

Multivariate age-related variations in quantitative MRI maps: Widespread age-related differences revisited.

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SUPPLEMENTARY MATERIAL

1 Data acquisition parameters

As mentioned in (Callagahn et al. 2014), participants were examined on two 3T whole body MR systems (Magnetom TIM Trio, Siemens Healthcare, Erlangen, Germany, 69 participants per scanner) each equipped with a standard 32 channel head coil for receive and RF body coil for transmission. The data were acquired as part of several cognitive neuroimaging studies at the Wellcome Trust Centre for Neuroimaging with approval from the local ethics committee.

A whole-brain quantitative MPM protocol was used. This consists of 3 spoiled multi-echo 3D fast low angle shot (FLASH) acquisitions with 1 mm isotropic resolution and 2 additional calibration sequences to correct for inhomogeneities in the RF transmit field (Lutti et al., 2010, 2012; Weiskopf et al., 2013). The FLASH volumes were acquired with predominantly proton density (PD), T1 or MT weighting, determined by the repetition time, and flip angle (α) (repetition time and flip angle were for the PD- and MT-weighted acquisitions: 23.7 ms/6°; and for the T1-weighted acquisition: 18.7 ms/20°). In the case of the MT-weighted acquisition, a Gaussian RF pulse with 4 ms duration and 220° nominal flip angle was applied 2 kHz off-resonance before nonselective excitation. Gradient echoes were acquired with alternating readout gradient polarity at 6 equidistant echo times between 2.2 ms and 14.7 ms. Two additional echoes were acquired for the PD-weighted acquisition at 17.2 ms and 19.7 ms. A high readout bandwidth of 425 Hz/pixel was used to reduce off-resonance artefacts (Helms and Dechent, 2009). To speed up data acquisition, parallel imaging with a speed up factor of 2 was used in the phase-encoded direction (anterior-posterior) using the generalized auto-calibrating partially parallel acquisition algorithm. A partial Fourier acquisition (6/8 sampling factor) was used in the partition direction (left-right). The total scanning time of the MPM protocol was approximately 25 minutes.

2 Multivariate SPM (mSPM) approach

2.1 Univariate GLM analyses

In this re-analysis, as recommended for MSPM (Gyger et al., 2021), the 8 resulting sets of qMRI maps were z-transformed per modality and across subjects to ensure comparability between maps, utilizing the mean and variance over each voxel. This procedure ensured comparability of different modalities for our multivariate analysis. The univariate general linear models were performed on each set of z-transformed maps using age, gender, total intracranial volume (TIV), and scanner as regressors.

2.2 Multivariate GLM (mGLM)

While a standard multivariate regression model is useful for predicting the values of the dependent variables, it doesn't directly address the question of how the entire set of dependent variables relates to the entire set of independent variables. In the context of this study, it is well-established that the brain undergoes simultaneous changes as we age. Therefore, a single independent variable may not adequately predict a single dependent variable; instead, the relationship might be more complex, where a combination of predictors may explain variance across multiple outcomes. Hence, a multivariate approach appears as a method of choice.

Here, we describe the multivariate GLM (mGLM) and its principles, including model and inference, as implemented in the MSPM toolbox are described here after (Gyger et al., 2021).

The multivariate GLM (mGLM) is specified using the design matrices of the 4 univariate models and the multivariate observations at each voxel as $Y = XB + E$, where $Y_{138 \times 4} = [Y_1, Y_2, Y_3, Y_4]$ is the multi-modal data matrix, each row of Y represents one participant, and each column represents one map; and $X_{138 \times 5} = [X_1, X_2, X_3, X_4, X_5]$ is the design matrix, the first column represents the mean over subjects, the rest of the columns represent mean-centered regressors (age, TIV, gender, scanner) respectively. Thus, B is a 5×4 matrix of regression coefficients estimated using an

ordinary least-square method, as $\hat{B} = (X^T X)^{-1} X^T Y$. And $\hat{E} = Y - \hat{Y} = Y - X \hat{B}$ is the residual matrix of size 138×4 . Importantly, the residuals are estimated on a per-voxel basis that allows for a straightforward determination of a unique covariance structure for each voxel. This feature is a significant advantage of mass multivariate approaches when dealing with dependent neuroimaging data. However, it is important to note that in this framework, the assumption of normality for the residuals implies that the covariance structure is assumed to be the same across different groups or conditions. There is an assumed degree of correlation between the columns of Y , this correlation is expressed by estimation of variance-covariance matrix $\hat{\Sigma} = \frac{1}{n-k} \hat{E}^T \hat{E}$, where n is the number of subjects and k is number of covariates.

The total sum-of-square-and-cross-product (SSCP) matrix of the model is $SSCP_{Total} = Y'Y - N\bar{y}\bar{y}'$ and can be partitioned into the regression and residual SSCP:

$$SSCP_{Total} = SSCP_{Regression} + SSCP_{Residual} = (\hat{y}'\hat{y} - N\bar{y}\bar{y}') + E'E$$

Hypothesis testing

Hypothesis testing in mGLM, relies on testing the linear contrast $CBL = 0$. This extension of the univariate scheme combines standard hypotheses on the rows of matrix B , i.e. on the regressor, represented by matrix C , with hypotheses on the columns of B , i.e. on the different modalities, represented by matrix L . In the context of multivariate ANOVA (MANOVA) models, contrasts of main effects and interactions are formulated by setting $L = I_t$, the $t \times t$ identity matrix, as the dependent variables in multimodal neuroimaging applications are not assumed to be directly proportional. This method is the most suitable for conducting hypothesis testing on multimodal neuroimaging applications.

