Decremental segregation of brain systems across the healthy adult lifespan

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Healthy aging has been associated with decreased specialization in brain function. This characterization has focused largely on describing age-accompanied differences in specialization at the level of neurons and brain areas. We expand this work to describe systems-level differences in specialization in a healthy adult lifespan sample (n = 210; 20–89 y). A graph-theoretic framework is used to guide analysis of functional MRI resting-state data and describe systems-level differences in connectivity of individual brain networks. Young adults’ brain systems exhibit a balance of within- and between-system correlations that is characteristic of segregated and specialized organization. Increasing age is accompanied by decreasing segregation of brain systems. Compared with systems involved in the processing of sensory input and motor output, systems mediating “associative” operations exhibit a distinct pattern of reductions in segregation across the adult lifespan. Of particular importance, the magnitude of association system segregation is predictive of long-term memory function, independent of an individual’s age.

Healthy adult aging is characterized by a progressive degradation of brain structure and function associated with gradual changes in cognition (see reviews in refs. 1, 2). Among the age-accompanied functional changes, one prominent observation is a reduction in the specificity with which distinct neural structures mediate particular processing roles [i.e., a reduction in functional specialization, or “dedifferentiation” (3)]. A reduction in functional specificity has been observed across multiple spatial scales of brain organization, ranging from the firing patterns of single neurons (e.g., refs. 4, 5) to the evoked activity of individual brain areas (6–10). (For additional discussion see ref. 11.)

Despite the compelling evidence for age-accompanied reductions in functional specialization across numerous brain areas, the relationship between neural specialization and cognition generally is weak. This likely is related to the fact that broad cognitive domains such as “long-term memory” and “executive control” are mediated by distributed and interacting brain systems, each consisting of multiple interacting brain areas. Thus, relating functional specialization in a single brain area to general measures of cognition likely will be unsuccessful. Such an argument is consistent with the view that severe impairment in cognitive function due to injury or insult typically is a consequence of damage to multiple brain locations (e.g., refs. 12, 13). Based on these considerations, it seems plausible that the cognitive decline evident even in healthy older adults may be related to decreased functional integrity at a systems level of organization. The present report approaches healthy aging from this systems-level perspective in an effort to relate systems-related functional specialization to age-accompanied differences in cognition.

Before proceeding, it is important to clarify the meaning of system. The term “system” often is used in relation to brain organization when referring to any group of areas that subserve common processing roles. For example, the visual system comprises brain areas primarily defined by their role in processing visual stimuli (e.g., ref. 14), and the frontal–parietal task control system consists of brain areas involved mainly in adaptive task control (15). Identifying distinct brain systems and defining their functional roles by examining how their constituent areas are modulated by experimental testing or are impaired by brain damage is not an easy endeavor; systems of brain areas typically mediate processing roles that span multiple stimuli and task demands. This reality makes assessing changes in the functional specialization of systems across cohorts of individuals extremely challenging.

An alternative formal and complementary approach to defining a brain system involves understanding how brain areas relate to one another via their patterns of shared functional or anatomical relationships in the context of a large-scale network (16, 17). Like many other complex networks, brain networks may be analyzed as a graph of connected or interacting elements. When a brain network graph represents the interaction of areas, one prominent feature is the presence of subsets of areas that are highly interactive with one another and less interactive with other subsets of areas. This pattern of organization reflects the presence of distinct “modules” or “communities” (e.g., ref. 18). Importantly, numerous connectivity-defined human brain modules have been shown to overlap closely with functional systems as defined by other methods of assessing information processing [e.g., task-evoked activity, lesion-mapping (19, 20)]. The close correspondence between differing methods of system identification provides a basis for using connectivity to understand the organization of brain systems and how they may differ with age.

Significance

The brain is a large-scale network, not unlike many social or technological networks. Just like social networks, brain networks contain subnetworks or systems of highly related or interacting nodes (in the case of brains, nodes may represent neurons or brain areas). Using functional MRI to measure functional correlations between brain areas during periods of rest, we describe differences in brain network organization in a large group of individuals sampled across the healthy adult lifespan (20–89 y). We characterize a measure of system segregation, reflecting the degree to which the systems share connections among one another. Increasing age is accompanied by decreasing segregation of brain systems. Importantly, system segregation is predictive of measures of long-term memory function, independent of age.

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Modular brain networks are characterized by a fine balance of dense within-system relationships among brain areas (nodes) that have highly related processing roles, as well as sparser (but not necessarily absent) relationships between areas in systems with divergent processing roles. This pattern of system segregation facilitates communication among brain areas that may be distributed anatomically but nevertheless are in the service of related sets of processing operations, and simultaneously reinforces the functional specialization of systems that perform different sets of processing operations (21). Importantly, segregated systems can communicate with one another via the presence of the sparser connections between them. As such, any deviation in the patterns of within- and between-system connectivity may be considered evidence for a change in the system’s specialization. Furthermore, if aging is associated with changes in functional specialization at the level of brain systems, this may be revealed by examining the differences in patterns of within- and between-system areal connectivity across age.

We use functional connectivity, as measured by blood oxygen-level-dependent (BOLD) functional MRI (fMRI) during rest (resting-state functional correlations [RSFCs], see ref. 22), to assess age-related differences in the organization of brain systems. Changes in RSFC patterns between sets of areas have been observed following extensive directed training (23–25), and differences in RSFC patterns also have been reported in cross-sectional comparisons spanning from childhood to older age (e.g., refs. 26–29). The extant data suggest that RSFCs are malleable and reflect sensitivity to a history of coactivation: changes in the processing roles of areas may be characterized by changes in their RSFCs with other areas (for discussion, see ref. 17). This feature makes RSFCs particularly useful in assessing differences in the organization and specialization of brain systems.

In the present study, the age-accompanied differences in the functional specialization of brain systems are revealed by examining patterns of within- and between-system areal RSFCs in a large healthy adult lifespan sample (n = 210; age range, 20–89 y). The inclusion of subjects distributed across each decade of adulthood not only allows us to assess how older and younger adults differ in their organization of brain systems, but also provides insight as to whether there is a critical stage of the adult lifespan when differences in system organization may appear. Previous research has attempted to address related questions by examining end points of the adult aging spectrum, focusing on the organization within specific systems (e.g., refs. 26, 28, 30), or using area nodes that are not representative of functional areas (e.g., structural parcels (31–34]). The latter feature likely contributes to the inconsistent findings observed in the relationship between summary network measures and age groups (e.g., refs. 31, 35 vs. refs. 30, 36). In addition to examining age-related differences in system organization developed from a biologically plausible cortical parcellation of the human brain network, we also relate systems-level differences in organization to differences in general measures of cognitive ability. To foreshadow the results that follow, we report that aging is associated with differences in patterns of connectivity within and between brain systems, that these differences are not uniform across all systems, and that the segregation of brain systems has a direct relationship to measures of cognitive ability independent of age.

Results

Defining Biologically Plausible Brain Network Nodes Using RSFC-Based Area Parcellation. Understanding the organization of brain systems and how areas within and between brain systems interact with one another mandates the identification of a set of nodes representing areas in the overall brain network. The identified nodes must be biologically constrained and represent meaningful areal distinctions (for discussion, see ref. 17). Network nodes were constructed by processing further (Experimental Procedures, Area Node Definition) a recently published RSFC-based area parcellation map. This parcellation map identified cortical locations where patterns of RSFCs exhibited abrupt transitions in a large group of subjects [i.e., putative area borders; Fig. 1L (37)]. Fixed-radius disks (3-mm radius) were built around putative area centers, defined in relation to the transitions, along the cortical surface (Fig. 1A).

A total of 441 nonoverlapping disks were created across the cortical surface of the two hemispheres; each of these disks served as a node in the construction of a graph representing an individual’s brain network. Each node was labeled according to a published functional system map, defined by consensus of system assignments using community detection of RSFCs across multiple thresholds (Fig. 1C) (19). The final system labels of each node are depicted in Fig. 1D, and SI Appendix, Table S6 lists the node count for each functional system. Using independent datasets to define nodes and assign system labels to these nodes allowed interrogation of connectivity within and between systems in an unbiased fashion. Brain graphs were constructed for each participant as a 441 x 441-node graph, labeled by RSFC-defined functional system. Edge weights were calculated as the Fisher z-transformed correlation (Pearson’s r) between each pair of nodes, and negatively weighted edges were removed from each correlation matrix to eliminate potential misinterpretation of negative edge weights (see Experimental Procedures for further details).

Healthy Adult Aging Is Accompanied by Decreasing Connectivity Within Functional Brain Systems, and Increasing Connectivity Between Functional Brain Systems. To explore whether the relationships between areas of distinct functional systems may differ across healthy adult aging, area-to-area relationships (graph edges) first were characterized according to whether they connected areas within a functional system or between distinct functional systems. Fig. 2 depicts the mean within-system and between-system correlations across age (r = 0.23; age range, 20–89 y). There is clear evidence that the mean within-system correlation is greater than the mean between-system correlation, independent of age. Importantly, we also observed two different patterns with respect to the types of relationships as a function of age: within-system correlations decreased with increasing age, and between-system correlations increased with increasing age. These observations were statistically supported in a general linear model demonstrating that although the mean correlation of all nodes in the network was predicted by age [F(1,208) = 4.16, P = 0.043], there was also a main effect of type of edge [within- vs. between-system; F(1,208) = 4114.99, P < 0.001] and an interaction between age and edge type [F(1,208) = 45.80, P < 0.001]. This interaction was a result of significant age-related decreases in within-system correlations (r = −0.28, P < 0.001) and significant age-related increases in between-system correlations (r = 0.32, P < 0.001), which are significantly different from one another: z = −7.77, P < 0.001.

The results depicted in Fig. 2 reflect node-to-node relationships collapsed across all functional systems identified by Power et al. (19). We focused on 10 consistently identified systems for subsequent analyses (system legend in Fig. 1C; for additional analyses, see SI Appendix, Supplemental Results and Table S5) and evaluated their age-related changes in within- and between-system correlations (SI Appendix, Fig. S3A and Table S7). Although varying in the degree of age-related change, almost all the systems demonstrated comparable patterns of decreasing correlations within systems and increasing correlations between systems, suggesting a general nature to this prominent pattern.

