Published in final edited form as:

Psychiatry Res. 2008 October 30; 164(1): 16–29. doi:10.1016/j.psychres.2007.12.015.

Interregional cerebral metabolic associativity during a continuous performance task Part I: Healthy adults

Mark W. Willis^{a,b,*}, Brenda E. Benson^a, Terence A. Ketter^c, Tim A. Kimbrell^d, Mark S. George^e, Andrew M. Speer^a, Peter Herscovitch^f, and Robert M. Post^a

^aBiological Psychiatry Branch, National Institute of Mental Health, Bethesda MD, USA

^bUniformed Services University of the Health Sciences, PHS, HHS, Bethesda, MD

^cStanford University School of Medicine, Stanford CA, USA

^dUniversity of Arkansas for Medical Sciences, Little Rock AR, USA

^eMedical University of South Carolina, Charleston SC, USA

^fPET Department, National Institutes of Health Clinical Center, Bethesda MD, USA

Abstract

One emerging hypothesis regarding psychiatric illnesses is that they arise from the dysregulation of normal circuits or neuroanatomical patterns. In order to study mood disorders within this framework, we explored normal metabolic associativity patterns in healthy volunteers as a prelude to examining the same relationships in affectively ill patients (Part II).

We applied correlational analyses to regional brain activity as measured with FDG-PET during an auditory continuous performance task (CPT) in 66 healthy volunteers. This simple attention task controlled for brain activity that otherwise might vary amongst affective and cognitive states. There were highly significant positive correlations between homologous regions in the two hemispheres in thalamic, extrapyramidal, orbital frontal, medial temporal and cerebellar areas. Dorsal frontal, lateral temporal, cingulate, and especially insula, and inferior parietal areas showed less significant homologous associativity, suggesting more specific lateralized function. The medulla and bilateral thalami exhibited the most diverse interregional associations. A general pattern emerged of cortical regions covarying inversely with subcortical structures, particularly the frontal cortex with cerebellum, amygdala and thalamus.

These analytical data may help to confirm known functional and neuroanatomical relationships, elucidate others as yet unreported, and serve as a basis for comparison to patients with psychiatric illness.

*Address correspondence reprint requests to: Brenda E. Benson, Ph.D., BPP, NIMH, 10 Center Drive MSC-1272, Bethesda, MD 20892-1272 (email: bbenson@mail.nih.gov Phone (301)496-6825; Fax (301)402-0052.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Potential reviewers:

Barry Horwitz, Ph.D., NIDCD/NIH, Bldg 10, Room 6C420, 9000 Rockville Pike, Bethesda, MD 20892-1591 USA, horwitz@helix.nih.gov

Jean-Luc Martinot, INSERM U334, Service Hospitalier Frédéric Joliot, DRM-CEA, 4 Place du Général Leclerc, 91406 Orsay, France, martinot@shfj.cea.fr

David Zald, Ph.D., Department of Psychology, Vanderbilt University, 325 Wilson Hall, 111 21st Ave. South, Nashville, TN 37203-0009, david.zald@vanderbilt.edu

Keywords

Positron emission tomography (PET); Cerebral glucose metabolism; FDG; Normal; Connectivity; Neural circuitry

1. INTRODUCTION

Functional imaging studies attempting to delineate the neural substrates of the mood disorders have been plentiful in recent years, but the findings have often proved disparate and conflicting. For instance, in depressed patients compared to matched controls, anterior cingulate activity has been reported as increased (Drevets et al., 1992; Mayberg et al., 1997 among responders) and decreased (Bench et al., 1992; Mayberg et al., 1997 among nonresponders), thalamic function increased (Brody et al., 2001) and decreased (Austin et al., 1992) and parietal increased (Abou-Saleh et al., 1999) and decreased (Mayberg et al., 1997). Even the most consistent finding in depression, decreased baseline dorsolateral prefrontal activity, has been contradicted by at least one recent study (Brody et al., 2001).

Similarly, few functional deficits specific to bipolar illness have been consistently identified (Strakowski et al., 2000). Much of the observed heterogeneity can be attributed to: methodological variation; the existence of multiple illness subtypes, severities, and co-morbidities; and state vs. trait distinctions. However, one hypothesis that could help explain divergent findings is that mean group differences between patients and controls do not fully characterize metabolic abnormalities in the illnesses in question, especially given the possibility of pathway-related alterations and compensatory mechanisms arising in illness (Sackeim, 2001). One region in particular, the thalamus, has been suggested to play a role in neural circuits gone awry in both bipolar disorder and schizophrenia (Buchsbaum et al., 1997). This is a key way station not only in somatomotor integration but also in the series of modulatory cortical-striatal-thalamic loops described by Alexander and DeLong (1990). Investigations are now beginning to focus on possible abnormalities in the interaction, or functional connectivity, among the various regions believed to play a part in the recognition, expression and regulation of affect in a variety of illnesses (Mallet et al., 1998). A necessary foundation for this area of study is the identification of functional circuits involved in normal mood regulation as well as emotional and cognitive processing of the environment.

Several methods have been introduced for this type of inquiry, among them eigenimage analysis (Friston et al., 1993), scaled subprofile modeling (Moeller et al., 1987), and direct covariance of regions (Horwitz, 1991a). Zald et al. (1998) have investigated the complementary information that can be derived from the latter paradigm using both nonsubtracted (single task) and subtracted (between task) correlational data in order to control for static influences (e.g., blood supply, gray/white ratios, etc.) that may dominate interregional covariances, and which should be a concern with any examination of this kind. In this regard, several studies have examined interregional covariance in major depression (Mallet et al., 1998; Anand et al., 2005), schizophrenia (Clark et al., 1984; Katz et al., 1996; Friston et al., 1996; Buchsbaum et al., 1999; Mallet et al., 1998; Andreason, 1999; Meyer-Lindenberg et al., 2001), obsessive-compulsive disorder (Horwitz et al., 1991b; Mallet et al., 1998), and Down's syndrome (Horwitz et al., 1990).