The SSCP matrices are defined as:

$$SSCP_{hypo} = (C\hat{B}L^T)^T (C(X^T X)^{-1}C^T)(C\hat{B}L^T)$$

And an SSCP matrix associated with the residuals:

$$SSCP_{residual} = L(\hat{E}^T E)L^T$$

Test statistics in mGLM

The global significance of the GLM, i.e. $H_0: B = 0$, one can simply be estimated as:

$$SSCP_{Regression}SSCP_{Residual}^{-1} = \frac{(\hat{y}'\hat{y} - N\bar{y}\bar{y}')}{E'E}$$

Then we can calculate the eigenvalues of the resulting matrix by solving $\det\left(\frac{(\hat{y}'\hat{y} - N\bar{y}\bar{y}')}{E'E} - \lambda I_m\right) = 0$ to get the $m = 4$ eigenvalues.

In hypothesis testing using the multivariate GLM, there are four standard test statistics available, which can be constructed based on the calculation of the SSCP matrices: Pillai's trace (Pillai, 1955), Wilks' lambda (Wilks, 1932), Hotelling-Lawley trace (Hotelling, 1951), and Roy's largest root (Roy, 1945). The Wilks Lambda statistic can be calculated based on the calculated m eigenvalues as $\Lambda = \prod_{i=1}^m \frac{1}{1+\lambda_i}$. This has an approximate F distribution with degrees of freedoms a and b :

$$F(a, b) = \frac{1 - \Lambda^{\frac{1}{t}}}{\Lambda^{\frac{1}{t}}} \cdot \frac{b}{a}$$

Where $a = lq$ and $b = rt - 2u$.

$$l = \text{rank}(L)$$

$$q = \text{rank}(C)$$

$$u = \frac{a - 2}{4}$$

$$r = N - q - \frac{a+1}{2}, \text{ N = sample size}$$

$$t = \begin{cases} \sqrt{\frac{l^2 q^2 - 4}{l^2 + q^2 - 5}} & , l^2 + q^2 - 5 > 0 \\ 1 & , l^2 + q^2 - 5 \leq 0 \end{cases}$$

If the minimum value between $l = 4$ and $q = 1$ is ≤ 2 , the distribution is exactly F.

The hypothesis $H_0: CBL = 0$ can be tested to assess the different potential co-occurrence of change between the modalities. To test the joint effect on the 4 quantitative maps in a specific tissue type, $L_{4 \times 4}$ is defined as an identity matrix corresponding to the number of dependent variables (semi-quantitative maps). As explained before, each column of L will perform a univariate analysis on each column of B . Here, C was defined as $[0 \ 1 \ 0 \ 0 \ 0]$ to only see the correlation between age and maps. In this case, Λ has an exact F distribution with $a = 4$ and $b = 130$ degrees of freedom.

These matrices are generalizations of the numerator and denominator sums-of-squares from the univariate GLM hypothesis-testing approach. When L is an identity matrix, the main diagonal of $SSCP_{hypo}$ contains the sums of squares for the hypothesis in C as applied to the estimated parameters for each dependent variable separately. And the $SSCP_{residual}$ matrix is an unscaled form of the estimated covariance matrix $\hat{\Sigma}$.

Construction of the test statistics rely on some linear combination of m eigenvalues $(\lambda_1, \dots, \lambda_q)$ of $SSCP_{residual}^{-1}SSCP_{hypo}$. Here, we will only focus on Wilks' lambda test statistics. It quantifies the proportion of variance not accounted for by the hypothesis compared to the total variance in the data.

2.3 Canonical Correlation Analysis

Canonical vectors are calculated under the assumption that matrix L involves multiple dependent variables ($l > 1$), to extract the contribution of each dependent variable to the test statistics Λ . This contribution corresponds to the eigenvectors of the eigen decomposition of $SSCP_{residuals}^{-1}SSCP_{hypo}$ (Tabachnick and Fidell, 2007). Once linear combinations of canonical variates for the dependent ($U_1 = a_1y_1 + a_2y_2 + a_3y_3 + a_4y_4$) and independent ($V_1 = b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5$) variables are formed, the first canonical correlation is calculated by solving $\det\left(\frac{(\hat{y}'\hat{y} - N\bar{y}\bar{y}')}{E/E} - \lambda I_m\right) = 0$ for λ_1 . After finding the first pair of canonical variates, the process continues. The analysis finds a second pair of variates (U_2 and V_2) that are also maximally

correlated with each other, but with the added constraint that they are uncorrelated with the first pair of variates. This process continues until a certain number of canonical variate pairs are extracted. The number of possible pairs is equal to the number of variables in the smaller of the two sets (in our case, 4).

