Intrinsic Connectivity Identifies the Hippocampus as a Main Crossroad between Alzheimer's and Semantic Dementia-Targeted Networks.

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HAL Id: inserm-00931685

https://www.hal.inserm.fr/inserm-00931685

Submitted on 16 Jan 2014

Inventory of Supplemental Information

- ## SUPPLEMENTAL DATA

    o **Supplemental Figure S1, related to Figure 2**
    Shows the stability of the main finding from Figure 2 when modifying the methodological procedure for the definition of the seeds.

    o **Supplemental Figure S2, related to Table 3**
    Provides statistical evidence that the specific correlation between episodic memory and the connectivity between the hippocampus and AD-derived seeds (see Table 3) is unlikely due to differences in the strength of intrinsic connectivity of the hippocampus to AD and SD-derived seeds or in the distribution of neuropsychological test scores.

    o **Supplemental Table S1, related to Table 1**
    Provides further neuropsychological data from patients with AD and SD, highlighting differential impairment of episodic versus semantic memory.

- ## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

    Provides details about the acquisition parameters of MRI and PET data as well as details on the pre-processing of resting-state functional MRI.

- ## SUPPLEMENTAL REFERENCES

    References related to the neuropsychological tests presented in Table S1.

SUPPLEMENTAL DATA

Figure S1 (next page), related to Figure 2

Influence of methodological changes regarding the definition of seed regions and identification of "crossroads" brain regions.

For the sake of comparison, results presented in the main article are also presented in this figure, including A) the comparison of AD and SD patients using partial volume effect corrected (PVEc) FDG-PET data (identical to Figure 1A), B) the selection of each significant cluster main peak as seeds, resulting in 6 seeds and iii) the analysis of spatial overlap of intrinsic connectivity maps in controls, showing a total overlap in the right hippocampus (identical to Figure 2C).

The first set of complementary analyses includes the use of additional seeds derived from the same PVEc FDG-PET comparison as in the main manuscript (A).
Because the cluster 1 (left anterior temporal lobe) was larger than the others clusters (see Table 2) and included several brain regions, the two main subpeaks from this clusterwere also used as seeds for intrinsic connectivity analyses in controls in addition to the main perirhinal peak. This consisted in a left superior temporal pole seed, and a left anterior middle temporal seed as shown in purple and pink (C). All 8 connectivity maps (the 6 seeds corresponding to each cluster peak + these 2 subpeaks) showed a spatial overlap on the right hippocampus.
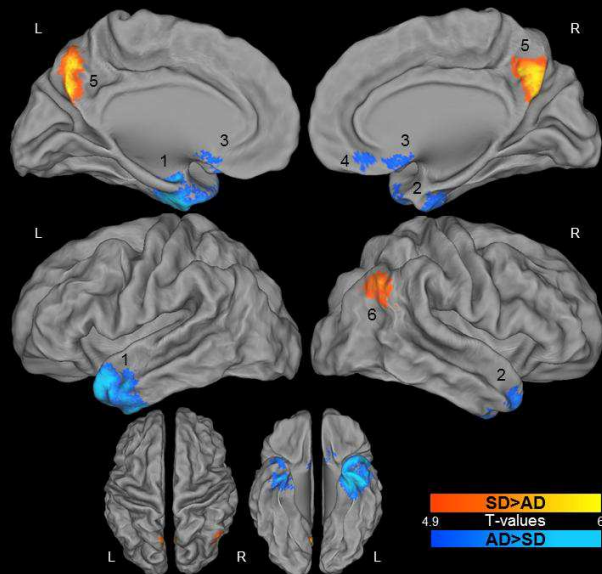
The second set of analyses consists in comparing FDG-PET data between AD and SD patients without the use of PVEc (D).
Using the same statistical threshold ($P_{FWE} < 0.05$; $k > 800$ mm^3), results appear to be very similar, though generally more significant than when using PVEc-FDG data and with clusters being generally larger. As a result, clusters 1 (L anterior temporal) and 3 (subgenual cortex) merged into one large cluster (size = 29,264 mm^3). In addition, the anterior cingulate cluster (cluster 4) was not significant anymore (at least at the stringent threshold of $P_{FWE} < 0.05$; $k > 800$ mm^3).
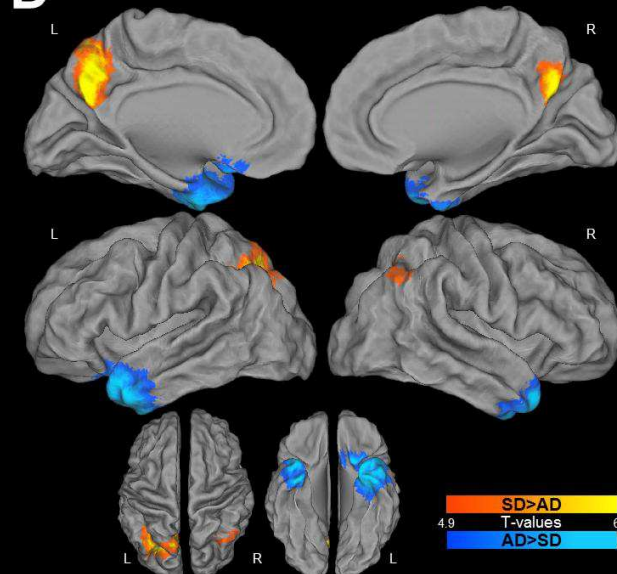Using the same criteria to define seeds as performed in the main manuscript (selecting the main peak in each cluster), 4 seeds were thus selected (E). The corresponding four intrinsic connectivity maps overlap in bilateral medial and lateral temporal lobes. The finding of additional "crossroad" regions in this analysis reflects the use of 4 seeds instead of 6 (due to the merging of some clusters), which results in being less selective to determine the crossroad regions. However, and despite this difference, it is worth highlighting that the same hippocampal crossroad was recovered in this analysis.

