SUPPLEMENTARY MATERIALS

Title: Brain Morphology Links Systemic Inflammation to Cognitive Function in Midlife Adults.

Authors: Anna L. Marsland†, Peter J. Gianaros, Dora C.-H. Kuan, Lei K. Sheu, Katerina Krajina, and Stephen B. Manuck.

SUMMARY

This supplementary information includes details regarding study methods and results.

TABLE OF CONTENTS

SUPPLEMENTARY METHODS

Assessment of Cognitive Function

Spatial reasoning. Spatial perception and reasoning were assessed using the Block Design and Matrix Reasoning subtests from the Wechsler Abbreviated Scale of Intelligence (WASI; (Coorporation, 1999; Wechsler, 1997a)). The Block Design test involves viewing a constructed model or picture and using red- and white-blocks to re-create the design within a specified time limit. The Matrix Reasoning test involves viewing an incomplete matrix and selecting the response option that completes the matrix.

Working Memory. Verbal and non-verbal working memory were assessed with the Digit Span subtest from the Wechsler Adult Intelligence Scale – III (WAIS-III, (Wechsler, 1997a) and the Spatial Span subtest from the Wechsler Memory Scale-III (WMS-III, (Wechsler, 1997b)), respectively. In the first instance, random number sequences of increasing length are presented orally to subjects, who are asked to repeat the number sequence either in the same or reverse order. The Spatial Span subtest requires subjects to repeat spatial patterns demonstrated on a 3-dimensional board, both in the order demonstrated and in reverse order. Both of these tests are used as measures of working memory.

Visuomotor Processing Speed. To assess processing speed, participants completed the first parts of the Trail Making Test (Reitan, 1985), and the Stroop Color-Word Test (Golden, 1978). Part A of the Trail Making Test requires subjects to draw a line connecting randomly arrayed, consecutively numbered circles as quickly as possible. The first 2 parts of the Stroop Color-Word Test require subjects to read a list of color names (i.e., red, green, blue) and to name the colors of the inks of congruous words (i.e., the word red printed in red ink) as quickly as possible.

Verbal Proficiency. The Vocabulary and Similarities subtests from the WASI were administered as tests of verbal comprehension and reasoning. The Vocabulary subtest requires subjects to define words that are presented orally. In the Similarities subtest, subjects are presented with two words that represent common objects of concepts and they are required to describe how they are similar.

Verbal Learning and Memory. Verbal memory was assessed with the Four Word Short-Term Memory Test (Kobayashi et al., 2010). On each trial of this test, four unrelated words are read to the subject, followed by a 3 digit number. The subject counts backwards in threes from this number for 5, 15, or 30 seconds. After this period, the subject is asked to recall the words. Participants were also administered the Rey Verbal Learning Test, a measure of auditory verbal learning and memory (Rey, 1958). Here, participants listen to a recorded list of 15 unrelated words and have to recall as many as possible. They complete 5 trials with the original list followed by an interference list of novel words and then recall of the original list.

Executive Function (mental flexibility, response inhibition). Participants were administered two tests of executive functioning: the Trail Making Test (Reitan, 1985), and the Stroop Color-Word Test (Golden, 1978). The Trail Making Test involves 2 parts. Part A, described above, provides a test of visuomotor speed. Part B of the test requires subjects to draw a line connecting numbered and lettered circles as quickly as possible, alternating numbers and letters (e.g., 1-A-2-B-3-C…). To derive a measure of cognitive function that is independent of psychomotor speed a difference score is calculated (i.e., Part B – Part A), with higher difference scores reflecting poorer performance. The Stroop Color-Word Test requires subjects to read aloud as quickly as possible from 3 pages of color word lists: Page 1 requires reading a list of color names (i.e., red, green, blue); page 2 requires naming the colors of the inks; and page 3 requires naming the color of the ink from a list of color names printed in incongruent colors (e.g., the word blue printed in yellow ink). Here, scores are the number of correct responses within a 45 second period, with higher scores indicating better performance.