Because we wished to generate normal baseline associativity maps to use in the subsequent investigation of couplings between arbitrary pairs of regions in affectively ill patients, and to keep the data accessible to the widest audience, we favored a simple correlational approach. In this report, we employ a single-task covariance approach in a study of 66 healthy volunteers to characterize in a descriptive fashion normal metabolic regional covariance during an

emotionally neutral and cognitively non-challenging auditory continuous performance task (CPT). We choose to measure metabolic activity during a simple attention task (a task is a prerequisite of connectivity studies) in attempt to entrain mental activity, with the goal of reducing the variable cognitive states that may be associated with REST condition. Furthermore, the REST condition is not considered a zero-activity period (Stark and Squire, 2001), but constitutes an uncontrolled condition that is difficult to assess and may be ambiguous as a baseline condition.

The interregional covariance method applied to a single condition scanning reveals correlative relationships that may arise from a variety of factors, including congruency in cytoarchitecture and gray/white ratios as well as actual neuronal communication (i.e., functional connectivity) between the regions examined. However, within the limits of this method and its interpretation, this exploratory analysis serves as a descriptive narrative to further define interregional associations of cerebral metabolism during a CPT task in normal volunteers. In conjunction with the companion paper, it also provides a normal baseline to which our unipolar and bipolar affectively ill patients can be compared (Benson et al., 2006, Part II).

2. METHODS

2.1 Subjects

Healthy adult volunteers were recruited from the surrounding community through the Clinical Research Volunteer Program at the National Institutes of Health (NIH). Applicants were excluded if they had any personal or first-degree relative history of psychiatric illness or substance abuse as determined by a structured interview using the on the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision (DSM-IV-TR). Other exclusionary criteria included personal history of significant head trauma, major surgery or medical conditions (such as hypertension, diabetes or asthma), prolonged prior medication use, or having taken any medications during the two weeks prior to the scan date. Thorough medical and neurologic exams and baseline laboratory screening including a thyroid profile and urine drug screen were performed prior to study entry.

Subjects gave oral and written consent prior to the scan, and were paid for their participation. Our study was approved by the NIH Institutional Review Board and Radiation Safety Committee. The 66 subjects in this study (mean age $\pm$ SD 39.3 ± 11.2 , range 20 to 69) included 28 women and 38 men, all of whom were right-handed by self-report. The cerebral glucose metabolism of this group has been characterized as to relationships with age, sex and laterality in a previous report (Willis et al., 2002).

2.2 PET Scan Procedure

We used a Scanditronix PC1024-7B scanner (Uppsala, Sweden) with seven tomographic slices, in-plane full width half-maximum (FWHM) resolution of 6.5 mm, and slice thickness of 10 mm at the center of the field of view. Subjects fasted for at least three hours prior to scans. Intravenous and radial arterial lines were placed for tracer injection and arterial blood sampling (22 samples; at times: 0, 0:15, 0:30, 0:45, 1:00, 1:15, 1:30, 1:45, 2:00, 2:30, 3:00, 3:30, 4:00, 5:00, 6:00, 7:00, 10:00, 15:00, 20:00, 30:00, 40:00). Ambient noise was minimized, and subjects wore patches over their eyes and individually molded thermoplastic masks to restrict head motion.

The PET gantry was aligned to the canthomeatal line, and a $^{68}\text{Ge}/^{68}\text{Ga}$ rotating pin source was used to obtain transmission scans to allow correction of emission data for photon attenuation. Four to five mCi of FDG was administered over one minute. This was followed by a 30-minute uptake period during which subjects performed an auditory continuous performance task (CPT)

with subjects indicating responses using their right hand in an attempt to reduce variability in cognitive and emotional processes (Cohen et al., 1988).

Then four interleaved emission scans of seven slices each were acquired over the next 30 minutes, resulting in 28 planes spaced 3.5 mm apart, starting 8.5 mm above the canthomeatal line. Serial arterial blood samples were obtained so that a time-activity curve for the determination of absolute regional cerebral metabolic rates of glucose (rCMRglc) could be constructed. Image voxel values were converted from nanocuries per cubic centimeter (nCi/cc) to milligrams of glucose per hundred grams of tissue per minute (mg/hg/min) (Kumar et al., 1992).

2.3 Data Analysis

Image processing and analysis were performed on UNIX workstations (Sun Microsystems Inc. and Silicon Graphics Inc., both of Mountain View, CA) using Matlab (Mathworks, Sherborn MA) and Statistical Parametric Mapping (SPM, courtesy of Functional Imaging Laboratory, Wellcome Department of Imaging Neuroscience, London UK) software. ANALYZE (Mayo Foundation, Rochester MN) and NIH- and NIMH-developed software was also used in the image processing, analysis and presentation. Correlations of regional means were performed on a Macintosh (Apple Computer, Cupertino, CA) using StatView (Abacus Software, Berkeley, CA) and Microsoft Excel (Redmond, WA).

To be compatible with our studies regarding treatment prediction (Ketter et al., 1999), characterization of baseline metabolism (Ketter et al., 2001; Kimbrell et al., 2002), co-morbid anxiety features (Osuch et al., 1999), and relative metabolic and perfusion coupling (Dunn et al., 2002), identical image processing methods were used. Scans were visually inspected for artifacts and stereotactically normalized using a histogram equalization-based non-linear transformation as described previously (Ketter et al., 1999; Willis et al., 2002) into a standard space corresponding to the human brain atlas of Talairach and Tournoux (1988). These images were then smoothed with a Gaussian low-pass filter of 10 mm in-plane and 6 mm axial FWHM (in order to minimize noise and improve between-subject spatial alignment) for a final resolution of approximately 12 mm isotropically. Images generated with this processing were not significantly different from more current methods. For gray-matter estimation, the background noise threshold (above which a voxel is presumed to be within the brain) was set at one-eighth of the mean of the entire image space, and the whole-brain mean calculated using only these suprathreshold voxels. Voxels exceeding 60% of the whole-brain mean were then assumed to be gray matter. Global metabolic rate for glucose (gCMRglc) was then calculated as the mean of these voxels.

