Supplementary Information for

Age-related alterations in functional connectivity along the longitudinal axis of the hippocampus and its subfields

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Supplementary Information Text

S1. Continuous Recognition Task

A total of 23 young (13F/10M; mean age = 27.5; range = 21-34 years) and 25 older adults (14F/11M; mean age = 70.2; range = 59-84 years). Five of these older adults also participated in the primary dataset but viewed a different set of objects during this scan session and it took place approximately 3 years later. Instead of performing the indoor/outdoor task, here participants performed and old, similar, new judgement on repeat, lure, and new items. The scan parameters and data processing pipeline paralleled our primary dataset.

To evaluate whether connectivity along the long axis of the hippocampus differed for young and older adults, we calculated FC along the 6 hippocampal segmentations to three separate medial temporal lobe regions, averaging across left and right hemispheres: ERC, PRC, and PHC. These data were entered into a 2x6 repeated-measures ANOVA with age (young or aging) and region (1,2,3,4,5,6) as variables for each region. All three regions (ERC, PRC, and PHC) showed a main effect across the six hippocampal subdivisions (main effect of region: ERC: $F(5,230) = 3.2$, $p<.01$; PRC: $F(5,230) = 5.9$, $p<.0001$; PHC: $F(5,230) = 8.5$, $p<.0001$). While ERC showed no main effect of age, there was a significant interaction ($F(5,230) = 3.2$, $p<.01$). However, Sidak's multiple comparisons tests on each of the 6 regions did not result in any significant age differences. In constrast, we observed greater connectivity between the Hx and PRC and PHC for younger than older adults (Supplemental Figure 2A-C) (PHC: $F(1,46) = 7.0$, $p <.02$). In addition, FC with PHC changed across the six hippocampal subdivisions with an interaction (PRC: $F(5,230) = 2.4$, $p<.05$; PHC: $F(5, 230) = 2.0$, $p=.08$ (marginal)). Sidak's multiple comparisons tests on each of the 6 PHC regions revealed greater FC in young than older adults for the first 3 regions (1: $t(276) = 3.1$, $p<.02$; 2: $t(276) = 2.6$, $p<.05$; 3: ($t(276) = 2.7$, $p <.05$), making up the

anterior portion of the hippocampus, but not the last 3 regions (4: t(276) = 2.0, p=.23; 5: t(276) = .64, p=.99; 6: t(276) = 1.0, p=.89). While Hx-PRC showed an interaction, none of the six subdivisions showed a reliable effect of age, consistent with the lack of a main effect of age.

S2. Scene Encoding Task

A total of 34 young (21F/13M; mean age = 28.3; range = 20-39 years) and 33 older adults (19F/14M; mean age = 76.2; range = 70-87 years). Twenty-seven young and 26 older adults also participated in the primary dataset within approximately 6 months as part of a larger study on the neural basis of age-related memory decline. Participants viewed pictures of scenes and determined if each one was oriented in a portrait or landscape orientation while in the scanner. They were later tested on their memory for these images, but these imaging data are from this encoding portion of the task only. The scan parameters and data processing pipeline paralleled our primary dataset.

Again, these data were entered into a 2x6 repeated-measures ANOVA with age (young or aging) and region (1,2,3,4,5,6) as variables for each region. All three regions (ERC, PRC, and PHC) showed a main effect across the six hippocampal subdivisions (main effect of region: ERC: $F(5,325) = 3.7$, $p<.01$; PRC: $F(5,325) = 7.4$, $p<.0001$; PHC: $F(5,325) = 8.4$, $p<.0001$). While ERC and PRC showed no main effect of age or interaction, we observed greater connectivity between the Hx and PHC for younger than older adults (Supplemental Figure 2E-F) (PHC: $F(1,65) = 6.7$, $p <.02$). In addition, FC with PHC changed across the six hippocampal subdivisions with an interaction (PHC: $F(5, 325) = 2.7$, $p<.05$). Sidak's multiple comparisons tests on each of the 6 PHC regions revealed greater functional connectivity in young than older adults for 2 of the first 3 regions (1: t(390) = 1.4, p=.69; 2: t(390) = 3.6, p<.01; 3: (t(390) = 2.9, p <.05), making up the anterior portion of the hippocampus, but not the last 3 regions (4: t(390) =

.89, p=.94; 5: t(390) = 1.7, p=.43; 6: t(390) = .03, p=.99), consistent with the other datasets demonstrating an age-related decrease in FC to the PHC in the anterior hippocampus relative to the posterior hippocampus.

