Neuroprotective effects of motherhood on brain function in late-life: a resting state fMRI study.

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Key words: Ageing, Neuroprotection, Parenthood, Resting State Functional Connectivity

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Abstract

The maternal brain undergoes structural and functional plasticity during pregnancy and the postpartum period. Little is known about functional plasticity outside caregiving-specific contexts, and whether changes persist across the lifespan. Structural neuroimaging studies suggest that parenthood may confer a protective effect against the ageing process, however it is unknown whether parenthood is associated with functional brain differences in late-life. We examined the relationship between resting state functional connectivity and number of children parented in 220 healthy older females (73.82±3.53years) and 252 healthy older males (73.95±3.50years). We compared patterns of resting state functional connectivity with three different models of age-related functional change to assess whether these effects may be functionally neuroprotective for the ageing human parental brain. No relationship between functional connectivity and number of children was obtained for males. For females, we found widespread decreasing functional connectivity with increasing number of children parented, with increased segregation between networks, decreased connectivity between hemispheres, and decreased connectivity between anterior and posterior regions. The patterns of functional connectivity related to the number of children an older woman has parented were in the opposite direction to those usually associated with age-related cognitive decline, suggesting that motherhood may be beneficial for brain function in late-life.

The transition to parenthood is associated with changes in brain structure and function across pregnancy and the post-partum period (Hoekzema et al., 2017; Hoekzema et al., 2020; Kim, 2016; Kim et al., 2010; Kim et al., 2014; Luders et al., 2018; Luders et al., 2020). Whilst the hormones of pregnancy return to pre-pregnancy levels within the postpartum period, the environmental changes and challenges of parenthood last a lifetime: as children grow and develop, new challenges continue to arise. Increased environmental complexity is compounded with additional children, requiring simultaneous care across different stages of dependency. However, the lasting impact of parenthood on the human brain is poorly understood.

It is not yet clear how long parenthood-related brain changes are sustained after childbirth (parturition) in humans, but evidence from preclinical models suggest that the changes may be permanent (Kinsley, Franssen, & Meyer, 2011). Strikingly, preclinical evidence suggests motherhood may convey a protective effect against dementia, with older mother rats showing reduced hippocampal amyloid deposits and attenuated memory decline, compared to older virgin rats (Gatewood et al., 2005). Thus, reproductive experience may benefit rodents into senescence, with hormonal and environmental changes that interact to produce a parental brain that is healthier, more flexible and resistant to age-related decline (Gatewood et al., 2005; Kinsley et al., 1999; Macbeth & Luine, 2010). Determining whether these neuroprotective effects are evident in humans will contribute to our understanding of the potentially protective advantages of parenthood on brain structure, function, and cognition in late-life.

Recent evidence has shown that reproductive experience may result in lifelong changes to the structure of the human brain. In middle-aged women in the UK Biobank, 'brain-age' – the difference between chronological age and estimated age based on structural magnetic resonance imaging (MRI; Franke & Gaser, 2019) - is associated with the number of children a woman has parented (de Lange et al., 2019). That is, mothers showed "younger-looking" brains with increasing number of children, suggesting a neuroprotective effect of motherhood on *brain-age*. This evidence of structural neuroprotection of parenthood has also been found in males (Ning et al., 2020). In addition, middle-aged parents in the UK Biobank have faster response times and fewer errors on visual memory tasks than childless men and women (Ning et al., 2020). These results suggest that the environmental

experience of parenthood is related to "younger-looking" brains, and improved cognitive performance, for both sexes. We have recently reported a relationship between cortical thickness and parenthood in healthy older adults (Orchard et al., 2019) in the Australian ASPREE sample (S. A. Ward et al., 2017). In both sexes, cortical thickness differed between parents and non-parents. Compared to women without children, mothers to one child had thinner grey matter in the dorsolateral prefrontal cortex. Compared to men without children, fathers to one child had thinner grey matter in the anterior cingulate cortex and thicker grey matter in the temporal pole. In addition, for mothers, number of children was associated with increased cortical thickness in the parahippocampal gyrus and improved verbal memory performance, suggesting that motherhood confers a cognitive benefit into late-life. Taken together, the results of de Lange et al. (2019), Ning et al. (2020), and Orchard et al. (2019) suggest a life-long effect of parenthood on the human brain, which, at least in mothers, may be neuroprotective in ageing.