The patterns described above may be appreciated further by examining the 441 node cross-correlation matrices. To do so, participants first were divided into four age-based cohorts. Cohorts were constructed with the aim of maintaining a relatively consistent
age range across subjects while ensuring a roughly equivalent number of participants in each cohort. The four adult lifespan categorical divisions were “younger” (YA; 20–34 y; \( n = 61 \)), “middle-early” (ME; 35–49 y; \( n = 46 \)), “middle-late” (ML; 50–64 y; \( n = 43 \)), and “older” adults (OA; 65–89 y; \( n = 60 \)). For each cohort, a mean matrix was constructed in which the nodes were ordered by their predefined community labels (from Fig. 1D). The mean matrices that depict the 10 systems (Fig. 2B) reinforce the two patterns we noted: (i) correlation values along the diagonal of the matrix reflect within-system relationships, and these decrease with increasing age, and (ii) correlation values off-diagonal reflect between-system relationships, and these increase with increasing age. It is important to note that these patterns are not homogenous across all systems or areas, suggesting that age-associated differences in connectivity may be specific to certain types of relationships. For example, the connectivity between the frontal-parietal control system and other systems increases strongly with age \( r = 0.36, P < 0.001 \), whereas the auditory system does not exhibit as strong an age-related increase in between-system connectivity \( r = 0.10, P = 0.156 \); see SI Appendix, Table S1 for details on the 10 systems. We revisit these important observations in a subsequent section.

**Age-Accompanied Differences in Brain Connectivity Reflect a Decreased Segregation of Functional Brain Systems.** The differences in the patterns of within- and between-system RSFCs suggest that aging is accompanied by decreased independence of brain systems (by way of exhibiting weaker connectivity among areas within systems and greater connectivity between areas of distinct systems). As a way of summarizing and quantifying the pattern of differences in the within-system correlations in relation to the between-system correlations, we created a measure of system segregation. This measure was calculated as the difference between the mean magnitudes of between-system correlations from the within-system correlations as a proportion of mean within-system correlation (see Experimental Procedures). Accordingly, values greater than 0 reflect relatively lower between-system correlations in relation to within-system correlations (i.e., stronger segregation of systems), and values less than 0 reflect higher between-system correlations relative to within-system correlations (i.e., diminished segregation of systems). This formulation of system segregation results in a theoretical maximal segregation value of 1, which would reflect a system with greater than 0 mean within-system connectivity but absent connectivity with all other systems.

Mean system segregation is plotted as a function of participant age in Fig. 2C. Across age, segregation values are greater than 0, demonstrating that mean within-system correlations are stronger than mean between-system correlations, regardless of age. Despite this commonality, older age is associated with decreasing system segregation \( r = -0.53, P < 0.001 \). Moreover, this pattern was observed in 8 of the 10 systems of interest when interrogated individually (SI Appendix, Fig. S3B and Table S7).

Our measure of segregation is intimately related to the graph-theoretic concept of participation coefficient. A node’s participation coefficient is a measure of the extent to which a given node connects to nodes in systems (communities) other than its own. Higher values indicate that the node is connected to many nodes in other systems, whereas lower values indicate that the node’s interactions are limited largely to its own system. Based on our findings of decreased segregation with age, we predicted that participation coefficients would increase with increasing age. The mean participation coefficient across all nodes increases with increasing age \( r = 0.46, P < 0.001 \); Fig. 2D; also see SI Appendix, Fig. S2 and Supplemental Results for further analyses on participation coefficients and discussion regarding the distinctions between multiple measures).

To summarize, evaluating multiple measures that quantify intersystem relationships reveals that older adults exhibit proportionally greater connectivity between nodes in different...
Older Adults (65-89 years)

SI Appendix defined; previously published system maps, cohort defined and individually multiple modalities (e.g., the frontal system, the motor system), whereas systems are engaged in neural coding and transformation of in-
mor associative processes (e.g., refs. 38, 39). Sensory-motor systems included the auditory, hand
acterizes functional systems and their constituent areas is whether "the
found to be robust in all instances.

Association and Sensory-Motor Systems Exhibit Distinct Patterns of Age-Related Differences in Segregation. Thus far, we have de-
dscribed system-related changes largely by collapsing measures across all systems. However, there also is evidence that different functional systems exhibit differences in their specific patterns of age-related changes in segregation. For example, it appears that areas in the frontal–parietal control system exhibit greater con-
nectivity to areas in several other systems, including the ventral visual-spatial attention system. Consistent
with their purported roles in coordinating diverse sets of operations with other systems, areas in association systems exhibit widespread and diverse anatomical projections with distributed brain systems (e.g., refs. 38, 40) and have been demonstrated to exhibit greater RSFC with areas in other systems compared with areas in sensory-motor systems (19).

We examined the sensory-motor/association system distinction to determine whether there were age-accompanied differences in segregation as a function of system type. Systems first were classified according to whether they primarily fit a sensory-motor or an as-

ation role. Sensory-motor systems included the auditory, hand somatomotor, mouth somatomotor, and visual systems. Association systems included the cingulo-opercular control, dorsal attention, frontal–parietal control, salience, ventral attention, and de-

fault systems.

Overall, the results show that aging is associated with decreasing segregation of both association and sensory-motor systems; however, their patterns of age-related changes in segregation differ (Fig. 3 A and B). When we applied linear and nonlinear fits (first-, second-, and third-degree polynomials) to sensory-motor system segregation, we found that the age function for these systems was fit significantly only by a linear model \[ R^2 = 0.24 \] and a third-degree polynomial \[ R^2 = 0.099 \], whereas the second-degree polynomials were not statistically reliable \[ n(208) = 1.13, P = 0.261 \] and \[ n(208) = 0.01, P = 0.099 \], respectively. In contrast, association system segregation was fit significantly by both a linear \[ (t = 8.55, P < 0.001) \] and a quadratic model \[ R^2 = 0.28, P < 0.001 \]. The linear and quadratic models of association system segregation were significantly different from one another \[ F(1,207) = 7.84, P = 0.006 \], with the quadratic model having a higher adjusted \[ R^2 \] than the linear model\[ (0.28 vs. 0.26, respectively) \]. The significant quadratic function is illustrated by nonparametric local smoothing (local

Fig. 2. Increasing adult age is associated with decreasing segregation of brain systems. (A) Mean within-system RSFC decreases with age, and mean between-system RSFC increases with age. (B) Mean node-to-node correlation matrix (10 systems) of each age cohort. Nodes are grouped according to system labeling (Fig. 1D); color bars along axes represent system labels (see legend to the right). Within-system RSFC (on matrix-diagonal) exhibits decreasing strength across cohorts, whereas between-system RSFC (off matrix-diagonal) exhibits increasing strength across cohorts. (C) With increasing age, the patterns are apparent for a subset of between-system relationships. For example, RSFCs of the frontal–parietal control or the ventral attention system with several other systems (e.g., default system; white arrows) are increasingly greater (yellow/orange colors) from younger to older adult cohorts. (D) Mean network participation coefficient exhibits an age-associated increase, supporting observations related to system segregation. For each scatterplot, a line reflecting the linear regression between age and the dependent variable is depicted.
significant age-related decrease was observed in episodic fluid processing, and verbal ability (emerged were related to measurements of episodic memory, the factor-loading structure revealed that the three factors that may represent distinct general functions. An examination of breadth of the cognitive measurements, participant scores first were submitted to a factor analysis to identify behavioral factors with a series of standardized behavioral tests that spanned multiple cognitive domains (see SI Appendix, Supplemental Experimental Procedures for exact cognitive measures). Given the breadth of the cognitive measurements, participant scores first were submitted to a factor analysis to identify behavioral factors that may represent distinct general functions. An examination of the factor-loading structure revealed that the three factors that emerged were related to measurements of episodic memory, fluid processing, and verbal ability (SI Appendix, Table S3). A significant age-related decrease was observed in the episodic memory ($r = -0.20$, $P = 0.003$) and fluid processing components ($r = -0.75$, $P < 0.001$), whereas the verbal ability component increased with age ($r = 0.32$, $P < 0.001$). This general pattern is consistent with previous characterizations of age-accompanied differences in cognition (41, 42).

We related the three cognitive factor scores to the measurements of system segregation. Critically, because age correlated with all measurements of interest, regression models were computed by first removing the effect of age on the measurements of cognition and segregation (i.e., partial correlations). After we controlled for age, segregation of association systems was significantly related to episodic memory ($r = 0.18$, $P = 0.007$): individuals with greater association system segregation exhibited higher episodic memory scores (Fig. 4A). Of particular interest, the high degree of segregation among different association systems was significantly related to memory function, whereas the relationship between memory and segregation of association from sensory-motor systems only approached significance (i.e., association-to-association segregation and memory: $r = 0.22$, $P = 0.001$; association-to-sensory-motor segregation and memory: $r = 0.13$, $P = 0.063$; Fig. 4B and C). The significant relationships between memory and association-to-association system segregation remained after Bonferroni correction for the 18 performed comparisons. After controlling for age, no relationship was found between sensory-motor system segregation and memory ($r = -0.09$, $P = 0.203$; see SI Appendix, Fig. S5, Table S8, and Supplemental Results for additional analyses).

Fig. 3. Sensory-motor and association systems exhibit distinct patterns of age-associated differences in segregation. Locally weighted scatterplot smoothing (LOESS) graphs depict (A) the linear association between decreasing sensory-motor system segregation and increasing age and (B) the quadratic association between decreasing association system segregation and increasing age. Decreases in association system segregation exhibit an inflection point reflecting accelerated reductions starting at an approximate age of 50 y (red dotted line). (C) Spring-embedded layouts of the 10 systems (4% edge density) of the four cohorts’ mean correlation matrices (Fig. 2B). Sensory-motor systems exhibit progressive age-accompanied reductions in both within-system correlations and segregation with other systems (e.g., the visual system, highlighted by the arrows). Association systems exhibit prominent and sudden decreases in segregation with other systems starting in middle-late adulthood [e.g., the frontal–parietal control (in yellow) and cingulo-opercular control systems (in purple) exhibit less within-system connectivity and greater between-system connectivity in middle late and older adult cohorts, highlighted by the circle).
adult lifespan. The organization and function of brain networks across the each of these observations and their implications for understanding association systems exhibited thepoorest memory ability. We consider even after controlling for age, individuals with less segregated as-

There is accumulating support that healthy aging is accompanied RSFC-Defined System Segregation as a Metric of Functional Specialization.