Each subject's image was then sampled in an automated manner with a set of 31 regional templates (see Figure 1), which had previously been delineated within a mask drawn from the Talairach atlas. This mask coincides well with cortical and subcortical structures as confirmed by overlaying the mask on a Talairach space structural MRI, and has been used in several prior FDG investigations (e.g., Ketter et al., 1999; Kimbrell et al., 1999; Dunn et al., 2002).

Fourteen regions were defined bilaterally and three in brainstem as single entities. The larger of these regions (cerebellum, orbital frontal, anterior and posterior temporal, dorsolateral prefrontal [DLPFC], anterior and posterior cingulate and insula) were sampled using a liberally-set SPM-style gray matter heuristic, counting only voxels falling within the template that had an intensity greater than 60% of whole-brain-mean (as defined above). The smaller brainstem (medulla, dorsal and caudal pons) and subcortical regions (amygdala, hippocampus, thalamus, caudate, and putamen), which have a higher gray/white ratio than cortical areas due to the neuropil-like nature, were sampled using all voxels within the template. The rationale for also sampling the inferior parietal in its entirety stemmed from the fact that its template is

a small sphere (of radius 6mm) contained completely within a gyrus and therefore likely to sample mostly gray matter.

The mean rCMRglc within the sampled area of each region was then divided by gCMRglc and the quotient multiplied by 100, resulting in mean normalized rCMRglc (MNRcmRglc). The purpose of this is to remove the confounding factor of intersubject differences in global metabolism, which might otherwise artifactually drive all brain regions to be positively correlated to varying degrees. The MNRcmRglc values across all subjects were correlated for every pair of regions, generating a metabolic associativity matrix (MAM), ordered roughly from phylogenetically and ontogenetically older structures to neocortical areas. Thirty-one regions produced 465 unique correlation tests. For this exploratory analysis, significant correlations are highlighted for both uncorrected ($p < .05$) and Bonferroni-corrected ($p < .0001$) rejections of the null hypothesis. P-values noted are all uncorrected.

Since age-related effects on normalized rCMRglc were seen in these 66 controls (Willis et al., 2001), an abbreviated analysis was performed similar to that above, except using age-gCMRglc regression corrected residuals of MNRcmRglc, to determine whether age effects could be acting as a confound in interregional correlations. No appreciable differences were seen, so our results are presented without age as a consideration.

For four regions of particular interest, spatially extended correlation maps of MNRcmRglc with rCMRglc (also proportionally normalized to gCMRglc) were produced showing more detailed brain-wide relationships to the region in question. These focus regions were the thalamus, amygdala, insula (defined as the area medial to the lateral sulcus in slices -4 mm through $+16$ mm) and inferior parietal (sampled by a sphere of radius 6 mm centered at $(-46, -50, 32)$ in the supramarginal gyrus). For this, voxel-wise Pearson correlations implemented by the general linear model (GLM) were performed with SPM95 (Friston et al., 1995). These resulted in Z-maps corresponding to the raw probabilities in each voxel of the null hypothesis ($r = 0$).

To correct for multiple comparisons within that regional SPM, these Z-maps were then submitted to cluster analysis (Friston et al., 1994) with a Z threshold of 1.96 (2-tailed $p = .05$) and a cluster probability threshold of $p = .05$. We chose liberal cluster parameters due to the exploratory nature of the study, and due to smaller sample sizes of the patient groups (Benson et al., 2007). Cluster analysis considers the smoothness of the Z-map, arising from the low-pass filtering applied to the input images as well as inherent spatial autocorrelation in the underlying brain function, in determining the effective independence of the multiple comparisons contained in the total brain volume analyzed. Raw two-tailed p-values were then displayed (for purposes of depicting topography) on axial slices for all voxels falling in clusters deemed significant. The atlas of Talairach and Tournoux (1988) was used to identify brain regions with significant associations. In addition to the metabolic associativity maps shown in the figures of this article, maps for the remaining regions may be found in the supplemental material online.

3. RESULTS

3.1 Metabolic Associativity Matrix

The metabolic associativity matrix (displayed in Figure 2) of 465 unique interregional correlations resulted in 131 (28% of all possible) relationships that were significant before Bonferroni correction (83 positive and 48 negative). After correction, 25 of these (all but one of which were positive) remained significant. The strongest positive association observed was between the left and right orbital frontal cortex ($r = .908$, $df = 64$, $p \ll .000001$ uncorrected) and the most negative between right DLPFC and right cerebellum ($r = -.470$, $p = .00007$). The

regions with the greatest number and diversity of associations were the medulla (with significant correlations to 18 regions before correction, 10 positive and 8 negative) followed by the left thalamus (10 positive and 5 negative) and the right thalamus (9 positive and 4 positive). The region with the fewest metabolic correlations was the right anterior temporal lobe, with just 3 positive associations.

Three general observations emerged: 1) structures or regions in similar developmental brain layers (i.e., subcortical or cortical) tended to correlate positively with each other; 2) cortical structures tended to correlate inversely with subcortical ones; and 3) homologous structures were positively correlated between the two hemispheres. Several regions which best illustrate these principles are discussed in more detail below. To test the significance of these observed general trends, we submitted each of the four groups of interaction types to combinatorial meta-analysis (Rosenthal, 1991). The 120 cortical-cortical, 105 subcortical-subcortical and 14 homologous associations demonstrated small but highly significant positive aggregate r -values of .081, .263 and .588 respectively with p -values of 3×10^{-9} or smaller. Conversely, the 240 cortical-subcortical associations showed a significant inverse aggregate $r = -.061$ ($p = 7 \times 10^{-14}$), providing additional support for the general trends beyond a simple number of positive vs. negative associations.

3.2 Examples of Regional Voxel-wise Correlations

The thalamus, which showed the second most prolific associativity, had robust positive associations bilaterally to the medulla and caudal (but not rostral) pons, entire caudate, hippocampal, and perihippocampal gray matter (Figure 3). Both left and right thalami also showed negative correlations with anterior cingulate, bilateral inferior frontal (left more than right) and middle temporal gyri. Correlations with the nearby portions of the basal ganglia (i.e., lentiform nuclei) were conspicuously absent (except for the most anterior portion near the head of caudate) despite strong positive correlations with the entire extent of the more distant caudate nuclei.

