively large, has a measureable [^{11}C]Ro15 4513 signal and has not been consistently implicated in nicotine addiction. Sampling was performed in Analyze 8.1 to obtain mean total [^{11}C]Ro15 4513 V_T values through all slices for all regions in the atlas.

Additionally, α1 and α5 $GABA_A$ receptor subtype spectral frequency components were extracted using the method described by Myers and colleagues to generate V_T values for these subtypes (Myers et al., 2012). Briefly, the "spectrum" of dissociation rates, each represented by one peak, can be divided into ranges that represent the different compartments of [^{11}C]Ro15 4513 binding. For example, in the case of the α1 subtype binding site, the range of decay values is approximately 0.0030–0.0040 s^{-1}. α1 and α5 $GABA_A$ receptor subtype V_T values were calculated by summing the peak height within the prescribed band for the subtype, divided by the radioactive decay constant subtracted from the pharmacological and physiological decay (Myers et al., 2012).

Statistical analysis

Group differences in [^{11}C]Ro15 4513 V_T values were assessed using a multivariate analysis of variance (MANOVA). Correlations between continuous data were assessed using Pearson's product moment correlation coefficient and discontinuous data with Spearman's rank correlation coefficient. All statistical comparisons were performed using SPSS 19.0 (SPSS, Chicago, Illinois, USA), all values are expressed as mean (SD) and the threshold for two tailed statistical significance was defined as $p<0.05$.

Results

Volunteer use of tobacco, alcohol and other substances

Volunteers with a history of cigarette smoking (n = 8) smoked an average of 14.8 (SD: 5.6) cigarettes per day whilst smoking. The mean duration of cigarette smoking was 18.1 (13.3) years, average number of lifetime cigarettes smoked was 102,798 (SD: 89,654, range: 11,125–277,765) and the mean number of cigarettes smoked per lifetime year was 2195 (SD: 1775). Two volunteers with a history of cigarette smoking were current smokers at the time of imaging and six were previous smokers who were now abstinent. Previous smokers were abstinent for an average of 13.1 years (SD: 13, range: 0.5–35). Volunteer tobacco use, alcohol use, use of other substances is shown in Table 1. There was no significant difference in age, current alcohol consumption, lifetime cannabis consumption, lifetime ecstasy consumption or lifetime stimulant consumption between volunteers with a history of cigarette smoking and non-smokers (all p values > 0.05).

Image analysis results

[^{11}C]Ro15 4513 binding in volunteers with a history of cigarette smoking

Volunteers with a history of cigarette smoking (n = 8) showed significantly higher total [^{11}C]Ro15 4513 V_T values in the presubgenual cingulate ($F_{1,8} = 7.3$, $p = 0.01$) and parahippocampal gyrus ($F_{1,8} = 4.3$, $p = 0.05$) compared to non-smokers (n = 12) (see Table 2 and Fig. 1). [^{11}C]Ro15 4513 V_T values were also higher at a trend significance level in the insula ($F_{1,8} = 3.8$, $p = 0.07$), nucleus accumbens ($F_{1,8} = 3.7$, $p = 0.07$) and subgenual cingulate ($F_{1,8} = 3.7$, $p = 0.07$). Excluding one volunteer with a history of cigarette smoking from the analysis, who had also smoked a comparatively high number of cannabis cigarettes over his lifetime (approximately 12,000), did not change the pattern of these results (presubgenual cingulate $F_{1,8} = 9.7$, $p = 0.006$; subgenual cingulate $F_{1,8} = 5.1$, $p = 0.04$; parahippocampal gyrus $F_{1,8} = 4.6$, $p = 0.05$; insula $F_{1,8} = 3.8$, $p = 0.07$; nucleus accumbens $F_{1,8} = 3.6$, $p = 0.07$). There were no significant differences between volunteers with a history of cigarette smoking and non-smokers in the total amount of [^{11}C]Ro15 4513 injected (mean (SD) MBq injected: 472 (22), 494 (30) respectively; $p > 0.05$).

