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Posteromedial cortex glutamate and GABA predict intrinsic functional connectivity of the default mode network

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Abstract

The balance between excitatory glutamatergic projection neurons and inhibitory GABAergic interneurons determines the function of cortical microcircuits. How these neurotransmitters relate to the functional status of an entire macro-scale network remains unknown. The posteromedial cortex (PMC) is the default mode network (DMN) node with the greatest functional connectivity; therefore, we hypothesized that PMC glutamate and GABA predict intrinsic functional connectivity (iFC) across the DMN. In 20 healthy men, we combined J-resolved magnetic resonance spectroscopy to measure glutamate and GABA in the PMC and resting fMRI followed by group Independent Components Analysis to extract the DMN. We showed that, controlling for age and GM volume in the MRS voxel, PMC glutamate and GABA explained about half of the variance in DMN iFC (represented by the network's beta coefficient for rest). Glutamate correlated positively and GABA correlated negatively with DMN iFC; in an alternative statistical model which included the glutamate/GABA ratio, the ratio correlated positively with DMN iFC. Age had no independent effect on DMN iFC. No other network was associated with PMC glutamate or GABA. We conclude that regional neurotransmitter concentrations in a network node strongly predict network but not global brain iFC.

Keywords

Default Mode Network; Excitation; Inhibition; Microcircuit; Brain network; Connectivity; precuneus

1. Introduction

The brain, in general, and, the cortex, in particular, are organized hierarchically: cortical neurons assemble locally into micro-scale circuits, which connect to each other to form nodes (underlying some modular brain function), which, in turn, assemble to constitute macro-scale networks (underlying some complex cognitive or emotional process or behavior). Uncovering associations spanning these successive levels of cortical organization is important for understanding not only normal brain function across the lifespan, but also the pathogenesis of neurological disorders (Friston, 2005; Mesulam, 2008).

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Macro-scale networks consist of distant brain regions that manifest synchronous activity fluctuations (i.e. functional connectivity, FC) during task performance and/or intrinsically, during experimentally unrestrained brain activity (“rest”) (i.e. intrinsic functional connectivity, iFC) (Buckner et al., 2009; He et al., 2009; Raichle, 2010; Tomasi and Volkow, 2011; Kelly et al., 2012). The function of these networks critically depends on few network-defining nodes with high connectivity (“epicenters”) (Bassett et al., 2006; Bullmore and Sporns, 2009; van den Heuvel et al., 2009). The archetypical brain network is the default mode network (DMN), the main epicenter of which is the posteromedial cortex (PMC), consisting of posterior cingulate, retrosplenial and precuneus cortices. PMC is functionally and structurally inter-connected with other DMN epicenters and with virtually every cortical area (Hagmann et al., 2008; Greicius et al., 2009). The DMN manifests its highest level of activity during “rest” (Pfefferbaum et al., 2011), the functional state of experimentally unrestrained, intrinsic brain activity. Moreover, DMN is involved in a range of trans-regional brain processes, including semantic processing, episodic memory encoding and retrieval, suppression of motor planning and visuospatial processing, as well as in the functional integration of large-scale cortical activity (Buckner et al., 2008; Tomasi and Volkow, 2010).

The basic processing unit throughout the cerebral cortex is the canonical microcircuit, which consists of excitatory glutamatergic projection neurons and GABAergic interneurons under functional modulation by thalamic, brainstem and basal forebrain projections (Douglas and Martin, 2004; Mesulam, 2008; Logothetis, 2008). The blood-oxygen level-dependent (BOLD) signal, the basis of functional magnetic resonance imaging (fMRI), is a surrogate for neuronal mass activity and is primarily determined by the balance between excitation and inhibition in microcircuits (Logothetis, 2008). Up to 85% of the energy consumed by the brain is used to support glutamatergic signaling (Hyder et al., 2006) and, as a result, regional oxygen consumption and BOLD signal generation are primarily determined from regional excitation (Magistretti and Pellerin, 1999; Smith et al., 2002; Raichle and Mintun, 2006). GABAergic interneurons likely modulate BOLD fMRI signal indirectly through inhibitory feedback signaling within microcircuits (Buzsaki et al., 2007; Logothetis, 2008).