In addition an interference score was calculated, indicating the participant's susceptibility to interference (i.e., difficulty inhibiting a primary verbal response). This score is derived by first calculating: (no. items/45 s on page 2 X no. items/45 s on page 1)/(no. items/45 s on page 2 + no. items/45 s on page 1). This provides a predicted score for page 3, which is then subtracted from the actual score for page 3 (no. items/45 s). This difference score reflects the degree of interference.

MRI data acquisition, preprocessing, and analysis

T1-weighted 3D magnetization-prepared rapid gradient echo (MPRAGE) neuroanatomical images were acquired over 7 min 17 sec by these parameters: FOV = 256×208 mm, matrix size= 256×208, TR = 2100 ms, inversion time (TI) = 1100 ms, TE = 3.29 ms, and FA = 8° (192 slices, 1mm thick, no gap).

Image Processing.

The FreeSurfer 5.3.0 software package (http://surfer.nmr.mgh.harvard.edu) was used to measure cortical surface area, cortical thickness, and volumetric data (Fischl and Dale, 2000). The surface-based cortical reconstruction included affine-registration to the Talairach atlas, intensity bias correction, intensity normalization, and skull stripping. White matter segmentation was based on the intensity and location of white matter voxels with neighboring intensity. The two hemispheres were separated to perform surface extraction to demarcate boundaries between white and gray matter (i.e. white surface) and gray and pia matter (i.e. pial surface). Cortical thickness was measured as the closest distance between the white and pial surface at each vertex on the tessellated surface. These surfaces were then inflated and registered to the spherical atlas that aligns cortical folding patterns to match cortical geometry on an average map for computing surface area. The cortical surfaces were further segregated into 34 regions of interest (ROIs) in each hemisphere based on the probabilistic information estimated from the Desikan-Killiany cortical atlas in Freesurfer. These ROIs were mapped onto the four cortical

lobes. Subcortical segmentation and volumetric determination were conducted using the volumetric segmentation stream in Freesurfer (Fischl et al., 2002) (See Figure S1). Intracranial volume (ICV) was estimated from the atlas-scaling factor based on the transformation of the full brain mask with the Talairach atlas (Buckner et al., 2004).

Regional Assessment of Cortical Lobe Structure

The 34 ROIs were mapped onto the four cortical lobes (see Figure S2). Here, the frontal lobe comprised left and right caudal anterior cingulate, rostral anterior cingulate, superior frontal gyrus, rostal and caudal middle frontal gyrus, inferior frontal gyrus (including subdivisions of pars opercularis, pars orbitalis, pars triangularis), lateral and medial orbitofrontal cortex, frontal pole, paracentral lobule, and precentral gyrus. The parietal lobe comprised the posterior and isthmus cingulate cortex, superior and inferior parietal cortex, postcentral gyrus, precuneus cortex, and supramarginal gyrus. The temporal lobe comprised the entorhinal cortex, fusiform gyrus, superior, middle, and inferior temporal gyrus, parahippocampal gyrus, temporal pole, and transverse temporal cortex. The occipital lobe comprised cuneus cortex, lateral occipital cortex, lingual gyrus, and pericalcarine cortex. The surface area and the average thickness for each ROI were calculated, with the volume being derived as the product of the surface area and the average thickness. The volumes and surface area of each lobe were computed as the sum of the volumes and surface of the ROIs in the lobe over the left and right hemispheres. The thickness of each cortical lobe was computed as the average over the left and right hemispheres.