There have been many studies investigating functional brain changes in relation to parenthood. The majority of these studies have used functional MRI (fMRI) to examine parents' brain response to infant pictures, movies, or sounds of infant cries (see Swain, Lorberbaum, Kose, & Strathearn, 2007 for review). Mothers show increased activation of brain networks including the reward/ motivation network, salience and fear networks, and theory of mind networks in response to images and videos of their own infant, compared to unfamiliar infant stimuli (Cardenas, Kujawa, & Humphreys, 2019; Leibenluft, Gobbini, Harrison, & Haxby, 2004; Lorberbaum et al., 2002; Naaz, Knight, & Depue, 2019). These studies have predominantly examined first-time parents, usually mothers, in early parenthood (first few years post-partum). Little is known about the cumulative hormonal and environmental impact of having multiple children, or parental brain function outside the context of responses to infant stimuli.

Resting state functional connectivity indexes the intrinsic connectivity of the brain when it is not engaged in a specific task. As an index of the 'baseline' connectivity of the brain, any change in resting-state functional connectivity in parenthood would suggest that parenting is associated with global, generalised changes, beyond responses to infant stimuli. To date, two studies have investigated resting-state functional connectivity in relation to parenthood. Using the amygdala as a seed, Dufford

Erhart, and Kim (2019) found increasing functional connectivity in mothers later in the postpartum period in 47 first-time mothers, 0 to 10 months postpartum. Specifically, mothers who were later into the postpartum period had increased connectivity between the left amygdala and the anterior cingulate cortex, nucleus accumbens, caudate and cerebellum, and between the right amygdala and the striatum. The connectivity between the left amygdala and nucleus accumbens was positively associated with positive maternal behaviour, i.e. the mothers' ability to scaffold appropriate interactions with her child. These findings suggest that connectivity between regions of reward/motivation and fear/salience may contribute to positive caregiving behaviours, and that the experiences of caregiving may influence this functional adaptation. Zheng et al. (2020) found reduced resting state functional connectivity within the default mode network in mothers at 3months postpartum, compared to non-mothers. Specifically, functional connectivity between the posterior cingulate cortex and the left medial prefrontal cortex was weaker in mothers than non-mothers. The default mode network is involved in outward, preventative aspects of self-relevant thought, including duties and responsibilities to others (Johnson et al., 2006), as well as theory of mind abilities (Mars et al., 2012). Altered default mode network connectivity may reflect the demands of early motherhood, as mothers would be expected to have a stronger focus on infant-related responsibilities, and be reflective on the thoughts, feelings and needs of their children during the resting state (Chase, Moses-Kolko, Zevallos, Wisner, & Phillips, 2014).

In the current study, we aimed to test the hypothesis that parenthood conveys resilience against age-related brain changes in humans. Healthy ageing is associated with widespread changes in functional brain activity. Task-related brain activity of healthy older adults is associated with reduced hemispheric lateralisation (hemispheric asymmetry reductions in older adults; HAROLD effect; Cabeza, 2002) and a posterior-to-anterior shift in fMRI activity (PASA effect; Davis, Dennis, Daselaar, Fleck, & Cabeza, 2008) compared to younger adults. HAROLD and PASA effects reflect compensatory recruitment of additional regions to support behavioural and cognitive function. In resting-state fMRI, older adults show lower within-network connectivity (reduced network integration), and higher between-network connectivity (decreased network segregation) compared to younger adults (Chan, Park, Savalia, Petersen, & Wig, 2014; Jamadar, 2019; Wig, 2017).

