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Imaging dopamine receptors in humans with [^{11}C]-(+)-PHNO: Dissection of D3 signal and anatomy

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ABSTRACT

[^{11}C]-(+)-PHNO is a D3 preferring PET radioligand which has recently opened the possibility of imaging D3 receptors in the human brain *in vivo*. This imaging tool allows characterisation of the distribution of D3 receptors *in vivo* and further investigation of their functional role. The specific [^{11}C]-(+)-PHNO signal is a mixture of D3 and D2 components with the relative magnitude of each component determined by the regional receptor densities. An accurate and reproducible delineation of regions of interest (ROI) is therefore important for optimal analysis of human PET data. We present a set of anatomical guidelines for the delineation of D3 relevant ROIs including substantia nigra, hypothalamus, ventral pallidum/substantia innominata, ventral striatum, globus pallidus and thalamus. Delineation of these structures using this approach allowed for high intra- and inter-operator reproducibility. Subsequently we used a selective D3 antagonist to dissect the total [^{11}C]-(+)-PHNO signal in each region into its D3 and D2 components and estimated the regional fraction of the D3 signal (f^{D3}_{PHNO}). In descending order of magnitude the following results for the f^{D3}_{PHNO} were obtained: hypothalamus = 100%, substantia nigra = 100%, ventral pallidum/substantia innominata = 75%, globus pallidus = 65%, thalamus = 43%, ventral striatum = 26% and precommissural-ventral putamen = 6%. An automated approach for the delineation of these anatomical regions of interest was also developed and investigated in terms of its reproducibility and accuracy.

Introduction

Since its discovery, dopamine has been implicated in the control of movement and cognition and has also emerged as a key factor in diverse brain diseases such as Parkinson's disease, schizophrenia and drug addiction (Cumming, 2009). Dopaminergic neurotransmission is transducted via five G-protein-coupled receptors (GPCRs), dopamine-D_1 (D_1R) to dopamine-D_5 (D_5R). The dopamine receptors (DRs) constitute two families, the D_1-like (D_1R and D_5R) and the D_2-like (D_2R, D_3R and D_4R) family. The D_2-like family of receptors are coupled to G_i/G_o G-proteins (as opposed to the G_s coupling of the D_1-like family), and the density of expression of D_2R is significantly higher than that of D_3R and D_4R. The D_3R were first characterised in 1990 by Sokoloff et al. (1990). Their distribution in brain areas linked with functional aspects of motivation and reward made them an attractive target for the treatment of addictive disorders.

Studies exploring the distribution of the D_3 receptors in the human brain using post-mortem tissue (Murray et al., 1994; Staley and Mash, 1996; Gurevich and Joyce, 1999) report a declining rostral to caudal gradient of D_3R in the striatum as well as the existence of D_3R in some extra-striatal locations, such as the substantia nigra (SN), and thalamus (TH). In the striatum, the D_3R are particularly enriched in the nucleus accumbens (NAC) and the precommissural ventral putamen (preVPU). The presence of D_3R sites and mRNA was demonstrated in the pallidum (Murray et al., 1994; Gurevich and Joyce, 1999) with the highest concentrations found in the internal segment of the globus pallidus (GP) and the ventral pallidum (VP). Gurevich and Joyce (1999) and Staley and Mash (1996) also documented the existence of D_3 receptors in the anterior ventral thalamus and throughout the hypothalamus (Hypo) though at considerably lower levels than in the striatum and pallidum. Gurevich stressed the relative abundance of the D_3 sites in the mammilothalamic tract (MMT) and the mammilary bodies (MB) of hypothalamus.