Mediation testing

Multiple meditator models were used to test whether the volumes of the four cortical lobes, individually and as a whole, indirectly mediated the associations of inflammation and cognitive function. The models can be described as follows:

$$Y = \mu_Y + c^{'} X + \sum_i b_i M_i + \sum_j q_j C_j + \varepsilon_Y \quad (Eq1)$$

$$M_i = \mu_i + a_i X + \sum_j p_j C_j + \varepsilon_i \quad (Eq2)$$

$$direct\ effect = c^{'}$$
$$indirect\ effect = a_i * b_i\ , \quad i = 1,..4,$$

where Y: cognitive function, X: inflammation score, M_i: individual mediator (brain volume), and C_j: covariates. The direct effect (c') of inflammation (X) on cognitive function (Y) was modeled by the associations of the inflammatory score with a given cognitive function, after controlling for age, sex, ICV (C_j), and the multiple mediator variables corresponding to the indirect paths $(M_i,\ i = 1,..4)$, see Eq1. The "indirect path" effect via a given mediator (M_i) was modeled as the product of the association of the inflammatory score with the mediating variable (controlling for the covariates, see Eq 2), a_i, and the associations of the mediating variable with the cognitive scores, controlling for inflammatory score, covariates, and other mediating variables b_i. The total indirect effect here corresponds to the sum of all indirect effects, $\sum(a_i b_i)$. Hence, for every indirect path (a*b path) model, the gray matter volume of all four lobes were treated simultaneously as multiple mediators. The *c'* effects were tested for significance assuming normality of these regression coefficients, while $a_i * b_i$ effects were tested using bootstrapping with 5000 iterations because of the non-normality of these product coefficients. The 95% confidence interval of the mediation effect, $a_i * b_i$, was determined from the bootstrapped distributions. This method enabled the examination of whether overall gray matter volume and/or the volumes of the four cortical lobes accounted for (mediated) the associations of inflammation with cognitive function.

SUPPLEMENTARY TABLES

Table S1. Partial correlations between total volumes of subcortical regions and inflammation measures covarying age, sex, and intracranial volume.

	Inflammatory Scores		**IL-6**		**CRP**	
	N=403		N=401		N=403	
Total Volume	*r*	*p*	*r*	*p*	*r*	*p*
Accumbens area	-.01	.84	.04	.46	-.02	.74
Amygdala	-.04	.46	-.04	.41	-.02	.68
Caudate	-.02	.69	-.07	.19	.03	.56
Hippocampus	**-.14***	**.005**	-.09	.08	**-.15****	**.002**
Pallidum	**-.16***	**.001**	**-.11**	**.02**	**-.16****	**.001**
Putamen	-.08	.10	-.03	.52	**-.11***	**.03**
Thalamus	**-.19***	**.001**	-.09	.07	**-.23****	**<.001**

**$p_{FDR\text{-}corrected} < .005$, *$p_{FDR\text{-}corrected} < .05$

Table S2. Results of analyses examining mediation of associations between inflammation and the domains of cognitive function by volume of the four cortical lobes, individually and as a whole.

		Path a	Path b	Indirect effect ab		
Dependent variable	**Mediator Variable**	**Point est. (SE)**	**Point est. (SE)**	**Point est. (SE)**	**95% CI Lower**	**95% CI Upper**
Spatial Reasoning						
	Total			-.0540* (.0185)	-.0954	-.0222
Direct effect c'	Frontal Lobe Volume	.000 (.000)	-331.3438 (181.5459)	-.0048 (.0060)	-.0228	.0028
Point est.						
(SE)	Parietal Lobe Volume	-.0016** (.0005)	5.6222 (8.5054)	-.0089 (.0143)	-.0428	.0156
-.1423** (.0520)	Temporal Lobe Volume	-.0016** (.0005)	17.6450* (8.1794)	-.0277* (.0168)	-.0738	-.0038
	Occipital Lobe Volume	-.0006* (.0003)	21.2392 (10.8503)	-.0126 (.0093)	-.0387	.0000
Short Term Memory						
	Total			-.0500* (.0190)	-0.0948	-0.0192
Direct effect c'	Frontal Lobe Volume	.0000 (.0000)	-233.3049 (198.9897)	-.0024 (.0052)	-0.0205	0.0034
Point est.						
(SE)	Parietal Lobe Volume	-.0016** (.0005)	.6018 (9.1380)	-.0010 (.0161)	-0.0356	0.0303
-.1029 (.0556)	Temporal Lobe Volume	-.0016** (.0005)	22.2803* (8.7777)	-.0363* (.0175)	-0.0836	-0.0104
	Occipital Lobe Volume	-.0007* (.0003)	15.5893 (12.0786)	-.0104 (.0097)	-0.0374	0.0023
Verbal Proficiency						
	Total			-.0353* (.0157)	-.0739	-.0101
Direct effect c'	Frontal Lobe Volume	.0000 (.0000)	-44.4438 (191.6126)	-.0006 (.0045)	-.0135	.0054
Point est.						
(SE)	Parietal Lobe Volume	-.0016** (.0005)	3.5664 (8.9770)	-.0057 (.0134)	-.0362	.0184
-.1952*** (.0549)	Temporal Lobe Volume	-.0016** (.0005)	14.1090 (8.6330)	-.0222 (.0152)	-.0613	.0000
	Occipital Lobe Volume	-.0006*	11.6461	-.0069	-.0289	.0041