3 Results

3.1 Univariate GLMs

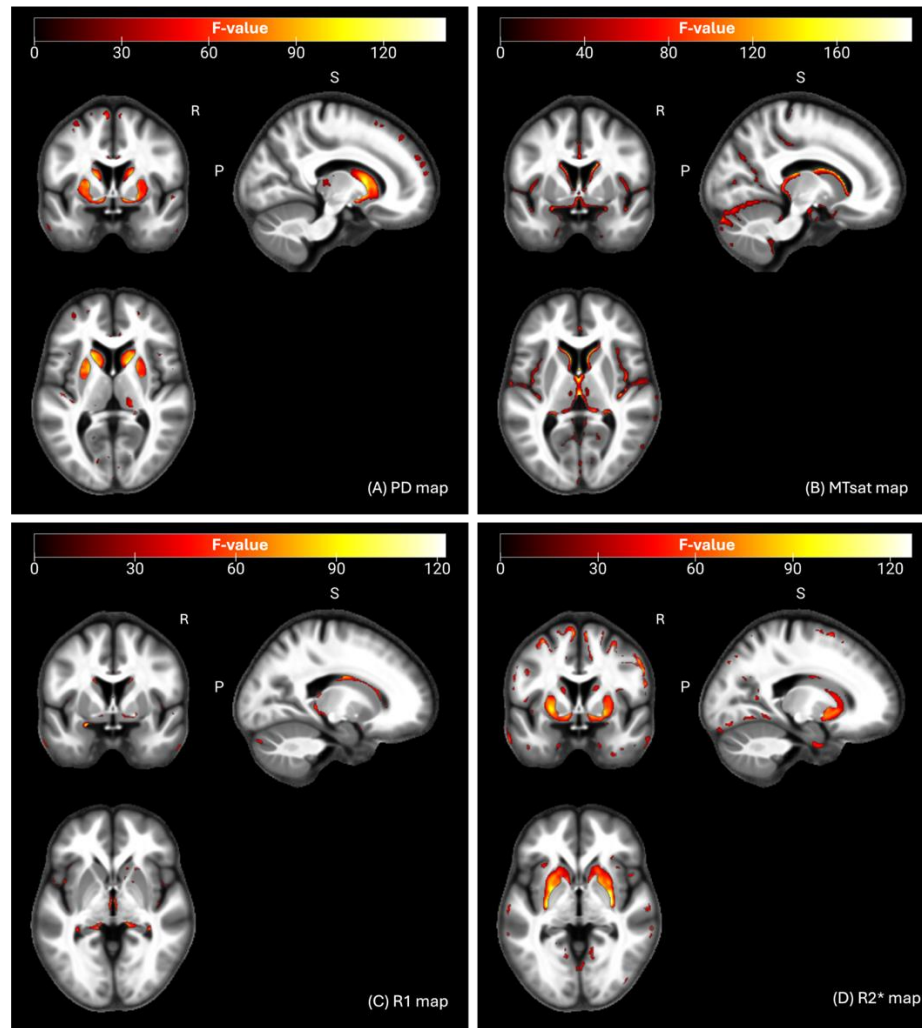


Figure 1. Statistical parametric maps for the uGLMs at a corrected threshold of $p < 0.05$ FWER in the GM; showing all the voxels with significant correlation with age, as detected by uGLMs for PD, MTsat, R1, and R2* maps. The F-tests were thresholded at $p < 0.05$ FWER corrected at voxel-level. The SPMs were overlaid on the mean MTsat map for the cohort in the MNI space. Abbreviation: GLM, general linear model; uGLM, univariate GLM; GM, gray matter; FWER, family-wise error rate; SPM, statistical parametric map.

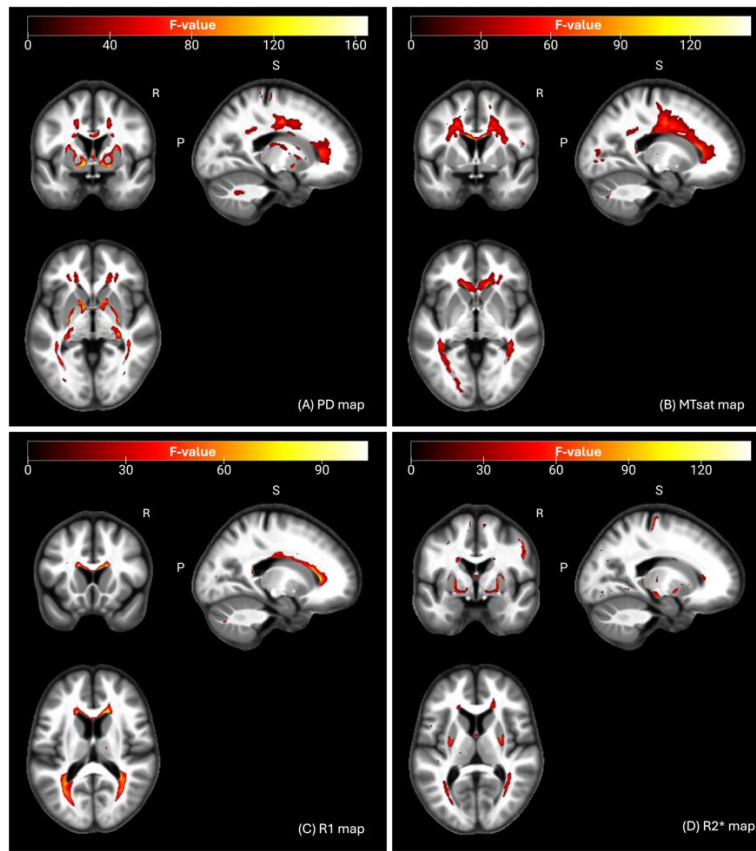


Figure 2. Statistical parametric maps for the uGLMs at a corrected threshold of $p < 0.05$ FWER in the WM; showing all the voxels with significant correlation with age, as detected by uGLMs for PD, MTsat, R1, and R2* maps. The F-tests were thresholded at $p < 0.05$ FWER corrected at voxel-level. The SPMs were overlayed on the mean MTsat map for the cohort in the MNI space. Abbreviation: GLM, general linear model, uGLM, univariate GLM, mGLM, multivariate GLM, WM, white matter, FWER, family-wise error, SPM, statistical parametric map.