The present findings indicate that increasing age is associated in segregation from sensory-motor systems, association systems exhibit greater decreases fined by patterns of RSFC between brain areas. Compared with

Discussion
The present findings indicate that increasing age is associated with decreasing segregation of functional brain systems, as defined by patterns of RSFC between brain areas. Compared with sensory-motor systems, association systems exhibit greater decreases in segregation from ~50 y of age onward. Of particular importance, even after controlling for age, individuals with less segregated association systems exhibited the poorest memory ability. We consider each of these observations and their implications for understanding the organization and function of brain networks across the adult lifespan.

RSFC-Defined System Segregation as a Metric of Functional Specialization.

There is accumulating support that healthy aging is accompanied by changes in the information processing of brain areas as evidenced by task-evoked activity. In some instances, these changes reflect quantitative young/old differences in comparable sets of areas (e.g., refs. 43-45), whereas in other instances, there is evidence for qualitative differences in the areas recruited across cohorts (e.g., refs. 6, 46). It is uncertain whether these changes reflect age-associated strategic differences in task engagement, or they are obligatory differences in response to the ongoing cascade of white-matter and gray-matter brain changes that occur in even very healthy older adults (47, 48). Further, it currently also is uncertain whether any of the qualitative changes are constrained by system distinctions. Despite these uncertainties regarding the cause and nature of changes in task-related activation, given the strong link between patterns of RSFC and task-related activity, we hypothesize that RSFC differences reflect a statistical marker of the activity changes that accompany aging. What is particularly intriguing is that RSFC differences were present both within and between systems.

We have suggested that the functional specialization of a system may be characterized by the balance of connections between areas within the system and limited interaction with areas in other systems. In younger adults, this modular architecture facilitates the common processing roles of areas within similar systems while allowing communication between systems with distinct processing roles (21). Consistent with this, there is evidence for a relationship between measures of modularity and changes in task performance and learning in healthy young adults (49, 50). Conversely, comparisons of younger and older adults have revealed differences in brainwide modularity in a direction consistent with the present observations (30, 32, 33, 36). Importantly, we believe our measure of segregation is more sensitive to age-related differences in network organization than modularity (SI Appendix, Fig. S6) and allows clearer insight regarding the underlying changes contributing to the measure. We hypothesize that age-associated decreases in system segregation may indicate decreased functional specificity of system-based processing roles. Brain areas in distinct systems exhibit greater interaction with continual aging, as reflected in patterns of RSFC. Although this may be an adaptive response to ongoing anatomical and biochemical alterations (1, 2), it does not appear that the increasing interaction between areas in distinct systems confers a benefit to the individual; rather, the increased “blurring” across systems and decreased communication within systems may reflect a fundamental age-related mechanism that negatively affects cognitive function. We return to this point in the following section.

Distinctions Between Association and Sensory-Motor System Segregation May Reflect Important Differences in Age-Accompanied Changes in Information Processing. Systems involved in “associative” operations exhibited greater age-accompanied decreases in segregation compared with systems involved in processing sensory input and motor output. This distinction was informed further by the observation of different patterns of age-related segregation: decreasing association system segregation was fit better by a quadratic than a linear function, distinguished by a sharper rate of decline from age 50 onward (Fig. 3). This distinction between association and sensory-motor systems may reflect different trajectories in the patterns of decreasing functional specialization. Operations mediated by association systems, including but not limited to the maintenance and

Fig. 4. Greater association system segregation is associated with superior long-term episodic memory, independent of age. (A) Episodic memory scores are predicted by participants’ association system segregation. Data points are color coded by participants’ age cohort to demonstrate that the relationship between memory and association system segregation is independent of age. (B) Relationship between episodic memory and segregation of association systems from sensory-motor systems. For each scatterplot, a line reflecting the linear regression between episodic memory scores and system segregation is depicted.
execution of task set, allocation of attention, controlled mnemonic retrieval, and executive control, likely are performed by multiple brain systems and require substantial interaction between areas in these systems (39, 51, 52). The decreasing executive ability that characterizes older age (42, 53, 54) may be a consequence of reduced functional specialization of systems that mediate these abilities, as indexed by decreasing association system segregation.

Although executive abilities exhibit a particular age-associated decline, there is substantial evidence that aging is associated with an acceleration of changes that affects both sensory and more associative or “cognitive” functions jointly (53). Lindenberger and Baltes (55) suggested that the interrelationships they observed among different cognitive and sensory behavioral systems in very old adults were mediated by some basic brain mechanism or “common cause” that was deteriorating with age. In fact, they speculated that the declining specificity of behavioral systems that occurs with age was the result of “dedifferentiation” of the brain. Based on our present findings, we speculate that the underlying substrate of the observations leading to the common-cause hypothesis may be the degree of between-system interactions occurring in the adult brain. Specifically, we suggest that the tighter link between sensory and cognitive function in older age is intimately related to the decreasing RSFC-defined system segregation observed here.

System segregation was predictive of a summary measure of memory. The age-invariant relationship between association system segregation and long-term memory scores suggests that our measurements of network properties exhibit a much broader relationship to behavior than that which simply characterizes differences present across adult aging. The direction of the relationship suggests that decreased system segregation has negative consequences for behavior (see SI Appendix, Supplemental Results and Discussion for additional analyses related to relationships between segregation and cognitive measures) and that measurements of segregation may be used as an important neural measure across a range of cross-sectional and longitudinal comparisons related to health and pathology (also see SI Appendix, Supplemental Discussion), but also experimental and interventional manipulations (e.g., refs. 50, 56).

The Use of Graph Theory to Study Brain Networks. Two authors of the present report (G.S.W. and S.E.P.) previously stressed the importance of examining brain network nodes that represent biologically meaningful entities, and our current efforts have attempted to satisfy this requirement by building brain networks from an area parcellation method that appears neurobiologically plausible [brain areas (37); also see SI Appendix, Supplemental Discussion]. Although relatively accurate node representation is an important constraint in generating valid brain networks, it also is important to understand the nature and interpretation of the derived network measures (e.g., refs. 37, 57). We briefly describe an illustrative and cautionary example.

We have described the use of a novel measure, termed system segregation, which characterizes the amount of within- and between-system connectivity in brain systems. This measure is strongly associated with many traditional graph measures (e.g., participation coefficient, modularity) but can capture the trend of aging more strongly than the other measures (SI Appendix, Supplemental Results and Fig. S6B). In addition, we also quantified a prominently examined measure, global efficiency (GE), in each of our participants (see SI Appendix, Supplemental Experimental Procedures for details on calculation). GE is a measurement related to the average shortness of paths between nodes of a network, where networks with shorter average paths have higher GE. Several previous studies examining differences in GE as a function of age cohort found greater GE in older vs. younger adults (31, 35). In the present study, examining the relationship between GE and age also reveals that increasing age is accompanied by increasing GE. Critically, this observation is trivial and logical when one recognizes that the introduction/strengthening of connections (edges) between systems (clusters) quickly decreases average path length (58). Given the negative relationship between age and system segregation, it is not surprising that GE increases with age. Importantly, accounting for system segregation eliminates the significant age–GE relationship (SI Appendix, Fig. S7). Observations related to differences in other summary measures, such as “small-worldliness” (e.g., ref. 33), may be similarly sensitive to these forms of basic underlying properties.

Concluding Comments. Using a network-based approach revealed that healthy aging is accompanied by decreased segregation of brain systems defined by their patterns of resting-state correlations. We hypothesize that system segregation is an important measure and guide toward understanding functional specialization of areas within distinct brain systems, and it will be important to examine how changes in segregation of specific systems affect the functional roles of their areas. The age vs. system segregation relationship was most prominent for association systems following 50 y of age. After controlling for age, we also found that the degree of association system segregation was predictive of offline measures of memory ability, suggesting that system segregation may be an age-invariant marker of individual differences in cognition.

Of additional interest, there is evidence for developmental differences in patterns of within- and between-system RSFC, wherein young adults appear more segregated than children and adolescents [(59), and this is also the case following more stringent movement correction (60)]. It will be important to examine system segregation in the context of early brain development more closely to understand how the mechanisms giving rise to changes in segregation differ across the entire lifespan. By measuring functional relationships in the absence of overt cognitive tasks, the present approach seems particularly well suited toward characterizing and understanding the complex organization of brain networks across various cohorts and species and how this organization develops, differs, and evolves in relation to behavior.

Experimental Procedures

Participant Demographics. Healthy adults from the Dallas Lifespan Brain Study (DLBS) who completed a resting-state fMRI scan were included in the present study (n = 268). Participants were recruited from the Dallas–Fort Worth community and provided written consent before participating. All study procedures were reviewed and approved by the Institutional Review Boards at The University of Texas at Dallas and The University of Texas Southwestern Medical Center. A final sample of 210 participants met the minimum requirements of RSFC data quality (See SI Appendix, Supplemental Experimental Procedures for details and additional exclusion criteria). Table 1 summarizes these participants’ demographics, broken down by age cohorts.

Experimental Design and Data Acquisition. The DLBS consists of multiple data acquisition sessions that include cognitive and neuropsychological testing and MRI scanning. The MRI scanning session consists of a series of anatomical MRI and fMRI scans acquired using a Philips Achieva 3T scanner. See SI Appendix, Supplemental Experimental Procedures for image acquisition details of the anatomical T1 scan and resting-state functional scan.

Data Preprocessing. Adult-lifespan atlas construction. Typical registration targets for MRI (e.g., MNI152) are based on a young adult sample, which systematically introduces greater registration error among older adults (61). To avoid this issue, following the procedure outlined in Buckner et al. (61), an adult-lifespan sample-representative template was created (see SI Appendix, Supplemental
### Table 1. Demographic information

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<th>Measure</th>
<th>Younger</th>
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<td>N</td>
<td>61</td>
<td>46</td>
<td>43</td>
<td>60</td>
<td>NA</td>
</tr>
<tr>
<td>Age range, y</td>
<td>20–34</td>
<td>35–49</td>
<td>50–64</td>
<td>65–89</td>
<td>NA</td>
</tr>
<tr>
<td>Female, %</td>
<td>77</td>
<td>65</td>
<td>72</td>
<td>55</td>
<td>ns</td>
</tr>
<tr>
<td>Education, y (SD)</td>
<td>14.90 (3.82)</td>
<td>14.67 (4.19)</td>
<td>15.49 (3.94)</td>
<td>13.25 (3.86)</td>
<td>0.026*</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.67 (1.19)</td>
<td>28.74 (1.12)</td>
<td>28.51 (1.16)</td>
<td>27.68 (1.17)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MMSE, mini-mental state examination; NA, not available; ns, not significant.