α1 subtype V_T values were higher at a trend significance level in volunteers with a history of cigarette smoking in the insula ($F_{1,8} = 4.1$, $p = 0.06$) but lower in the presubgenual cingulate ($F_{1,8} = 3.9$, $p = 0.06$). α5 subtype V_T values were not significantly different in any region studied (all p values > 0.05). There was no significant correlation in any region studied between total, α1 or α5 subtype [^{11}C]Ro15 4513 availability and either number of lifetime cigarettes smoked or the mean number of cigarettes smoked per lifetime year (all p values > 0.05).

[^{11}C]Ro15 4513 binding in abstinent previous smokers

In an exploratory analysis we went on to compare whether there were differences in total, α1 and α5 subtype [^{11}C]Ro15 4513 V_T values between abstinent previous smokers ('ex-smokers'), defined as no reported cigarette use in the previous six months, and non-smokers (see Table 2). Ex-smokers had significantly higher total [^{11}C]Ro15 4513 V_T values than non-smokers in all regions studied except the superior parietal gyrus: insula ($F_{1,8} = 10.3$, $p = 0.005$), parahippocampal gyrus ($F_{1,8} = 10.8$, $p = 0.005$), presubgenual cingulate ($F_{1,8} = 9.2$, $p = 0.008$), amygdala ($F_{1,8} = 8.2$, $p = 0.01$), nucleus accumbens ($F_{1,8} = 6.9$, $p = 0.02$), hippocampus ($F_{1,8} = 5.1$, $p = 0.02$), subgenual cingulate ($F_{1,8} = 5.0$, $p = 0.04$) and anterior cingulate ($F_{1,8} = 5.0$, $p = 0.04$). α1 subtype V_T values were significantly lower in ex-smokers compared to non-smokers in the nucleus accumbens ($F_{1,7} = 5.0$, $p = 0.04$) and the presubgenual cingulate ($F_{1,8} = 4.6$, $p = 0.05$). α5 subtype V_T values were significantly higher in ex-smokers in the amygdala ($F_{1,8} = 10.6$, $p = 0.005$), anterior cingulate gyrus ($F_{1,8} = 8.4$, $p = 0.01$), nucleus accumbens ($F_{1,8} = 6.5$, $p = 0.02$) and presubgenual cingulate ($F_{1,8} = 6.5$, $p = 0.02$) (see Fig. 2). There was no correlation between

Table 1
Volunteer tobacco use, alcohol use and use of other substances.

	Volunteers with a history of cigarette smoking (n = 8): mean (SD)	Non-smokers (n = 12): mean (SD)	p-value
Number of cigarettes smoked over lifetime	102,798 (89,654)	6.2 (21.6)	0.001
Number of cigarettes smoked per lifetime year	2195 (1775)	0.2 (0.6)	<0.001
Number of cigarettes smoked per day	14.8 (5.6)	0 (0)	<0.001
Duration of smoking (years)	18 (13)	–	
Last cigarette smoked (years)	9.8 (12.5)	–	
Alcohol unit consumption per week	10.8 (6.7)	10.2 (7.4)	0.85
Number of cannabis cigarettes smoked over lifetime	1612 (4264)	85.7 (206)	0.38
Number of cannabis cigarettes smoked per lifetime year	43	2	0.22
Total lifetime MDMA use sessions	3 (8)	0.3 (0.7)	0.40
Total lifetime stimulant use sessions	0.3 (0.7)	1 (2.9)	0.40

Table 2
Comparison of mean total, α1 and α5 [^{11}C]Ro15 4513 V_T values between volunteers with a history of cigarette smoking, ex-smokers, and non-smokers. ( ) = Standard deviation.