3.2 Canonical Vectors

Table 1. Canonical vector sizes for different modalities from the mGLM model, representing the contribution of each modality in each voxel. The coordinates correspond to the peak voxels at the selected ROIs.

ROI name	Peak Coordinate (mm)	PD	MTsat	R1	R2*
Left					
Putamen	(-28,-11,1)	1.000	0.007	0.001	0.002
Thalamus	(-6,-8,15)	0.953	0.012	0.302	0.022
Hippocampus	(-32,-39,-5)	0.884	0.422	0.107	0.173
Mid-Frontal g	(-27,-3,49)	0.996	0.051	0.016	0.066
Precentral	(-30,-24,64)	0.832	0.137	0.247	0.478
Heschl g	(-38,-22,12)	0.946	0.024	0.322	0.027
Supp-motor	(-7,-2,59)	0.993	0.040	0.048	0.100
Caudate	(-8,15,8)	0.977	0.126	0.079	0.155
Pallidum	(-28,-13,-4)	1.000	0.006	0.001	0.001
Right					
Putamen	(12,10,-3)	0.922	0.285	0.079	0.248
Thalamus	(10,-17,17)	0.984	0.119	0.136	0.009
Hippocampus	(33,-34,-3)	0.951	0.027	0.302	0.063
Mid-Frontal g	(32,1,52)	0.980	0.158	0.010	0.125
Precentral	(54,-1,42)	0.894	0.397	0.084	0.190
Heschl g	(37,-20,7)	0.945	0.110	0.306	0.029
Supp-motor	(5,-10,57)	0.984	0.086	0.049	0.149
Caudate	(10,16,10)	0.985	0.075	0.141	0.063
Pallidum	(11,8,-4)	0.973	0.146	0.012	0.181

Key: MTsat, magnetization transfer saturation; PD, proton density; R2*, effective transverse relaxation rate; R1, longitudinal relaxation rate.

3.3 Univariate and multivariate model evaluation

Table 2. Summary statistics for significant voxels in uGLMs and mGL in CV1. “United” rows show the union of significant voxels in SPMs for all modalities. Univariate GLMs were thresholded at $p < 0.05$ FWER corrected per tissue class (GM or WM). Abbreviation: GLM, general linear model; uGLM, univariate GLM; mGLM, multivariate GLM; GM, gray matter; WM, white matter.

Map name	#clusters	Cluster size range	#voxels
uGLMs $p < 0.05$ FWE corrected within GM			
MTsat	196	1-3177	9183
PD	75	1-2359	6322
R1	27	1-102	297
R2*	84	1-2117	5789
United	304	1-4053	18794
mGLM $p < 0.05$ FWE corrected within GM			
All maps in mGLM	277	1-1302	11081
uGLMs $p < 0.05$ FWE corrected within WM			
MTsat	32	1-8087	14026
PD	57	1-1384	8298
R1	23	1-3126	6268
R2*	36	1-446	2349
United	92	1-9519	22817
mGLM $p < 0.05$ FWE corrected within WM			
All maps in mGLM	80	1-4079	13758

Table 3. Summary statistics for significant voxels in uGLMs and mGLM in CV1. “United” rows show the union of significant voxels in SPMs for all modalities. Univariate GLMs were thresholded at $p < 0.0125 = .05/4$ FWER corrected, to account for the multiplicity of maps tested (4) per tissue class (GM or WM). The mGLM was thresholded at $p < 0.05$ FWER corrected. Abbreviation: GLM, general linear model; uGLM, univariate GLM; mGLM, multivariate GLM; SPM, statistical parametric maps; GM, gray matter; WM, white matter.

Map name	#clusters	Cluster size range	#voxels
uGLMs $p < 0.0125$ FWE corrected within GM			
MTsat	163	1-1767	5872
PD	45	1-1046	3950
R1	14	1-69	142
R2*	43	1-1584	3787
United	214	1-2657	12141
mGLM $p < 0.05$ FWE corrected within GM			
All maps in mGLM	277	1-1302	11081
uGLMs $p < 0.0125$ FWE corrected within WM			
MTsat	33	1-4347	8352
PD	42	1-1027	5493
R1	23	1-2001	3798
R2*	24	1-354	1432
United	69	1-5237	14400
mGLM $p < 0.05$ FWE corrected within WM			
All maps in mGLM	80	1-4079	13758

Table 4. **Summary statistics for significant voxels in uGLMs and mGL in CV2.** “United” rows show the union of significant voxels in SPMs for all modalities. Univariate GLMs were thresholded at $p < 0.05$ FWER corrected per tissue class (GM or WM). Abbreviation: GLM, general linear model; uGLM, univariate GLM; mGLM, multivariate GLM; GM, gray matter; WM, white matter.