In summary, while the method slightly impacted the identification of "crossroad" regions, the hippocampal cluster highlighted in the main manuscript was found in both complementary analyses and can therefore be considered as a strong and stable finding.

A FDG analysis with PVEc (same as Figure 1A)

L 5 3 1 3 4 R
5
1 2

L 6 R

L R

SD>AD
4.9 T-values 6
AD>SD

D FDG analysis without PVEc

L R

L R

L R

SD>AD
4.9 T-values 6
AD>SD

B maximal significance peak for each cluster

L perirhinal
-34 -10 -34
R temporal pole
38 14 -30
L subgenual
-4 21 -14
R anterior cingulate
4 34 -10
R precuneus
2 -66 36
R angular
46 -62 38
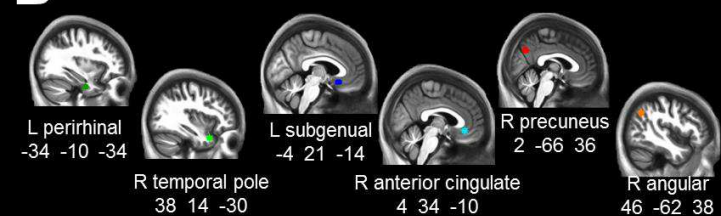
Overlap of connectivity maps in controls (same as Fig 2C)

1 2 3 4 5 6
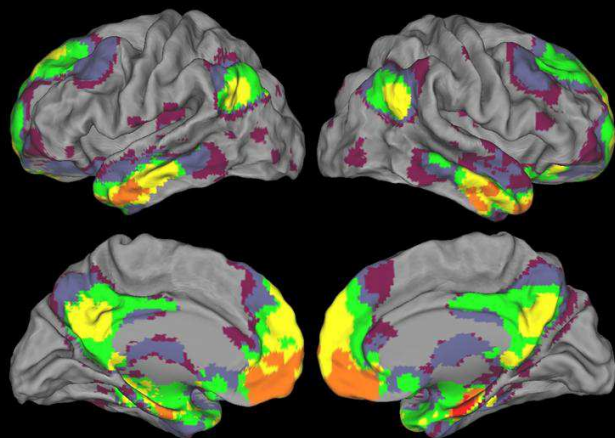
C maximal significance peak for each cluster
+ 2 secondary peaks from cluster 1
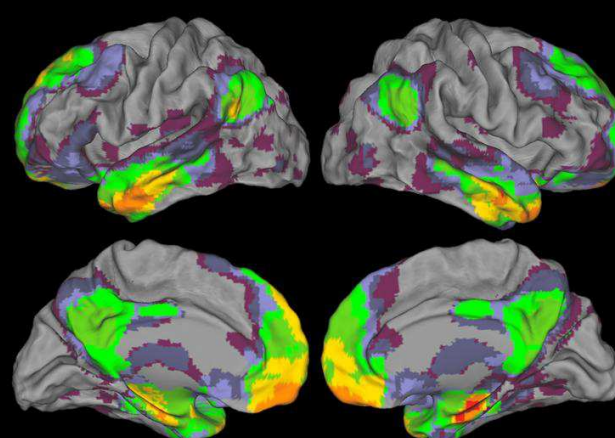
L superior temporal pole
-48 12 -20
L anterior middle temporal gyrus
-56 0 -28