Associations between regional neurotransmitter concentrations measured with magnetic resonance spectroscopy (MRS) within regions of interest (ROIs), BOLD signal within and functional connectivity between ROIs are progressively being discovered. Differential regional serotonin-1A receptor binding predicted BOLD signal change in three different DMN nodes (retrosplenial, posterior cingulate and dorsomedial prefrontal cortices) (Hahn et al., 2012). Studies combining MRS and fMRI reported correlations between GABA, glutamate (represented by the combined concentration of glutamate and glutamine, Glx) and stimulus-or task-induced BOLD signal changes (Northoff et al., 2007; Donahue et al., 2010; Falkenberg et al., 2012; Muthukumaraswamy et al., 2012). Reported associations have been consistently negative for GABA (Northoff et al., 2007; Donahue et al., 2010; Muthukumaraswamy et al., 2012), but for glutamate the direction of the association has been variable depending on task demands (Falkenberg et al., 2012). Furthermore, there is evidence suggesting that Glx in a ROI may modulate functional connectivity (Horn et al., 2010) and stimulus-induced BOLD signal change (Duncan et al., 2011) in distant ROIs; reported interactions have been unidirectional suggesting effective (i.e. cause and effect) connectivity between nodes of a network rather than some global modulation of network activity (Duncan et al., 2011). These findings raise the possibility that neurotransmitter concentrations within a node may predict the functional connectivity across an entire brain network.

In this study, we combined fMRI with MRS to examine the relationship between DMN iFC and the concentrations of GABA and glutamate in the PMC, the DMN node with the highest

level of activity (Jiao et al., 2011). DMN iFC was assessed using resting state fMRI followed by group-level Independent Component Analysis (ICA) (Calhoun et al., 2009). For MRS acquisition, we placed a voxel over the bilateral PMC (Fig. 1). GABA is difficult to quantify using standard one-dimensional MRS acquisition methods at clinical field strengths because of its relatively low concentration and substantial overlap with other metabolites in the chemical shift spectrum. To obtain unambiguous quantification of GABA and glutamate simultaneously, we acquired two-dimensional J-resolved spectra (Schulte et al., 2006b), which permit better separation of overlapping metabolites by spreading them in an additional dimension sensitive to j-modulations (Fig. 1d). Metabolite concentrations were resolved from these two-dimensional spectra as a linear combination of two-dimensional basis spectra (Schulte and Boesiger, 2006).

2. Subjects and Methods

Subjects/Task

This study was performed under the Clinical Research Protocol 2008–204, “Development of 3T Magnetic Resonance Research Methods for NIA Studies”, approved by the MedStar Institutional Review Board and subjects provided written informed consent. Clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. 20 healthy men (mean age $\pm$ SD, 47 $\pm$ 16 years) free of neuropsychiatric disease and not taking any neuromodulatory medications, participated in this research. Women were excluded to avoid the unaccounted effect of variable levels of circulating neurosteroids on cortical inhibition (Belelli and Lambert, 2005; Benarroch, 2007) that could potentially mask associations with DMN iFC. Subjects were instructed to lay still with their eyes open during both fMRI and MRS acquisition, to maintain the same functional state of the brain.

MRS

Single voxel ^1H MRS data were acquired using a Philips Achieva 3T whole-body MR scanner equipped with an 8-channel SENSE head coil. A volume of interest of $25 \times 18 \times 20 \text{ mm}^3$ was placed at midline between the two posteromedial cortices (PMC). For each participant, the MRS voxel was placed on the 3D MPRAGE image under direct visual inspection by one of the authors (DK). For voxel placement, we consulted the Talairach atlas (Talairach et al., 1993) to ensure maximum inclusion of precuneus, BA 7, in the voxel; depending on individual anatomical variability, portions of BA 23 have also been included in the voxel. In order to unambiguously measure GABA – whose chemical shift resonance substantially overlaps with several other metabolites in a standard one-dimensional spectrum (Rothman et al., 1984; Behar and Ogino, 1991; Rothman et al., 1993) – we acquired a two-dimensional j-resolved spectrum using a JPRESS sequence with maximum-echo sampling (Aue et al., 1976; Schulte et al., 2006b). This method acquires a dynamic series of PRESS spectra incrementing the echo time to encode J-modulations in the second (indirect) dimension; this additional dimension permits the discrimination and quantification of resonances that overlap in the directly detected chemical shift direction but have different J couplings. The echo times in this study were incremented from 31 ms to 229 ms using 100 echo steps with a step size of 2ms. Other JPRESS acquisition parameters consisted of a repetition time = 1600 ms, eight averages per echo time, bandwidth in the direct dimension = 2kHz, 1024 sample points, for a total scan duration of 21 min and 20 s. Linewidths for the water resonance were monitored for intra-subject scan reliability and were (mean $\pm$ SD) 7.3 $\pm$ 1.7 Hz. Relative metabolite concentrations were determined using the Prior-Knowledge Fitting procedure (ProFit), which fits linear combinations of simulated two-dimensional basis metabolite spectra (Schulte and Boesiger, 2006; Schulte et al., 2006a); metabolite amounts are reported as their ratio to creatine. Although creatine has been shown