Map name	#clusters	Cluster size range	#voxels
uGLMs $p < 0.05$ FWE corrected within GM			
MTsat	196	1-3887	11155
PD	42	1-3236	5861
R1	68	1-170	931
R2*	126	1-1006	6689
United	324	1-7321	21760
mGLM $p < 0.05$ FWE corrected within GM			
All maps in mGLM	326	1-2125	13280
uGLMs $p < 0.05$ FWE corrected within WM			
MTsat	36	1-2821	6973
PD	55	1-1116	10089
R1	30	1-390	861
R2*	54	1-1860	5364
United	111	1-3183	18875
mGLM $p < 0.05$ FWE corrected within WM			
All maps in mGLM	148	1-2488	18042

Table 5. **Summary statistics for significant voxels in uGLMs and mGLM in CV2.** “United” rows show the union of significant voxels in SPMs for all modalities. Univariate GLMs were thresholded at $p < 0.0125 = .05/4$ FWER corrected, to account for the multiplicity of maps tested (4) per tissue class (GM or WM). The mGLM was thresholded at $p < 0.05$ FWER corrected. Abbreviation: GLM, general linear model; uGLM, univariate GLM; mGLM, multivariate GLM, SPM, statistical parametric maps, GM, gray matter, WM, white matter.

Map name	#clusters	Cluster size range	#voxels
uGLMs $p < 0.0125$ FWE corrected within GM			
MTsat	163	1-2080	7339
PD	24	1-2286	3925
R1	37	1-123	440
R2*	87	1-1006	3903
United	247	1-3224	13971
mGLM $p < 0.05$ FWE corrected within GM			
All maps in mGLM	326	1-2125	13280
uGLMs $p < 0.0125$ FWE corrected within WM			
MTsat	29	1-1616	3780
PD	55	1-1116	6780
R1	9	1-233	369
R2*	42	1-1199	3689
United	91	1-2021	12290
mGLM $p < 0.05$ FWE corrected within WM			
All maps in mGLM	148	1-2488	18042