Overlap of connectivity maps in controls

1 2 3 4 5 6 7 8

E maximal significance peak for each cluster

L perirhinal
-34 -2 -40
R temporal pole
36 18 -34
L precuneus
-4 -66 28
R angular
51 -64 37
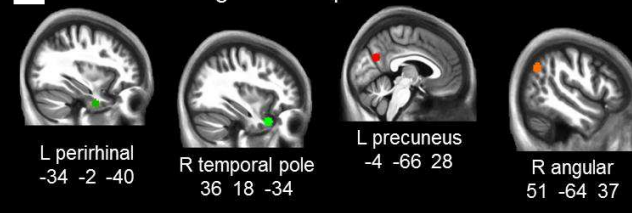
Overlap of connectivity maps in controls

1 2 3 4

Figure S2 (next page), related to Table 3

Supplemental analyses assessing the validity of the connectivity-behavioral correlations presented in Table 3, i.e. checking that the specificity of the correlations were not due to differences in the strength of connectivity or in the distribution of cognitive performances.

A. Comparison of the intrinsic connectivity of the hippocampus with the 6 patient-derived seeds in the healthy control group. Black bars represent SEM. Repeated-measure ANOVA was significant: $F_{(5,285)}=6.55$, $p<10^{-3}$. Post hoc analyses were performed using Fisher's LSD test: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. Although differences were observed, hippocampal connectivity values with AD regions were neither the strongest, nor the weakest.
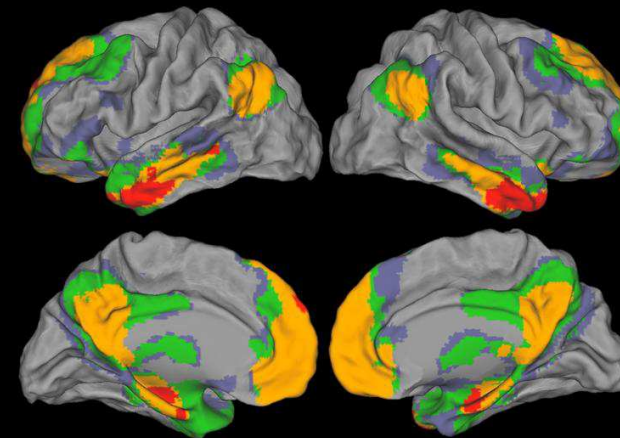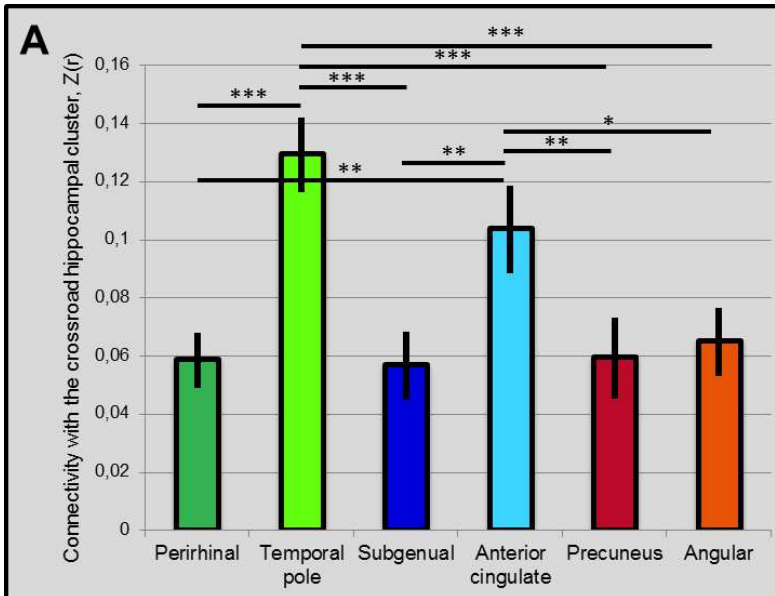
B. Statistical distribution of the cognitive scores used for cognitive-connectivity correlations. For each test raw score, the coefficient of variation (CV) is indicated; all values are in a similar range [0.12, 0.63] and episodic memory tests have intermediate values (0.24 and 0.31). Further indices were calculated on the four composite scores that were actually used to assess cognition-connectivity correlations (skewness, Kurtosis) and Shapiro-Wilk test was used to assess the normality of each composite score distribution. As a conclusion, episodic memory subtests and composite score did not show any specific feature as compared to other tests.