Area	Volunteers with a history of cigarette smoking (n = 8)			Ex-smokers (n = 6)			Non-smokers (n = 12)		
	Total	α1	α5	Total	α1	α5	Total	α1	α5
Nucleus accumbens	13.4 (2.5)	1.5 (0.6)	11.9 (3.8)	14.2 (2.4)*	1.3 (0.6)*	13.2 (3.6)*	11.6 (1.8)	1.9 (0.4)	9.5 (2.4)
Insula	6.8 (1.0)	2.0 (0.6)*	3.8 (1.6)	7.2 (0.9)**	1.8 (0.6)	4.3 (1.4)	6.1 (0.5)	1.3 (0.7)	4.2 (0.9)
Anterior cingulate	7.4 (1.2)	1.1 (1.0)	6.1 (1.6)	7.7 (1.3)*	1.2 (0.9)	6.8 (0.8)*	6.8 (0.5)	1.0 (0.7)	5.5 (0.9)
Subgenual cingulate	7.5 (1.1)	0.8 (0.3)	6.1 (1.4)	7.7 (1.1)*	0.8 (0.3)	6.4 (1.4)	6.8 (0.7)	0.8 (0.4)	5.2 (1.0)
Presubgenual cingulate	9.4 (1.0)*	0.2 (0.3)*	8.5 (2.0)	9.6 (1.1)**	0.2 (0.2)*	9.2 (1.5)*	8.3 (0.8)	0.7 (0.6)	7.1 (1.6)
Parahippocampal gyrus	8.8 (1.4)*	0.6 (0.5)	6.1 (1.6)	9.3 (1.3)**	0.7 (0.6)	6.9 (1.0)	7.8 (0.7)	0.8 (0.6)	5.8 (1.1)
Hippocampus	9.4 (1.1)	1.0 (0.4)	6.3 (1.7)	9.9 (1.0)*	1.0 (0.5)	7.1 (0.9)	8.7 (0.8)	0.7 (0.5)	6.6 (1.2)
Amygdala	8.7 (1.5)	1.0 (0.7)	7.3 (2.0)	9.3 (1.3)*	0.9 (0.8)	8.2 (1.3)**	7.8 (0.9)	1.1 (0.8)	6.2 (1.2)
Superior parietal gyrus	3.8 (0.7)	2.5 (0.6)	1.2 (0.9)	4.1 (0.6)	2.4 (0.5)	1.6 (0.6)	3.7 (0.3)	2.6 (0.5)	1.0 (0.6)

* $p<0.05$.
** $p<0.01$.

the duration of abstinence in ex-smokers and total, α1 or α5 subtype V_T values in any region studied (all p values > 0.05).

Association with alcohol use, cannabis use or MRI scanner parameters

There was no association between current weekly alcohol consumption and total, α1 or α5 subtype V_T values in any region studied (all p values > 0.05). There was also no association between a history of previous cannabis use and total, α1 or α5 subtype V_T values (all p values > 0.05) except for α1 V_T values in the amygdala ($F_{1,8} = 4.8$, $p = 0.04$) and anterior cingulate ($F_{1,8} = 4.5$, $p = 0.05$). MRI scanner magnet strength was not associated with significant differences in total [^{11}C] Ro15 4513 V_T values in any region studied (all p values > 0.05).

