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Evaluation of (–)-[¹⁸F]Flubatine-specific binding: Implications for reference region approaches

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Abstract

We aimed to characterize changes in binding of (–)-[¹⁸F]Flubatine to $\alpha_4\beta_2^*$ -nicotinic acetylcholine receptors ($\alpha_4\beta_2^*$ -nAChRs) during a tobacco cigarette smoking challenge. Displacement of (–)-[¹⁸F] Flubatine throughout the brain was quantified as change in (–)-[¹⁸F]Flubatine distribution volume (V_T), with particular emphasis on regions with low V_T . Three tobacco smokers were imaged with positron emission tomography (PET) during a 210 min bolus-plus-constant infusion of (–)-[¹⁸F]Flubatine. A tobacco cigarette was smoked in the PET scanner ~125 min after the start of (–)-[¹⁸F] Flubatine injection. Equilibrium analysis was used to estimate V_T at baseline (90–120 min) and after cigarette challenge (180–210 min), at the time of greatest receptor occupancy by nicotine. Smoking reduced V_T by $21 \pm 9\%$ (average $\pm SD$) in corpus callosum, $17 \pm 9\%$ in frontal cortex, $36 \pm 11\%$ in cerebellum, and $22 \pm 10\%$ in putamen. The finding of displaceable (–)-[¹⁸F]Flubatine binding throughout the brain is an important consideration for reference region-based quantification approaches with this tracer. We observed displacement of (–)-[¹⁸F]Flubatine binding to $\alpha_4\beta_2^*$ -nicotinic acetylcholine receptors in corpus callosum by a tobacco cigarette challenge. We conclude that reference region approaches utilizing corpus callosum should first perform careful characterization of displaceable (–)-[¹⁸F]Flubatine binding and nondisplaceable kinetics in this putative reference region.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Keywords

$\alpha_4\beta_2^*$ -nicotinic acetylcholine receptors; positron emission tomography; reference region; tobacco smoking; white matter

1. | INTRODUCTION

In vivo imaging of $\alpha_4\beta_2$ -containing nicotinic acetylcholine receptors ($\alpha_4\beta_2^*$ -nAChRs) with positron emission tomography (PET) has important clinical applications for studying addiction and other neuropsychiatric disorders (Cosgrove et al., 2009; Esterlis et al., 2014; Hannestad et al., 2013; Saricicek et al., 2012). (–)-[¹⁸F]Flubatine is an effective PET tracer for in vivo imaging of $\alpha_4\beta_2^*$ -nAChRs in nonhuman primates (Bois et al., 2015) and was shown to have favorable kinetic properties in humans (Sabri et al., 2015).

Previous PET studies quantified (–)-[¹⁸F]Flubatine binding with distribution volume (V_T), using the gold standard of measuring radiotracer concentrations in arterial plasma for kinetic modeling analysis. Reference tissue modeling approaches eliminate the need for invasive arterial sampling, simplifying logistical and analytic aspects of outcome parameter estimation. An ideal reference region contains negligible amounts of specifically bound radiotracer, and is used as a proxy of nondisplaceable radiotracer kinetics in target regions. A previous study of nicotine occupancy in nonhuman primates with (–)-[¹⁸F]Flubatine observed that V_T in cerebellum, a potential reference region, was greater than nondisplaceable volume of distribution (V_{ND}), suggesting specific binding in cerebellum (Bois et al., 2015). To date, however, there has been no study in humans assessing (–)-[¹⁸F]Flubatine reference region approaches by systematically examining specific binding throughout the brain.

The goal of this study was to evaluate displaceable binding of (–)-[¹⁸F]Flubatine in multiple brain regions in humans. We estimated the displacement of (–)-[¹⁸F]Flubatine by measuring the change in V_T across several regions before and after a tobacco cigarette challenge. Previous studies have shown that nicotine delivered from smoking cigarettes (number of cigarettes varying from 1.5 to 4) occupies from 55% to 95% of $\alpha_4\beta_2^*$ -nAChRs throughout the brain (Brody et al., 2006; Esterlis et al., 2010) and can be used to measure nondisplaceable binding. We had particular interest in whether the corpus callosum, a region previously found to have a low volume of distribution (V_T) for (–)-[¹⁸F]Flubatine (Sabri et al., 2015), may also have negligible or low specific binding and therefore be suitable as a reference region. To further characterize (–)-[¹⁸F]Flubatine binding profile across regions, measured V_T values were compared to values of V_{ND} in each subject, which were estimated from Lassen plots (Cunningham, Rabiner, Slifstein, Laruelle, & Gunn, 2010).