C. Scatterplots showing the cognitive-connectivity correlations (same as Table 3).

A

Connectivity with the crossroad hippocampal cluster, Z(r)

y-axis: 0, 0,02, 0,04, 0,06, 0,08, 0,1, 0,12, 0,14, 0,16

x-axis: Perirhinal | Temporal pole | Subgenual | Anterior cingulate | Precuneus | Angular

B

	CV	Skewness	Kurtosis	Shapiro-Wilk test (p value)
Episodic memory retrieval (n=56)		0.23	0.102	0.94
verbal recall	0.24			
visual recall	0.31			
Verbal knowledge (n=54)		-0.034	0.103	0.29
semantic fluency	0.29			
Mill Hill Vocabulary test	0.16			
Processing speed (n=54)		-0.216	-0.226	0.90
TMT A (time)	0.30			
Stroop: reading (time)	0.23			
Stroop: naming (time)	0.12			
Executive functions (n=55)		0.259	-0.102	0.84
TMT B-A (time)	0.63			
Stroop interference-naming (time)	0.44			
phonemic fluency	0.29			

C

Intrinsic connectivity between the crossroad hippocampal cluster and:

1. L perirhinal | 2. R temporal pole | 3. L subgenual | 4. R ant cingulate | 5. R precuneus | 6. R angular

Episodic memory retrieval
r = -0.23 | r = 0.10 | r = -0.09 | r = 0.03 | r = 0.41*** | r = 0.35**

Verbal knowledge
r = -0.05 | r = 0.01 | r = -0.18 | r = 0.04 | r = -0.05 | r = 0.05

Executive functions
r = -0.08 | r = 0.14 | r = -0.00 | r = 0.03 | r = 0.23 | r = 0.10

Processing speed
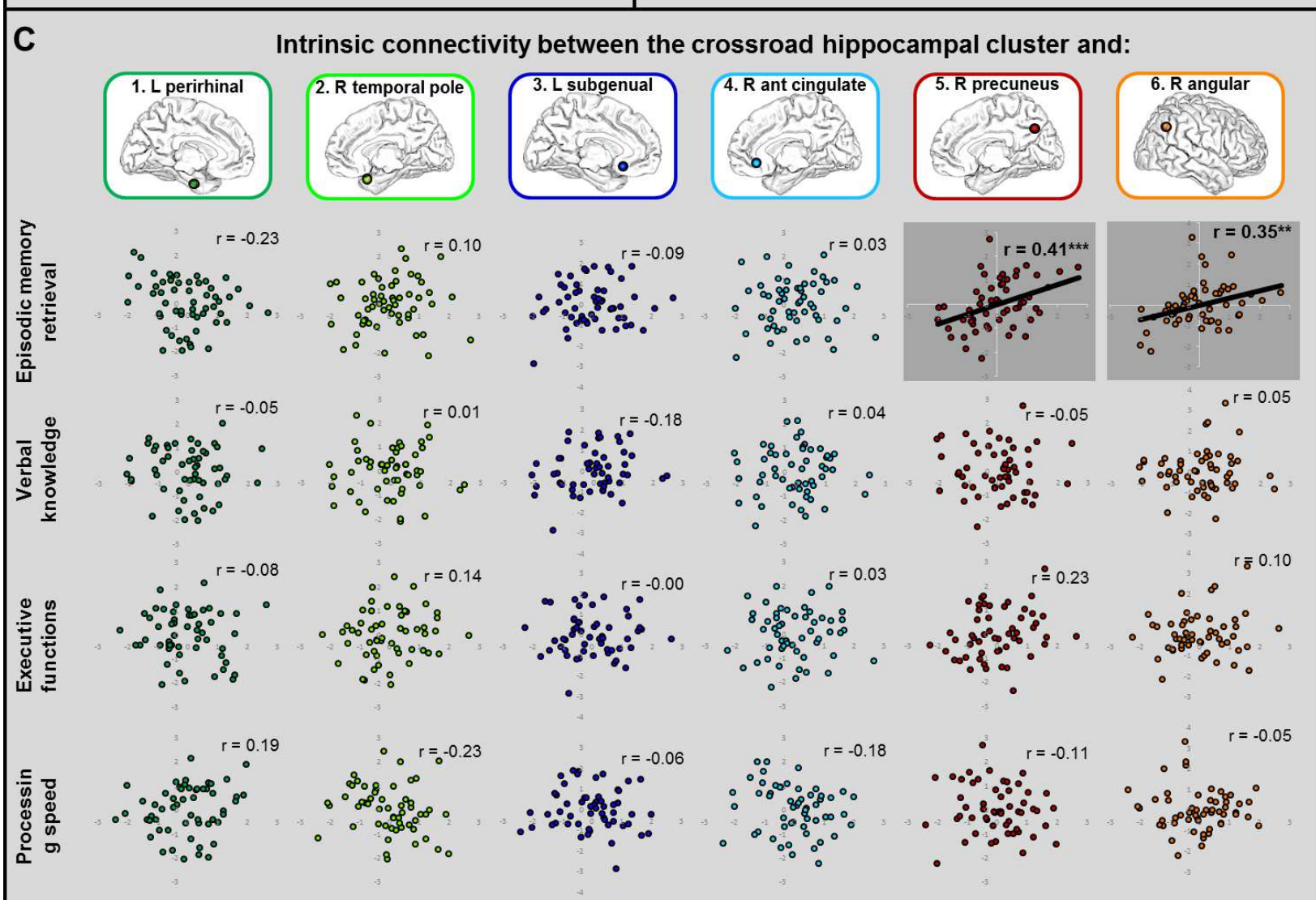r = 0.19 | r = -0.23 | r = -0.06 | r = -0.18 | r = -0.11 | r = -0.05