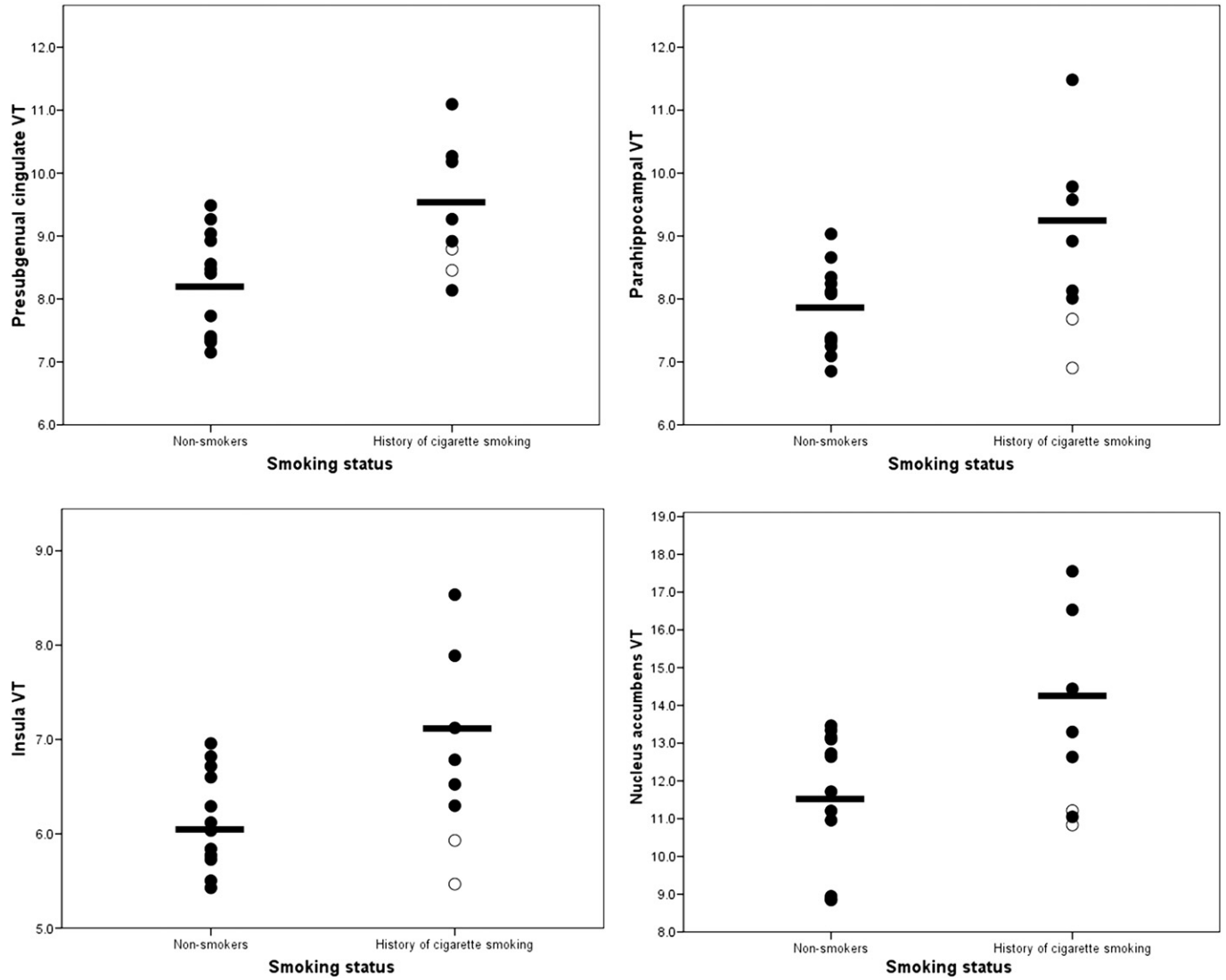

Fig. 1. Mean total [^{11}C]Ro15 4513 V_T values for the presubgenual cingulate, parahippocampal gyrus, insula and nucleus accumbens in volunteers with a history of cigarette smoking and non-smokers (bar indicates mean group value, unfilled circles indicate current smokers).