2. | MATERIALS AND METHODS

2.1. | Subjects

Two male (51 years and 55 years) and one female (27 years) tobacco smokers participated in the study. Smoking status was confirmed with average ($\pm SD$) CO levels of 30.0 ± 28.4 ppm and urine cotinine levels of 783.3 ± 375.3 ng/mL on the day of screening. Smokers were

moderately dependent on nicotine (scores of 5.3 ± 2.5 on the Fagerström Test for Nicotine Dependence (FTND)) (Heatherton, Kozlowski, Frecker, & Fagerstrom, 1991). A physical examination, routine blood tests, medical and psychiatric histories, drug screen (urine), ECG, and a pregnancy test (for female participants) were performed as part of the subject screening and were found to be normal or not clinically significant to continue. Screening was completed using the Structured Clinical Interview for the DSM-IV to rule out Axis I disorders other than nicotine dependence. Exclusion criteria also included prescription or illegal psychotropic medication use within the past year and >21 alcoholic drinks per week for women and 35 drinks per week for men. Subjects were instructed to abstain from cigarettes and any form of nicotine, for example, nicotine replacement therapy, e-cigarettes, for 5 consecutive days before their scan. Abstinence was confirmed with CO levels <10 ppm and urine cotinine levels of <100 ng/mL on all 5 days prior to scan and on scan day.

2.2. | Imaging

Structural magnetic resonance imaging (MRI) was performed on a 3-T Trio system (Siemens, Erlangen, Germany) for purposes of excluding individuals with brain abnormalities, anatomically co-registering with PET images, and delineation of regions of interest. (–)-[¹⁸F]Flubatine was prepared as previously described (Bois et al., 2015). (–)-[¹⁸F]Flubatine (199.6 ± 74.5 MBq; 0.38 ± 0.26 mg/kg) was injected as a bolus plus constant infusion (B/I) for 210 min with K_{BoI} 5360 min. A 6-min transmission scan was acquired for attenuation correction after the PET scan. PET data were acquired in list mode with a high-resolution research tomograph (HRRT; Siemens/CTI) started 90 min after the beginning of radiotracer administration, the time when tissue radiotracer levels reach equilibrium (Hillmer et al., 2016), and continued for an additional 120 min. Subjects smoked a cigarette of their preferred brand starting at 125 min after radiotracer injection, taking 1 puff every 30 s for 5 min while in the scanner, following a previously described paradigm (Morris et al., 2013). Arterial blood samples were acquired to measure the parent input function as previously described (Hillmer et al., 2016). Briefly, discrete blood samples were drawn from the radial artery at 90, 105, 120, 135, 150, 165, 180, 195, and 210 min following radiotracer injection. Plasma was extracted following centrifugation and assayed for radioactivity. The parent fraction was determined by column-switching high-performance liquid chromatography (Hilton et al., 2000) of discrete arterial samples at 90, 120, 180, and 210 and fit with an inverted gamma function. The parent (–)-[¹⁸F]Flubatine input function was calculated as the product of radioactivity concentration in plasma and parent fraction of radiotracer in plasma. The plasma-free fraction was also measured in arterial blood samples taken immediately before radiotracer injection.

PET data were reconstructed with ordered-subsets expectation maximization-based MOLAR algorithm correcting for motion on the event level (Polaris Vicra Tracking System, Northern Digital) and for scatter, attenuation, dead-time, normalization, scanner geometry, and point-spread function (Carson et al., 2003). Emission data were binned into 5-min frames. PET images were aligned to the subject's MRI and then to a standard MRI template. Regional TACs were computed using the AAL atlas (Tzourio-Mazoyer et al., 2002). Regions defined included the cerebellum (83.9 mm³), frontal (255.9 mm³), temporal (172.4 mm³), occipital (80.6 mm³), and parietal (64.6 mm³) cortices, hippocampus (15.0 mm³), caudate (15.7

mm³), and putamen (16.6 mm³). These delineated regions were comprised of predominantly gray matter and are hereafter referred to as gray matter regions. The thalamus was not included since equilibrium is not reached in this region with the given infusion protocol. The corpus callosum region of interest (11.5 mm³) was manually drawn on consecutive sagittal slices of the same human MRI template and a subject-specific white matter mask was applied to compute the regional TAC (Figure 1a).