The amygdalae exhibited positive associations with each other and bilateral extended amygdala, as well as ipsilateral posterocaudal portions of the ventral striatum and insula (see Figure 4). The right amygdala had an inverse metabolic relationship with inferior, mid and superior frontal regions bilaterally in addition to dorsal portions of the anterior cingulate, which were not present on the left.

In contrast to the thalamus (Figure 3) and most other cortical regions, the associative connections of the left and right insula tended to be more unilateral (Figure 5). Each insula showed only limited positive couplings with its contralateral homologue, reflecting the cross-hemispheric r -value of just .35 in the MAM. Each insula was positively correlated only with its ipsilateral amygdala, globus pallidus, inferior/middle frontal gyrus, and medial temporal area. While the right insula correlated inversely with the right anterior cingulate, the left insula varied inversely with its ipsilateral posterior cingulate instead and positively with a more dorsal portion of anterior cingulate.

Metabolism in the inferior parietal regions showed unique associations not seen on the other side (Figure 6). The left inferior parietal region showed inverse correlations with the cerebellum bilaterally and the contralateral superior putamen, and positive correlation with dorsal medial anterior cingulate (see Figure 6, left side). None of these relationships were present with the homologous right inferior parietal lobule. The right inferior parietal area correlated negatively with bilateral thalamus and contralateral fusiform gyrus and posterior cingulate. Both inferior parietal regions correlated positively with the areas extending contiguously around themselves from about $Z = 0$ mm to +44 mm, traversing the middle and superior temporal gyri and the supramarginal gyrus (where it was sampled) and stopping just short of the somatosensory strip.

4. DISCUSSION

4.1 Metabolic Associativity Matrix

A salient feature of the metabolic associativity matrix is the coupling of most bilateral regions with their contralateral homologues with few exceptions (see boxed coefficients along the diagonal in Figure 2). This was also observed in healthy volunteers in three prior studies (Horwitz et al., 1984; Mallet et al., 1998; Metter et al., 1984). The orbital frontal cortex, which was the most strongly coupled ($r = .91$) homologous pair, was also highly correlated ($r = .76$) in resting blood flow in Zald et al. (1998). While some of this may be attributable to genetically-coded parallel development of the structures within a given subject and to smoothing issues for midline regions (see Limitations below), it also likely expresses a degree of symmetry in the hemispheric functions of homologous areas, in addition to direct yoking of activity via anterior commissure and callosal fibers.

It is interesting to note, however, that while some regions (chiefly frontal, thalamic, extrapyramidal and cerebellar areas) were strongly interhemispherically coupled, this was much less robust for others (anterior and posterior temporal and cingulate, insula, and inferior parietal areas). This distinction was not related to the specific spatial extent of the area sampled and might suggest more specific lateralized functions in these latter regions. Another general pattern evident in the matrix is that both cortical regions and subcortical structures correlated positively amongst themselves, but subcortical covaried inversely with neocortical regions (note predominance of yellow and gold near diagonal and blue in upper right and lower left quadrants of Figure 2). While only one of the latter inverse relationships survived Bonferroni correction, combinatorial meta-analysis showed the overall observation to bear out significantly. The positive associations amongst cortical regions may be a metabolic reflection of the extensive cortical to cortical networks, especially among secondary association areas. The positive relationship among subcortical areas is not unexpected, given the strong interconnections across extrapyramidal and limbic areas. The inverse relationships of activity in cortical and subcortical structures are consistent with previous concepts of anatomical, developmental, and functional divergence of these structures (MacLean, 1973; Deutch, 1992). Positive correlations could be related to excitatory glutamatergic projections, while negative correlations could be related to inhibitory gabaergic interneuron connections. These putative cortical-subcortical loops (Alexander and DeLong et al, 1990) were the basis for our development of prefrontal rTMS as a treatment for depression, where periodic and repeated prefrontal stimulation would re-regulate subcortical structures over time.

It is of interest that the thalamus (after the medulla) is the structure with the most numerous and diverse positive and negative regional associations, in light of the work of Llinás (1998), suggesting that the thalamus is a sensory integration and polling “hub” through which different areas of cortex communicate with other areas. The anterior thalamus appears to have connections with limbic areas, which could permit a role in affective processing. In Llinás’ view, this thalamocortical communication is a likely substrate of cognitive and perceptual states, and even consciousness. What is unclear from our data is the significance of the predominantly inverse correlations between thalamus and cortical areas. It is possible that inhibitory GABA projections from the thalamus (Wallenstein, 1994) are involved, however the metabolic valence of inhibitory pathways in PET measurements is still not fully understood (Tagamets and Horwitz, 2001). The robust and balanced nature of thalamic functional associations are also consistent with the critical role of this structure in the striatal-thalamic-cortical loops crucial to the modulation of motor, cognitive and emotional behavior in the models of Alexander and DeLong (1990). Recently, Mitelman et al (2006) also observed extensive metabolic associations between major thalamic nuclei and nearly all cortical Brodmann areas during a verbal learning task, underscoring the abundance of pathways to and from this structure.

Also of note were inverse relationships between frontal cortical areas and cerebellum, and that metabolism in the cerebellum was differentially correlated with anterior cingulate (negatively) and posterior cingulate (positively). These opposing relationships may be pertinent to the recent emphasis of the role of the cerebellum in emotional and cognitive coordination (Schmahmann and Sherman, 1998), in addition to its more classically understood role in the timing of motor function.

Finally, the inverse relationships between anterior and posterior regions noted by Metter, et al (1984) were not evident in our data (with the exception of a strong inverse coupling between cerebellum and frontal regions), and were also not prominent in any of the other previous studies cited.

Regional SPMs—The voxel-wise regional associativity maps aid in elucidating finer-grained associations between and within the regions (some of which are rather large) represented in the metabolic associativity matrix (MAM). In some cases, these associative patterns may describe more intricate topologies of functional circuits active during an emotionally neutral, cognitively nonchallenging task, such as the auditory CPT we used.