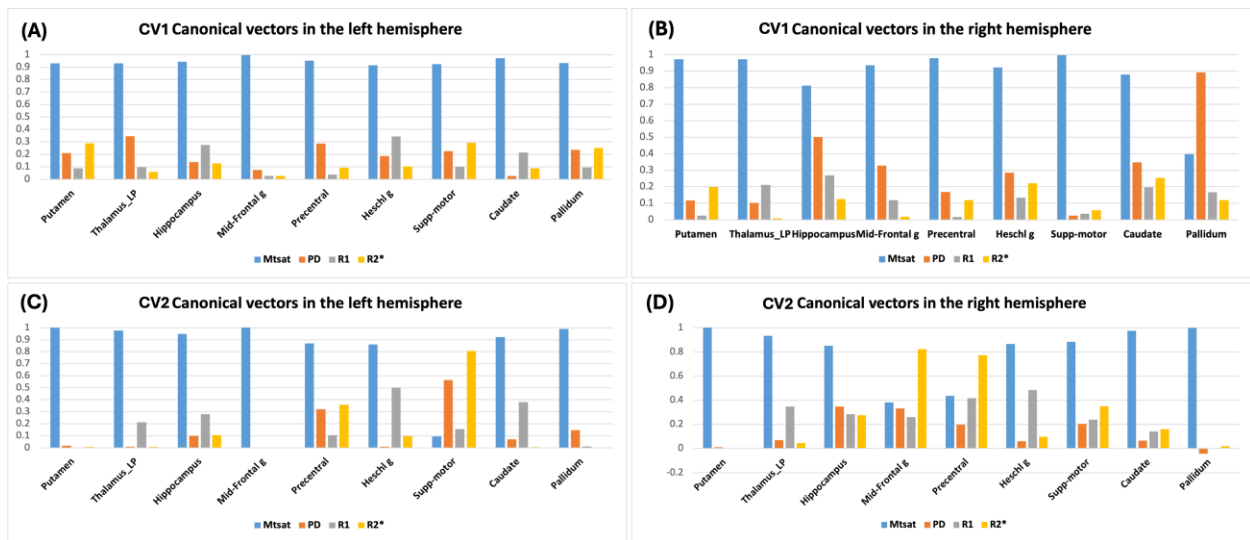


Figure 3. Canonical Vectors for left and right hemispheres for CV1 and CV2

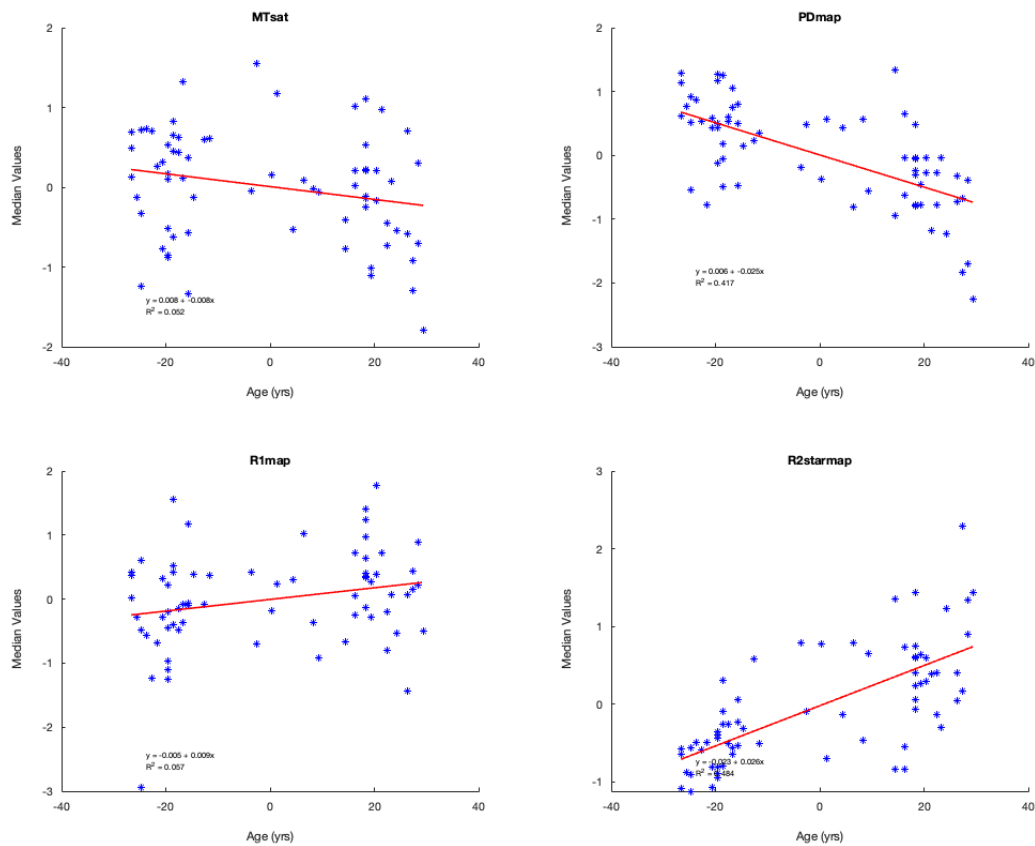


Figure 4. Median voxel values bilaterally in the Putamen in CV1. The red lines depict the linear model fit. These data are shown for illustration purposes only and were not used for any additional analyses.

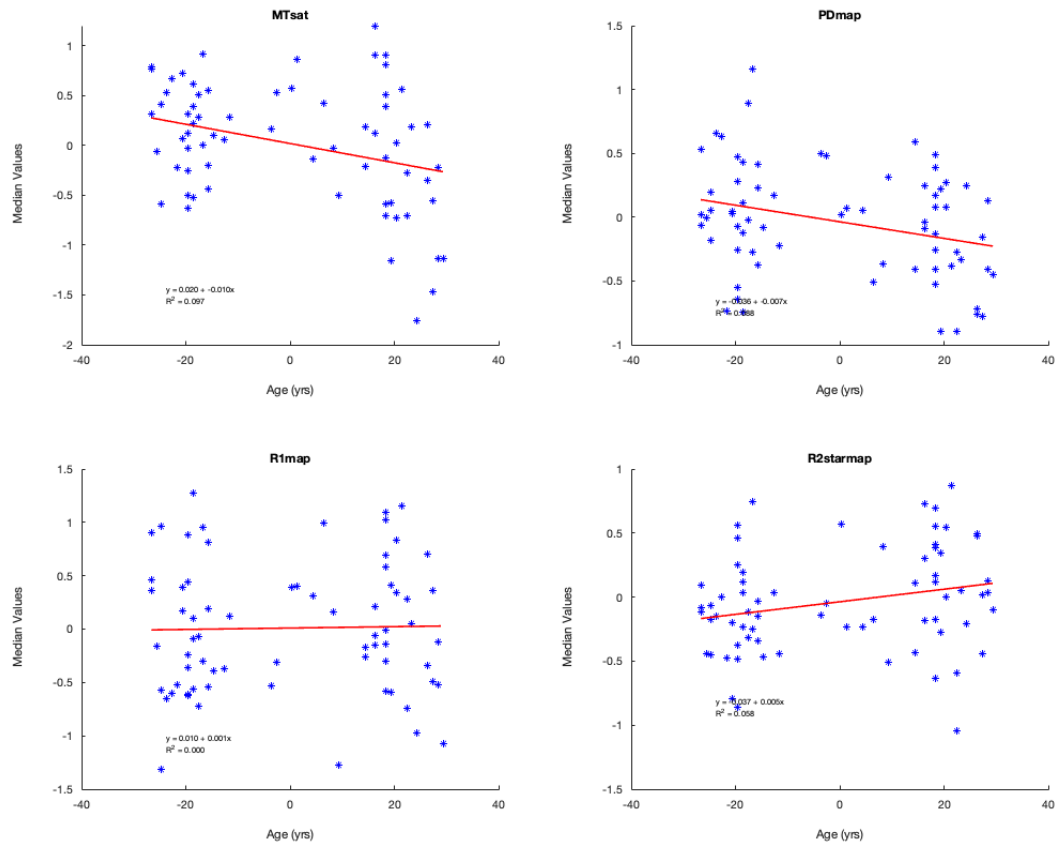


Figure 5. Median voxel values bilaterally in the Hippocampi in CV1. The red lines depict the linear model fit. These data are shown for illustration purposes only and were not used for any additional analyses.