2.3. | Analysis

The outcome measure of (–)-[¹⁸F]Flubatine V_T was estimated in target regions with V_T defined as the ratio at equilibrium of total radioactivity concentration in tissue to parent radiotracer in arterial plasma (Innis et al., 2007). Previous studies have shown that bolus plus constant infusion of (–)-[¹⁸F]Flubatine (K_{BoI} 5360 min) achieved equilibrium in most gray matter regions of interest by 90 min (Hillmer et al., 2016) motivating the use of the equilibrium analysis method here. Quality of equilibrium was evaluated as percent increase in tissue activity of (–)-[¹⁸F]Flubatine per hour. Tissue concentrations were calculated as the average of activities from two 5-min frames before and after each arterial sample time point (Carson et al., 1997). $V_{T \text{ baseline}}$ was measured as an average from 90 to 120 min and $V_{T \text{ postcigarette}}$ was measured as an average from 180 to 210 min.

Displacement was estimated as the percent difference in V_T from baseline to after cigarette smoking. Occupancy by nicotine and nondisplaceable binding (V_{ND}) were estimated from Lassen plots (Cunningham et al., 2010) including all identified gray matter regions.

To estimate the bias associated with a reference region approach, we calculated the percent difference in BP_{ND} resulting from two different V_{ND} estimates. BP_{ND} was calculated across gray matter regions as $(V_{T \text{ baseline}} - 2 V_{ND})/V_{ND}$, first with V_{ND} estimated from a Lassen plot and second with V_{ND} estimated as $V_{T \text{ baseline}}$ in corpus callosum as a putative reference region.

3. | RESULTS

Equilibrium quality was good; average percent increase per hour ($\pm SD$) in radioactivity across subjects was $3.8\% \pm 2.6\%$ in gray matter and $1.0\% \pm 11.5\%$ in metabolite-corrected plasma assessed at 90–120 min. Percent increase per hour in corpus callosum was $13.5\% \pm 10.2\%$ at 90–120 min. The quality of the new equilibrium achieved after cigarette challenge from 180 to 210 min was also assessed. Percent increase per hour ($\pm SD$) in activity at 180–210 min was $3.6\% \pm 3.4\%$ averaged across gray matter regions, suggesting that a new equilibrium was established at this time after cigarette smoking, and $5.4\% \pm 15.4\%$ in corpus callosum. Percent increase per hour in metabolite-corrected radiotracer concentration at 180–210 min as an average ($\pm SD$) across subjects was $6.7\% \pm 9.2\%$.

Time–activity curves of corpus callosum and cerebellum from a representative subject (Figure 1b) demonstrated a 31.5% and 48.5% decrease in (–)-[¹⁸F]Flubatine activity after the cigarette challenge, in each region respectively. Across all three subjects, the average ($\pm SD$) of $V_{T \text{ baseline}}$ at 90–120 min in corpus callosum was $6.7 \pm 1.1 \text{ mL/cm}^3$. The cigarette challenge reduced (–)-[¹⁸F]Flubatine V_T throughout the brain for all three subjects at 180–

210 min (Table 1). Percent reduction from V_T baseline to V_T post-cigarette was $21.4\% \pm 9.3\%$ in corpus callosum, $17.3\% \pm 8.7\%$ in frontal cortex, $36.1\% \pm 11.1\%$ in cerebellum, and $22.3\% \pm 10.1\%$ in putamen across three subjects (Figure 1c).

V_{ND} and occupancy were calculated for each subject as the x -intercept and slope, respectively, of the Lassen plot (Cunningham et al., 2010) (Supporting Information, Figure 1). V_T baseline of corpus callosum (6.7 ± 1.1 mL/cm³) was not significantly different from the average V_{ND} of 6.9 ± 0.9 mL/cm³ across subjects. Occupancy ranged from 55.2% to 93.7% across the three subjects.

BP_{ND} values estimated using V_T from corpus callosum were lower in Subjects 1 and 2 and greater in Subject 3 than BP_{ND} values estimated using V_{ND} from Lassen plots, with percent underestimation ranging from 210.5% in cerebellum to 245.7% in temporal cortex between Subjects 1 and 2 (Supporting Information, Table 1).

4. | DISCUSSION

In this study, we evaluated the extent of specific (–)-[¹⁸F]Flubatine binding throughout the brain with a cigarette smoking challenge. We measured an average ($\pm SD$) $21.4\% \pm 9.3\%$ decrease in (–)-[¹⁸F]Flubatine V_T following cigarette challenge in corpus callosum, the region with lowest observed V_T at baseline. The observed change in V_T in the corpus callosum following a cigarette challenge suggests significant specific binding in this region. The average ($\pm SD$) decrease of $36.1\% \pm 11.1\%$ in cerebellum V_T is consistent with observed differences in cerebellum specific binding across species from previous studies using other $\alpha_4\beta_2^*$ -nAChR radiotracers (Chefer et al., 2003; Lotfipour, Mandelkern, Alvarez-Estrada, & Brody, 2012; Mukhin et al., 2008).