to be a stable metabolite in healthy individuals (Soher et al., 1996) and thus is commonly used as an internal reference in brain spectroscopy, absolute creatine concentrations were determined from coincident voxels in a subset ($n=10$) of our participants using a standard short echo time PRESS acquisition and LCModel's internal water reference method (Provencher, 1993) showing values and intra-subject variation consistent with those published in the literature (mean $\pm$ SD, [Cr] = 5.85 $\pm$ 0.40 mM).

ProFit assesses goodness of fit in terms of Cramer-Rao lower bounds (crlb %), which were used as an estimate of measurement error. To determine the crlb% for the ratio of glutamate to GABA, we performed a standard error propagation using the data reduction equation ($r = (\text{Glu/Cr})/(\text{GABA/Cr})$), where the crlb% for the ratio is calculated using the square root of the sum of squares of the product of the partial derivatives (i.e. $r/(\text{Glu/Cr})$ and $r/(\text{GABA/Cr})$) and the individual crlb% measures for Glu/Cr and GABA/Cr (Coleman and Steele, 1999).

MRS voxel volumetrics—Standard T1-weighted three-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo (3D MPRAGE) images were acquired. Images were normalized to Montreal Neurological Institute (MNI) space using the VBM8 toolbox with default parameters (<http://dbm.neuro.uni-jena.de/vbm/download/>). This procedure uses high-dimensional Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) (Ashburner, 2007) to align the native space image to an MNI template of 550 normal adult brains. VBM8 tissue probability map priors were then used to segment the normalized brain into probability maps of gray matter (GM), white matter (WM), cerebrospinal fluid (CSF), bone, and unspecified tissue. This process is simultaneously used to de-skull the image, remove tissue artifacts, and de-noise the data. The GM, WM, and CSF images were then taken back into native space using the inverse DARTEL deformation field. Next, MarsBaR toolbox (Brett et al., 2002) was used to construct a volume of interest (VOI) from the original MRS voxel in native space. Finally, partial volume estimates for GM, WM, and CSF were extracted within the VOI.

fMRI

The same Philips Achieva 3T whole body MRI scanner and 8-channel SENSE head coil were used to acquire single-shot, 2-dimensional gradient-echo with echo-planar readout. The acquisition parameters included an echo time = 30 ms, repetition time = 2000 ms, FOV = 20 $\times$ 23 cm, acquisition matrix = 68 $\times$ 76, interpolated matrix size = 128 $\times$ 128, voxel size = 2.9 $\times$ 3.0 $\times$ 3.0 mm³, flip angle = 75°, EPI factor = 41, number of dynamics = 211, and a total acquisition time of 7 min and 30 s. The entire brain and cerebellum were imaged in a single shot using 37 contiguous slices oriented parallel to the anterior-posterior commissure plane. We performed standard EPI image preprocessing using SPM8 (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL), including realignment and unwarping, slice-timing correction, co-registration to the MPRAGE image, normalization (to a 3 $\times$ 3 $\times$ 3 voxel size), and spatial smoothing with a Gaussian kernel full-width-at-half-maximum of 6 mm.