Additionally, the covariance of a region or structure with areas extending contiguously beyond the sampled area (which were delineated from the Talairach & Tournoux atlas) may augment current attempts to cytoarchitectonically re-define the Brodmann areas (e.g., Zilles and Palomero-Gallagher, 2001) into more appropriate isofunctional regions of the brain. One particularly robust example of this may be in the peri-regional correlations with amygdala metabolism. While the sampled area for the amygdala was limited to the classical almond-shaped structure drawn from four axial slices of the Talairach atlas, the regional “autocorrelation” map indicates its functional covariance (at least during the CPT) with areas extending not only medially and dorsally through the extended amygdala, but also laterally to insula and more lateral portions of the temporal lobes. This covarying area extends outward several times as far as the half-power distance of the Gaussian smoothing kernel used on the images, and is not likely due to inter-subject variance in the stereonormalization process. Thus, these association patterns are convergent with current concepts of the extended amygdala (Heimer, 2004) and may augment other anatomical and physical methods for redefining the extent of the periamygdalar functional territory.

Similar definition of the extent of isofunctional mapping might be gleaned from the regional associativity patterns for inferior parietal metabolism. While the covariate MNR_{CMR}glc was sampled from a small sphere in the supramarginal gyrus (intersecting three axial slices), this metabolic rate covaried across the 66 subjects with a large region around it that extended for eleven slices. The functional continuity during this simple task extends from the inferior parietal lobules through the angular gyrus around into the temporal lobes.

A more striking finding of differential lateralized associativity was the robust metabolic coupling of left parietal cortex with the cerebellum bilaterally, while the right parietal region showed no such relationships. While this may be specific to the CPT task during the scan, it may also yield inferences about underlying differences in normal parieto-cerebellar connectivity in the two hemispheres.

Lateral specificity in parietal function is additionally suggested by cross-hemispheric associations seen in the regional maps. The left parietal area correlated inversely with a region of the right putamen, while the right parietal area correlated inversely with the left posterior cingulate. The overall divergence in associativity patterns between left and right parietal regions was more striking than in most other cortical regions, and may indicate a noteworthy laterality in the basic functions subserved by these anatomically homologous regions. This

would be consistent with the very different clinical syndromes associated with functional lesion of right versus left parietal cortex in man (e.g., Coghill et al., 2001; Rumsey et al., 1999).

The relative unilaterality and potential autonomy of each insula is especially evident in their regional associativity maps. The paucity of cross-hemispheric correlations seen in the MAM (Figure 2) are revealed as a relative “hemispheric disconnect” in normal volunteers at even the voxel level (Figure 5). Not only is there only limited coherence with the contralateral homologue for each insula, but also few voxels of either positive or negative correlation with either insula appear in the opposing half of the brain. This relative independence may suggest a degree of hemispheric specialization of insular function not seen in most other structures or regions studied. Given the prominent role of the insula in autonomic regulation and emotion, further study and clarification of these differences in hemispheric associativity as they related to differences in lateralized insula function appear indicated. It is noteworthy in this regard that the severity of the psychomotor and anhedonic clusters in the Beck Depression inventory correlated inversely with metabolic activity in the left (but not the right) insula in patients with both unipolar and bipolar illness (Dunn et al., 2002).

These interregional correlations may illuminate pathways of normal baseline neural connectivity, thus providing a standard against which psychiatrically ill patients engaged in the same task may be assessed, which reported in the accompanying paper (Benson et al., 2005). The detection of divergences from these normal patterns of association in patients with affective and other illnesses may aid in uncovering functional circuits abnormally active or inactive in patients, as well as others of a compensatory nature arising from the illness and not present in healthy adults.

Limitations of the study—A noteworthy limitation of our study is the uncertainty of the source of the interregional correlations observed. There are several alternative explanations of interregional covariance beyond that of functional connectivity, including similarities in: intracellular mechanism of glucose metabolism, gray/white or neuronal/glia percentages, local tissue density, or CSF volume contribution. Additionally, smoothing across the interhemispheric fissure causes some overstatement of the metabolic coupling between homologues of structures close to the midline. It is also possible that functional covariance between homologous areas is partially an expression of genetically coded roughly similar patterns of neural activity rather than actual communication between the regions via the corpus callosum (Zald et al., 1998). Moreover, the very different patterns of neural activity pathways in the left versus right parietal lobes suggest that even homologous areas putatively connected by the commissures and corpus callosum do not always function in parallel.

In contrast, the lack of certain relationships could also be due to the larger size of structures with internally diverse functions (e.g., cerebellum), the proximity to anatomically distinct but spatially close areas, and/or irregular shapes (e.g., insula) of the ROIs, rather than functional disconnection. For example, the precise location of the template is more critical for the insula, which has a long narrow shape, proximity to the claustrum and anatomically disjoint but spatially close temporal lobes, and the possibility of CSF from the Sylvian Fissure contributing to artifacts in relationships. These attributes increase the likelihood of a ROI incorporating voxel values that are quite variable and possibly functionally unrelated, thus generating diluted mean values that result in null relationships with other voxels across the brain. These confounds reduce the possibility of reflecting actual anatomical connections. In defense of these results, the existence of reciprocal pathways between the DLPFC and the cerebellum, and vice-versa, is a viable explanation of the inverse relationships between these two areas.

In addition, functional connectivity in the brain is generally considered to be context-dependent. While the CPT performed by our volunteers was chosen for its lack of major

affective content or cognitive stress, therefore providing a consistent mental domain in which to quantify cerebral baseline activity in both patients and controls, it is likely that some or many of the associativity patterns demonstrated here are not generalizable to other tasks or mental states. Moreover, this task may not be the ideal one in which to elucidate pathological circuitry in mood disorder patients compared to that in healthy controls. A task that engages more cognitive or emotional processes may show very different patterns of associativity. Nonetheless, very striking differences in associativity were observed between these healthy controls and affectively ill individuals, and as well between those with bipolar and unipolar illness as discussed in the accompanying paper (Benson et al., 2007), suggesting that even this neutral task reflects meaningful differences among groups. These might arise from chronically over- or under-used pathways or those related to attentional processes, such that they become engaged differently in patients and healthy subjects even during a simple task.