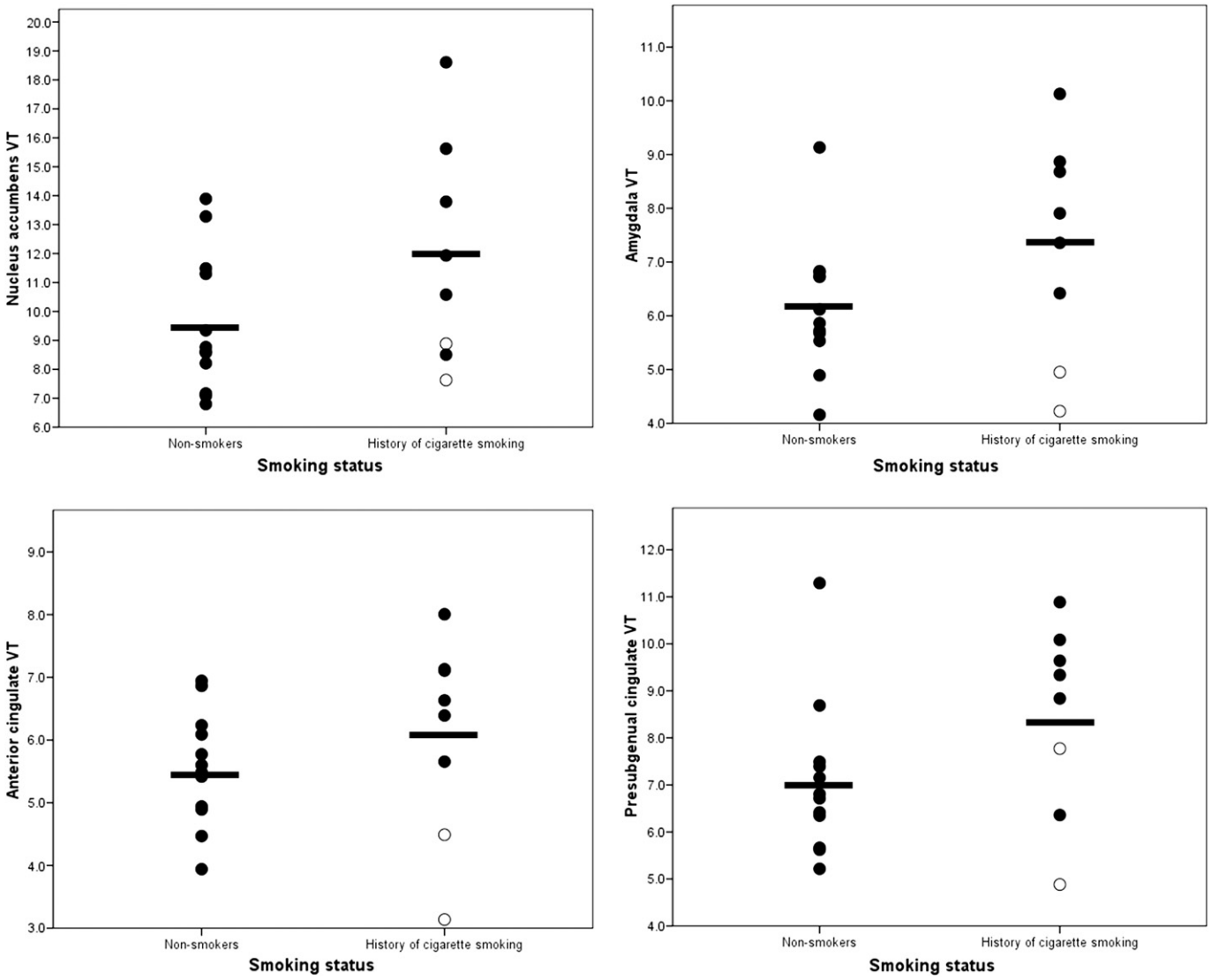

Fig. 2. Mean α5 subtype [^{11}C]Ro15 4513 V_T values for the nucleus accumbens, anterior cingulate, amygdala and presubgenual cingulate in volunteers with a history of cigarette smoking and non-smokers (bar indicates mean group value, unfilled circles indicate current smokers).

Discussion

In this study we found that volunteers with a history of cigarette smoking have higher total [^{11}C]Ro15 4513 binding, indicating higher $GABA_A$ receptor availability, in several limbic brain regions compared to non-smokers. This is the first time, to our knowledge, that differences in limbic $GABA_A$ receptor availability have been demonstrated in smokers without co-morbid alcohol dependency. Our results contrast with previous [^{123}I]iomazenil SPET studies which report no differences in $GABA_A$ availability between smokers and non-smokers or between active and abstinent smokers (Esterlis et al., 2009; Esterlis et al., 2012; Staley et al., 2005). However these studies examined cortical rather than limbic $GABA_A$ availability in smokers and included both male and female participants. The contrast in the findings of this present study with previous [^{123}I]iomazenil SPET studies may also reflect the higher resolution of PET compared to SPET imaging and the higher affinity of [^{11}C]Ro15 4513 for the $GABA_A$ α5 subtype which is particularly highly expressed in limbic regions.

