BJPO/2015/001339
Data supplement

METHOD

Participants

A total of 108 participants presenting with subjective memory complaints (SMC) with and without accompanying mild cognitive changes were recruited into the Aibl Active study. Eight of these participants were deemed not eligible for undergoing an MRI scan. Three participants did not have resting-state data acquired. Of the remaining 97 eligible participants, 10 were excluded due to poor image coregistration and/or normalization to the study-specific template, 5 participants had missing APOE genotype information, one had both missing APOE genotype information and poor imaging data and one participant was excluded due to an abnormality on the brain. Our final sample of 80 participants fulfilled the criteria below.

All participants completed the Alzheimer's Disease Assessment Scale-cognition (ADAS-Cog) to assess cognitive impairment [1]. As stated in the main text, all participants recruited in this study were part of the Aibl Active trial, a randomized controlled trial assessing the effects of physical activity (PA) on cerebrovascular disease. To be included in the Aibl Active trial, all individuals were required to be over 60 years of age at their last birthday and have the presence of at least one of the following cardiovascular risk factors: hypertension, diabetes, dislipademia, obesity or be a current smoker. Exclusion criteria for all participants included: baseline Mini Mental State Examination MMSE; [2] score < 24, diagnosis of dementia, unable to undergo MRI scans, baseline Geriatric Depression Scale GDS-15; [3] score > 6, unstable or life threatening medical condition, medical condition that contraindicates physical

activity (PA), severe visual or hearing impairment, history of chronic alcohol abuse within past 5 years, unable to attend follow-up visits.

APOE genotyping

For APOE genotyping, white blood cells were isolated from whole blood collected in the same EDTA tubes used for plasma isolation. DNA was purified using the midi-prep kit (QIAGEN Sciences, Maryland, USA). For the analysis, if a participant had at least one APOE ε4 allele they were categorized as APOE ε4+ and if no APOE ε4 allele they were categorized as APOE ε4-.

Image acquisition

The functional sequence consisted of a single-shot, T2* weighted gradient-echo planar imaging (EPI) sequence: (TR= 2500ms TE= 30ms, 90° pulse angle, FOV= 220mm, 64 x 64 matrix, slice thickness= 3mm with 3mm gap). Thirty-four interleaved axial slices were acquired parallel to the anterior-posterior commissure to provide complete brain coverage. The total sequence time was 7 minutes 35sec, yielding 180 whole brain echo planar imaging volumes. Participants were instructed to relax, stay awake and lie still without moving. A high-resolution T1-weighted anatomical image was acquired using the Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence: (176 contiguous sagittal slices, TR= 1900ms TE= 2100ms, 9° pulse angle, FOV= 256mm, 256 x 256 matrix; 1mm isotropic voxels (no gap).

Study specific template

This script first performs an affine registration of the input images to one brain and intensity averages the result to produce a new averaged brain. Then each of the original images is nonlinearly registered (using SyN) to this averaged brain to produce

a new shape-based average brain. This step is then repeated four times with each iteration updating the average brain until an optimal study-specific template is obtained. Finally, the study-specific template is registered to MNI space using a 12-parameter affine linear registration (FLIRT) in FSL 5.06 [4 5]. This final template is then used as an alternative template in the SPM 8 processing pipeline. The script was run on a 64-core Dell PowerEdge C6145 with 256Gb of RAM using the Sun Grid Engine (SGE) and was completed within 3 hours. Default settings were used for the template script (initial N4 bias field corrections of images, cross-correlation similarity metric, 60x90x40 non-linear iterations using a Greedy-SyN transformation model with step-size 0.25).

Image preprocessing

Motion correction was performed by aligning each participant's time series to the first image using least squares minimization and a six-parameter (rigid body) spatial transformation. Participants' data were excluded if movement in the translational or rotational planes exceeded 2mm or 2°, respectively. These realigned functional images were then corrected for slice-dependent time shifts and co-registered to each participants' T1 anatomical scan, which had been co-registered to the study-specific template. The co-registered T1 image for each participant was subsequently segmented into grey matter, white matter and cerebrospinal fluid (CSF) using the SPM new segmentation tool implemented in SPM8, which provides a robust registration over the unified segmentation algorithm. Finally, functional images were spatially smoothed with a 6-mm half width full maximum Gaussian filter.

Functional connectivity analysis

Placement of each of the four PCC seeds corresponded to the following cytotectonic sulcal landmarks: 1) dorsal PCC (rostral): located in the middle of the splenium in the y-axis, within the cingulate gyrus; 2) dorsal PCC (caudal): located ventral to the subparietal sulcus and slightly caudal to seed 1; 3) ventral PCC (rostral): located along the parietal-occipital fissure; 4) ventral PCC (caudal) located ventral and caudal to seed 3 along the parietal-occipital fissure.

Functional connectivity analysis & brain-behaviour correlations

Cluster-sizes for between-group differences and brain-behaviour correlations with each PCC seed were determined using AlphaSim implemented in the REST toolbox. Using this method, AlphaSim simulates the data based on the smoothness of the data, and assigns a cluster-extent value above which results are considered significant and not due to chance. The size of the cluster reflects the extent to which each of the PCC subregions are functionally and anatomically connected, and therefore, larger ROIs will yield a larger connectivity profile. For between-group effects a global conjunction mask that consisted of the within-subjects effects calculated for both groups, thresholded at $P<.05$ (FDR) corrected, for the combined left and right hemisphere was used in AlphaSim. For brain-behaviour correlations, the between-group statistical map for each respective seed, thresholded at $P<.001$, was used in AlphaSim.