*Mean differences were tested with χ² test for sex distribution and ANOVA for years of education and MMSE.

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**Experimental Procedures** for details to incorporate typical structural variation present across our healthy adult lifespan sample (n = 268, age range, 20–89 y).

**Standard fMRI preprocessing.** Functional images first were processed to reduce artifacts (62), register each individual’s magnetization-prepared rapid gradient echo (MP-RAGE) image to the adult-lifespan sample atlas, and resampled to 3-mm isotropic atlas space. See SI Appendix, Supplemental Experimental Procedures for details.

**RSFC preprocessing.** Several additional preprocessing steps were used to reduce spurious variance unlikely to reflect neuronal activity in RSFC data, which included in the following order: (i) multiple regression of the BOLD data to remove variance related to the whole brain signal (cf. ref. 63), ventricular signal, white matter signal, six detrended head realignment parameters, rigid-body correction, and spatial order derivative terms for all aforementioned nuisance variables; (ii) band-pass filtering (0.009–0.08 Hz); and (iii) volumetric spatial smoothing (6-mm full width at half maximum in each direction). To reduce the effect of motion artifact on RSFCs, data were processed following the recently described “scrubbing” procedure (64). See SI Appendix, Supplemental Experimental Procedures for details and Supplemental Results for additional analyses related to the present findings.

**Surface preprocessing.** There is growing evidence that landmark-based registration of a participant’s cortical surface exhibits more accurate alignment of cortical anatomy than either linear or nonlinear volume-based registration techniques (65). As this may confer particular benefits toward minimizing variation in anatomical registration in the present adult-lifespan sample, FreeSurfer 5.0 was used to process volumetric images into fsaverage surface images. The left and right hemisphere fsaverage surfaces were brought into register with each other by mapping onto a hybrid left–right fsaverage surface ("fs_LR") (66). See SI Appendix, Supplemental Experimental Procedures for details.

**Area node definition.** Network nodes were constructed by processing further a recently published RSFC-based area parcellation map. This parcellation map identified cortical locations where patterns of RSFC exhibited abrupt transitions in a large group of subjects (i.e., putative area borders; Fig. 1A) (37). There is evidence that RSFC transitions are least pronounced at the identity, locations where RSFC maps exhibited high local stability, local minima of RSFC transition, were identified in the RSFC parcellation map (in fs_LR space) (37). Minima were required to be at least 8 mm apart along the cortical surface, and minima identified within the FreeSurfer medial wall were excluded. Fixed-radius disks (3-mm geodesic radius) were built around each of the minima locations (Fig. 1B). By representing nodes as fixed-radius disks around the probabilistically defined center of an area, this method of node definition avoids locations of greater uncertainty. In addition, the small fixed-radius disks have the benefit of ensuring equivalent node size across the entire node set and minimizes the likelihood of creating nodes that capture information from multiple adjacent areas (for discussion, see refs. 17, 67, 68). Small fixed-size disks also are beneficial if the nodes are intended to sample information across individuals who may have variation in their area parcellation (69–71). This technique, by definition, limits use of information from the full extent of a given area, but the many advantages noted above outweighed this concern. A total of 441 nonoverlapping disks were created across the two cortical hemispheres (L = 221, R = 220). Visual inspection confirmed that the disks did not cross any strong RSFC boundaries on the original parcellation map.

Disks were labeled according to a published functional system map defined by voxelwise community detection of RSFC, in which systems were assigned based on consensus across multiple thresholds (19) (Fig. 1C). As the RSFC parcellation and system maps both are represented on the fs_LR cortical surface, we could combine information from both approaches and interrogate DLBS data in the same cortical space. All fs_LR vertices first were labeled according to RSFC system membership. The system labels of all vertices within a disk then were identified, and each disk was labeled with a system by a winner-take-all approach. The final system labels of each disk are depicted in Fig. 1D, and SI Appendix, Table S6 lists the disk count for each functional system. Single-system analyses in this study focused on 10 consistently identified systems (see legend in Fig. 1C) and include at least four nodes per system.

**Basic RSFC and Graph-Theoretic Analysis.**

**Preparing RSFC data for connectivity analysis.** For each participant, the resting-state fMRI time series of vertices within each of the 441 nodes was extracted, and the vertex–mean time course was computed for each node.

The cross-correlation of each node’s time course with every other node’s time course was calculated, forming a node-to-node correlation matrix. Correlation coefficients were converted into z-values using Fisher’s equation (72). The resulting Fisher z-transformed r-matrix (z-matrix) is a fully connected, weighted relatedness graph. Although it is possible to include negative ties in a network analysis (73), because of the present ambiguity regarding the meaning of negative correlations (63, 74, 75), negative z-values were excluded from the data matrix. The final data matrix for each participant was a 441 × 441 z-matrix with the diagonal and negative values set to zero.

**Within-system and between-system connectivity.** Nodes were labeled by functional systems (Fig. 1D). For a given system, within-system connectivity was calculated as the mean node-to-node z-value of all nodes of that system to each other (e.g., the mean of the z-values between all default system nodes to all other default system nodes). Conversely, between-system connectivity was calculated as the mean node-to-node z-value between each node of a system and all nodes of all other systems (e.g., mean of all z-values between all default system nodes and all other nodes in the brain).

**System segregation.** A measure of system segregation was computed to summarize values of within-system correlations in relation to between-system correlations. Specifically, this measure takes the differences in mean within-system and mean between-system correlation as a proportion of mean within-system correlation, as noted in the following formula:

$$\text{System segregation} = \frac{Z_{\text{within}} - Z_{\text{between}}}{Z_{\text{within}}}$$

where $Z_{\text{within}}$ is the mean Fisher z-transformed r-betweens within the same system and $Z_{\text{between}}$ is the mean Fisher z-transformed r-betweens between nodes of one system to all nodes in other systems. Importantly, our measure of system segregation retains the weight of all positive edges in a graph, allowing weak connections to contribute to the characterization of system interactions.

**Participation coefficient.** A node’s participation coefficient measures to what extent a node interacts with nodes in other systems, in relation to the total number of connections it possesses (total degree). Participation coefficient results presented here and in the main manuscripts were calculated from each subject’s z-matrix, where negatives and the diagonal are coded as 0. Participation coefficients for each node were calculated according to the following equation (76, 77):

$$p_i = 1 - \sum_{k \in \text{nodes}} \left( \frac{k^{\text{in}}(m)}{k^{\text{in}}(m)} \right)^2,$$

where $k^{\text{in}}(m)$ is the weighted connections of node $i$ with nodes in system $m$ (a system to which node $i$ does not belong) and $k^{\text{in}}(m)$ is the total weighted connections node $i$ exhibits. Higher participation coefficient values indicate proportionally greater communication with nodes in other systems.
The tasks that contributed to each factor are detailed in SI Appendix, Table S3. Factor scores were produced by using the regression method of Thomson (78).

ACKNOWLEDGMENTS. This work was supported by NIH Grant 5R37AG006265-30 (to D.C.P.), a McDonnell Foundation Collaborative Action Award (to S.E.P.), and a research support fund from The University of Texas at Dallas (to G.S.W.).
SUPPORTING INFORMATION (SI) APPENDIX

DECREASED SEGREGATION OF BRAIN SYSTEMS ACROSS THE HEALTHY ADULT LIFESPAN

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3Department of Neurology, Washington University School of Medicine, St. Louis, MO, 63110, USA

SI APPENDIX

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   1.2. Age-accompanied differences in brain connectivity reflect a decreased segregation of functional brain systems: choice of node-set and system labels (Table S1)
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SUPPLEMENTAL RESULTS

Age-accompanied differences in brain connectivity reflect a decreased segregation of functional brain systems: community labels based on age-cohorts and individuals

Any conclusion related to measurements quantifying within- and between-system connectivity and their differences (or changes) with age is predicated on how the systems are initially labeled. In order to have an unbiased characterization of the patterns of within- and between-system node-to-node connectivity, the system labels were based on a previously published report that focused on healthy young adults (1).

A number of points are important to consider: (i) it is not necessary that the Power et al. system labels correspond to system labels that would emerge from community-detection analysis of RSFC of younger adults in the present data set, and (ii) since aging is associated with differences in connectivity between areas and systems, this may also lead to differences in the community divisions themselves. To account for these possibilities, community detection was performed on the correlation matrix of each of the 4 age cohorts (3-10% edge density) to define cohort-specific systems (see Supplemental Experimental Procedures), and the cohort-specific system labels were used to examine differences in segregation and participation coefficient as a function of age. Mean system segregation demonstrates a negative relationship with age across the range of densities for which community detection was examined. This observation holds true for thresholded (Fig. S1b) or unthresholded z-matrices ($rs > -0.49$, $ps < 0.001$). Likewise, participants’ mean participation coefficients continued to demonstrate a positive relationship with age, irrespective of choices on how system labels were defined or thresholding (Fig. S2).