		(.0003)	(11.4520)	(.0079)		
Verbal Learning						
	Total			-.0264*	-.0595	-.0004
				(.0149)		
Direct effect c'	Frontal Lobe Volume	.0000	64.6075	.0007	-.0041	.0159
Point est.		(.0000)	(209.4876)	(.0041)		
(SE)	Parietal Lobe Volume	-.0016**	-3.5794	.0057	-.0222	.0412
-.1437*		(.0005)	(9.6711)	(.0155)		
(.0582)	Temporal Lobe Volume	-.0016**	12.4925	-.0202	-.0604	.0057
		(.0005)	(9.2104)	(.0166)		
	Occipital Lobe Volume	-.0007*	19.0809	-.0127	-.0408	.0007
		(.0003)	(12.5853)	(.0102)		
Executive Function						
	Total			-.0376*	-.0783	-.0115
				(.0165)		
Direct effect c'	Frontal Lobe Volume	.0000	-85.9199	-.0007	-.0154	.0048
Point est.		(.0000)	(208.5572)	(.0044)		
(SE)	Parietal Lobe Volume	-.0016**	3.1763	-.0050	-.0430	.0253
-.0881		(.0005)	(9.5778)	(.0167)		
(.0583)	Temporal Lobe Volume	-.0016**	15.7699	-.0258	-.0718	.0019
		(.0005)	(9.1310)	(.0181)		
	Occipital Lobe Volume	-.0006*	9.5434	-.0061	-.0330	.0073
		(.0003)	(12.5973)	(.0094)		

References

Buckner, R.L., Head, D., Parker, J., Fotenos, A.F., Marcus, D., Morris, J.C., Snyder, A.Z., 2004. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. NeuroImage 23, 724-738.

Coorporation, T.P., 1999. Wechsler Abbreviated Scale if Intelligence (WASI) manual., San Antonio, TX.

Fischl, B., Dale, A.M., 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proceedings of the National Academy of Sciences of the United States of America 97, 11050-11055.

Fischl, B., Salat, D.H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., van der Kouwe, A., Killiany, R., Kennedy, D., Klaveness, S., Montillo, A., Makris, N., Rosen, B., Dale, A.M. 2002. Whole Brain Segmentation: Automated Labeling of Neuroanatomical Structures in the Human Brain, Neuron 33, 341-355.

Golden, J.C., 1978. Stroop color and word test, Chicago, IL.

Kobayashi, N., Nakano, K., Tago, H., Niwa, S., 2010. Development of a simplified Short-Term Memory recall Test (STMT) and its clinical evaluation. Aging clinical and experimental research 22, 157-163.

Reitan, R.M., Wofson, D., 1985. THe Halstead-Reitan Neuropsychological Test Battery, Tuscon, AZ.