A final consideration in interpreting these data is that we chose a cross-sectional, rather than within-subjects, approach to studying metabolic associativity. Owing to the capability of collecting many time points during the scanning of each subject, fMRI paradigms allow for a potentially richer and more robust way to measure coupling of activity between brain regions. Unfortunately absolute measurement of metabolic activity using fMRI is still not available, and within-subject analysis using PET is impractical due to dosimetry limitations. However, since our goal was to compare coupling patterns between one presumed relatively homogenous group (of normal volunteers, to contrast with other patient groups in Benson et al. 2007) rather than to investigate heterogeneity within a given group, we believe that the power supplied by our large sample compensates for this shortcoming.

A further limitation concerning these data pertains to the possibility of gender dissociations in metabolic associativity. Due to the overwhelming nature of the data to be presented here we did not exhaustively investigate this variable, but it is likely that important differences would be uncovered in future study. For instance, Kilpatrick et al. (2006) demonstrated significant sex-related differences in amygdala functional connectivity during a resting state.

Conclusions

This exploratory study demonstrates numerous correlative metabolic associations existing within the healthy adult brain during a simple attentional task chosen to be largely devoid of affective content. The associative patterns demonstrate both expected and novel types of functional connectivity in normal adults. The positive correlations among cortical regions as well as among subcortical regions, but the inverse relationships between cortical and subcortical structures are also particularly noteworthy and may reveal fundamental patterns of normal regional neural function and connectivity. The extensive positive associations of the amygdala with surrounding structures are consistent with the concept of the close anatomical and functional connectivity of the extended amygdala.

Metabolism in homologous brain regions in each hemisphere was usually highly intercorrelated, but the insular and parietal cortices were notable exceptions. Differential anatomy and valence of connectivity of the left versus right parietal cortex is evident as are the striking unilateral hemispheric associativity of both the left and right insula, perhaps suggesting greater than previously recognized differences in specialization and function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the intramural program of the NIMH, NIH, DHHS. The work of Mark Willis, Terence Ketter, and Mark George was supported by Ted and Vada Stanley of the Stanley Foundation. The authors wish to thank the PET technologists and cyclotron/radiochemistry staff of the NIH Clinical Center PET Department for their expertise. Software provided by Neil Weisenfeld and José M. Maisog, M.D. of the NIMH was used in the processing and analysis of our data. These data were presented in abstract and poster form at the Society of Biological Psychiatry 55th Annual Scientific Convention, May 11–13, 2000, Chicago, Illinois.

Work was conducted in the intramural program of NIMH; Salary support for MWW was provided by the Stanley Medical Research Institute and Henry M. Jackson Foundation for the Advancement of Military Medicine

References

- Abou-Saleh MT, Al Suhaili AR, Karim L, Prais V, Hamdi E. Single photon emission tomography with 99m Tc-HMPAO in Arab patients with depression. *Journal of Affective Disorders* 1999;55:115–123. [PubMed: 10628880]
- Alexander GE, Crutcher MD, DeLong MR. Basal ganglia-thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. *Progress in Brain Research* 1990;85:119–146. [PubMed: 2094891]
- Andreason NC, Nopoulos SP, O'Leary DS, Miller DD, Wassink T, Flaum M. Defining the phenotype of schizophrenia: cognitive dysmetria and its neural mechanism. *Biol Psychiatry* 1999;46:908–920.
- Austin MP, Dougall N, Ross M, Murray C, O'Carroll RE, Moffoot A, Ebmeier KP, Goodwin GM. Single photon emission tomography with 99mTc-exametazime in major depression and the pattern of brain activity underlying the psychotic/neurotic continuum. *Journal of Affective Disorders* 1992;26:31–43. [PubMed: 1430666]
- Bench CJ, Friston KJ, Brown RG, Scott LC, Frackowiak RS, Dolan RJ. The anatomy of melancholia--focal abnormalities of cerebral blood flow in major depression. *Psychological Medicine* 1992;22:607–615. [PubMed: 1410086]
- Brody AL, Saxena S, Stoessel P, Gillies LA, Fairbanks LA, Alborzian S, Phelps ME, Huang S, Wu H, Ho ML, Ho MK, Au SC, Maidment K, Baxter LR. Regional brain metabolic changes in patients with major depression treated with either paroxetine or interpersonal therapy. *Archives of General Psychiatry* 2001;58:631–640. [PubMed: 11448368]
- Buchsbaum MS, Someya T, Wu JC, Tang CY, Bunney WE. Neuroimaging bipolar illness with positron emission tomography and magnetic resonance imaging. *Psychiatric Annals* 1997;27:489–495.
- Buchsbaum MS, Hazlett EA, Hazenedar MM, Spiegel-Cohen J, Wei TC. Visualizing fronto-striatal circuitry and neuroleptic effects in schizophrenia. *Acta Psychiatr Scand* 1999;99:129–137.
- Clark CM, Kessler R, Buchsbaum MS, Margolin RA, Holcomb HH. Correlational methods for determining regional coupling of cerebral glucose metabolism: A pilot study. *Biol Psychiatry* 1984;19(5):663–677. [PubMed: 6610442]
- Coghill RC, Gilron I, Iadarola MJ. Hemispheric lateralization of somatosensory processing. *Journal of Neurophysiology* 2001;85:2602–2612. [PubMed: 11387404]
- Cohen RM, Semple WE, Gross M, Holcomb HH, Dowling SM, Nordahl TE. Functional localization of sustained attention: comparison to sensory stimulation in the absence of instruction. *Neuropsychiatry, Neuropsychology and Behavioral Neurology* 1988;1:3–20.
- Diagnostic and Statistical Manual of Mental Disorders. Text Revision. Vol. 4th Ed. The American Psychiatric Association; 2000.
- Deutch AY. The regulation of subcortical dopamine systems by the prefrontal cortex: interactions of central dopamine systems and the pathogenesis of schizophrenia. *Journal of Neural Transmission Supplementum* 1992;36:61–89. [PubMed: 1527521]
- Drevets WC, Videen TO, Price JL, Preskorn SH, Carmichael ST, Raichle ME. A functional anatomical study of unipolar depression. *Journal of Neuroscience* 1992;12:3628–3641. [PubMed: 1527602]
- Dunn RT, Kimbrell TA, Ketter TA, Frye MA, Willis MW, Luckenbaugh DL, Post RM. Principle components of the Beck depression inventory and regional cerebral metabolism in unipolar and bipolar depression. *Biol Psychiatry* 2002;51:387–399. [PubMed: 11904133]