Despite these in-vitro findings, the in-vivo examination of D_3R has been limited due to the lack of a selective PET ligand. The introduction of [^{11}C]-(+)-PHNO (Wilson et al., 2005) has opened the possibility of imaging the D_3R. [^{11}C]-(+)-PHNO was initially introduced as a potent

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agonist radioligand suitable for imaging the high affinity state of the D_2R (D_2^{high}) (Willeit et al., 2006). At the same time, Narendran and colleagues (2006) demonstrated that [^{11}C]-(+)-PHNO is a D_3 preferring radioligand, using the D_3 preferring blocker BP-897 in non-human primates. Further characterisation of the binding of [^{11}C]-(+)-PHNO *in vivo* in non-human primates and D_2R and D_3R knock-out mice confirmed the preferential selectivity of [^{11}C]-(+)-PHNO for D_3R over D_2R, and characterised the D_2R and D_3R components of [^{11}C]-(+)-PHNO binding in different brain areas (Rabiner et al., 2009). A formal investigation of the relative D_2R and D_3R affinities of [^{11}C]-(+)-PHNO *in vivo* in the primate brain found a ~20-fold selectivity for D_3R over D_2R (Gallezot et al., 2009). In humans, using selective D_3R antagonists, the [^{11}C]-(+)-PHNO binding profile has been shown to be consistent with that found in non-human primates. (Searle et al., 2010).

The [^{11}C]-(+)-PHNO binding is a mixture of D_3 and D_2 components whose relative contribution varies regionally. An accurate and reproducible delineation of regions of interest (ROIs) is therefore important for optimal analysis. The aim of this present study is to optimise the methodology for the analysis of human [^{11}C]-(+)-PHNO studies and to explore the D_3R distribution in the human brain *in vivo*. Firstly, we present a set of criteria for the anatomical delineation of D_3 relevant ROIs. Secondly, manual and automated approaches for the delineation of these ROIs are investigated in terms of their reproducibility and accuracy. Thirdly, the regional [^{11}C]-(+)-PHNO signal is dissected into its D_3 and D_2 components using a selective D_3 antagonist.

Materials and methods

Subjects

Nineteen healthy male volunteers, free from clinically significant illness or disease as determined by their medical history and standard laboratory tests, had [^{11}C]-(+)-PHNO PET and MRI scans. All PET scans were conducted at the Centre of Addiction and Mental Health in Toronto, Canada. Subjects were scanned at baseline and following the administration of a selective D_3 blocker (GSK598809) at doses of 5 mg to 175 mg. GSK598809 is a selective D_3R antagonist which exhibits a 500-fold selectivity for the D_3R over the D_2R in vitro (Searle et al., 2010) The data presented here are obtained from the cohort described previously by Searle et al. (2010).

PET data

In total, 48 PET scans were acquired on a Siemens Biograph HiRez XVI PET tomograph (Siemens Healthcare). Subjects were positioned in the scanner and movement was minimised by using a custom-made thermoplastic facemask together with a head-fixation system (Tru-Scan Imaging, Annapolis, Maryland). Subjects were injected with a single intravenous bolus of [^{11}C]-(+)-PHNO between 235 and 368 MBq (mean = 307.2, SD = 40.6) with specific activity ranging between 23.3 and 38.6 GBq/μmol (mean = 32.24, SD = 4.58) at the time of injection. The mass of injected [^{11}C]-(+)-PHNO was between 2 and 2.6 μg (mean = 2.36, SD = 0.13) and the radiochemical purity of the compound was 97.7–99.9% (mean = 99.4, SD = 0.6). Following bolus administration of [^{11}C]-(+)-PHNO, dynamic emission data were acquired for 90 minutes (1×30s, 8×15 s, 3×1 min, 5×2 min, 5×5 min, and 5×10 min). A low-dose CT scan (effective dose = 0.2 mSv) was also acquired and used for attenuation correction and model-based scatter correction. The dynamic images were reconstructed using Fourier rebinning and a 2D filtered back projection algorithm with a ramp filter at Nyquist cut off frequency (Defrise et al., 1997).

