Neuroscience 235 (2013) 226–231

GREY MATTER DENSITY AND $GABA_A$ BINDING POTENTIAL SHOW A POSITIVE LINEAR RELATIONSHIP ACROSS CORTICAL REGIONS

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Abstract—Voxel based morphometry (VBM) is a widely used technique for studying the structure of the brain. Direct comparisons between the results obtained using VBM and the underlying histology are limited, however. To circumvent the problems inherent in comparing VBM data *in vivo* with tissue samples that must generally be obtained post-mortem, we chose to consider $GABA_A$ receptors, measured using ^{18}F-flumazenil PET (18F-FMZ-PET), as non-invasive neural markers to be compared with VBM data. Consistent with previous cortical thickness findings, $GABA_A$ receptor binding potential (BP_{ND}) was found to correlate positively across regions with grey matter (GM) density. These findings confirm that there is a general positive relationship between MRI-based GM density measures and $GABA_A$ receptor BP_{ND} on a region-by-region basis (i.e., regions with more GM tend to also have higher BP_{ND}).

Key words: VBM, receptor density, benzodiazepine receptor, flumazenil, anatomical imaging, brain morphometry.

INTRODUCTION

Voxel-based morphometry (VBM) (Ashburner and Friston, 2000) is a widely used MRI technique for studying brain structure. It has been used in many different research contexts, including social neuroscience (Kanai et al., 2011), memory (Kanai and Rees, 2011), depression (Bora et al., 2011), and Alzheimer's disease (Ferreira et al., 2011). However, the relationship between VBM measures and actual neural structure has not been well defined.

One approach to linking VBM measures and the underlying histology would be to compare *in vivo* MRI scans with post-mortem histology. However, given the obvious practical issues involved in such an approach, a non-invasive measure of neural structure is required that can then be compared with VBM data. In this context it has been suggested that ^{18}F-flumazenil PET (Ryzhikov et al., 2005) (18F-FMZ-PET) could be utilised as an indicator for neuronal density and integrity (Heiss et al., 1998; Hammers et al., 2001; la Fougère et al., 2011). This technique allows the imaging of $GABA_A$ receptors *in vivo* in humans (Hammers et al., 2003; Salmi et al., 2008) as flumazenil binds to the benzodiazepine site on the $GABA_A$ receptor (Sigel, 2002). With the $GABA_A$ receptor found widely across the human cortex, playing a major role in neural inhibition, the inference can potentially be drawn between $GABA_A$ receptor density and the density of neurons.

Adapting this approach, la Fougère et al. (2011) compared cortical thickness, as obtained through the analysis of MRI images, with $GABA_A$ receptor density, finding a correlation between these measures across the regions studied. This suggests that the link between $GABA_A$ receptors and morphological measures is valid in the context of cortical thickness analyses. It is not clear, however, if these findings can be extended to VBM.

We thus compared $GABA_A$ receptor density and VBM measures to establish whether this approach can be applied to VBM also. It was hypothesised that receptor density would correlate with VBM measurements across cortical regions. As a second, exploratory, aim we also investigated whether grey matter (GM) measurements within individual regions of interest correlated with $GABA_A$ receptor density within the same region.

EXPERIMENTAL PROCEDURES

Participants

Twenty-five healthy subjects (10 female; mean age 22.67 years, range 18–32 years) underwent both PET and MRI scanning. PET (Montreal Neurological Institute, McGill) and MRI (Unité de neuroimagerie fonctionelle, Université de Montréal) scans took place on different days (mean time between scans = 2.44 days, range = 1–6 days). PET scan sessions began at either 11 am or 1 pm; MRI scans were made at approximately 3 pm. Subjects were screened for psychiatric or neurological disorders, recreational drug use, and depression, the latter using the Beck Depression Inventory-II with a cut-off of four (Beck et al., 1996). All subjects gave their written informed

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Abbreviations: BP_{ND}, binding potential; CSF, cerebrospinal fluid; 18F-FMZ-PET, ^{18}F-flumazenil PET; GM, grey matter; WM, white matter; VBM, Voxel based morphometry.

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http://dx.doi.org/10.1016/j.neuroscience.2012.12.075

consent and were compensated financially for their participation. Approval was obtained from the ethics committees of both McGill University and the Université de Montréal. Image analyses were carried out using the FSL suite of tools (Smith et al., 2004).

MRI

T1-weighted anatomical images were acquired on a 3T Siemens Trio scanner using a 16-channel headcoil (MPRAGE, resolution = $1 \times 1 \times 1\,mm^3$). Anatomical images were processed in accordance with the FSL-VBM pipeline (Ashburner and Friston, 2000), as follows: Anatomical images were brain extracted and segmented into GM, white matter (WM), and cerebrospinal fluid (CSF) partial volume images. The GM images were aligned with the MNI template using a non-linear registration and averaged across subjects to produce a study-specific template. A non-linear registration was then performed between the original GM images and this study-specific template. Finally, the registered partial volume images were modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field.

PET

^{18}F-FMZ-PET data were acquired using a Siemens ECAT HRRT PET scanner. ^{18}F-flumazenil was synthesised as previously described (Massaweh et al., 2009). Head movement was minimised with a head-restraining adhesive band. A 6-min transmission scan (^{137}C-point source) was first acquired for attenuation correction followed by an intravenous tracer injection (over 60 s) of 260.7 MBq (±21.24 SD) of ^{18}F-flumazenil. Subjects were instructed to close their eyes and remain awake.