We found significantly higher total $GABA_A$ receptor availability in volunteers with a history of cigarette smoking in the presubgenual cingulate and parahippocampal gyrus, and at a trend significance level in the insula, nucleus accumbens and subgenual cingulate. All of these limbic areas have been previously implicated in neuroimaging studies of nicotine addiction, providing face validity to our results. We also found no differences in availability in the superior parietal comparator region which suggests that differences in limbic availability may be specifically associated with a history of cigarette smoking. The pre/subgenual cingulate gyrus is an area involved in emotional regulation (Fan et al., 2011; Yu et al., 2011) and associated with the generation of depressive symptoms (Walter et al., 2009) which are more common in nicotine dependence (Boden et al., 2010). The pre/subgenual cingulate gyrus becomes more metabolically active when smokers are exposed to cigarette related cues (Brody et al., 2002) and is functionally connected to the anterior insula (Taylor et al., 2009). The parahippocampal gyrus plays an important role in smoking related attentional biases (Janes et al., 2010a), the insula in mediating nicotine addiction (Naqvi et al., 2007) and the accumbens in mediating smoking related reward (Brody et al., 2009; David et al., 2005) or mood changes (Montgomery et al., 2007).

Higher $GABA_A$ receptor availability in volunteers with a history of cigarette smoking may reflect higher expression of the receptor in limbic areas and/or lower endogenous GABA levels as a consequence

of the higher affinity of [^{11}C]Ro15 4513 for the receptor as it is an inverse agonist. This interpretation is supported by a previous primate autoradiographic study demonstrating that [^{11}C]Ro15 4513 binding was markedly reduced by increased GABA levels in multiple brain regions (Onoe et al., 1996). It is also possible that higher [^{11}C]Ro15 4513 binding could be a consequence of increased competition with endogenous benzodiazepines (Katsura et al., 1994).

We suggest that our data showing higher total $GABA_A$ receptor availability indicates that the limbic $GABA_A$ system is dysregulated in volunteers with a history of cigarette smoking. $GABA_A$ receptor mediated inhibition is important in regulating a number of other neurotransmitter systems. In the striatum, an area critically involved in mediating reward processes, 90–95% of all neurones consist of either GABAergic projection or interneurons (Kawaguchi et al., 1995). Although the interplay between the human striatal dopaminergic and GABAergic systems has not been as extensively studied as in the ventral tegmental area, it is likely to be similar, with the activation of GABA neurons playing a crucial role in the control of nicotine-elicited dopaminergic activity (Tolu et al., 2012). Preclinical studies indicate that activation of striatal $GABA_A$ pathways is a prerequisite for dopaminergic systems to facilitate conditioned behaviour (Dixon et al., 2010) and is also responsible for modulating the late phase of striatal dopamine release after an ethanol challenge (Lof et al., 2007). We would therefore speculate that dysregulation of GABAergic inhibition in the nucleus accumbens could result in enhanced dopamine release during cigarette smoking.

In a further exploratory analysis we found that ex-smokers show significantly higher $GABA_A$ availability compared to non-smokers in all limbic areas studied, including the presubgenual cingulate and parahippocampal gyrus. For example availability was 22% higher in the nucleus accumbens and 16% higher in the insula. Moreover higher $GABA_A$ availability in ex-smokers did not correlate with the duration of abstinence. Higher $GABA_A$ availability in ex-smokers suggest that this may be a trait marker for future cigarette smoking or that alterations in the GABAergic system as a result of cigarette smoking may not resolve with abstinence.

Interestingly, from the subtype analysis, we found that ex-smokers had significantly higher $GABA_A$ $\alpha5$ subtype availability in the amygdala, anterior cingulate, nucleus accumbens and presubgenual cingulate. We also found lower $GABA_A$ $\alpha1$ subtype availability in the nucleus accumbens and presubgenual cingulate, although these results need to be interpreted with caution due to lower [^{11}C]Ro15 4513 binding to $GABA_A$ $\alpha1$ subtype receptors in limbic regions. In animal studies, the $GABA_A$ $\alpha1$ subtype is localised within GABAergic synapses and mediates synaptic neurotransmission whereas the $GABA_A$ $\alpha5$ subtype is localised in extrasynaptic areas and mediates tonic inhibitory currents (Brunig et al., 2002; Caraiscos et al., 2004). Our subtype results therefore suggest, for the first time, that ex-smokers show differential alterations in both synaptic and extrasynaptic limbic $GABA_A$ transmission. These results however do require confirmation in further participant cohorts. There have been no published human genetic or post-mortem studies investigating the expression of $GABA_A$ $\alpha1$ or $\alpha5$ subtypes in nicotine addiction and given our findings we would suggest that this may be an important area to explore.