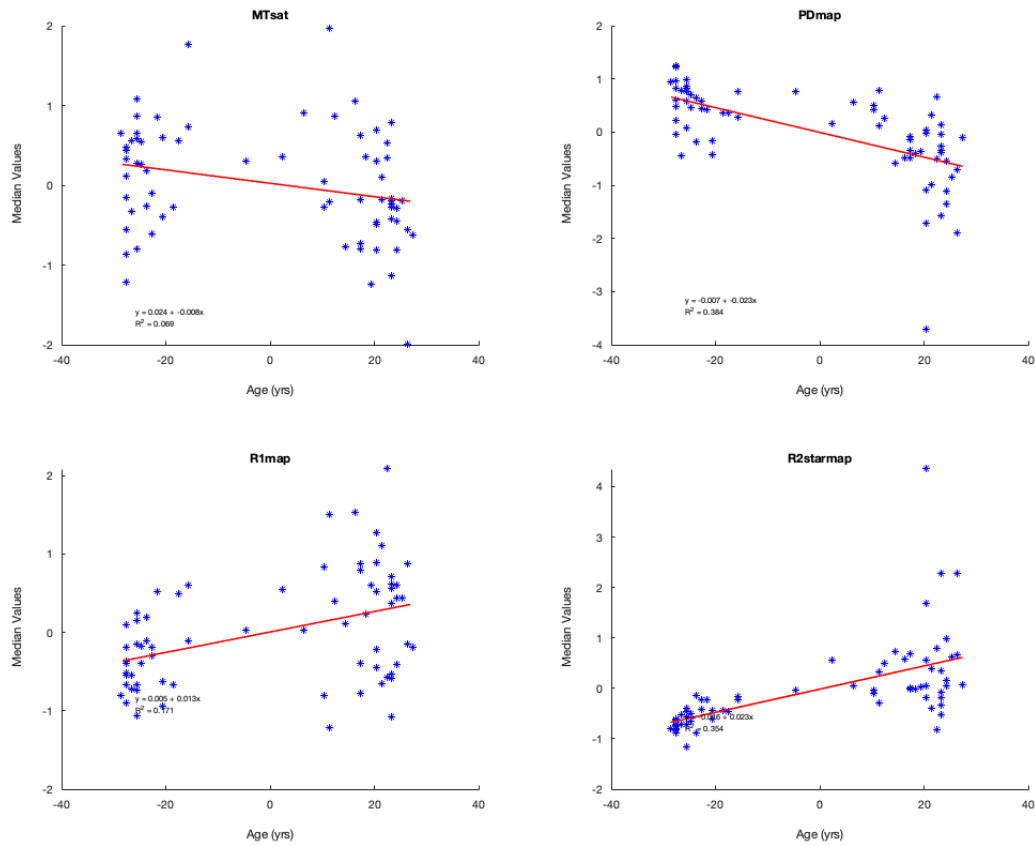


Figure 6. Median voxel values bilaterally in the Putamen in CV2. The red lines depict the linear model fit. These data are shown for illustration purposes only and were not used for any additional analyses.

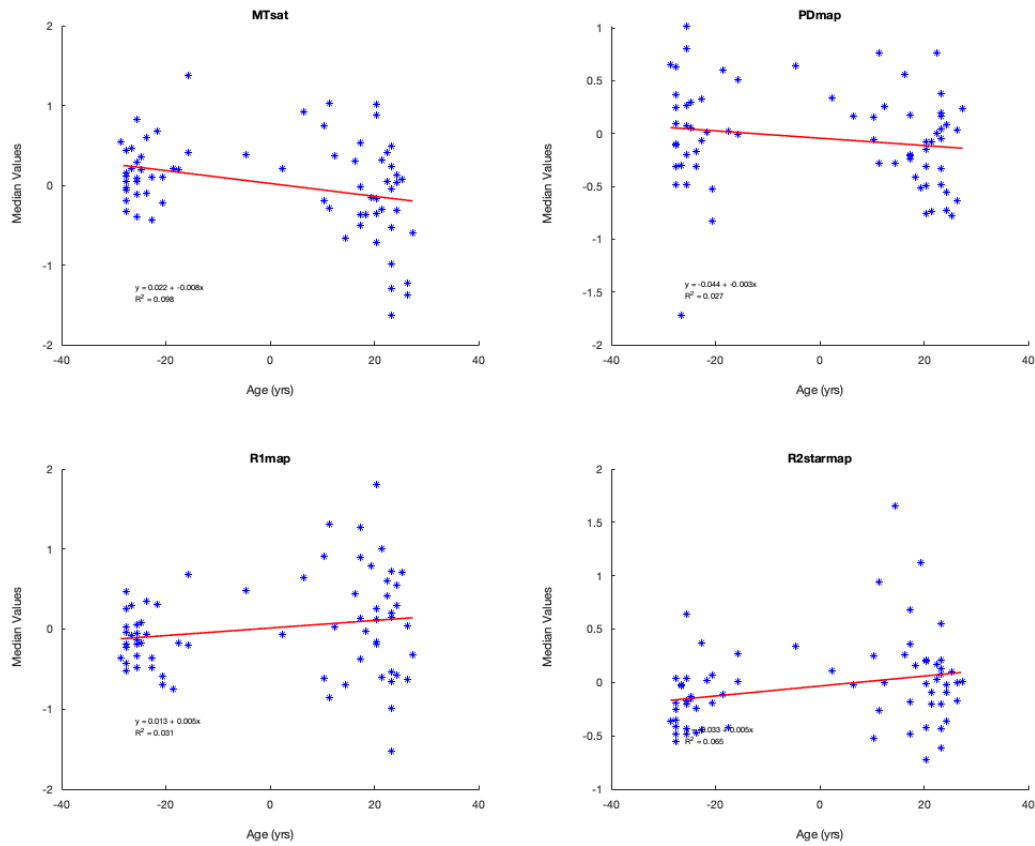


Figure 7. Median voxel values bilaterally in the Hippocampi in CV2. The red lines depict the linear model fit. These data are shown for illustration purposes only and were not used for any additional analyses.

Table 6. Pearson Partial Correlations in CV1 on the median voxel values within different regions of interest.