S3. Global Signal Included in Object Encoding Task

To evaluate whether regressing the global signal out of the data induced these findings, these data without the global signal regressed out were entered into a 2x6 repeated-measures ANOVA with age (young or aging) and region (1,2,3,4,5,6) as variables for each region (see Supplemental Figure 7). Both PRC, and PHC showed a main effect across the six hippocampal subdivisions (main effect of region: PRC: $F(5,300) = 7.8$, $p<.0001$; PHC: $F(5,300) = 8.9$, $p<.0001$). There was greater functional connectivity for younger adults than older adults (main effect of age: PRC: $F(1,60) = 3.2$, $p=.08$; PHC: $F(1,60) = 11.8$, $p<.001$). Finally, the interaction across the longitudinal axis and age was marginally significant with PHC ($F(5, 300) = 2.0$, $p = .07$) and not reliable for PRC ($F(5, 300) = .87$, $p = .50$). These results are all consistent with our findings with the global signal regression reported in *Section 3.2*.

S4. Framewise Displacement in Object Encoding Task

To evaluate the role of motion in inducing these findings, we calculated the mean framewise displacement (FD) for the unscrubbed/non-censored data and found greater mean FD for older (mean = .20 mm) than younger (mean = .14 mm) adults ($t(60) = 2.9$, $p<.01$). Likewise, even the censored data also showed greater mean FD for older (mean = .19 mm) than younger (mean = .13 mm) adults ($t(60) = 3.3$, $p<.01$). Thus, there is some difference in motion between the two groups, even once we have censored the data.

However, the question is whether a difference of ~.06mm is inducing the age x long-axis interaction in the PHC. To address this question, we matched the mean FD for the young and aging group by removing 7 young participants with the lowest FD and 7 older participants with the highest FD. Once the groups were matched (N = 24 per group; mean FD = .14 for both), we conducted the analyses in *Section 3.2*. These data were entered into a 2x6 repeated-measures ANOVA with age (young or aging) and region (1,2,3,4,5,6) as variables for each region. Both PRC, and PHC showed a main effect across the six hippocampal subdivisions (main effect of region: PRC: $F(5,230) = 5.1$, $p<.0001$; PHC: $F(5,230) = 7.1$, $p<.0001$). There was greater functional connectivity for younger adults than older adults (main effect of age: PRC: $F(1,46) = 6.9$, $p<.02$; PHC: $F(1,46) = 11.8$, $p<.001$). Finally, the interaction across the longitudinal axis and age was marginally significant with PHC ($F(5,230) = 2.1$, $p = .07$) and not reliable for PRC ($F(5,230) = 1.0$, $p = .39$). These results again replicate our previous findings, showing that motion is not inducing the age, region, or age x long-axis relationship in these ROIs.

S5. Hemispheric Asymmetry in Object Encoding Task

We explored whether there was hemispheric asymmetry in these findings by examining each FC relationship within hemisphere: left Hx to left PHC, PRC, ERC and right Hx to right PHC, PRC, ERC. Again, FC changed across the 6 hippocampal regions for both left and right hemispheres for all MTL cortices (main effect of region: all p's<.05). The Hx-PHC showed an age related shift in both hemispheres (main effect of age: left: $F(1,60) = 8.7$, $p<.005$; right: $F(1,60) = 9.4$, $p<.005$) and the Hx-PRC showed a marginal age-related shift only in the right hemisphere ($F(1,60) = 3.4$, $p = .07$) with greater FC for young adults than older adults. Therefore, there is some evidence for hemispheric asymmetry, with larger effects present in the

right than left hemisphere, but both hemispheres consistent in showing an age-related decrease in Hx-PHC functional connectivity.