Table S1 (next page), related to Table 1

Neuropsychological performances (z-scores) in episodic versus semantic memory in Alzheimer's disease (AD) versus semantic dementia (SD).

For each patient, score in each test was compared to available normative data and Z-scores were obtained to allow comparisons between the different tests. # For these tests, Z-scores were computed from scaled scores using the following transformation: $Z = [(\text{patient's scaled score} - 10) / 3]$.
Depending on the test and corresponding references, 'impaired' patients were identified as having either i) scores ≤ 5th percentile, ii) Z-scores ≤ -1.65 or iii) scaled scores ≤ 5.
When the same test was available in both patient groups, Z-scores and the percentage of impaired patients were compared using Mann-Whitney test or Khi-square with Yate's correction respectively; *: $p < 0.05$, t: $p < 0.06$.
FCSRT: Free and Cued Selective Reminding Test.
For normative values, see:Mattis scale (Lucas et al., 1998), FCSRT (Van der Linden et al., 2004), Logical Memory (Weschler, 2001), BEM figure recall (Signoret, 1991), Rey figure recall (Fasteneau et al., 1999), test de la Ruche (Violon and Wijns, 1984), picture naming (Deloche and Hannequin, 1997), verbal fluency (Cardebat et al., 1990).

Conclusion:
Patients with SD showed relatively preserved episodic memory function when using visuo-spatial tasks whereas they had low scores on verbal episodic memory tests, probably at least partly because of their severe language deficits. By contrast, AD patients were strongly impaired on episodic memory tests independently of the type of material, likely reflecting genuine and global episodic memory deficits. Lastly, even though AD patients showed very mild to moderate impairment on tests of semantic memory, deficits were much more marked in SD patients.
Overall, it confirms the existence of a stronger impairment of semantic memory in SD and a stronger impairment of episodic memory in AD, especially when considering non-verbal episodic memory tasks. It is true however that the deficits are not isolated, i.e. that episodic versus semantic memory is not perfectly preserved in SD versus AD respectively. Yetthis was expected as patients are in the stage of mild to moderate dementia. In an earlier (e.g. prodromal) stage, the double dissociation would probably have been more clear-cut (see Nestor et al., 2006, where patients had a mean MMSE score ≈ 26, versus ≈ 21 in the present study).