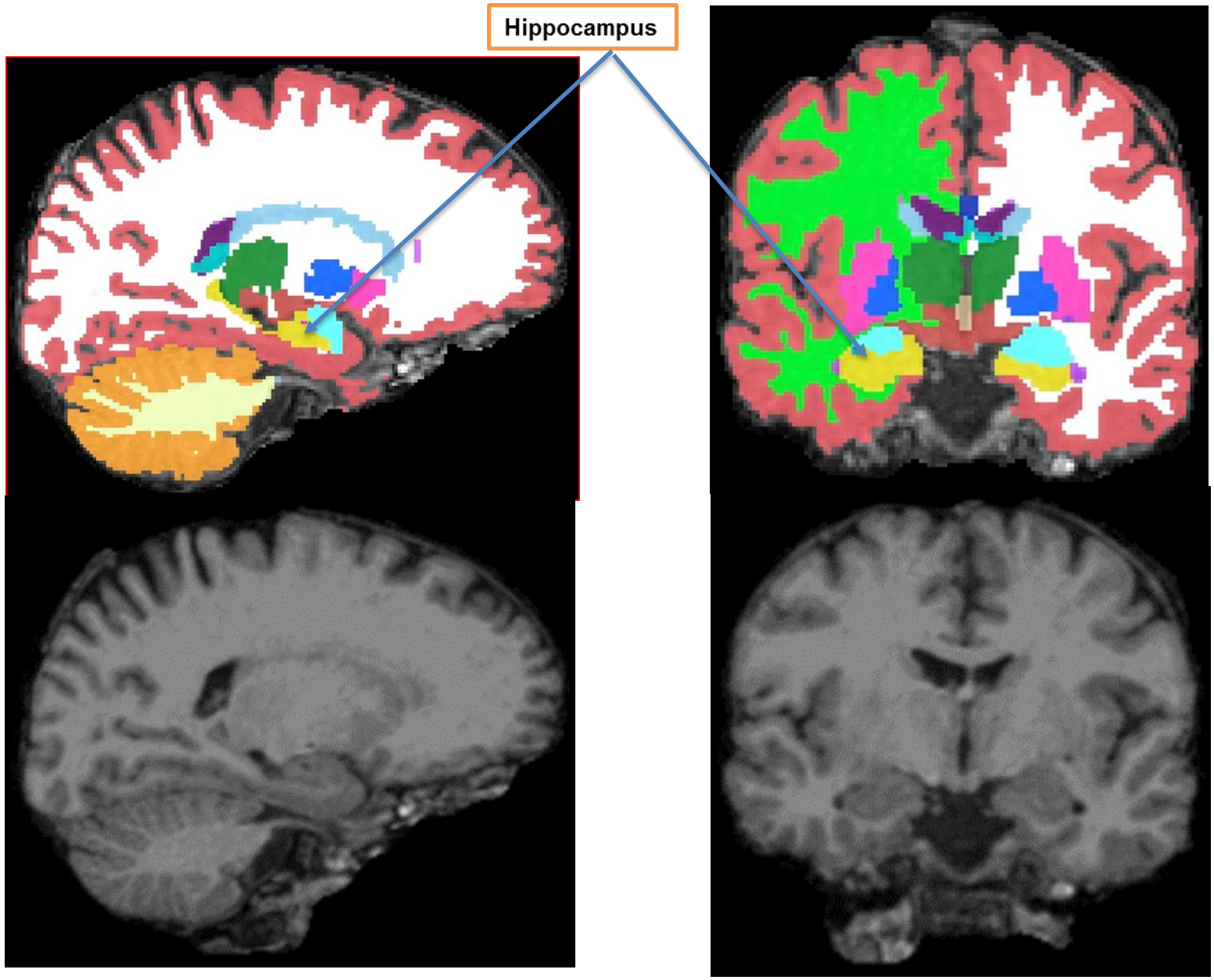
Rey, A., 1958. L'Examin Clinique en Psychologie, Paris, France.