In the current study, we examined associations between resting-state functional connectivity and parenthood in healthy older adults. To test the hypothesis that parenthood conveys a neuroprotective effect against age-related neural changes, we correlated whole-brain functional connectivity matrices of mothers and fathers with the number of children parented. We compared the obtained matrices to three models of age-related neural change: HAROLD, PASA, and network integration/segregation (Figure 1). Since the HAROLD and PASA models are most commonly studied using task-related fMRI, we operationalised these patterns based on connectivity matrices sorted by anatomy (i.e., sorted by hemisphere, then by lobe). We defined a HAROLD effect as increased functional connectivity between lateral hemispheres, evident in the connectivity matrix as stronger connections edges between nodes in the left and right hemispheres (Figure 1a). We defined the PASA effect as increased connectivity between anterior and posterior brain regions, evident as stronger connections between frontal and posterior brain regions in the connectivity matrix (Figure 1b). Lastly, the network integration/segregation pattern is defined as changes in connectivity patterns within, and between, known resting-state networks. Therefore to test this model, we sorted brain regions into canonical networks as classified by Yeo et al. (2011). The network integration/segregation pattern is evident as lower connectivity along the matrix diagonal (i.e., within-network connectivity) and higher connectivity off-diagonal (i.e., between-network connectivity Figure 1c).

Importantly, because we hypothesise that parenthood confers resilience to the ageing process, we hypothesised that as the number of children parented increases, older adults would show *reduced* HAROLD, PASA and network integration/segregation effects. In other words, that as number of children parented increases, older adults will show decreased connectivity between hemispheres (HAROLD), between frontal and posterior regions (PASA), and between networks (integration/segregation). Such a pattern of results would be consistent with the argument that older parents have 'younger-looking' brains (de Lange et al., 2019; Ning et al., 2020).

Methods

This data was acquired by the ASPREE Investigator Group, under the ASPREE-Neuro sub-study (S. A. Ward et al., 2017). Data are available on application from the ASPREE International

Investigator Group (https://aspree.org). All methods for the ASPREE-Neuro clinical sub-trial were approved by the Monash University Human Research Ethics Committee, in accordance with the Australian National Statement on Ethical Conduct in Human Research (2007). This data was used to explore effects of parenthood on cortical thickness in late adulthood in our previous manuscript (Orchard et al., 2019).

Participants

Baseline characteristics of the full ASPREE sample are reported in McNeil et al. (2017). We included data from baseline, prior to study medication (aspirin). Healthy older adults in Australia were eligible to participate if aged 70-years and over, had no history of occlusive vascular disease, atrial fibrillation, cognitive impairment or disability; were not taking antithrombotic therapy, and did not have anaemia or high risk of major bleeding, or a diagnosis likely to cause death within five years. At study entry, each participant had a Modified Mini Mental Status Examination (3MS; Teng & Chui, 1987) score of at least 78/100, and were able to perform all six of the Katz activities of daily living (ADLS; Katz & Akpom, 1976).

MRI data from the ASPREE-Neuro sub-trial, *N*=472 participants aged 70-87 years (mean age [+/-stdev] = 73.9+/-3.5years) were used, comprising n=252 males (73.9 ±3.5 years), and n=220 females (73.8 ±3.5 years). As part of a health outcomes questionnaire, ASPREE-Neuro participants were asked "How many children do you have?". More detailed parenthood data (e.g. biological/adopted children, grandparenthood, primary/secondary caregiver, absence from children, age at first/last pregnancy etc.) is not available. The number of children parented by males and females in our sample is shown in Table 1.

Image Acquisition and Preparation

All MRI scans were obtained on a 3T Siemens Skyra MR scanner (Erlangen, Germany) with a 20-channel head and neck coil at Monash Biomedical Imaging, Melbourne, Australia. Here, we used T1 and resting state functional magnetic resonance imaging (rsfMRI) data. T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) images were acquired (TR=2300ms, TE=2.13ms, TI=900ms,

matrix size=256x240x192, bandwidth=230 Hz/pixel) and used for anatomical reference. Resting state data were obtained using multiband echo planar imaging (EPI) sequence (TR=754ms; multiband acceleration factor = 3; matrix size = 64x64; number of slices = 42; TE=21ms; number of time points=400; bandwidth=1474 Hz/pixel; 3-mm isotropic voxel (Xu et al., 2018)). Prior to functional data acquisition, a gradient echo-based field map was acquired to correct rsfMRI scan for geometric distortions. Additional sequences were acquired but not used in the present study; full details in Ward et al. (S. A. Ward et al., 2017).