- Friston KJ, Frith CD, Liddle PF, Frackowiak RS. Functional connectivity: the principal-component analysis of large (PET) data sets. *Journal of Cerebral Blood Flow and Metabolism* 1993;13:5–14. [PubMed: 8417010]
- Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping* 1995;2:189–210.
- Friston KJ, Worsley KJ, Frackowiak RSJ, Mazziotta JC, Evans AC. Assessing the significance of focal activations using their spatial extent. *Human Brain Mapping* 1994;1:210–220.
- Friston KJ, Frith CD, Fletcher P, Liddle PF, Frackowiak RS. Functional topography: multidimensional scaling and functional connectivity in the brain. *Cereb Cortex* 1996;6(2):156–164. [PubMed: 8670646]
- Heimer L. A new anatomical framework for neuropsychiatric disorders and drug abuse. *Am J Psychiatry* 2003;160(10):1726–1739. [PubMed: 14514480]Review
- Horwitz B, Schapiro MB, Grady CL, Rapoport SI. Cerebral metabolic pattern in young adult Down's syndrome subjects: altered intercorrelations between regional rates of glucose utilization. *J Ment Deficiency Res* 1990;34:237–252.
- Horwitz B. Functional interactions in the brain: use of correlations between regional metabolic rates. *Journal of Cerebral Blood Flow and Metabolism* 1991a;11:A114–A120. [PubMed: 1997479]
- Horwitz B, Swedo SE, Grady CL, Pietrini P, Schapiro MB, Rapoport JL, Rapoport SI. Cerebral metabolic pattern in obsessive-compulsive disorder: Altered intercorrelations between regional rates of glucose utilization. *Psychiatry Research: Neuroimaging* 1991b;40:221–237.
- Horwitz B, Duara R, Rapoport SI. Intercorrelations of glucose metabolic rates between brain regions: application to healthy males in a state of reduced sensory input. *Journal of Cerebral Blood Flow and Metabolism* 1984;4:484–499. [PubMed: 6501442]
- Ketter TA, Kimbrell TA, George MS, Willis MW, Benson BE, Danielson A, Frye MA, Herscovitch P, Post RM. Baseline cerebral hypermetabolism associated with carbamazepine response, and hypometabolism with nimodipine response in mood disorders. *Biological Psychiatry* 1999;46:1364–1374. [PubMed: 10578451]
- Ketter TA, Kimbrell TA, George MS, Dunn RT, Speer AM, Benson BE, Willis MW, Danielson A, Frye MA, Herscovitch P, Post RM. Effects of mood and subtype on cerebral glucose metabolism in treatment-resistant bipolar disorder. *Biol Psychiatry* 2001;49(2):97–109. [PubMed: 11164756]
- Kilpatrick LA, Zald DH, Pardo JV, Cahill LF. Sex-related differences in amygdala functional connectivity during resting conditions. *Neuroimage* 2006;30:452–461. [PubMed: 16326115]
- Kimbrell TA, Little JT, Dunn RT, Frye MA, Greenberg BD, Wassermann EM, Repella JD, Danielson AL, Willis MW, Benson BE, Speer AM, Osuch E, George MS, Post RM. Frequency dependence of antidepressant response to left prefrontal repetitive transcranial magnetic stimulation (rTMS) as a function of baseline cerebral glucose metabolism. *Biological Psychiatry* 1999;46:1603–1613. [PubMed: 10624541]
- Kumar A, Braun A, Schapiro M, Grady C, Carson R, Herscovitch P. Cerebral glucose metabolic rates after 30 and 45 minute acquisitions: a comparative study. *Journal of Nuclear Medicine* 1992;33:2103–2105. [PubMed: 1460500]
- Kimbrell TA, Ketter TA, George MS, Little JT, Benson BE, Willis MW, Herscovitch P, Post RM. Regional cerebral glucose utilization in patients with a range of severities of unipolar depression. *Biol Psychiatry* 2002;51(3):237–252. [PubMed: 11839367]
- Llinas R, Ribary U, Contreras D, Pedroarena C. The neuronal basis for consciousness. *Philosophical Transactions of the Royal Society of London Series B -Biological Sciences* 1998;353:1841–1849.
- MacLean, PD. A triune concept of the brain and behavior. In: Boag, TJ.; Campbell, D., editors. *The Clarence M. Hicks Memorial Lectures*, 1969. Toronto: University of Toronto Press; 1973. p. 6–66.
- Mallet L, Mazoyer B, Martinot JL. Functional connectivity in depressive, obsessive-compulsive, and schizophrenic disorders: an explorative correlational analysis of regional cerebral metabolism. *Psychiatry Research* 1998;82:83–93. [PubMed: 9754451]
- Mayberg HS, Brannan SK, Mahurin RK, Jerabek PA, Brickman JS, Tekell JL, Silva JA, McGinnis S, Glass TG, Martin CC, Fox PT. Cingulate function in depression: a potential predictor of treatment response [see comments]. *Neuroreport* 1997;8:1057–1061. [PubMed: 9141092]