	Pearson's r	p-value	Effect size (Fisher's z)	SE Effect size		Pearson's r	p-value	Effect size (Fisher's z)	SE Effect size
Caudate					Pallidum				
MTsat	-0.482*	< .001	-0.526	0.123	MTsat	-0.247*	.040	-0.253	0.123
PD map	-0.656*	< .001	-0.786	0.123	PD map	-0.645*	< .001	-0.767	0.123
R1 map	0.059	.628	0.059	0.123	R1 map	0.276*	.022	0.283	0.123
R2s map	0.508*	< .001	0.561	0.123	R2s map	0.684*	< .001	0.836	0.123
Cerebellum					Precentral Gyrus				
MTsat	-0.553*	< .001	-0.622	0.123	MTsat	-0.450*	< .001	-0.485	0.123
PD map	-0.403*	< .001	-0.427	0.123	PD map	-0.508*	< .001	-0.560	0.123
R1 map	-0.170	0.163	-0.171	0.123	R1 map	-0.028	.822	-0.028	0.123
R2s map	0.359*	0.002	0.324	0.123	R2s map	0.319*	.007	0.331	0.123
Heschl Gyrus					Putamen				
MTsat	-0.543*	< .001	-0.608	0.123	MTsat	-0.229	.058	-0.233	0.123
PD map	-0.222	.066	-0.226	0.123	PD map	-0.646*	< .001	-0.768	0.123
R1 map	-0.330*	.006	-0.343	0.123	R1 map	0.238*	.049	0.242	0.123
R2s map	0.067	.586	0.067	0.123	R2s map	0.695*	< .001	0.858	0.123
Middle Frontal Gyrus					Superior motor cortex				
MTsat	-0.141	.247	-0.142	0.123	MTsat	-0.205	.091	-0.208	0.123
PD map	-0.576*	< .001	-0.656	0.123	PD map	-0.529*	< .001	-0.589	0.123
R1 map	0.112	.360	0.112	0.123	R1 map	0.119	.329	0.120	0.123
R2s map	0.374**	.002	0.393	0.123	R2s map	0.555*	< .001	0.626	0.123
Hippocampus					Thalamus				
MTsat	-0.312*	.009	-0.323	0.123	MTsat	-0.517***	< .001	-0.572	0.123
PD map	-0.296*	.013	-0.305	0.123	PD map	-0.405***	< .001	-0.430	0.123
R1 map	0.021	.864	0.021	0.123	R1 map	-0.240*	.047	-0.245	0.123
R2s map	0.241*	.046	0.245	0.123	R2s map	-0.240*	.047	-0.245	0.123

Note. Conditioned on variables: Gender, TIV, TRIO. * $p < .05$

Table 7. **Pearson Partial Correlations in CV2 on the median voxel values within different regions of interest.**

	Pearson's r	p-value	Effect size (Fisher's z)	SE Effect size		Pearson's r	p-value	Effect size (Fisher's z)	SE Effect size
Caudate					Pallidum				
MTsat	-0.498*	< .001	-0.546	0.123	MTsat	-0.214	.078	-0.217	0.123
PD map	-0.638*	< .001	-0.755	0.123	PD map	-0.727*	< .001	-0.922	0.123
R1 map	0.071	.560	0.071	0.123	R1 map	0.421*	< .001	0.449	0.123
R2s map	0.457*	< .001	0.494	0.123	R2s map	0.689*	< .001	0.846	0.123
Cerebellum					Precentral Gyrus				
MTsat	-0.586*	< .001	-0.671	0.123	MTsat	-0.345*	.004	-0.359	0.123
PD map	-0.250*	.039	-0.255	0.123	PD map	-0.414*	< .001	-0.440	0.123
R1 map	0.074	.544	0.074	0.123	R1 map	0.012	.925	0.012	0.123
R2s map	0.444*	< .001	0.477	0.123	R2s map	0.541*	< .001	0.605	0.123
Heschl Gyrus					Putamen				
MTsat	-0.512*	< .001	-0.565	0.123	MTsat	-0.263*	.029	-0.269	0.123
PD map	-0.207	.088	-0.210	0.123	PD map	-0.619*	< .001	-0.724	0.123
R1 map	-0.050	.681	-0.050	0.123	R1 map	0.413*	< .001	0.440	0.123
R2s map	0.358*	.003	0.374	0.123	R2s map	0.595*	< .001	0.685	0.123
Middle Frontal Gyrus					Superior motor cortex				
MTsat	-0.050	.685	-0.050	0.123	MTsat	-0.042	.730	-0.042	0.123
PD map	-0.448*	< .001	-0.483	0.123	PD map	-0.519*	< .001	-0.575	0.123
R1 map	0.221	.068	0.225	0.123	R1 map	0.214	.077	0.218	0.123
R2s map	0.574*	< .001	0.653	0.123	R2s map	0.644*	< .001	0.764	0.123
Hippocampus					Thalamus				
MTsat	-0.314*	.009	-0.325	0.123	MTsat	-0.435*	< .001	-0.467	0.123
PD map	-0.164	.179	-0.165	0.123	PD map	-0.233	.054	-0.237	0.123
R1 map	0.177	.145	0.179	0.123	R1 map	-0.172	.159	-0.173	0.123
R2s map	0.254*	.035	0.260	0.123	R2s map	-0.122	.320	-0.122	0.123

Note. Conditioned on variables: Gender, TIV, TRIO. * p < .05