T1- and T2-weighted MR images were segmented using the 'recon-all' function of FreeSurfer v5.3.0 (Dale, Fischl, & Sereno, 1999). Cortical thickness values were extracted for 41 regions for each hemisphere using the Desikan-Killiany Tourville atlas, producing 82 cortical thickness values in total (Desikan et al., 2006).

fMR images were corrected for geometry distortions (FUGUE) and the brain extracted (BET) (Smith, 2002). Intra-scan movements were corrected using 3dvolreg; both high frequencies (above 0.1Hz) and the temporal mean (and first- and second-order polynomials) were removed from each voxel's time series using 3dTproject (AFNI; Cox, 1996). Filtered images were entered into a first-level independent component analysis with automatic estimation of the number of components using MELODIC (Beckmann, DeLuca, Devlin, & Smith, 2005). All the extracted ICA maps were then automatically labelled by FSL-FIX (Salimi-Khorshidi et al., 2014), which was previously manually trained on 25 random subjects. Temporal trends from noise-labelled ICA components were linearly regressed out of the 4D MR images using ordinary least squares (OLS) regression as implemented in FSL FIX. The cleaned images were then normalized to the MNI template (2mm isotropic resolution); the first volume of the EPI time series was registered to the T1-weighted image using linear registration (with 6 degrees of freedom). Each T1 was then non-linearly registered to the MNI template using the symmetric normalization algorithm in ANTs (Avants, Epstein, Grossman, & Gee, 2008). The brain was parcellated into 82 regions using the Desikan-Killiany-Tourville atlas (Desikan et al., 2006; Fischl et al., 2002) and then applied to the EPI. Finally, the normalized and cleaned file was smoothed with a 5mm FWHM Gaussian kernel.

Data Analysis

Functional connectivity

fMRI timeseries were extracted from each of the 82 Desikan-Killiany-Tourville regions for each individual. An individual functional connectivity matrix was calculated as an 82x82 correlation matrix, formed by calculating temporal correlations (Pearson) between each pair of regions. A single entry in the correlation matrix is referred to as an 'edge' in the connectivity matrix; each region is referred to as a 'node'.

Effects of parenthood on functional connectivity

As the number of children parented is discrete, ordinal data, with a non-normal distribution, all analyses used were non-parametric. Mass univariate testing using a two-step procedure was performed to compute Spearman's correlation between the number of children parented and functional connectivity at each edge. In the first step, we used a GLM to adjust functional connectivity values for the effects of education, haemoglobin (P. G. Ward et al., 2019), and cortical thickness (Damoiseaux, 2017) on parenthood-connectivity relationships (Equation 1). In the second step, we computed a Spearman's correlation coefficient between the residualised functional connectivity and number of children.

For statistical inference and family-wise error correction, the Network Based Statistic (NBS) was used (Zalesky, Fornito, & Bullmore, 2010). The extent (number of edges) of any connected-components in the observed data that survived a predetermined primary component-forming threshold of $p<0.01$ were compared to a null-distribution of maximal component sizes from 10,000 permutations. At each permutation, the number of children was randomly shuffled between subjects, without replacement. Then, the same mass-univariate two-step procedure which was used to compute the correlation between functional connectivity and number of children parented in the observed data, was applied to each edge. This provided a null distribution of Spearman's Rho (ρ) values at each edge, which were then used to obtain non-parametric edge-level *p*-values for the observed data. For component-level family-wise-error correction, each permutated 82 x 82 matrix of ρ values was

thresholded at a critical ρ value equivalent to $p<0.01$. This critical value was determined by combining the ρ values from all permutations into one large null distribution and selecting the value at the 99th percentile as the critical equivalent to $p<0.01$, which in the current study was ρ= .174. The extent of the largest connected component that survived this threshold at each permutation was recorded. This resulted in an FWE-corrected null distribution of maximum component sizes. Any connected component in the observed data that survived the primary threshold and was larger than the 99th percentile (*i.e.* $p< 0.01$ FWE-corrected) was deemed statistically significant (Zalesky et al., 2010).