Reference region approaches for estimating binding potential rely on the following equation:

$$BP_{ND} = (V_T - V_{ND})/V_{ND} \quad 1$$

and implicitly assume that the distribution volume in the reference region is representative of nondisplaceable radiotracer (i.e., $V_{T(ref)} \approx V_{ND}$). If this assumption is not met, then the biased estimation of BP_{ND} becomes

$$BP_{ND} = (V_T - V_{T(ref)})/V_{T(ref)} \quad 2$$

and the estimated value of BP_{ND} will be less than the true value since $V_{T(ref)} > V_{ND}$. Moreover, if the specific binding distribution volume in an imperfect reference region ($V_{S(ref)}$) is affected differently between the population groups or states studied, this will bias subsequent statistical analyses. Here, the $21.4\% \pm 9.3\%$ decrease in (–)-[¹⁸F]Flubatine V_T following cigarette challenge in corpus callosum suggest that analysis approaches using this region as a reference is subject to these effects. Indeed, we estimate that quantification of

(-)-[¹⁸F]Flubatine BP_{ND} using corpus callosum as a reference region produced an underestimation of 36.6% ± 6.8% in Subject 1 and 15.0% ± 3.6% in Subject 2.

A further assumption of reference region estimation of BP_{ND} is that values of V_{ND} are comparable between the reference and target regions (i.e., $V_{ND(ref)} \approx V_{ND(ROI)}$). However, it is possible that differences in vascularization or tissue composition affect the regional ratios of transfer of parent radiotracer in plasma to and from the non-displaceable compartment (i.e., K_1/k_2), particularly in white versus gray matter. The observation that corpus callosum was not characterized by the same linear occupancy relationship as other regions, that is, these points did not stay on the occupancy line (Supporting Information, Figure 1) suggests that the nondisplaceable properties of (-)-[¹⁸F]Flubatine may be different between corpus callosum and gray matter regions. $V_{T \text{ postcigarette}}$ values in corpus callosum were 15.4%–32.5% lower than Lassen plot-estimated V_{ND} values across subjects. These observations are consistent with differences in (-)-[¹⁸F]Flubatine nondisplaceable properties between white and gray matter regions, specifically, that $V_{ND(WM)} < V_{ND(GM)}$. Interestingly, combined with the presence of specific binding in corpus callosum, the net effect is V_T values in corpus callosum that were similar to the estimated V_{ND} in gray matter regions. Taken together, these findings indicate that careful consideration must be used if white matter is used as a reference region with (-)-[¹⁸F]Flubatine.

One limitation of this study was the small sample size. Additionally, in Subject 3, the rate of change of (-)-[¹⁸F]Flubatine tissue activity in corpus callosum during the 90–120 min equilibrium period was 16.3% per hour, indicating equilibrium was not yet established in this region. The lack of equilibrium in this region at 90–120 min likely caused underestimation of baseline V_T estimates, which would quantitatively underestimate (-)-[¹⁸F]Flubatine displacement and occupancy. Future studies designed to measure (-)-[¹⁸F]Flubatine V_T in white matter following radiotracer infusion should make the baseline measurement at steady state, which is later than the 90–120 min period optimized for gray matter regions. In addition, we observed a range of receptor occupancies (55.2%–93.7%) across the three subjects due to smoking a single cigarette. This variation could be due to bias in the $V_{T(postcigarette)}$ measurements if new equilibrium was not yet established, or individual differences in smoking topography such as puff volume, which were not controlled.

The definition of white matter regions is another important consideration for this work. Although we used corpus callosum as the primary white matter region, we conducted an exploratory analysis in the region of centrum semiovale, which yielded an average decrease of (-)-[¹⁸F]Flubatine V_T of 24.4 ± 10.3% following cigarette smoking. This observation suggests that the corpus callosum is representative of white matter regions in the context of (-)-[¹⁸F]Flubatine kinetics. Another possible consideration is that V_T measurements in corpus callosum could be affected by partial volume spill-in from surrounding gray matter regions, which we minimized by white matter masking of corpus callosum.