List-mode data were acquired for a period of 60 min and then binned into a series of 26 sequential sets of fully 3D sinograms of increasing duration, ranging from 30 s to 5 min. PET data were reconstructed using a 3D OP-OSEM algorithm (10 iterations and 16 subsets) with correction for scatter, random coincidences, attenuation, decay, dead-time, and frame-based motion correction (Hudson and Larkin, 1994; Hong et al., 2007; Costes et al., 2009). The resulting images were composed of voxels of $1.22 \times 1.22 \times 1.22\,mm^3$ ($256 \times 256 \times 207$ voxels). $GABA_A$ binding potential (BP_{ND}) maps were then calculated according to the simplified reference tissue method, using the cerebral WM as the reference tissue region (Logan et al., 1996).

The following steps were adopted to minimise partial volume effects: WM and CSF maps (where each voxel has a value of between 0 and 1, representing the estimated proportion of that voxel that can be assigned to the relevant tissue type) were first each thresholded at a tissue proportion of 0.95 and used to produce binary masks. The masks were eroded by two voxels to ensure that only the tissue type of interest (and not GM) was covered, and the mean BP_{ND} within these WM and CSF regions calculated. The original, non-eroded, WM and CSF maps were then convolved with a 2.5-mm FWHM Gaussian kernel to simulate the scanner resolution and were multiplied by the mean BP_{ND} value for the appropriate tissue (WM or CSF). These WM and CSF BP_{ND} maps were then subtracted from the original BP_{ND} maps to give GM BP_{ND} images corrected for WM and CSF signal spill over. To further reduce partial volume effects the atlas ROIs were eroded to produce a separation between each one (see below). The BP_{ND} values obtained were broadly comparable to those obtained in other studies (Odano et al., 2009; la Fougère et al., 2011).

BP_{ND} images were aligned to the study GM template in a two-step process. Firstly, the GM map in anatomical space was convolved with a 2.5-mm FWHM kernel to produce a "simulated PET" image. BP_{ND} images were then linearly aligned to this image. This linear transform was then combined with the previously calculated non-linear anatomical-to-template warp to transfer the BP_{ND} images into the template space. Alignment between the BP_{ND} and GM images can be seen in Fig. 1.

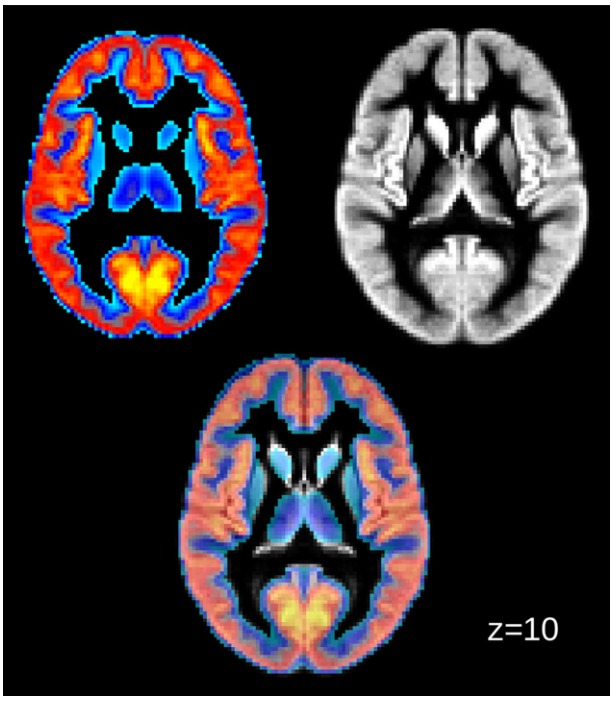

Fig. 1. Sample flumazenil BP_{ND} map and GM density image, plus the super-imposition of these to demonstrate alignment between modalities.

Regions of interest

Regions of interest were taken from the Jülich histological atlas (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html). This atlas consists of 52 GM regions (and 10 WM regions, not included here), bilaterally, and was created using post-mortem cyto-architectural segmentations. Following the creation of all 104 ROIs, the masks were eroded by one voxel in order to reduce partial volume effects, the voxel size (2 mm) corresponding reasonably well to the resolution of the PET scanner (FWHM approx 2.5 mm). This erosion also served to reduce the effects of misalignment at the borders of regions. Atlas ROIs were then masked with the study-specific brain template to ensure that no out-of-brain voxels were included in the calculations. ROIs consisting of fewer than 50 voxels after erosion were excluded from the analysis, leaving 59 out of 104 ROIs (see Table 1 for list of ROIs included).

The GM volume for each ROI from each subject was extracted from the registered GM maps. Mean BP_{ND} values were calculated for each ROI across subjects in the same manner.

Comparison of GM and BP_{ND}

Analyses of GM and BP_{ND} values were carried out using MATLAB 7.12 (The Mathworks, Natick, MA, USA). In the first step, mean BP_{ND} and GM values within each region (i.e., a mean value across subjects for each region was calculated and a single correlation done using regions as data-points) were compared using partial correlations, controlling for ROI size.

In an exploratory second step, the relationship between BP_{ND} and GM was then tested across subjects within each region independently (i.e., subject BP_{ND} and GM values were compared in 59 independent tests, one test per region, where