ICA

We performed group-level ICA using the Infomax algorithm as implemented by the Group ICA Toolbox GIFT (<http://www.nitrc.org/projects/gift>) (Calhoun et al., 2009; Allen et al., 2011). This technique is able to reconstruct highly stable networks across individuals (Buckner et al., 2009; Allen et al., 2011) and sessions (Chen et al., 2008), but also to reveal between subject variability and the effect of aging (Koch et al., 2010). Data reduction was implemented in two steps, first producing 30 Principal Components (PCs) per subject and session using a standard economy-size decomposition (retaining 99% of the variance of the

data) and, subsequently, producing 45 ICs (retaining 88.66% of the total variance). The Infomax ICA algorithm was repeated 3 times using the ICASSO algorithm to increase the reliability of the decomposition (Allen et al., 2011). ICASSO orthogonalizes source estimates of resting state activity into eigenvectors using a nonlinear, Bayesian approach and excluding voxels that likely represent noise (Sarela and Valpola, 2005). Back-reconstruction of these ICs for individual subjects was performed using the GIGA3 algorithm. ICs were regressed by both spatial and temporal templates and sorted accordingly. For spatial regression, we used the DMN template mask provided by GIFT to identify the IC with the highest spatial correlation with DMN (IC22). Visual inspection confirmed that IC22 matches the spatial pattern of activation typical of the DMN (Fig. 2) and, thereby, IC22 will be referred to as the “DMN” component. Then, we performed fitting (temporal regression) of the time-courses of all ICs to the condition of rest. In a general linear model in SPM8, rest was modeled as a series of events (each volume of acquisition comprising a single event) and the six movement parameters (i.e. linear movement in x, y, z, and rotation in yaw, roll, pitch) were used as additional (nuisance) regressors. From this temporal regression, beta coefficient values expressing the degree of modulation of the ICs time-courses by the condition of rest were generated. The beta coefficients for the DMN component (IC22) modulation by rest were used in subsequent between subjects comparisons as a measure of DMN iFC attributable to the condition (Calhoun et al., 2009).

MRS – fMRI associations

To examine the association between MRS measures and DMN iFC, we performed a series of linear regression analyses in SPSS 20. In all regressions, the beta coefficient expressing the modulation of the DMN component by the condition of rest was used as the dependent and MRS measures, age and MRS voxel GM volume as independent variables. We deemed a linear model acceptable, after examining the distribution of the independent variables (which was acceptably close to normal) and plotting the dependent by each independent variable. All independent variables were entered in a single step (method enter) and all models included a constant. The significance of each model was assessed in terms of F statistic and the portion of the variance explained (fit) in terms of R^2 statistic. Zero-order, partial and part correlations were calculated. The strength of the association for individual regressors was assessed in terms of standardized coefficients (Beta). Possible collinearity was assessed in terms of tolerance and variable inflation factor (VIF).

Three different models were examined in this analysis. Model 1 describes the linear regression of the beta coefficient of the DMN for rest by glutamate/GABA, age, and MRS voxel GM volume. Model 2 describes the linear regression of the beta coefficient of the DMN for rest by glutamate/GABA, age, and MRS voxel GM volume weighted by the least-squares of the inverse of $\text{crlb}\%$ for glutamate/GABA. In this model, more uncertain data points (i.e. data points with greater measurement uncertainty derived from larger $\text{crlb}\%$ for glutamate and/or GABA) are given less influence. Model 3 describes the linear regression of the beta coefficient of the DMN for rest glutamate/creatine, GABA/creatine, age and MRS voxel GM volume.

3. Results

MRS

Reliable glutamate and GABA spectra were obtained, as revealed by Cramer-Rao lower bounds, a measure of goodness of fit, for: glutamate/creatine = $11 \pm 10.7\%$; GABA/creatine = $19.2 \pm 10.2\%$; and glutamate/GABA = $24.5 \pm 10.9\%$. The amount of GM within the 9000 mm^3 voxel was $5463.5 \pm 536.5 \text{ mm}^3$ (or the percentage of GM within the voxel was $61 \pm 6\%$). Figure 1d shows a representative 2D JPRESS spectrum (real channel) demonstrating the

reduction of spectral overlap with the additional J evolution dimension and the benefit of maximum-echo sampling, which tilts the residual tail of the water peak away from the metabolites of interest avoiding unwanted distortions.

