NeuroImage 155 (2017) 209–216

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Protein synthesis is associated with high-speed dynamics and broad-band stability of functional hubs in the brain

Peter J. Hellyer[a,b], Erica F. Barry[a,1], Alberto Pellizzon[c,1], Mattia Veronese[a], Gaia Rizzo[c], Matteo Tonietto[c], Manuel Schütze[d], Michael Brammer[a,d], Marco Aurélio Romano-Silva[d,e], Alessandra Bertoldo[c], Federico E. Turkheimer[a,*]

[a] Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK
[b] Department of Bioengineering, Imperial College London, Royal School of Mines, Room 4.35, South Kensington Campus, SW7 2AZ, UK
[c] Department of Information Engineering, University of Padova, Padova, Italy
[d] Instituto Nacional de Ciência e Tecnologia em Medicina Molecular, Federal University of Minas Gerais, Belo Horizonte, Brazil
[e] Mental Health Department, Faculty of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil

ARTICLE INFO

Keywords:
Resting state
Protein synthesis
Functional connectivity
Graph theory
Dynamics

ABSTRACT

L-[1-^{11}C]leucine PET can be used to measure *in vivo* protein synthesis in the brain. However, the relationship between regional protein synthesis and on-going neural dynamics is unclear. We use a graph theoretical approach to examine the relationship between cerebral protein synthesis (rCPS) and both static and dynamical measures of functional connectivity (measured using resting state functional MRI, R-fMRI). Our graph theoretical analysis demonstrates a significant positive relationship between protein turnover and static measures of functional connectivity. We compared these results to simple measures of metabolism in the cortex using [^{18}F]FDG PET). Whilst some relationships between [^{18}F]FDG binding and graph theoretical measures was present, there remained a significant relationship between protein turnover and graph theoretical measures, which were more robustly explained by L-[1-^{11}C]Leucine than [^{18}F]FDG PET. This relationship was stronger in dynamics at a faster temporal resolution relative to dynamics measured over a longer epoch. Using a Dynamic connectivity approach, we also demonstrate that broad-band dynamic measures of Functional Connectivity (FC), are inversely correlated with protein turnover, suggesting greater stability of FC in highly interconnected hub regions is supported by protein synthesis. Overall, we demonstrate that cerebral protein synthesis has a strong relationship independent of tissue metabolism to neural dynamics at the macroscopic scale.

Introduction

To effectively interact with the external world, the brain must build rich representations of environmental inputs received from sensory systems and update these representations ensuing action plans to effectively interact with a dynamic environment (Turkheimer et al., 2015). Modern interpretation of brain function describes the brain as a broad network of functionally interacting regions (the functional connectome) which adapts in both space and time in response to both to external (environmental) and internal (cognitive) demands (Corbetta and Shulman, 2002; Fornito et al., 2012; Fox et al., 2005; Hellyer et al., 2015; Vincent et al., 2008). One powerful framework for exploring the functional connectome and how the disparate regions of the brain interact is graph theory. Such approaches have provided strong hypotheses about the structural organization of the cortex, identifying sets of regions that are critically important for enabling efficient neuronal signalling and communication (the so-called 'hubs' regions) (Hagmann et al., 2007; Sporns et al., 2004; Sporns and Honey, 2013; Sporns and Kotter, 2004; van den Heuvel et al., 2012; van den Heuvel and Sporns, 2013; van den Heuvel et al., 2013), and describing large scale connectivity networks which neatly mirror the functional connectivity of slow neural dynamics (Beckmann et al., 2005; De Luca et al., 2006; Fox et al., 2009). Fluorodeoxyglucose (^{18}FDG) PET also reveals that these highly connected 'hub' regions are amongst the most metabolically active regions of the brain (Raichle, 2015; Raichle et al., 2001; Raichle and Snyder, 2007) supporting the hypothesis that these hubs are key, metabolic nodes responsible for large-scale 'functional integration' of information.