MRI data

T1-weighted and proton density (PD) MRI images were acquired on a GE Medical system Signa EXCITE HD, 1.5 T. The structural T1-weighted images (acquired in the axial plane: FSPGR-IR PREPPED, TR = 12.008, TE = 5.1160, flip angle = 20°, slice thickness = 0.78 × 0.78 × 1.5 mm) were acquired to aid in the definition of the subcortical ROIs. The PD images (FSE PD, TR = 6000, TE = 12.1920, flip angle = 90°, slice thickness = 0.86 × 0.86 × 3 mm) were acquired to help define the size and orientation of the SN ROI.

Image analysis

The MR and PET images of each subject underwent a series of spatial processing steps. The non-brain voxels from the MR images were removed using the FSL Brain Extraction Tool (Smith, 2002) and the extracted brain was rigidly registered to the nonlinear ICBM152 template (http://www2.bic.mni.mcgill.ca/) (MNI space) using SPM5 (Wellcome Trust Centre for Neuroimaging) to bring subjects into a similar orientation and facilitate manual ROI delineation. PET images were corrected for motion by realigning each frame to a reference frame, using mutual information as a cost function, and then these were registered to the T1-MRI.

Regional analysis was performed with both manual and automatically delineated ROIs (see below MAN I, MAN II and AUTO). For the manual ROI delineation, the MRI was made isotropic to the smallest voxel size of the original image (0.79 × 0.79 × 0.79 mm) in order to obtain the best possible visual quality. For the automated method, the nonlinear ICBM152 template was non-linearly warped with SPM5 (Wellcome Trust Centre for Neuroimaging) to the high-resolution T1-MRI of each individual. The deformation parameters derived were then applied to an anatomical atlas (detailed below) to bring this into the individual subject's space. Finally, the MRI image, the ROIs and the warped anatomical atlas were resampled to match the PET image resolution (2 × 2 × 2 mm).

Derivation of the parameters of interest—BP_{ND} and D3 contribution to the total [^{11}C]-(+)-PHNO specific signal (f_{PHNO}^{D3})

[^{11}C]-(+)-PHNO binding potential (BP_{ND}) estimates were obtained by three separate analysis approaches: (A1) ROIs were applied to the dynamic PET data to derive regional time activity curves (TACs) before applying the simplified reference tissue model (SRTM) (Lammertsma and Hume, 1996), with the cerebellum as a reference region, to derive regional BP_{ND} estimates. (A2) The basis function implementation of SRTM (Gunn et al., 1997) was applied to the dynamic PET data to derive parametric images of BP_{ND} (parameter bounds in terms of non-decay corrected data: $\theta_3^{min} = 0.0008\ s^{-1}$ and $\theta_3^{max} = 0.5\ s^{-1}$; cf. Gunn et al., 1997). ROIs were then applied to the BP_{ND} images to derive regional BP_{ND} estimates. (A3) The parametric BP_{ND} images generated in A2 were nonlinearly warped onto the ICBM152 template and then ROIs defined on this template were applied to derive regional BP_{ND} estimates.

Using a single site competition model for the binding of [^{11}C]-(+)-PHNO (Searle et al., 2010), the BP_{ND} obtained following administration of the selective D_3 antagonist, GSK598809, can be described by:

$$BP_{ND}^{GSK598809} = BP_{ND}^{Baseline}\left[\frac{f_{PHNO}^{D3}}{1+\frac{C_P^{GSK598809}}{EC_{50}^{D3}}} + \left(1-f_{PHNO}^{D3}\right)\right] \quad (1)$$

where $BP_{ND}^{Baseline}$ and $BP_{ND}^{GSK598809}$ are the BP_{ND} values at baseline and following dosing with GSK598809, respectively, $C_P^{GSK598809}$ is the plasma concentration of GSK598809 and EC_{50}^{D3} is the half saturation concentration of GSK598809. Regional f_{PHNO}^{D3}were obtained by fitting the competition model in Eq. (1) to the BP_{ND} values obtained from methods A1 and A2 under the assumption that EC_{50}^{D3} was constant across all regions. All regional f_{PHNO}^{D3} derived from regional and parametric analyses were obtained from manually delineated ROIs