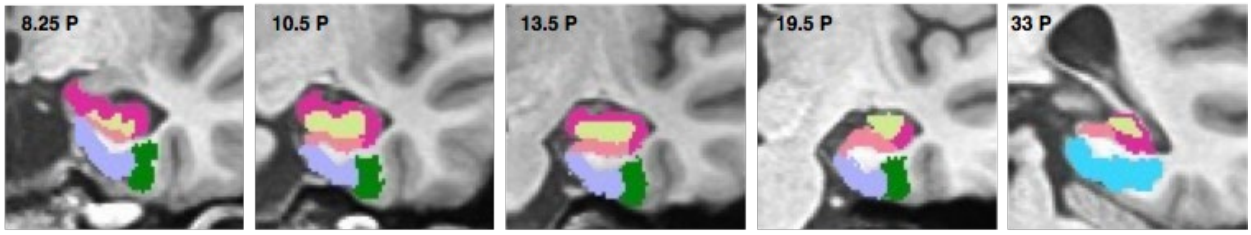

Figure S1. Representative slices (and corresponding anterior-posterior position in Talairach space) demonstrating the segmentation of the hippocampus and surrounding medial temporal lobe cortex. Hippocampal subfields: CA1 (fuschia), DGCA3 (yellow), and subiculum (pink). Medial Temporal Lobe: entorhinal cortex (purple), perirhinal cortex (green), and parahippocampal cortex (blue).

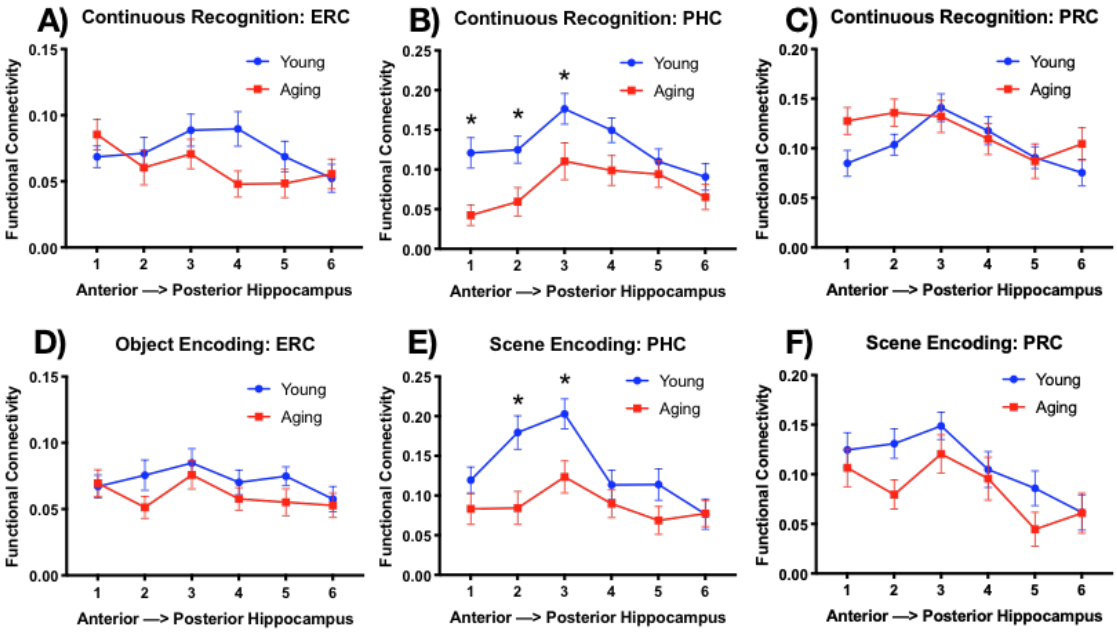

Figure S2. There was no effect of age on functional connectivity with the ERC in any of the datasets, including the continuous recognition task (A) and object encoding task (D). However, functional connectivity across the longitudinal axis of the hippocampus with the PHC shows an age-related decline in the anterior portions during both a continuous recognition task (B) and an incidental scene encoding task (E). There was an overall age-related decline in FC for Hx-PRC during the incidental scene encoding task (F) that was not observed during the continuous recognition task (C), suggesting that task demands may be able to modulate this effect.