* Correspondence to: Centre for Neuroimaging Sciences, Institute of Psychiatry, Psychology & Neuroscience, King's College London, 16 De Crespigny Park, London SE5 8AF, UK.
E-mail address: federico.turkheimer@kcl.ac.uk (F.E. Turkheimer).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.neuroimage.2017.04.062
Received 23 November 2016; Accepted 26 April 2017
Available online 02 May 2017

Complementary computational and experimental approaches have also provided broad evidence that the brain is functionally organized as a complex system possessing a critical attractor (Fagerholm et al., 2015; Haimovici et al., 2013; Kitzbichler et al., 2009; Meisel et al., 2013; Scott et al., 2014; Shew and Plenz, 2013; Tagliazucchi et al., 2012). A system at a 'critical state' is finely balanced in a position between robust ordered and chaotic dynamics. Such dynamics are attractive as a model for neural function, as they provide a mechanistic framework for storage and processing information in a fluid dynamic system (Shew and Plenz, 2013; Shew et al., 2009; Shew et al., 2011). Moreover, 'critical' dynamics in the brain may emerge not just by the interaction of activity between highly connected cortical regions, but also through the action of local plasticity. Homeostatic plasticity, alongside a range of other adaptive or plastic approaches, have been proposed as a potential tuning mechanism for maintaining 'critical' neural dynamics (Cowan et al., 2013a). In the brain, these plastic mechanisms may not only induce 'critical' dynamics, but also enhance the emergence of functional connectivity networks (Hellyer et al., 2016). At the same time, computational approaches have demonstrated that the removal of 'peripheral' and 'hub' nodes, defined using graph theoretical approaches in computational simulations of the brain, have differential effects on the overall dynamics of the brain (Vasa et al 2015). Thus, not only do computational accounts suggest that plasticity is key to forming flexible brain dynamics, but the network topology underlying those dynamics is important for selecting and maintaining a flexible set point for activity.

Whilst a range of works in human explores the relationship between de-novo metabolism, e.g. measured using [^{18}F]FDG PET and measures of functional integration (described using graph theoretical means) – the relationship between functional integration and protein turnover which is undoubtedly associated with local cortical plasticity (such as the subtle re-organisations underlying a flexible dynamic state at rest proposed by computational accounts), has not yet been explored.

In this work, we use a graph theoretical approach (Rubinov and Sporns, 2010) to examine static and dynamic measures of functional integration and network topology at rest using functional connectivity (measured using fMRI) and measures of protein synthesis. The L-[1-^{11}C]leucine PET method allows the quantitative determination of local rates of protein synthesis in the central nervous system in-vivo (Smith et al., 1988). This assay uses L-[1-^{11}C]leucine as a tracer to measure the rate of incorporation of leucine, one of the nine essential amino acids, into protein. Leucine is very attractive for this kind of assay because its only pathway of degradation is transamination followed by carboxylation; here the 11C label is quickly transferred to labelled CO2 which is quantitatively minimal and negligibly re-incorporated as heavily diluted by the large pool of unlabelled CO2 resulting from carbohydrate metabolism. Hence brain radioactivity is mostly due to free L-[1-^{11}C]leucine and labelled protein defining a sympathetic measure of "de novo" cerebral protein synthesis (*rCPS*) (Bishu et al., 2008; Schmidt et al., 2005; Smith et al., 2005). Applied to the human-brain this approach allows the quantification of regional plasticity in the cortex (Veronese et al., 2012).

We explore the possibility that large-scale dynamics of the brain are in part constrained empirically by plasticity at a broad range of temporal scales using both a multi-scale decomposition and dynamic functional connectivity approach. Furthermore, we compare and contrast these results with a simpler description of functional processing using ^{18}FDG-PET as a measure of local metabolic demand. Whilst our analysis is broadly exploratory in nature, we test the hypothesis that functional network hubs are associated with high levels of protein synthesis, independent of basal levels of metabolism. For network hubs to maintain their stability and allow integration of flexible neural dynamics over time (predicted by computational approaches) likely requires dynamic reorganisation of the local neural architecture, thus we predict that highly interconnected hubs will be stable over time – associated with highly active protein turnover.