Higher limbic [^{11}C]Ro15 4513 binding in volunteers with a history of cigarette smoking are the reverse direction of the results from our previous study in alcohol addiction where lower limbic [^{11}C]Ro15 4513 binding was found in abstinent alcohol dependent volunteers (Lingford-Hughes et al., 2012). This suggests that lower $GABA_A$ availability, and particularly lower $GABA_A$ $\alpha5$ subtype receptor availability, cannot be a universal marker for addiction. The contrast between $GABA_A$ availability in nicotine and alcohol addiction was also reported by Staley et al. (2005) who found that cigarette smoking suppressed higher cortical $GABA_A$ receptor availability in alcohol dependent volunteers during early abstinence. Given the high rates of cigarette smoking in alcohol dependence and the reduced levels of intoxication reported by alcohol dependent smokers after an alcohol challenge (Madden et al., 1995), we would speculate from our results that cigarette smoking may reverse some of the alterations in $GABA_A$ availability found in alcohol dependency and withdrawal. We would suggest that future studies investigate the interaction between the effects of nicotine and alcohol addiction on the $GABA_A$ system.

There are a number of limitations to this present study. Firstly our sample size is relatively small, although similar in size to previous [^{123}I]iomazenil SPET studies in nicotine addiction (Esterlis et al., 2009; Staley et al., 2005). Secondly our cohort was comprised of only male participants which may restrict the generalisability of our results to male cigarette smokers only and limits comparisons to previous [^{123}I]iomazenil SPET studies where both sexes were included. Participant gender is relevant to the interpretation of our results as recently sex differences have been described both in cortical $GABA_A$ receptor availability and in the relationship between cortical $GABA_A$ receptor availability and tobacco craving (Esterlis et al., 2012). Thirdly, PET images were obtained from a database and volunteer smoking histories obtained by their self-report at the time of imaging and from retrospective questionnaire results. We were therefore not able to confirm self-reports by objective measures of nicotine dependence such as carbon monoxide monitoring and measurement of cotinine levels. Finally, there were only two current smokers in our cohort which meant that we were not able to determine whether this group was different in binding to non-smokers or abstinent previous smokers. Given these limitations, we would suggest that it is important that our findings are confirmed in further prospective [^{11}C]Ro15 4513 PET studies of nicotine use and addiction.

In summary, we found higher limbic $GABA_A$ receptor availability in volunteers with a history of cigarette smoking potentially indicating dysregulation of the limbic $GABA_A$ receptor system. These differences were more pronounced in ex-smokers and were reflected by higher $GABA_A$ $\alpha5$ availability in these volunteers. The differences in $GABA_A$ receptor availability in ex-smokers could potentially imply that limbic $GABA_A$ receptor dysregulation is either a trait marker for nicotine addiction or a persistent adaptation to cigarette smoking which does not resolve with abstinence.

Disclosure/conflict of interest

The authors declare no conflicts of interest. Dr. Stokes has shares in Smith & Nephew. Dr. Kalk has received PhD funding from GlaxoSmithKline. Prof. Nutt has served on the advisory boards for Lundbeck, Servier, Pfizer, Reckitt Benkiser, D&A Pharma, and has also received honoraria from Bristol Myers Squibb, Glaxo Smith Kline and Schering-Plough. He has received research funding from P1vital, has share options with P1vital, and receives editorial honoraria from Sage. Prof. Lingford-Hughes has received honoraria from Janssen-Cilag, Pfizer, Servier, Lundbeck and from the British Association for Psychopharmacology. She has provided consultancy to NET Device Corp, received research funding from Archimedes, Pfizer and Schering, and holds research grants with GlaxoSmithKline. Profs. Lingford-Hughes and Nutt are both members of the Lundbeck International Neuroscience Foundation. Ms Benecke, Mr. Myers, Dr. Watson, Dr. Erritzoe, Dr. Riano Barros and Prof. Hammers have no financial interests to declare.

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