An alternate concern relates to using a set of system labels derived from the mean of a group of participants: any observed differences in system segregation for one group versus another may be a consequence of greater variability in the community structure of that group. To directly account for this possibility, we conducted community detection on each participant’s own z-matrix (3-10% edge-densities) to compute community assignments for every participant. System segregation was then calculated for each participant on his or her corresponding thresholded matrices for each given edge-density. Across the range of densities examined, the negative relationship between age and system segregation remained significant (Fig. S1c; note that using unthresholded matrices to calculate system segregation yielded similar results, $rs = -0.15$ to $-0.31$, $ps = 0.032$ to $< 0.001$).
Fig. S1 – Mean system segregation decreases with age, across edge-densities and community (system) definitions. Participants’ mean system segregation is negatively correlated with age across system label definitions and edge densities. (a) For each node, system labels were based on a Power system map (1), and the z-matrices were thresholded to 3-10%. (b) For each node, system labels were based on age cohort-specific community assignments applied to thresholded z-matrices (3-10%) and (c) Participant-specific community assignments applied to thresholded z-matrices (3-10%).
Fig. S2 – Mean network participation coefficient increases with increasing age, across edge-densities and community (system) definitions. Participants’ mean participation coefficients are positively correlated with age across all depicted edge-densities (3-10%). (a) Positive age-correlation with participation coefficients that were calculated based on system labels from the consensus system labeling of a previously published map (1) on thresholded matrices (3-10%). (b) Positive age-correlation with participation coefficients that were calculated based on system labels derived from age-group specific community detection (3-10% edge-density; current DLBS sample) on thresholded matrices of corresponding edge-densities. (See Fig. S4 and Supplemental Experimental Procedures for details on DLBS cohort-defined community detection).
Age-accompanied differences in brain connectivity reflect a decreased segregation of functional brain systems: choice of node-set and system labels

In efforts to maximize the precision of anatomical registration across participants, surface-based registration procedures were employed, and all RSFC data were analyzed on the cortical surface. By using the fs_LR surface as a common reference point, we generated surface-based nodes using the most recent RSFC-based parcellation maps, which are in the same fs_LR surface space, include divisions not previously identified (e.g., V1 vs. V2), and exhibit close alignment with task-defined divisions (see ref. 2).

It is useful to that establish that our results are not dependent on the choice of node set and system definitions. Therefore, we re-analyzed the data in volume space using the 264 nodes presented in Power et al. (1), which also includes subcortical nodes. Nodes consisted of spherical ROIs with 5mm radius. Paralleling our primary analyses with surface-based nodes, for each participant, the mean time course of each ROI was extracted and cross-correlated to create a 264 × 264 nodes correlation matrix, and transformed into a z-matrix using Fisher’s z-transform. We used the consensus system labels of Power et al. (1) and examined participant’s unthresholded z-matrices. The age vs. within- and between-system connectivity relationships remained significant, as do the age vs. system segregation relationships.

Furthermore, to demonstrate that the results are also not contingent on the labeling of our 441 surface-based nodes using the Power et al. system maps, we also re-analyzed our data using fs_LR surface-based system labels available in Yeo et al. (3) (17 clusters). Here too the relationships remain, demonstrating that the results are robust to choices of node set and system labels (Table S1).

| Node Set | Power et al. (2011) 264 volumetric nodes | Wig et al. (2014) defined 441 surface nodes |
| System Labeling | Power et al. (2011) consensus systems | Yeo et al. (2011) systems (17 clusters) |
| Age correlation | | |
| Within-System Connectivity | $0.28$ | $< 0.001$ | $-0.34$ | $< 0.001$ |
| Between-System Connectivity | $0.37$ | $< 0.001$ | $0.30$ | $< 0.001$ |
| System Segregation | $-0.53$ | $< 0.001$ | $-0.57$ | $< 0.001$ |

**Table S1** – Relationships between age and within/between system connectivity and system segregation using alternate node sets (volume-based) & system labeling. All values of system-to-system correlations and system segregation were calculated using participant’s unthresholded z-matrices.
Age-accompanied differences in brain connectivity reflect a decreased segregation of functional brain systems: thresholding considerations and inclusion of negative edges

Throughout the primary report, unthresholded (positive edges only) matrices were used to compute measures of connectivity and system segregation. In the supplemental analyses, thresholded/sparsified graphs (3-10% edge-density) have been primarily used in cohort- and individually-defined community detection analyses and accompanying analyses dependent on specific community assignments (e.g., participation coefficient), as these analyses are more robust when conducted on sparse matrices (Newman 2010). To this end, we have found that graphs of greater density (e.g., >10%) contain too few plausible communities, and graphs of too low density contain too many. Consistent with this, modularity values (Q) fell below .50 approximately after 10% edge density in the majority of our analyses, and graphs of less than 3% contained too many unconnected nodes and communities consisting of very few nodes (i.e., 1-2 node/community). It is important to note however, that inclusion of weaker edges to calculate measures of segregation do not alter the nature of the conclusions in the supplemental analyses. This is consistent with our primary observations that use unthresholded matrices.

Although negative edges were removed to reduce interpretation ambiguity, it is important to consider the impact of negative edges on segregation observations. We evaluated how exclusion of negative edges may impact the segregation by age relationship in two ways. We re-calculated within-system connectivity, between system connectivity, and system segregation with participant's data where (1) both positive and negative edges are retained in each participant's z-matrix, or (2) when the absolute values of positive and negative edges were included in each participant's z-matrix. The relationship between age and within-system connectivity, between-system connectivity, and system segregation do not qualitatively differ from the original results (see Table S2).

<table>
<thead>
<tr>
<th>Age Correlation</th>
<th>Positive &amp; negative relationships retained in each participant’s z-matrix</th>
<th>Absolute values of positive &amp; negative relationships included in each participant’s z-matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )  ( p )</td>
<td>( r )  ( p )</td>
</tr>
<tr>
<td>Within-System Connectivity</td>
<td>-0.32 &lt; 0.001</td>
<td>-0.21 &lt; 0.001</td>
</tr>
<tr>
<td>Between-System Connectivity</td>
<td>0.40 &lt; 0.001</td>
<td>0.23 &lt; 0.001</td>
</tr>
<tr>
<td>System Segregation</td>
<td>-0.38 &lt; 0.001</td>
<td>-0.56 &lt; 0.001</td>
</tr>
</tbody>
</table>

Table S2 – Relationships between age and within/between system connectivity and system segregation with information from negative edges retained.
Association and sensory-motor systems exhibit different patterns of age-related differences in segregation: analysis of interaction between age-cohort and system-type segregation

To compliment and expand on the observations regarding different patterns of relationship between age and system-segregation, we employed a mixed ANOVA with age group (YA, ME, ML, OA) as a between-subject factor, and system type (sensory-motor vs. association) as the within-subject factor. As expected, the ANOVA revealed main effects of age group [$F(3,206) = 30.86$, $p < 0.001$], system type [$F(1, 206) = 242.82$, $p < 0.001$], and an age-by-system type interaction [$F(3,206) = 4.02$, $p = 0.008$]. Older age groups exhibited less segregation than younger groups, sensory-motor systems were more segregated than association systems, and the interaction indicated that the two types of systems exhibited a different pattern of age-related decline [mean sensory-motor system segregation: YA = 0.62 ($SD = 0.07$), ME = 0.60 ($SD = 0.07$), ML = 0.54 ($SD = 0.08$), OA = 0.52 ($SD = 0.07$); mean association system segregation: YA = 0.51 ($SD = 0.08$), ME = 0.52 ($SD = 0.07$), ML = 0.47 ($SD = 0.08$), OA = 0.40 ($SD = 0.09$)]. Consistent with our observations related to distinct patterns (linear/non-linear) of differences in segregation as a function of system type across age, prominent reductions in association system segregation appear in the later part of adulthood, between ME and ML [$t(208) = 2.91$, $p = 0.005$], and between the ML and OA age cohorts [$t(208) = 3.81$, $p < 0.001$], whereas the difference between ML and OA in sensory-motor system segregation was not significant [$t(208) = 0.89$, $p = 0.376$].
Segregation of association systems predicts memory function across the adult lifespan: behavioral correlates with sensory-motor and association system segregation based on a priori cognitive constructs

To compliment the data driven behavioral factor scores in the main text, we also correlated sensory-motor and association system segregation with 7 cognitive construct scores that were composed a priori using the same tasks used in the exploratory factor analysis (see Table S3). The a priori constructs were based on theoretically distinct cognitive constructs and task designs (e.g., processing speed was intended to be tested by the digit comparison and digit symbol task, and operation span was designed to detect working memory span).

Construct scores were calculated by averaging the z-scores of cognitive tasks within a construct. All constructs that contained multiple tasks, with the exception of mental control, had high reliability (Cronbach’s $\alpha > 0.78$), and mental control still had moderate reliability (Cronbach’s $\alpha = 0.49$).

Consistent with data-driven results, the episodic memory construct was significantly correlated with association system segregation ($r = 0.19$, $p = 0.007$); this relationship was stronger with association-to-association system segregation ($r = 0.22$, $p = 0.001$) than association-to-sensory-motor system segregation ($r = 0.13$, $p = 0.056$). Additionally, association-to-association system segregation also significantly correlated with the processing speed construct ($r = 0.14$, $p = 0.038$), and sensory-motor-to-sensory-motor segregation correlated with the reasoning construct ($r = 0.15$, $p = 0.027$). After correcting for the multiple tests that were performed in this analysis, the only significant relationship that remained was that between association-to-association system segregation and episodic memory scores.
<table>
<thead>
<tr>
<th><strong>Exploratory Factor Construct</strong></th>
<th><strong>A Priori Constructs</strong></th>
<th><strong>Tasks</strong></th>
<th><strong>Age Correlation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Episodic Memory</strong></td>
<td>Episodic Memory</td>
<td>Hopkins Verbal Learning Test (4)</td>
<td>-.20**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CANTAB Verbal Recognition Memory (5)</td>
<td></td>
</tr>
<tr>
<td><strong>Processing Speed</strong></td>
<td></td>
<td>Digit Comparison (6)</td>
<td></td>
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<tr>
<td><strong>Fluid Processing</strong></td>
<td></td>
<td>WAIS Digit Symbol (7)</td>
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<tr>
<td><strong>Verbal Fluency</strong></td>
<td></td>
<td>FAS (8)</td>
<td></td>
</tr>
<tr>
<td><strong>Working Memory</strong></td>
<td></td>
<td>Letter Number Sequencing</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CANTAB Spatial Working Memory</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CANTAB Spatial Recognition Memory</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CANTAB Delayed Matching to Sample</td>
<td>-.75***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Operation Span (9)</td>
<td></td>
</tr>
<tr>
<td><strong>Mental Control</strong></td>
<td></td>
<td>CANTAB Stop Signal Task</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ETS Card Rotation (10)</td>
<td></td>
</tr>
<tr>
<td><strong>Reasoning</strong></td>
<td></td>
<td>Raven’s Progressive Matrices (11)</td>
<td></td>
</tr>
<tr>
<td><strong>Verbal Ability</strong></td>
<td>Verbal Ability</td>
<td>ETS Letter Sets</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CANTAB Stocking Of Cambridge</td>
<td></td>
</tr>
<tr>
<td><strong>Verbal Ability</strong></td>
<td></td>
<td>ETS Advanced Vocabulary</td>
<td>.32***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shipley (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CANTAB Graded Naming Test</td>
<td></td>
</tr>
</tbody>
</table>

**p < 0.01, ***p < 0.001

Table S3 – Behavioral Tasks grouped by exploratory factor analysis and *a priori* constructs.
Inclusion of ‘pre-scrubbing’ movement parameters does not alter the nature of the observed relationships

Despite the particular attention paid towards removing potential movement-related artifact, it is possible that our data censoring procedure (‘scrubbing’) may not completely remove all movement-related variance. As mentioned, older adults exhibited greater head movement across resting-state scans. To further ensure that the observed segregation relationships were not related to potential differences in head movement, mean ‘pre-scrubbing’ frame-by-frame displacement (FD) values was included as a covariate for each participant in the segregation by age comparisons presented throughout the manuscript. Although a slight decrease in t-value was observed, all previously significant results remained significant with FD as a covariate in the model (see Table S4).