ICA

Group ICA generated 45 ICs that collectively explained 88.73% of the variance in the data. We identified the IC with the highest spatial correlation with the DMN template image, thereby referred to as “DMN” (Fig. 2). Temporal regression was used to fit IC time-courses to a general linear model (modeling rest as a single block) and generate beta coefficient values expressing the degree of modulation of the IC time-courses by the condition (rest). The beta coefficients for the DMN were used in subsequent between subjects comparisons as a measure of DMN iFC during rest (Calhoun et al., 2009).

MRS – fMRI

Then, we examined the relationship between resting DMN iFC and the glutamate/GABA ratio, an expression of the balance between excitation and inhibition at the PMC, as well as GABA and glutamate considered individually (Table 1, Fig. 3). The linear regression of DMN beta for rest by glutamate/GABA, age, and MRS voxel GM volume revealed that glutamate/GABA positively correlated with DMN iFC, while age and MRS voxel GM volume showed no correlation with it (Fig. 3a; 3b). Next, to take into account the intrinsic uncertainty of MRS measures, we performed a linear regression of DMN beta for rest by glutamate/GABA, age, and MRS voxel GM volume weighted by the least-squares of the inverse of the crlb % for glutamate/GABA. This way, more uncertain data points (i.e. data points with greater measurement uncertainty derived from larger crlb % for glutamate and/or GABA) are given less influence. The positive correlation between glutamate/GABA and DMN iFC remained essentially unchanged. Finally, to examine the effects of glutamate and GABA individually, we performed a linear regression of DMN beta for rest by glutamate/creatine, GABA/creatine, age, and MRS voxel GM volume. Glutamate/creatine positively correlated with DMN iFC, GABA/creatine negatively correlated with it, while age and MRS voxel GM volume showed no correlation with it (Fig. 3c; 3d).

We repeated the analysis for the beta coefficients for rest of each one of the remaining 44 ICs. No other network was significantly associated with PMC glutamate/GABA, glutamate/creatine or GABA/creatine.

4. Discussion

Our findings reveal a previously unknown association between glutamate and GABA levels in a network node and the functional connectivity of the entire network at the same functional state. Specifically, glutamate levels and the glutamate/GABA ratio in the PMC were positively correlated with DMN iFC, whereas GABA levels were negatively correlated with it. The power of models based on regional MRS measures in predicting DMN iFC is robust, explaining half of its variance, even after controlling for age and MRS voxel tissue composition and taking into account the uncertainty of spectroscopic measurements.

In this study we considered concentrations of glutamate and GABA as surrogates for the regional balance between excitation and inhibition. Even though these neurotransmitters occupy compartments beyond the spatial resolution of MRS (intracellular vs. extracellular, cytosolic vs. intravesicular, neuronal vs. astrocytic), their concentrations in these compartments are balanced and their total concentrations are neurophysiologically relevant. Glutamate and GABA are continuously recycled between neurons and nearby astrocytes via re-uptake from the extracellular space (Hyder et al., 2006). Increased rate of neurotransmission of either glutamate or GABA shifts the balance in the bidirectional

reactions taking place in astrocytes and neurons from glutamine towards glutamate or GABA synthesis (Hyder et al., 2006). Moreover, it has recently been appreciated that, besides synaptic transmission, volume transmission (i.e. transmission via diffusion in the extracellular fluid and occupancy of extrasynaptic receptors) of glutamate and GABA plays an important role in modulating regional microcircuits and the hemodynamic response of a region (Vizi and Mike, 2006; Logothetis, 2008).

The various forms of regional excitatory and inhibitory transmission and their multifaceted modulation determine the BOLD signal (Logothetis, 2008). Regional glutamate release increases the metabolic rate of not only post-synaptic neurons (Smith et al., 2002), but also of surrounding astrocytes (DiNuzzo et al., 2011), quite unambiguously leading to regional BOLD signal increases (Logothetis, 2008). In contrast, the effects of regional GABAergic inhibition on BOLD signal are complex and might even be regionally-specific (Buzsaki et al., 2007; Logothetis, 2008): some studies show inhibition increasing a region's metabolic rate (Jueptner and Weiller, 1995), while other studies demonstrate a BOLD signal decrease (Stefanovic et al., 2004). The closest neurophysiologic correlates of BOLD signal are fluctuations of local field potentials that reflect the input to a region and intracortical processing comprising both excitation and inhibition (Logothetis, 2008; Magri et al., 2012). In this study, we expanded upon the findings of previous studies that revealed associations between neurotransmitter concentrations and BOLD signal changes and iFC concerning ROIs (Northoff et al., 2007; Donahue et al., 2010; Horn et al., 2010; Duncan et al., 2011; Falkenberg et al., 2012; Hahn et al., 2012; Muthukumaraswamy et al., 2012), by examining their relationship to the iFC of an entire network. Our results, showing a positive correlation for glutamate and a negative for GABA are in good agreement with these studies. Even though FC is a theoretical construct that emerges from mathematical analysis of synchronous BOLD signal changes in distant brain regions, its basis is neuronal and not hemodynamic synchronization (Brookes et al., 2011). Given that one of the main functions of GABAergic interneurons is to orchestrate synchrony between microcircuits (Bonifazi et al., 2009; Rutishauser et al., 2012), the regional balance between glutamate and GABA may determine the synchronized portion of neuronal activity in that region and, therefore, correlate with FC.