	AD			SD		
	n	Z-score	Impaired: n (%)	n	Z-score	Impaired: n (%)
VERBAL EPISODIC MEMORY						
Mattis memory subscale #	15	-2.31 ± 0.70	13 (87%)	13	-1.62 ± 1.06 *	8 (62%)
Delayed free recall FCSRT	11	-3.73 ± 0.93	11 (100%)	-	-	-
Recognition FCSRT	11	-	10 (91%)	-	-	-
Logical memory: delayed recall #	-	-	-	11	-1.06 ± 1.01	5 (45%)
VISUAL EPISODIC MEMORY						
BEM figure recall	12	-4.19 ± 2.04	11 (85%)	-	-	-
REY figure recall #	-	-	-	13	0.05 ± 1.37	2 (15%)
Test de la ruche: delayed recall	-	-	-	8	0.62 ± 0.79	0 (0%)
Test de la ruche: recognition	-	-	-	8	0.49 ± 0.20	0 (0%)
SEMANTIC MEMORY						
Mattis concept subscale #	15	-0.38 ± 0.97	2 (13%)	13	-1.03 ± 0.67 *	4 (44%)
Picture naming	16	-	3 (19%)	13	-	13 (100%) *
Verbal fluency: animal	18	-1.57 ± 0.95	11 (61%)	12	-2.43 ± 0.41 *	11 (92%)
Verbal fluency: letter	18	-0.86 ± 0.94	3 (17%)	12	-1.56 ± 0.56 (t)	8 (73%) *

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

MRI data acquisition

A Philips (Eindhoven, The Netherlands) Achieva 3.0 T scanner was used for MRI data acquisition. Different images were acquired during the same MRI session, either for preprocessing or for analysis purposes. A high-resolution T1-weighted anatomical volume was first acquired using a 3D fast field echo sequence (3D-T1-FFE sagittal; repetition time = 20 ms; echo time = 4.6 ms; flip angle = 20°; 170 slices with no gap; slice thickness = 1 mm; field of view = 256 x 256 mm^2; in-plane resolution = 1 x 1 mm^2). Then, a high-resolution T2-weighted spin echo anatomical acquisition (2D-T2-SE sagittal; SENSE factor = 2; repetition time = 5500 ms; echo time = 80 ms; flip angle = 90°; 81 slices with no gap; slice thickness = 2 mm; field of view = 256 x 256 mm^2; in-plane resolution = 1 x 1 mm^2) and a non-Echo-Planar Imaging (EPI) T2* volume (2D-T2*-FFE axial; SENSE factor=2; repetition time = 3505 ms; echo time = 30 ms; flip angle = 90°; 70 slices with no gap; slice thickness = 2 mm; field of view = 256 x 256 mm^2; in-plane resolution = 2 x 2 mm^2) were obtained. Lastly resting-state functional volumes were obtained using an interleaved 2D T2* SENSE EPI sequence designed to reduce geometric distortions using parallel imaging, short echo time, and small voxels (2D-T2*-FFE-EPI axial, SENSE = 2; Time Repetition = 2382 ms; Time Echo = 30 ms; flip angle = 80°; 42 slices with no gap; slice thickness = 2.8 mm; field of view = 224 x 224 mm^2; in-plane resolution = 2.8 x 2.8 mm^2; 280 volumes). Subjects were equipped with earplugs and their head was stabilized with foam pads to minimize head motion. During this acquisition, which was the last of the MRI scanning session, subjects were asked keep their eyes closed while not falling asleep.

PET data acquisition.

FDG-PET scans were acquired on a Discovery RX VCT 64 PET-CT device (General Electric Healthcare) with a resolution of 3.76 x 3.76 x 4.9 mm (field of view= 157mm). Forty-seven planes were obtained with a voxel size of 2.7 x 2.7 x 3.27 mm. Participants were fasted for at least 6h before scanning. After a 30-min resting period in a quiet and dark environment, ≈180 MBq of FDG were intravenously injected as a bolus. A transmission scan was performed for attenuation correction and a 10-min PET acquisition scan began 50 min post-injection.

Resting-state fMRI data pre-processing

The first 6 volumes were discarded because of saturation effects. The EPI volumes were corrected for slice timing and realigned to the first volume. Data were then spatially normalized using a technique designed to reduce geometric distortion effects. This procedure includes for each individual i) a coregistration of the mean EPI volume, non-EPI T2*, T2, and T1 volumes; ii) a warping of the mean EPI volume to match the non-EPI T2* volume; iii) a segmentation of the T1 volume using the VBM5 procedure; iv) a normalization of the coregistered T1, EPI, and non-EPI T2* volumes using the parameters obtained from the T1 segmentation; and v) a 4mm full-width at half-maximum (FWHM) smooth of the EPI volumes. Finally, a binary mask was created from the group segmented mean grey matter T1 volume in conjunction with the mean non EPI-T2* volume in the MNI space (including only voxels with values greater than 0.25 in both mean volumes). Eventually, data were temporally band-passed filtered (0.01–0.08 Hz).

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