<table>
<thead>
<tr>
<th>Regression Models</th>
<th>Without FD</th>
<th></th>
<th>With FD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>p</td>
<td>t</td>
<td>p</td>
</tr>
<tr>
<td>System segregation vs. Age</td>
<td>-9.05</td>
<td>&lt;0.001</td>
<td>-6.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sensory-motor system segregation vs. Age</td>
<td>-8.27</td>
<td>&lt;0.001</td>
<td>-6.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Association system segregation vs. Age</td>
<td>-8.55</td>
<td>&lt;0.001</td>
<td>-6.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Memory (age-regressed) vs. Association system segregation (age-regressed)</td>
<td>2.71</td>
<td>0.007</td>
<td>2.57</td>
<td>0.011</td>
</tr>
<tr>
<td>Memory (age-regressed) vs. Association-to-Association system segregation (age-regressed)</td>
<td>3.33</td>
<td>0.001</td>
<td>3.21</td>
<td>0.002</td>
</tr>
<tr>
<td>Memory (age-regressed) vs. Association-to-Sensory-motor system segregation (age-regressed)</td>
<td>1.87</td>
<td>0.063</td>
<td>1.70</td>
<td>0.091</td>
</tr>
</tbody>
</table>

Table S4 – Comparison of regression statistics with and without mean un-scrubbed FD as an additional covariate.
Evaluation of system segregation while including additional functional systems

In the main report, we focused our primary analyses on systems that have been consistently defined and characterized across studies of task-related activity but also community detection of RSFC. This was particularly important for clear classification of system type (sensory-motor vs. association), as the identity and reliability of the remaining systems are currently uncertain (e.g., see refs 1, 3). To demonstrate that the results are not contingent on this choice, we added the several remaining communities that could possibly fall under the umbrella of association systems given their anatomical locations (i.e., Superior Temporal, Medial Temporal, Memory retrieval, & Nelson et al. (2010) ‘retrieval’ community), excluding the communities that either fell in locations of known signal artifact (grey colored systems in Power et al. system map; Fig. 1c) or communities with few voxels (white colored systems in Power et al. system map; Fig. 1c) and re-evaluated the results. As noted in Table S5, doing so does not qualitatively alter relationships between age and system segregation, and the relationships between segregation and memory.

<table>
<thead>
<tr>
<th>Age correlations</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association Segregation</td>
<td>-0.53</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Association - Association Segregation</td>
<td>-0.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Association - Sensory-motor Segregation</td>
<td>-0.54</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sensory-motor - Association Segregation</td>
<td>-0.49</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age-regressed correlations</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory vs. Association Segregation</td>
<td>0.14</td>
<td>0.042</td>
</tr>
<tr>
<td>Memory vs. Association - Association Segregation</td>
<td>0.15</td>
<td>0.029</td>
</tr>
<tr>
<td>Memory vs. Association - Sensory-motor Segregation</td>
<td>0.12</td>
<td>0.092</td>
</tr>
</tbody>
</table>

Table S5 – Age- and memory-relationships with association system segregation including additional systems. Additional association systems include: Superior Temporal, Medial Temporal, Memory retrieval, and Nelson et al. (2010) ‘retrieval’ community.
### ADDITIONAL SUPPLEMENTAL FIGURES AND TABLES

**Table S6 – System node distribution**

*NOTE:* Systems marked with asterisks are the 10 systems used in single-system analyses and association/sensory-motor system segregation.

<table>
<thead>
<tr>
<th>Functional Systems</th>
<th>Number of Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral frontal temporal</td>
<td>18</td>
</tr>
<tr>
<td>Default*</td>
<td>78</td>
</tr>
<tr>
<td>Hand somato-motor*</td>
<td>46</td>
</tr>
<tr>
<td>Visual*</td>
<td>72</td>
</tr>
<tr>
<td>Frontal-parietal control*</td>
<td>30</td>
</tr>
<tr>
<td>Ventral attention*</td>
<td>10</td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td>1</td>
</tr>
<tr>
<td>Superior temporal gyrus</td>
<td>11</td>
</tr>
<tr>
<td>Inferior temporal pole</td>
<td>14</td>
</tr>
<tr>
<td>Orbitofrontal cortex</td>
<td>10</td>
</tr>
<tr>
<td>Inferior anterior insula</td>
<td>1</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>6</td>
</tr>
<tr>
<td>Cingulo-opercular control*</td>
<td>37</td>
</tr>
<tr>
<td>Dorsal attention*</td>
<td>21</td>
</tr>
<tr>
<td>Mouth somato-motor*</td>
<td>15</td>
</tr>
<tr>
<td>Lateral temporal pole</td>
<td>13</td>
</tr>
<tr>
<td>Lateral occipital-temporal</td>
<td>1</td>
</tr>
<tr>
<td>Salience*</td>
<td>4</td>
</tr>
<tr>
<td>Unknown medial-temporal-parietal</td>
<td>9</td>
</tr>
<tr>
<td>Unknown memory retrieval</td>
<td>7</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1</td>
</tr>
<tr>
<td>Auditory*</td>
<td>20</td>
</tr>
<tr>
<td>Inferior insula</td>
<td>6</td>
</tr>
<tr>
<td>Unknown similar to Nelson et al. (2010)</td>
<td>10</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>441</strong></td>
</tr>
</tbody>
</table>
Fig. S3 – Increasing adult age is associated with decreasing segregation of RSFC brain systems
(a) Mean within-system RSFC (red) decreases significantly with increasing age in 6/10 consistently identified systems (the hand and mouth somato-motor systems, dorsal attention system and salience system∗ did not exhibit a significant age vs. within-system RSFC relationship); in contrast, between-system RSFC (blue) increases significantly in 9/10 systems (the auditory system did not exhibit a significant age vs. between-system RSFC relationship). (b) Participant’s mean system segregation for 8/10 systems decreases significantly with increasing age (the hand somato-motor system and salience system did not exhibit a significant age vs. segregation relationship), reflecting proportionally greater increasing between-system correlations relative to decreasing within-system correlations. Mean correlation values and segregation scores that were 3SD above or below the mean value were excluded from each analysis. Linear regression lines for age and within/between system correlations, and age vs. segregation are plotted in each single-system plot. See Table S7 for detailed statistical relationships.

∗Note that the salience system only contains 4 nodes, and exhibited lower within-system connectivity than other systems.
<table>
<thead>
<tr>
<th>Brain system</th>
<th><strong>Within-</strong></th>
<th></th>
<th><strong>Between-</strong></th>
<th></th>
<th><strong>System</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>System</td>
<td></td>
<td>System</td>
<td></td>
<td>Segregation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Hand somato-motor</td>
<td>-0.03</td>
<td>0.629</td>
<td>0.24</td>
<td>&lt; 0.001</td>
<td>-0.11</td>
<td>0.110</td>
</tr>
<tr>
<td>Visual</td>
<td>-0.30</td>
<td>&lt; 0.001</td>
<td>0.19</td>
<td>0.007</td>
<td>-0.36</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mouth somato-motor</td>
<td>-0.08</td>
<td>0.255</td>
<td>0.29</td>
<td>&lt; 0.001</td>
<td>-0.30</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Auditory</td>
<td>-0.24</td>
<td>&lt; 0.001</td>
<td>0.10</td>
<td>0.156</td>
<td>-0.39</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Default</td>
<td>-0.22</td>
<td>0.002</td>
<td>0.33</td>
<td>&lt; 0.001</td>
<td>-0.33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Frontal-parietal control</td>
<td>-0.14</td>
<td>0.043</td>
<td>0.36</td>
<td>&lt; 0.001</td>
<td>-0.35</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ventral attention</td>
<td>-0.22</td>
<td>0.002</td>
<td>0.25</td>
<td>&lt; 0.001</td>
<td>-0.29</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cingulo-opercular control</td>
<td>-0.22</td>
<td>0.002</td>
<td>0.25</td>
<td>&lt; 0.001</td>
<td>-0.43</td>
<td>&lt; 0.001</td>
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<tr>
<td>Dorsal attention</td>
<td>-0.08</td>
<td>0.255</td>
<td>0.29</td>
<td>&lt; 0.001</td>
<td>-0.30</td>
<td>&lt; 0.001</td>
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<tr>
<td>Salience</td>
<td>-0.07</td>
<td>0.296</td>
<td>0.22</td>
<td>0.001</td>
<td>-0.10</td>
<td>0.168</td>
</tr>
</tbody>
</table>