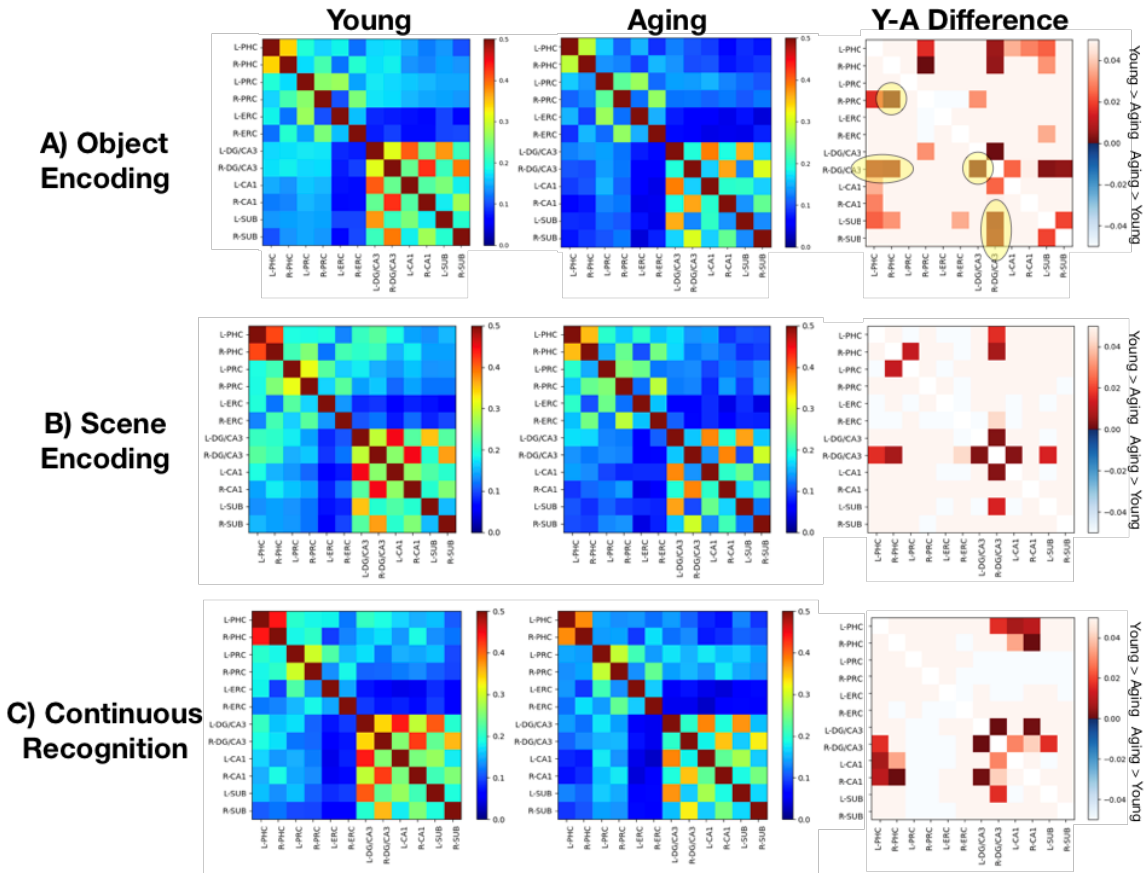

Figure S3. Functional connectivity matrices for young and aging adults and the difference matrix mapping p-values for each of the 3 datasets (object encoding data is identical to Figure 4 but shown again here for direct comparison). Difference matrix testing whether regions showed greater connectivity in young than old or vice-versa. The regions that passed multiple comparisons thresholding in the Object Encoding dataset are circled in yellow, but we did not impose that strict comparison on the other two datasets since we were looking for replication of those a priori regions.