Methods

Positron emission tomography (PET)

[1-11C]Leucine

High-Resolution [1-^{11}C]Leucine PET images were acquired from 9 healthy awake subjects (9 male, age 20–24). These data were kindly provided by the authors of a previously published study. Detailed acquisition parameters and inclusion criteria are described in detail in the original work (Bishu et al., 2008). In brief, all studies were performed on a High-Resolution Research Tomograph (HRRT) (CPS Innovations, Knoxville, TN, USA). The dynamic 90-min scan was initiated coincident with a 2-min intravenous infusion of 20–30 mCi of L-[1-^{11}C]leucine. Images were reconstructed using motion-compensated three-dimensional ordinary Poisson ordered subset expectation maximization as 42 frames (16×15, 4×30, 4×60, 4×150, 14×300 s); voxel size was 1.21×1.21×1.23 mm. Concentrations of unlabelled and labelled leucine in plasma and total ^{11}C and $^{11}CO_2$ in whole blood were estimated using continuous arterial blood sampling. Voxel-level estimates of rCPS [nmol/gr/min] were determined by spectral analysis with an iterative filter (SAIF) (Veronese et al., 2010; Veronese et al., 2012). The resulting rCPS maps were then spatially normalized to the MNI-152 (2mm) co-ordinate system (Grabner et al., 2006) and a population average rCPS template image was calculated for further analysis. Finally, the rCPS PET template was segmented into 82 regions of interest (ROIs) according to the Desikan-Kilaney+Aseg atlas (Fischl, 2012; Fischl et al., 2002) projected into the MNI-152 coordinate space. An overview of this atlas is available in (Fig. S1), resulting in a single 82(n)x1 vector of average rCPS in each region across the cortex and sub-cortex.

[^{18}F]-FDG (Fludeoxyglucose)

High resolution [^{18}F]FDG PET images were collected from 11 healthy volunteers (5 female, age 30.5 ± 7.1 years). [^{18}F]FDG PET/CT brain images were acquired in a GE Discovery 690 (GE Healthcare, Milwaukee, Wisconsin, USA), at the Centro de Tecnologia em Medicina Molecular, Faculdade de Medicina da UFMG, Belo Horizonte, Brazil. Fifty minutes prior to scanning, each subject received an intravenous bolus injection of 5.18 MBq/kg of [^{18}F]FDG before resting in a quiet, dark room with minimum stimuli. PET images were subsequently acquired over 10 minutes, and reconstructed in a 192x192x47 matrix using the OSEM (Ordered Subsets Expectation Maximization) algorithm, with 2 iterations and 20 subsets. CT images were used to perform attenuation correction. The resulting standardized uptake value (SUV) maps normalised by body weight and injected dose were then spatially normalized to the MNI-152 (2mm) co-ordinate system (Grabner et al., 2006) and a population average SUV template image was calculated for further analysis. Like the analysis described for the rCPS data above, this atlas was subsequently divided into the same 82 cortical and subcortical ROIs resulting in a single 82(n)x1 vector of average SUV in each region across the cortex and sub-cortex.

Magnetic resonance imaging

Resting state fMRI (R-fMRI)

High temporal and spatial resolution R-fMRI data was obtained from 20 subjects (10 female, age 30.1 ± 3.3 years) released as part of the Human Connectome Project (humanconnectome.org/). In order to explore the effect of gender and specific subject selection bias on the reported results, we randomly selected a cohort of a further 20 subjects (10 female, age: 30.2+3.46 years), as well as 20 male (age: 29.1+3.79 years) and 20 female subjects (age: 29.76+3.07 years) In brief, all HCP subjects were scanned on a customized Siemens 3T "Connectome Skyra" housed at Washington University in St. Louis, using a standard 32-channel Siemens receive head coil and a "body" transmission coil designed by Siemens specifically for the smaller space available using