Table S7 – Correlation coefficients of age vs. within/between system RSFC, and age vs. system segregation for 10 consistently identified brain systems.
Community detection applied to younger adults from the DLBS sample is highly similar to community detection results from Power et al. (2011). The color plot in (a) displays the assignment of each node across edge-densities of 3-10% when the Infomap community detection algorithm (13, 14) was applied to the DLBS YA cohort (age = 20-34) mean 441 × 441 z-matrix. The leftmost column of this plot depicts each node’s community assignment based on Power et al. (1). (b) The red line indicates the normalized mutual information (NMI), a measure of similarity between two sets of assignments, between the Power et al. (1) assignments, 1st column in (a), and the DLBS YA community assignment across all edge-densities [all other columns in (a)]. The grey line indicates the NMI between the Power et al. (1) assignment and 1000 randomly rewired DLBS YA matrices for each edge-density, with the error bar reflecting the SD. It is important to note that the Power et al. (1) community assignment labels do not correspond to a specific edge-density threshold, but rather, are based off a consensus labeling across multiple thresholds. When our YA network community assignments are considered across densities in aggregate [panel (a)], the correspondence between the two sets of assignments is evident. Furthermore, the similarity between the community assignments, as compared to the randomly rewired graphs, can be attributed to unique connections within and between nodes of different systems (i.e., the brain network’s graph structure), and not simply the general connectedness of a graph.
Fig. S5 – Long-term episodic memory performance does not exhibit a significant relationship with sensory-motor system segregation. In contrast to association system segregation (main text, Fig. 4), (a) participant’s mean sensory-motor system segregation does not exhibit a relationship with his or her episodic memory factor score. Age has been regressed from both variables and data points are color-coded by the participant’s age cohort. A significant relationship does not exist between (b) memory scores and the mean segregation of sensory-motor systems from other sensory-motor systems, nor between (c) memory scores and the mean segregation of sensory-motor systems from association systems. For each scatterplot, a line reflecting the linear regression between episodic memory scores and the dependent variable is depicted.
System segregation across the adult lifespan – SI Appendix

**Fig. S6** – Age exhibits a significant relationship with multiple measures sensitive to between-system correlations. (a) Modularity (Q), which represents the ease with which subgraphs of a network can be detected (see Supplemental Experimental Procedures for details), decreases with increasing age across edge-density thresholds. This analytic feature may be a consequence of the decreased system segregation observed in older age. (b) Measures of system segregation, participation coefficient and Q are all theoretically related and each exhibits a significant relationship with age independent of edge-density (refer to Fig. S2 for details on participation coefficient and age). Consistent with this, the three measures are all strongly related with one-another across 3-10% edge-densities: system segregation (calculated based on Power et al. (2011) system labels, on 3-10% sparse matrices) and Q are highly positively related (all rs > 0.45, all ps < 0.001), and both system segregation and Q exhibit strongly negative relationships with participation coefficient (all rs < -0.26, all ps < 0.001). To determine if segregation uniquely predicts age independent of Q and participation coefficient, a semi-partial correlation between age and segregation was performed (participation coefficient and system-segregation based on Power et al. (2011) systems and sparse z-matrices 3-10%). After accounting for mean participation coefficient and Q in each participant’s segregation score, the significant relationship between age and segregation persists. The same effect does not occur between age and participation coefficient nor between age and Q when the other two network measures are regressed out (all rs = -0.09 to 0.05, all ps = 0.210 to 0.748). This observation demonstrates that system segregation provides unique information about brain organization in predicting age, and reinforces its selection as the measure examined throughout the present report.
Fig. S7 – The relationship between ‘global efficiency’ and age, before and after controlling for participant’s mean system segregation. (Figure legend on subsequent page)
Fig. S7 – The relationship between ‘global efficiency’ and age, before and after controlling for participant’s mean system segregation. (a) A significant positive relationship between mean global efficiency (GE; see Supplemental Experimental Procedures for details) and age is observed at 7/8 edge-densities. After controlling for mean system segregation, GE no longer exhibits a significant relationship with age, suggesting that system segregation mediates the relationship between age and average minimum path length (see Discussion in the main text). The age correlation between global efficiency and segregation-regressed global efficiency are significantly different across all edge-densities, \( z \approx 7.77, p < 0.001^\dagger \). (b) Scatterplots of age and GE and (c) age and segregation-regressed GE.

\^All comparisons between r values were calculated according to Steiger’s method, which accounts for correlation between all three variables (jk, jh, and kh) (15).
<table>
<thead>
<tr>
<th>System Segregation</th>
<th>Fluid Processing (age-regressed)</th>
<th>Episodic Memory (age-regressed)</th>
<th>Verbal Ability (age-regressed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$</td>
<td>$r$</td>
</tr>
<tr>
<td>Sensory-motor</td>
<td>0.07</td>
<td>0.329</td>
<td>-0.09</td>
</tr>
<tr>
<td>Sensory-motor-Sensory-motor</td>
<td>0.13</td>
<td>0.057</td>
<td>-0.06</td>
</tr>
<tr>
<td>Sensory-motor-Association</td>
<td>0.01</td>
<td>0.847</td>
<td>-0.08</td>
</tr>
<tr>
<td>Association</td>
<td>0.01</td>
<td>0.939</td>
<td>0.18</td>
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<tr>
<td>Association-Association</td>
<td>0.00</td>
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<td>0.22</td>
</tr>
<tr>
<td>Association-Sensory-motor</td>
<td>0.01</td>
<td>0.865</td>
<td>0.13</td>
</tr>
</tbody>
</table>

** $p < 0.01$

Table S8 – Sensory-motor & association system segregation correlation with age-regressed behavioral factor scores.
SUPPLEMENTAL DISCUSSION

Methodological choices & current limitations in node definition
It is important to consider and evaluate some of the present choices that were made to build brain network graphs and relate them to aging and behavior. Given the potential anatomical and artifact-related functional data variability that may be present in an adult lifespan sample, particular attention was paid towards ensuring anatomical alignment of participants’ cortical surfaces and minimizing movement-related artifacts in the resting-state signal, respectively. Two authors of the present report (GSW and SEP) have previously stressed the importance of examining brain network nodes that represent biologically meaningful entities (in the present case, brain areas; 16). By building brain networks from an area parcellation method that identifies locations where patterns of RSFC exhibit a high probability of transitioning (2, 17), we have attempted to create brain networks that have neurobiological plausibility and view the present effort as a step towards valid characterization of aging brain networks. We believe subsequent work will continue to refine and improve areal parcellation maps which will certainly improve the accuracy of resultant networks that are constructed (for discussion and caveats of the parcellation method, see Wig et al. (2)). For example, although we have used a single parcellation to represent area centers for all participants in our adult-lifespan sample, it is possible that area parcellation differs in older cohorts. However, preliminary work suggests this may not be the case (18).

Cognitive factor scores
The lack of relation between association system segregation and our ‘fluid processing’ factor scores may be because this factor contained tasks spanning multiple cognitive domains (e.g., processing speed, visual-spatial processing, etc.). In support of this, Cronbach’s alpha (which reflects the internal consistency among the tasks (dependent variables) within a construct) of each factor construct (i.e., episodic memory, fluid processing, and verbal ability) was calculated based on the z-score of original task’s score, and the alpha of fluid processing is 0.72, in comparison to 0.82 and 0.81 for episodic memory and verbal ability. Theoretically, tasks such as the digit comparison task (6) and Raven’s Progressive Matrices (11) belong to distinct cognitive domains of processing speed and reasoning, respectively. However, the exploratory factor analysis employed here combined these tasks together, possibly due to shared age variance. In line with this, results from a priori constructs shown in Supplemental Results showed that processing speed is also significantly correlated with association-to-association segregation. It will be important for subsequent work to explore this possibility further. To this regard, it will also be important to determine whether specific tasks or cognitive domains relate to segregation of specific systems or collections of systems beyond our initial description.

Cross-sectional versus longitudinal data
We recognize that a caveat to the present report is that our speculations are based on cross-sectional rather than longitudinal data. The evidence suggests that each increasing decade of recruited participants in DLBS is increasingly elite, due to health factors and medication usage playing a significant screening role in admitting a participant into an fMRI study, as does diagnosis of alcoholism, major depression, concussion, or neurological disease diagnosis. As one’s life progresses, more of these exclusion factors are likely to occur, resulting in increasing sample selectivity confounded with age. We recognize that by exploring changes in an individual’s system segregation with age would be beneficial to understanding the role of neural system organization in relation to changes in cognitive function; therefore, we are avidly pursuing longitudinal data collection of DLBS participants. However, the present data converge on the likelihood that greater change in neural segregation over time would likely be strongly predictive of age-relate decline in cognitive abilities.
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Participation exclusion criteria
All participants were native English-speaking and right-handed with no self-reported history of neurological or psychiatric disorders. Participants with Mini-Mental State Examination (MMSE) scores below 26, a history of chemotherapy in the past five years, coronary bypass, major substance abuse, disorders of the immune system, loss of consciousness for more than 10 minutes, or any MRI safety contraindications were excluded in the recruitment phase. All participants had normal or corrected-to-normal visual acuity of 20/30 or better on a Snellen eye chart.

Following a rigorous procedure for participant and data screening that removed data points that may be contaminated by movement-related artifact (see RSFC preprocessing), RSFC data from a total of 210 participants (131 females; age-range = 20-89 years) met the minimum inclusion requirements.

Participants
All participants completed multiple data acquisition sessions that included two two-hour sessions of cognitive and neuropsychological testing and a session of MRI scanning. Cognitive testing was done at the Center for Vital Longevity at the University of Texas at Dallas. The MRI scanning session took place at the Advanced Imaging Research Center at the University of Texas Southwestern Medical Center, located about 1.5 miles from the Center for Vital Longevity. The scanning session consisted of a series of anatomical- and functional-MRI (fMRI) scans acquired using a Philips Achieva 3T scanner. See Imaging Data Acquisition section for image acquisition details of the anatomical T1 scan and resting-state functional scan.

Imaging data acquisition
Brain images were acquired with a Philips Achieva 3T whole-body scanner (Philips Medical Systems, Bothell, WA) and a Philips 8-channel head coil at the University of Texas Southwestern Medical Center using the Philips SENSE parallel acquisition technique. A T1-weighted sagittal magnetization-prepared rapid acquisition gradient echo (MP-RAGE) structural image was obtained (TR = 8.1 ms, TE = 3.7 ms, flip-angle = 12°, FOV = 204 x 256 mm, 160 slices with 1 x 1 x 1mm voxels). Functional imaging was performed in a single run using a blood oxygenation level-dependent (BOLD) contrast sensitive gradient echo echo-planar sequence (TR = 2000 ms, TE = 25 ms, flip angle = 80°, FOV = 220 mm, 43 interleaved axial slices per volume, 3.5/0 mm (slice-thickness/gap), in-plane resolution = 3.4 x 3.4 mm). Five volumes were acquired at the beginning of the run and discarded to allow the MR signal to reach steady-state. The functional run consisted of 154 BOLD acquisitions; during this time participants were instructed to relax while fixating on a black crosshair against a white background. The experimenter verified that participants complied with the instructions and did not fall asleep during the functional scan via verbal confirmation.