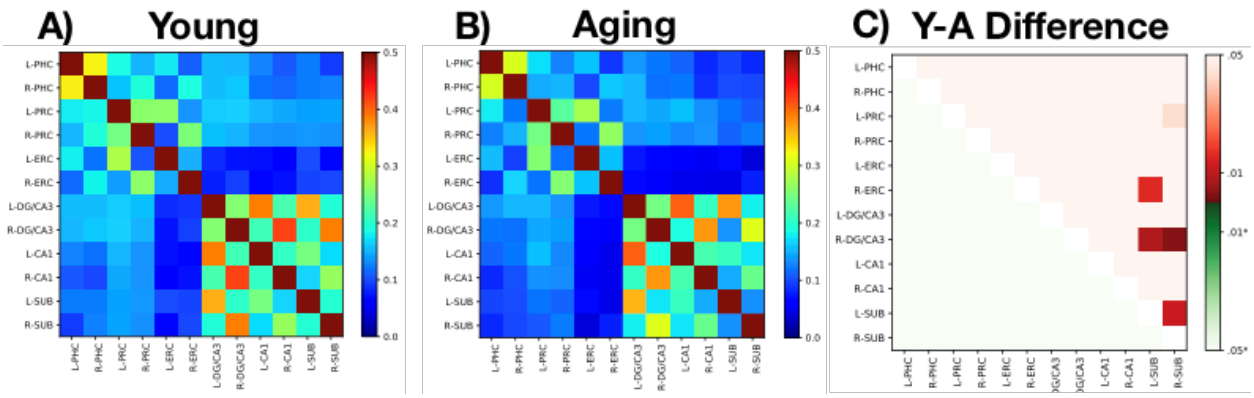

Figure S4. Functional connectivity matrices for young (A) and aging (B) adults and the difference matrix (C) mapping p-values for the object encoding data when regional volumes are regressed out of the correlations. Difference matrix testing whether regions showed greater connectivity in young than old or vice-versa. Values above the diagonal (reds) are uncorrected p-values while values below the diagonal (greens) reflect corrections for multiple comparisons.

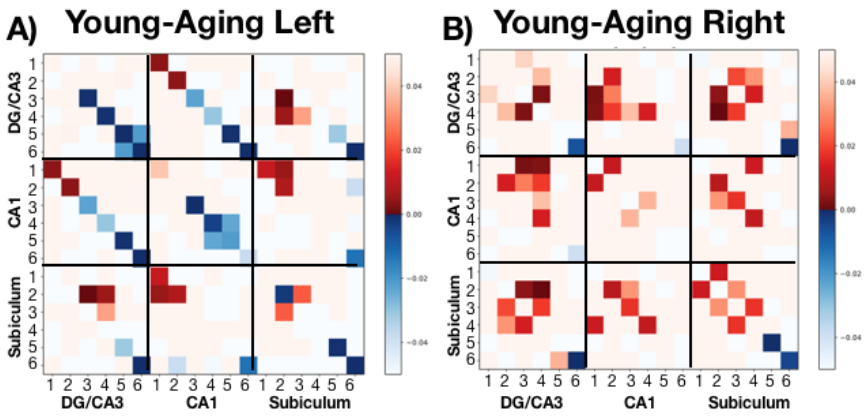

Figure S5. Young-Aged (Y-A) differences in the functional correlation matrices among the 6 segmentations of the hippocampus along the longitudinal axis, reflecting uncorrected p-values with a threshold of $p<.05$. There is a striking pattern of greater anterior FC in young adults and a reverse pattern in posterior FC across each of the subfields.