- Metter EJ, Riege WH, Kuhl DE, Phelps ME. Cerebral metabolic relationships for selected brain regions in healthy adults. *Journal of Cerebral Blood Flow and Metabolism* 1984;4:1–7. [PubMed: 6607258]
- Mitelman SA, Byne W, Kemether EM, Newmark RE, Hazlett EA, Haznedar MM, Buchsbaum MS. Metabolic thalamocortical correlations during a verbal learning task and their comparison with correlations among regional volumes. *Brain Research* 2006;1114:125–137. [PubMed: 16935269]
- Moeller JR, Strother SC, Sidtis JJ, Rottenberg DA. Scaled subprofile model: a statistical approach to the analysis of functional patterns in positron emission tomographic data. *Journal of Cerebral Blood Flow and Metabolism* 1987;7:649–658. [PubMed: 3498733]
- Osuch EA, Ketter TA, Kimbrell TA, George MS, Benson BE, Willis MW, Herscovitch P, Post RM. Regional cerebral metabolism associated with anxiety symptoms in affective disorder patients. *Biol Psychiatry* 2000;48:1020–1023. [PubMed: 11082477]
- Rosenthal, R. *Meta-Analytic Procedures for Social Research* (revised ed.). Newbury Park, CA: Sage; 1991.
- Rumsey JM, Horwitz B, Donohue BC, Nace KL, Maisog JM, Andreason P. A functional lesion in developmental dyslexia: left angular gyrus blood flow predicts severity. *Brain and Language* 1999;70:187–204. [PubMed: 10550226]
- Sackeim HA. Functional brain circuits in major depression and remission. *Archives of General Psychiatry* 2001;58:649–650. [PubMed: 11448370]
- Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain* 1998;121:561–579. [PubMed: 9577385]
- Stark CE, Squire LR. When zero is not zero: the problem of ambiguous baseline conditions in fMRI. *Proc Natl Acad Sci U S A* 2001;98(22):12760–12766. [PubMed: 11592989] Epub 2001 Oct 9
- Strakowski SM, DelBello MP, Adler C, Cecil DM, Sax KW. Neuroimaging in bipolar disorder. *Bipolar Disorders* 2000;2:148–164. [PubMed: 11256682]
- Tagamets MA, Horwitz B. Interpreting PET and fMRI measures of functional neural activity: the effects of synaptic inhibition on cortical activation in human imaging studies. *Brain Research Bulletin* 2001;54:267–273. [PubMed: 11287131]
- Talairach, J.; Tournoux, P. *Co-planar Stereotaxic Atlas of the Human Brain*. New York, NY: M. Rayport, translator, Thieme; 1988.
- Wallenstein GV. Simulation of GABAB-receptor-mediated K⁺ current in thalamocortical relay neurons: tonic firing, bursting, and oscillations. *Biological Cybernetics* 1994;71:271–280. [PubMed: 7918804]
- Willis MW, Ketter TA, Kimbrell TA, George MS, Herscovitch P, Danielson AL, Benson BE, Post RM. Age, sex and laterality effects on cerebral glucose metabolism in healthy adults. *Psychiatry Research: Neuroimaging* 2002;114(1):23–37.
- Zald DH, Donndelinger MJ, Pardo JV. Elucidating dynamic brain interactions with across-subjects correlational analyses of positron emission tomographic data: the functional connectivity of the amygdala and orbitofrontal cortex during olfactory tasks. *Journal of Cerebral Blood Flow and Metabolism* 1998;18:896–905. [PubMed: 9701351]
- Zilles K, Palomero-Gallagher N. Cyto-, myelo-, and receptor architectonics of the human parietal cortex. *Neuroimage* 2001;14:S8–S20. [PubMed: 11373127]

FIGURE 1.

Templates used for sampling mean normalized regional cerebral metabolic rate for glucose (MNR_rCMR_{glc}) in 14 bilateral and 3 brainstem regions. These regions were delineated from a mask corresponding to the Talairach atlas and the stereonormalization image template. MNR_rCMR_{glc} values calculated for each subject were used to generate the Metabolic Associativity Matrix (MAM) and as the covariate of interest for the regional associativity maps (see subsequent figures).

FIGURE 2.

Metabolic Associativity Matrix (MAM) showing interregional correlations of mean normalized metabolism (MNCMRglc). Homologous structures and regions were all positively coupled (boxes along diagonal). MNCMRglc values in subcortical structures tended to correlate positively with those in other subcortical structures (upper left portion of matrix) and negatively with those in cortical regions (upper right or lower left). Cortical regions tended to correlate positively with other cortical areas (lower right).

FIGURE 3.

Statistical parametric maps showing clusters of significant correlations with left thalamic (left half of figure) and right thalamic (right half) MNRcmrglc. Patterns were similar for both sides, showing positive associations (red-orange-yellow) with brainstem, cerebellum, caudate and hippocampal gyrus. Inverse associations (shades of blue) were principally cortical: anterior cingulate, posterior temporal, parietal, and some inferior frontal cortex.

FIGURE 4.

Statistical parametric maps showing MNRcmrglc associations with left and right amygdala. Each amygdala positively correlated metabolically with its contralateral homologue as well as with a region spreading superiorly through extended amygdala and into insula and posterocaudal portions of ipsilateral putamen. The right amygdala showed an inverse metabolic relationship with inferior, mid and superior frontal regions bilaterally, in addition to dorsal portions of ipsilateral anterior cingulate.

FIGURE 5.

Statistical parametric maps showing MNRcmrglc associations with left and right insula. These regions demonstrated a high degree of hemispheric autonomy, with only weak positive correlation with each other and only limited cross-hemispheric associations. Each insula positively correlated with its ipsilateral globus pallidus, inferior/middle frontal gyrus and temporal lobe. The right insula showed inverse association with its ipsilateral amygdala, anterior cingulate, while the left insula had a weak positive association with that same area.

Normalized Correlations of rCMRglu with Mean Inf. Parietal CMRglu in 66 Controls

FIGURE 6.

Statistical parametric maps showing MNRcmrglc associations with left and right inferior parietal lobules. Parietal coupling is almost entirely laterally specific. The left inferior parietal lobule has an inverse relationship that spans most of the cerebellum, while the right shows none. The left also uniquely relates in an inverse manner to right posterior putamen and positively to medial anterior cingulate, while the right parietal alone correlates inversely to left fusiform gyrus and posterior cingulate.