Data preprocessing

Adult-lifespan atlas constructions
Individual structural images were first registered to the Talairach template (19) following the procedure of Lancaster et al. (20) and then averaged to create a temporary group-average. The group average was then registered to the Talairach template once more to be as close to the Talairach template as possible, creating a temporary sample-averaged template. An adult-lifespan template was created over multiple iterations of averaging individual structural images. First (i), the individual native structural images were registered to the temporary sample-
averaged template, which resulted in a new affine transform matrix (12-parameter affine, 4 x 4 matrix). Second (ii), an average was created with these newly transformed structural images, creating a new group-average image. Third (iii), this new group-average image was registered to the Talairach template to reduce deviation from the Talairach template, which resulted in an affine transform matrix for the group-average image. This resulted in a set of individual affine transform matrices from the first step (registering native MP-RAGEs to the temporary sample-average template) and a group affine transform matrix. Fourth (iv), we multiplied each of the individual affine matrices with the group affine matrices to create a new individual affine matrix, which was then used to transform the individuals’ MP-RAGEs, resulting in an updated transformed MP-RAGE for each individual. Steps 2 through 4 were repeated until the sample-average template achieved convergence in all transforms (up to four decimal spaces).

**Standard fMRI preprocessing**

Functional images were first processed to reduce artifacts (21). These steps included: (i) correction of odd vs. even slice intensity differences attributable to interleaved acquisition without gaps, (ii) correction for head movement within and across runs and (iii) across-run intensity normalization to a whole brain mode value of 1000. Atlas transformation of the functional data was computed for each individual using the adult-lifespan sample atlas registered MP-RAGE scan. Each run was then re-sampled to an isotropic 3-mm atlas space, combining movement correction and atlas transformation in a single cubic spline interpolation (20, 22). This single interpolation procedure eliminates blurring that would be introduced by multiple interpolations. All subsequent operations were performed on the atlas-transformed volumetric time series.

**Micro-movement correction: “Scrubbing”**

Temporal masks were created to flag motion-contaminated frames so that they could be ignored during subsequent nuisance regression and correlation calculations. Motion-contaminated volumes were identified by frame-by-frame displacement (FD, calculated as the sum of absolute values of the differentials of the 3 translational motion parameters and 3 rotational motion parameters). Volumes with FD > 0.3mm were flagged. In addition, the frames acquired immediately prior and immediately after each of these frames were also flagged to account for temporal spread of artifactual signal resulting from the temporal filtering in the first RSFC preprocessing iteration.

The RSFC preprocessing steps outlined above (steps i–iii; including nuisance regression, temporal filtering, and volumetric smoothing) were applied in the second iteration on RSFC data that excluded volumes flagged during motion scrubbing. Following data scrubbing, any participant with less than 75 frames of remaining data was removed from subsequent analyses (N = 56). In addition, 6 participants were also removed when it was revealed that their mean RSFC timecourse across all grey-matter voxels was more than 2 SD away from the group mean. The mean percent of frames excluded from the remaining participants (N = 210) was 16% (range: 1% – 51%). All participants had a minimum of 75 frames remaining after RSFC preprocessing (mean = 129 frames). With increasing age, adults tended to exhibit greater frame-to-frame head movement, and proportionally more frames were removed from their data. As such, all analyses in the present study were repeated using 75 randomly selected frames of data (after scrubbing) from each participant to ensure age-associated relationships were not related to systematic differences in the number of frames contributing to the analyses. No qualitative differences were observed in any of the major results reported, as a consequence of this additional constraint.
Surface preprocessing from fsaverage to the fs_LR surface
Following volumetric registration and data “scrubbing,” each participant’s MP-RAGE image was processed to generate anatomical surfaces using FreeSurfer’s default recon-all processing pipeline (version 5.0). This pipeline included brain extraction, segmentation, generation of white matter and pial surfaces, inflation of the surfaces to a sphere, and surface shape-based spherical registration of the participant’s ‘native’ surface to the fsaverage surface (23-27). The fsaverage-registered left and right hemisphere surfaces were brought into register with each other using deformation maps from a landmark-based registration of the left and right fsaverage surfaces to a hybrid left–right fsaverage surface (fs_LR; 28) and resampled to a resolution of 164,000 vertices (164k fs_LR) using Caret tools (29). Finally, each participant’s 164k fs_LR surface was down-sampled to a 32,492 vertex surface (fs_LR 32k), which allowed for analysis in a computationally tractable space while still oversampling the underlying resolution of BOLD data used in subsequent analyses. The various deformations from the ‘native’ surfaces to the fs_LR 32k surface were composed into a single deformation map allowing for one step resampling.

The above procedure results in a surface space that allows for quantitative analysis across participants as well as between hemispheres. Importantly, transforming participants RSFC data into fs_LR surface space also enabled time course extraction from nodes that were defined and generated on the same surface.

Cognitive testing
Each participant in the DLBS completed two sessions of cognitive testing. The battery of tests contains standardized tasks that are generally used for measuring processing speed (Digit Comparison & WAIS Digit Symbol; 6, 7), verbal fluency (FAS; 8), working memory (Letter Number Sequencing, Operation Span, CANTAB Spatial Working Memory, Spatial Recognition Memory and Delayed Matching to Sample; 5, 7, 9), episodic memory (Hopkins Verbal Learning Test and CANTAB Visual Recognition Memory; 4, 5), reasoning (Raven’s Progressive Matrices, ETS Letter Sets, and CANTAB Stocking of Cambridge; 5, 10, 11), crystallized abilities (ETS Advanced Vocabulary, Shipley, and CANTAB Graded Naming Test; 5, 10, 12), and mental control (CANTAB Stop Signal Task and ETS Card Rotation; 5, 10). There were 18 separate tasks, with a total of 22 dependent variables.

Parallel analysis
The present study employed a quantitative approach using parallel analysis (simulation data = 10000; 30) to determine the number of factors in the exploratory factor analysis, which is recommended over Cattell’s scree test (31) and Kaiser-Guttman rule (32) based on reduced variability and increased sensitivity to different factors (33, 34).

Graph measures
Participation coefficient
A node’s participation coefficient measures to what extent a node interacts with nodes in other systems, in relation to the total number of connections it possesses (total degree). In supplemental analyses, graph connections are first defined by thresholding the graph’s edge-density to create sparse matrices, where a graph with 10% edge-density retains only the strongest 10% of connections out of its total possible number of connections. Thresholded matrices were mainly used when cohort- or participant-defined community detection-based system labels were used, which was derived from sparse matrices (see Community Detection section below), or to supplement analyses done with unthresholded matrices (e.g., system segregation with Power et al. system labels). To obtain a given edge-density, all edges that fall
below the corresponding correlation threshold are assigned values of '0', resulting in a weighted sparse matrix. Since there is no set rule for an appropriate threshold of density, we explored results across a range of edge-densities (3-10%).

Using weighted sparse matrices (thresholded from 3-10% edge-density) participation coefficients for each node were calculated according to the following formula (35):

\[ p_i = 1 - \sum_{m \in M} \left( \frac{k_i^w(m)}{k_i^w} \right)^2 \]

where \( k_i^w(m) \) is the weighted connections of node \( i \) with nodes in system \( m \) (a system that node \( i \) does not belong to), and \( k_i^w \) is the total weighted connections node \( i \) exhibits. Higher participation coefficient values indicate proportionally greater communication with nodes in other systems. Each node’s system assignment was determined either \textit{a priori} (see Area Node Definition) or using a community detection algorithm (see below). Mean participation coefficients were calculated across all nodes at a given edge-density.

\textbf{Modularity}

The measure of mean participant coefficient is conceptually similar to system segregation in that they both in part characterize between-system relationships. Relatedly, modularity (Q) represents the ease in which modules (communities) of a network can be detected (36), and has previously been used to infer system connectedness (e.g., (37)). Modularity is defined by the following formula:

\[ Q = \frac{1}{l^w} \sum_{i,j \in N} \left[ w_{ij} - \frac{k_i^w k_j^w}{l^w} \right] \delta_{m_i, m_j} \]

where \( l^w \) is the sum of all weighted edges in a network, \( w_{ij} \) is the weighted edge between node \( i \) and \( j \), and \( k_i^w k_j^w \) are the weighted edges of node \( i \) and \( j \), \( m_i \) is the module containing node \( i \), and \( \delta_{m_i, m_j} = 1 \) if \( m_i = m_j \), and '0' otherwise.

\textbf{Global efficiency}

Global efficiency is the average inverse shortest path length, where disconnected nodes are defined as having a path of infinite length, and therefore, an efficiency of zero (38). Weighted global efficiency (GE) is calculated based on the following formula (39):

\[ E^w = \frac{1}{n} \sum_{i \in N} \frac{\sum_{l \in N, j \neq i} (d_{ij})^{-1}}{n - 1} \]

where \( d_{ij}^w \) is the shortest weighted path length, and \( n \) is the number of nodes.

GE was calculated for each participant on weighted sparse matrices (3-10% edge-densities).

\textbf{Community detection}

A community detection algorithm, Infomap (13, 14), was used to detect system assignments based on the DLBS data sample. Infomap is an algorithm that detects clusters by analyzing random walk trajectory within a graph; this method capitalizes on the fact that a random walker will statistically spend a longer period of time within clusters of highly connected nodes. Community detection was performed on weighted graphs of 3-10% edge densities (i.e., only a percentage of the edge values were retained). For additional discussion of this choice see supplemental results. Next, the mean-matrix of each of the four age cohorts was created using participants’ thresholded graphs. Each cohort’s mean matrix, for each edge-density, was submitted to Infomap community detection. The resulting community assignments served as
system assignments for each age cohort separately, as a comparison to the system assignments based on the Power et al. map (1).

**Computation and visualization**

The Connectome Workbench (V 0.83; 40) was used to compute areal centers, create geodesic disks, and visualize brain surfaces and nodes (Fig. 1). MATLAB (2013a, The MathWorks, Natick, MA) was used to create and visualize each participant’s z-matrix. Graph metrics were computed with the BCT toolbox (35). The spring-embedded graphs were visualized using SoNIA (42) and Cytoscape (43). Statistical analyses were performed in R (2.15.3; R Core Team, 2013), and the remaining figures were visualized with the R-package, ggplot2 (45).
REFERENCES


44. R Core Team (2013) R: A language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria), 2.15.3.