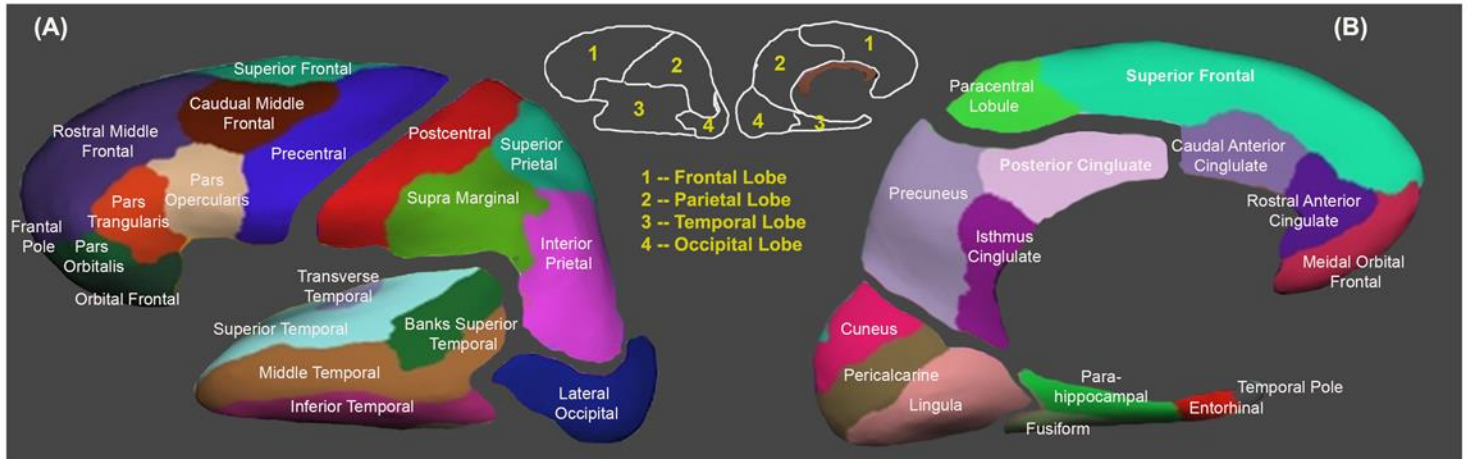
Wechsler, D., 1997a. Wechsler Adult Intelligence Scale - Third Edition: Administration and scoring manual, San Antonio, TX.
Wechsler, D., 1997b. THe WMS-III administration and scoring manual, San Antonio, TX.

Figure Legend

Figure S1: Hippocampus overlay of single subject. The left illustrates the sagittal view of hippocampus while the right illustrates the coronal view of the bilateral hippocampus. The bottom two images are (unsegmented) structural views of the same subject.

Figure S2. Mapping of 34 labeled ROIs (Desikan-Killiany) to the four cortical lobes. (A) Lateral view, and (B) Medial view.


Hippocampus


(A)
(B)
Superior Frontal
Caudual Middle Frontal
Rostral Middle Frontal
Precentral
Pars Opercularis
Pars Trangularis
Frantal Pole
Pars Orbitalis
Orbital Frontal
Postcentral
Superior Prietal
Supra Marginal
Interior Prietal
Transverse Temporal
Superior Temporal
Banks Superior Temporal
Middle Temporal
Inferior Temporal
Lateral Occipital
1 -- Frontal Lobe
2 -- Parietal Lobe
3 -- Temporal Lobe
4 -- Occipital Lobe
Paracentral Lobule
Superior Frontal
Posterior Cingluate
Caudal Anterior Cinglulate
Precuneus
Isthmus Cinglulate
Rostral Anterior Cingulate
Meidal Orbital Frontal
Cuneus
Pericalcarine
Lingula
Para-hippocampal
Temporal Pole
Entorhinal
Fusiform