The strength of the associations observed does not necessarily imply that PMC neurotransmission “drives” DMN activity; indeed, there is evidence that other DMN nodes may drive PMC activity (Uddin et al., 2009; Jiao et al., 2011). Nevertheless, PMC may reflect the functional state of the network, since it shows tight anatomical and functional connectivity with all other DMN nodes (Hagmann et al., 2008; Greicius et al., 2009), and the highest level of activity among DMN nodes during rest (Jiao et al., 2011). PMC glutamate and GABA did not show any association with any of the remaining 44 ICs, suggesting that regional neurotransmitter concentrations do not predict global iFC and, therefore, demonstrating a simple dissociation. Due to practical considerations related to scanning time, this study did not collect MRS data from a node of some other network, which might have allowed us to demonstrate a double dissociation (neurotransmitters measured within a node of another network predicting iFC of that network, but not of the DMN).

Although low spatial resolution is inherent to brain MRS studies, the spectroscopic technique we used has been shown to provide the most stable and accurate measures for glutamate and GABA (Schulte and Boesiger, 2006). Moreover, we implemented an anisotropic midline voxel to maximize PMC sampling and limit the amount of white matter and CSF contained in it; this required using a relatively small voxel volume (9cm³) compared with the original work (15 cm³) using this jPRESS methodology (Schulte and Boesiger, 2006). While our reduction of voxel volume resulted in an increase in the Cramer-

Rao lower bounds estimates obtained from our fits, it did not negate the observed trends in the metabolites of interest in this work. Furthermore, It has been shown that variability between individuals of MRS-measured cortical GABA concentrations are dominated by differences in physiology rather than micro-anatomical differences in relative volume, thickness or tissue composition (Muthukumaraswamy et al., 2012).

Our findings might have important implications for future research on the aging brain and Alzheimer's disease (AD). Here we showed that age has no effect on DMN iFC independently of PMC glutamate and GABA levels. This raises the possibility that the alterations that are known to occur with both glutamatergic and GABAergic signaling during aging (Gleichmann et al., 2010) may underlie the decreased iFC of the DMN seen with aging (Koch et al., 2010). In addition, early and preferential amyloid beta-peptide (A β) deposition associated with network dysfunction occurs in PMC in AD (Buckner et al., 2009; Sperling et al., 2009; Hedden et al., 2009). Regional A β deposition is also known to induce an imbalance between excitation and inhibition (Busche et al., 2008; Bero et al., 2011). Based on the findings of this study, we hypothesize that, in AD, changes in the balance between excitation and inhibition in the PMC predict FC changes in the DMN.

Novel glutamatergic signaling modulators are therapeutic candidates for a range of important neuropsychiatric diseases (including schizophrenia, depression, anxiety, autism spectrum disorders, substance abuse and AD), but their development has been stalled by the lack of suitable biomarkers (Javitt et al., 2011). Applying the combined fMRI/MRS methodology described here to various brain regions and networks, future studies may be able to associate drug-induced changes in regional neurochemistry with changes in network function and clinical outcomes and aid in the development of new treatments (Javitt et al., 2011).

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Highlights

Posteromedial cortex glutamate positively correlates with the intrinsic functional connectivity of the default mode network. Posteromedial cortex GABA negatively correlates with the intrinsic functional connectivity of the default mode network. Age has no association with the intrinsic functional connectivity of the default mode network independent of Glutamate and GABA. No other network is associated with posteromedial cortex neurotransmitters.