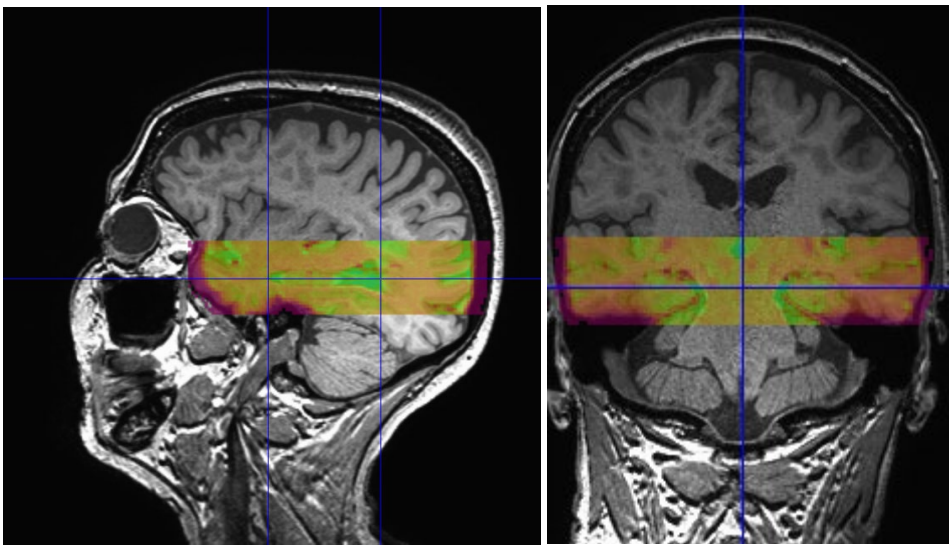

Figure S6. Example from a single subject with an underlay of the T1 structural with an overlay of the mean EPI following T1/EPI registration in the sagittal and coronal planes to demonstrate adequate coverage of the anterior, middle, and posterior segments of the hippocampus.

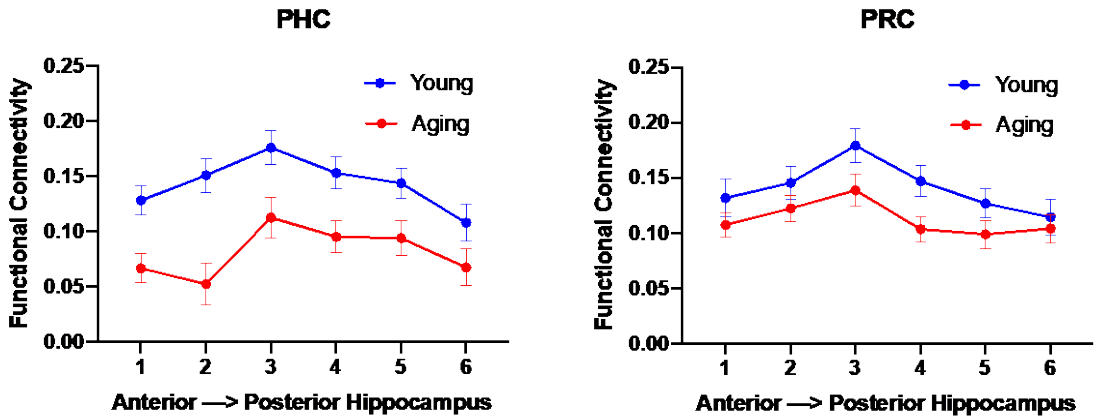

Figure S7. Consistent with the data from the main manuscript, functional connectivity with the global signal not regressed out between the hippocampus and PHC is greater for young than aging adults in the anterior portions of the hippocampus, while there was an age-related decrease in FC between Hx-PRC, but no difference across the longitudinal axis of the hippocampus.