These findings corroborate growing evidence to suggest measureable amounts of $\alpha_4\beta_2^*$ -nAChRs in white matter, which may even serve a functional role. Studies in mice provide evidence for the presence of $\alpha_4\beta_2^*$ -nAChRs on myelinated axons of thalamocortical tracts

with the observed function of increasing the axonal excitability of auditory thalamocortical projections in response to endogenous acetylcholine (Kawai, Lazar, & Metherate, 2007). In addition, Mukhin et al. (2008) measured higher $\alpha_4\beta_2^*$ -nAChR availability with the PET radiotracer 2-[^{18}F]-A-85380 (2-FA) across regions in smokers relative to nonsmokers, suggesting upregulation of $\alpha_4\beta_2^*$ -nAChRs in smokers, with the greatest increase in $\alpha_4\beta_2^*$ -nAChR availability being observed in corpus callosum. Our observation that corpus callosum V_T postcigarette was lower than V_{ND} in gray matter regions is consistent with previous work. Brody et al. (2006) suggested that postcigarette levels of 2-FA radioactivity in target regions were higher than postcigarette levels in corpus callosum. Additionally, specific binding to $\alpha_4\beta_2^*$ -nAChRs in corpus callosum was estimated as 30% of 2-FA total distribution volume in a paradigm using a combination of low-dose varenicline and smoking to satiety to occupy 2-FA binding sites (Lotfipour et al., 2012).

In conclusion, this study demonstrates the need for careful characterization of specific binding and nondisplaceable kinetics in a putative reference region. Our observation of displaceable ($-$)-[^{18}F]Flubatine binding in corpus callosum supplements the existing literature on $\alpha_4\beta_2^*$ -nAChR binding in corpus callosum, indicating limitations for use of corpus callosum and other white matter reference regions for quantification of ($-$)-[^{18}F]Flubatine-specific binding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Changes in $\alpha_4\beta_2^*$ -nAChR availability in corpus callosum before and after smoking one cigarette. (a) Region of interest for corpus callosum indicated in green on sagittal section of human MRI template. (b) Time activity curves in corpus callosum and cerebellum of $\alpha_4\beta_2^*$ -nAChR availability in Subject 1. Cigarette was given at 125 min as indicated by the red arrow. (c) Decrease in $\alpha_4\beta_2^*$ -nAChR availability after cigarette challenge (average of 180–210 min) from baseline (average of 90–120 min) in all three subjects in corpus callosum ($21.4\% \pm 9.3\%$) and representative regions including frontal cortex ($17.3\% \pm 8.7\%$), cerebellum ($36.1\% \pm 11.1\%$), and putamen ($22.3\% \pm 10.1\%$)

TABLE 1

V_T values (mL/cm^3) before and after cigarette smoking in 9 regions and V_{ND} and Occupancy estimated from V_T values

		Subject 1	Subject 2	Subject 3	Mean ($\pm SD$)
Corpus callosum	Baseline V_T	8.0	6.2	5.9	6.7 ± 1.1
	Postcigarette V_T	5.5	4.9	5.2	5.2 ± 0.3
Cerebellum	Baseline V_T	14.4	11.2	11.9	12.5 ± 1.6
	Postcigarette V_T	7.4	7.6	8.7	7.9 ± 0.7
Frontal cortex	Baseline V_T	10.1	9.2	8.7	9.3 ± 0.7
	Postcigarette V_T	7.4	7.7	7.9	7.7 ± 0.3
Temporal cortex	Baseline V_T	9.4	8.7	8.3	8.8 ± 0.6
	Postcigarette V_T	7.1	7.4	8.0	7.5 ± 0.4
Occipital cortex	Baseline V_T	10.2	8.8	8.6	9.2 ± 0.9
	Postcigarette V_T	7.6	7.5	8.1	7.8 ± 0.3
Parietal cortex	Baseline V_T	10.2	8.5	8.9	9.2 ± 0.9
	Postcigarette V_T	7.7	7.2	8.2	7.7 ± 0.5
Caudate	Baseline V_T	9.9	8.3	8.2	8.8 ± 1.0
	Postcigarette V_T	6.7	6.5	7.3	6.8 ± 0.4
Putamen	Baseline V_T	11.1	11.1	10.0	10.7 ± 0.6
	Postcigarette V_T	7.5	8.6	8.8	8.3 ± 0.7
Hippocampus	Baseline V_T	10.0	7.8	8.1	8.6 ± 1.2
	Postcigarette V_T	6.9	6.3	7.7	6.9 ± 0.7
V_{ND}		7.1	5.9	7.6	6.9 ± 0.9
Occupancy (%)		93.7	55.2	69.5	72.8 ± 19.5