Brain-behaviour correlations

The correlations with each seed were masked with the between-group connectivity map for each respective seed, to limit correlations with brain regions that were significantly different between groups. All results were covaried for the presence or absence of mild cognitive changes, age and gender. The cluster-wise corrected

minimum estimated cluster-sizes were: dorsal PCC (rostral), 18 voxels; dorsal PCC (caudal), 25 voxels; ventral PCC (rostral), 44 voxels; ventral PCC (caudal), 15 voxels.

RESULTS

The table below contains the between-group differences for each of the four dorsal and ventral PCC seeds when the presence or absence of mild cognitive changes was not included in the 2nd level statistical models. The Figures illustrate each of the brain regions where significant between group differences in functional connectivity were found, and the corresponding MNI coordinates. Overall, the results did not change; between-group differences in functional connectivity with each of the four PCC regions were found in the same regions as our main analysis, when the presence of mild cognitive changes were covaried for using a continuous approach through analysis of covariance (see main text for comparisons).

Table DS1. Regions showing significant between-group differences in functional connectivity with the posterior cingulate seed regions-of-interest

			Difference			
Seed	**Connected region**	**Between-group difference**	**Coordinates (x,y,z)**	**Statistic (Peak z-score)**	**Cluster size**	**BA**
dorsal PCC (r)						
	Middle frontal gyrus	APOE ε4+>APOE ε4-	-34, 44, 8	4.15	255	9
	Medial frontal gyrus	APOE ε4+>APOE ε4-	4, 58, 6	3.95	348	10
	Parrahippocampal gyrus	APOE ε4->APOE ε4+	32, -26, -16	4.19	33	

dorsal PCC (c)						
	Middle frontal gyrus	APOE ε4+>APOE ε4-	42, 0, 32	3.77	78	46
	Precentral gyrus	APOE ε4+>APOE ε4-	-50, 2, 28	4.17	39	4
	Lateral occipital cortex	APOE ε4+>APOE ε4-	-56, -62, -6	4.83	550	37
ventral PCC (r)						
	Supplementary motor area	APOE ε4+>APOE ε4-	-2, -20, 54	5.13	364	6
ventral PCC (c)						
	Middle frontal gyrus	APOE ε4->APOE ε4+	-34, -4, 54	4.13	88	6
	Lateral occipital cortex	APOE ε4+>APOE ε4-	18, -84, 16	4.91	349	18
	Inferior temporal gyrus	APOE ε4+>APOE ε4-	46, -46, -6	4.83	100	

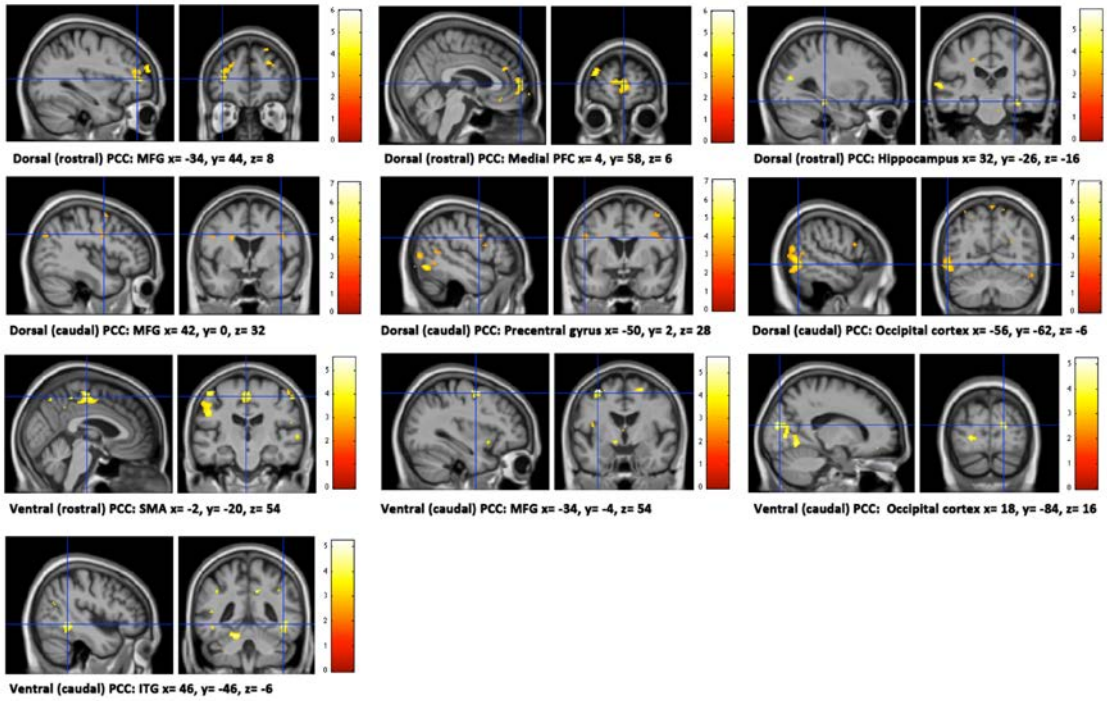

Fig. DS1. Regions showing significant between-group differences in functional connectivity with the four dorsal and ventral posterior cingulate seed regions. Note: the direction of significant between-group differences (i.e., APOE ε4+ >APOE ε4- and APOE ε4- > APOE ε4+) for each of the regions shown did not change (please see Figure 2 and Table 2 in main text for details). Abbreviations: MFG, middle frontal gyrus; SMA, supplementary motor area; ITG, inferior

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