Figure 1. MRS

a., b., c. Mid-sagittal, axial and coronal views of the anisotropic voxel (25×18×20mm) placed between the bilateral PMCs. d. J-PRESS generated MR spectra showing multiple metabolites overlapping with GABA, which are readily resolved with the addition of the f1 (J coupling) dimension. Glu = glutamate; Gln = glutamine; Cre = creatine; GSH = glutathione; NAA = N-acetyl-aspartate; GABA = -aminobutyric acid.

Figure 2. ICA

a. Mid-sagittal view the mean IC22, the IC with the greatest spatial correlation with the DMN. b. Coronal view of the mean IC22. c. Serial axial views of the mean IC22.

Figure 3. DMN iFC and MRS measures

Partial regression plots from the (unweighted) linear regression analyses of the beta coefficient for rest of the DMN by PMC spectroscopic measures and age. The beta coefficient for rest of the DMN (y-axis) is plotted against (1) the ratio of Glu/GABA; (2) age; (3) the ratio Glu/Cr; and (4) the ratio GABA/Cr. Glu/GABA and Glu/Cr correlate positively, whereas GABA/Cr correlate negatively with DMN iFC. Age shows no association with DMN activity. iFC = intrinsic functional connectivity; Glu = glutamate; Cr = creatine.

Table 1
Linear regression of DMN beta for rest by Age, voxel GM volume and PMC metabolites

a. Model Summary b. Coefficients. Model 1: linear regression of the beta coefficient of the DMN for rest by Age, voxel GM volume, and Glu/GABA. Model 2: linear regression of the beta coefficient of the DMN for rest by Age, voxel GM volume, and Glu/GABA, weighted by the inverse of the $crlb\%$ for Glu/GABA. Model 3: linear regression of the beta coefficient of the DMN for rest by Age, voxel GM volume, Glu/Cr and GABA/Cr.

a.									
Model	R	R ²	Adjusted R ²	Std. Error of the Estimate	Change Statistics				
					R ² Change	F Change	df1	df2	Sig. F Change
1	0.713	0.508	0.416	2.55492	0.508	5.503	3	16	0.009
2	0.738	0.545	0.459	0.49760	0.545	6.382	3	16	0.005
3	0.733	0.538	0.415	2.55676	0.538	4.365	4	15	0.015

b.											
Model	Unstandardized Coefficients		Standardized Coefficients		t	Sig.	Correlations			Collinearity Statistics	
	B	Std. Error	Beta	Zero-order			Partial	Part	Tolerance	VIF	
1 (Constant)	6.115	9.260		0.660	0.518						
Age	-0.039	0.048	-0.185	-0.820	0.424	-0.224	0.201	-0.144	-0.607	1.647	
GM volume	-0.001	0.001	-0.209	-0.959	0.352	-0.083	-0.233	-0.168	0.646	1.547	
Glu/GABA	0.741	0.208	0.651	3.555	0.003	0.690	0.664	0.623	0.918	1.089	
2 (Constant)	12.375	8.552		1.447	0.167						
Age	-0.059	0.043	-0.298	-1.372	0.189	-0.310	-0.325	-0.232	0.604	1.655	
GM volume	-0.002	0.001	-0.359	-1.748	0.100	-0.107	-0.400	-0.295	0.675	1.481	
Glu/GABA	.0782	0.229	0.616	3.413	0.004	0.672	0.649	0.576	0.872	1.146	
3 (Constant)	-1.700	10.048		-0.169	0.868						
Age	-0.048	0.052	-0.228	-0.935	0.365	-0.224	-0.235	-0.164	0.520	1.924	
GM volume	5.403 E-005	0.001	0.009	0.037	0.971	-0.083	0.009	0.006	0.561	1.782	
Glu/Cr	11.904	3.590	0.672	3.316	0.005	0.465	0.650	0.582	0.750	1.334	
GABA/Cr	-7.691	3.454	-0.457	-2.227	0.042	-0.383	-0.498	-0.391	0.730	1.370	

Glu = glutamate; GABA = -aminobutyric acid; R = multiple correlation coefficient; R² = coefficient of determination; F = F statistic; df = degrees of freedom; t = t-statistic; VIF = variable inflation factor.