	YOUNG			AGING		
REGION	**MEAN**	**StDEV**	**SNR**	**MEAN**	**StDEV**	**SNR**
CA1	85.9 (7.1)	0.23 (.04)	388.5 (74.9)	84.2 (7.9)	0.24 (.03)	360.1 (69.5)
CA1_1	88.4 (7.7)	0.51 (.08)	177.9 (30.9)	86.0 (8.9)	0.51 (.08)	176.3 (36.5)
CA1_2	89.4 (8.2)	0.58 (.12)	161.3 (31.9)	86.2 (8.9)	0.58 (.08)	154.35 (31.8)
CA1_3	85.5 (8.6)	0.59 (.09)	150.6 (34.4)	82.4 (8.9)	0.64 (.09)	132.9 (29.7)
CA1_4	82.4 (7.6)	0.63 (.10)	133.7 (33.1)	79.6 (8.3)	0.66 (.09)	124.4 (26.9)
CA1_5	82.1 (7.3)	0.67 (.11)	126.9 (30.9)	81.5 (7.7)	0.68 (.08)	123.9 (21.6)
CA1_6	86.7 (6.4)	0.41 (.06)	220.2(31.9)	85.2 (7.2)	0.44 (.07)	202.2 (42.8)
DGCA3	85.4 (7.4)	0.35 (.05)	249.4 (46.1)	82.4 (7.9)	0.37 (.05)	229.0 (43.6)
DGCA3_1	87.2 (7.8)	1.30 (.46)	74.6 (23.4)	83.3 (8.8)	1.18 (.36)	77.0 (25.0)
DGCA3_2	89.7 (8.0)	0.68 (.10)	135.9 (24.5)	84.6 (8.8)	0.69 (.08)	125.3 (25.2)
DGCA3_3	87.8 (8.2)	0.64 (.08)	140.9 (25.7)	83.9 (9.2)	0.69 (.08)	122.9 (24.4)
DGCA3_4	85.7 (8.2)	0.71 (.10)	124.4 (26.5)	83.2 (8.7)	0.74 (.09)	114.2 (22.7)
DGCA3_5	84.2 (7.5)	0.72 (.10)	119.6 (24.7)	82.8 (7.9)	0.78 (.12)	108.8 (23.2)
DGCA3_6	87.6 (7.0)	0.69 (.14)	132.8 (33.1)	85.4 (7.9)	0.86 (.29)	107.15 (29.6)
SUBICULUM	79.6 (7.0)	0.34 (.05)	240.3 (45.1)	77.8 (7.9)	0.35 (.05)	226.8 (48.8)
Sub_1	79.2 (7.6)	0.88 (.22)	95.6 (21.3)	75.9 (9.6)	0.86 (.14)	91.9 (23.6)
Sub_2	78.9 (7.7)	0.89 (.16)	91.4 (19.6)	75.2 (8.9)	0.95 (.13)	81.3 (18.4)
Sub_3	81.4 (8.2)	0.80 (.11)	104.7 (20.9)	78.8 (9.1)	0.83 (.12)	98.5 (23.6)
Sub_4	81.9 (8.1)	0.71 (.11)	119.4 (25.4)	79.5 (8.7)	0.71 (.10)	114.9 (25.2)
Sub_5	82.2 (7.4)	0.62 (.08)	135.8 (27.4)	81.2 (7.8)	0.69 (.11)	121.5 (27.2)
Sub_6	83.8 (7.0)	0.87 (.16)	101.2 (25.8)	82.4 (7.8)	1.08 (.22)	80.0 (19.7)
ERC	62.4 (8.7)	0.43 (.10)	155.0 (44.2)	63.0 (9.3)	0.46 (.09)	145.8 (41.0)
PRC	67.7 (6.7)	0.23 (.06)	302.9 (69.0)	67.1 (7.8)	0.23 (.05)	302.5 (77.0)
PHC	78.8 (7.5)	0.30 (.09)	281.0 (67.3)	77.8 (7.3)	0.30 (.09)	275.0 (68.6)
HIPPO_1	86.2 (7.3)	0.48 (.09)	186.9 (34.8)	84.0 (8.9)	0.46 (.09)	187.6 (43.2)
HIPPO_2	86.9 (7.7)	0.46 (.07)	193.9 (33.5)	83.5 (8.5)	0.46 (.05)	185.1 (33.8)
HIPPO_3	84.4 (7.8)	0.45 (.06)	189.9 (35.1)	81.3 (8.3)	0.48 (.06)	172.5 (32.8)
HIPPO_4	81.6 (7.5)	0.46 (.06)	180.6 (33.5)	79.3 (8.1)	0.47 (.06)	174.5 (35.7)
HIPPO_5	81.0 (7.7)	0.45 (.06)	185.8 (38.0)	80.8 (8.2)	0.47 (.07)	175.2 (36.6)
HIPPO_6	85.6 (7.8)	0.38 (.06)	233.9 (42.6)	84.6 (7.7)	0.42 (.07)	208.0 (44.1)

Table S1. Table of the mean signal, standard deviation (post detrending) and resulting timeseries signal-to-noise (SNR) computed at the ROI level and averaged across subjects within the group.