To examine whether the pattern of parenthood-connectivity was consistent with the three patterns of age-related functional brain changes, we created a matrix of significant edges, and ordered nodes in terms of their anatomical organisation (HAROLD and PASA) or functional networks (integration/segregation).

To quantify agreement with these models, we first defined the patterns of connectivity consistent with each model (Figure 1). We then calculated the number of observed edges consistent with each (i.e. inter-hemispheric connections, frontal lobe connections, between network connections). We then randomly permuted a random network of the same size 10,000 times and calculated the number of model-consistent edges in each permutation. Comparing the number of model-consistent edges between observed and permuted data allowed non-parametrically computation of significance, i.e., the likelihood of observing these patterns in networks with edges randomly distributed across the brain.

The HAROLD effect was defined as increased functional connectivity between lateral hemispheres, evident in the connectivity matrix as stronger connections edges between nodes in the left and right hemispheres (Figure 1a). To test the HAROLD model, the ROIs were sorted by hemisphere (left, right) then anatomical division (frontal, parietal, subcortical, temporal, occipital); and then calculated whether parenthood-related connectivity appears as increased functional connectivity between the left and right hemispheres (consistent with age-related change) or decreased functional connectivity between the left and right hemispheres (consistent with resilient ageing).

The PASA effect was defined as increased connectivity between anterior and posterior brain regions, evident as stronger connections between frontal and posterior brain regions in the connectivity

matrix (Figure 1b). To test the PASA hypothesis, the ROIs were sorted by anatomical division (by hemisphere then lobe: frontal, parietal, subcortical, temporal, occipital) and calculated whether parenthood-related connectivity appears as increased connectivity between frontal and posterior brain regions (consistent with age-related change) or decreased functional connectivity between frontal and posterior brain regions (consistent with resilient ageing).

Lastly, the network integration/segregation pattern is defined by changes in connectivity patterns between regions (nodes) of canonical resting-state neural networks To test the network integration/segregation hypothesis, the ROIs were sorted by network (visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, default mode, and subcortical; as classified by Yeo et al. (2011), and calculated whether parenthood-functional connectivity relationships showed greater within- or between-network connectivity. Two networks are 'segregated' if they have low/negative between-network functional connectivity, as their patterns of activation are not highly correlated.

Additionally, we were interested in determining which networks showed the largest effect of parenthood. In order to do this, we first had to correct for network size bias. The ROIs are not evenly distributed between networks, such that some networks (e.g. default mode network) contain a higher number of nodes than other networks. As such, there is a higher probability that significant edges would occur within networks with more nodes by chance. This bias was accounted for by normalising the count of significant edges by the network capacity, i.e., the total number of possible edges of each network.

Results

Functional connectivity

We examined the relationship between late-life brain function and number of children parented in healthy older females and males (Figure 2). Older males showed strong resting state functional connectivity within anatomical sub-divisions, particularly within the frontal, parietal and subcortical regions, both within and between hemispheres (Figure 2a). A number of long-range connections between anatomical sub-divisions were also evident, including fronto-parietal, parietal-subcortical and

frontal-subcortical regional connectivity. These long-range connections were also evident in older females (Figure 2b), but were of smaller magnitude than in males. Females showed strong connectivity within subcortical regions, and strong connectivity between subcortical regions and frontal and parietal regions. Occipital regions showed negative functional connectivity with frontal, parietal, subcortical and temporal regions, both within and between hemispheres in both males and females.

Effects of parenthood on functional connectivity

No significant connected component was found to be related to fatherhood for the men, so the following results pertain to the mothers only.

We obtained a 47-edge connected component related to the number of children a woman has mothered (1 to 6 children; $p<.01$ FWE-corrected). As a general observation, all edges in the maternal network are negatively weighted, which indicates *decreasing* functional connectivity between nodes with increasing number of children.

Hypothesis 1: Increased hemispheric asymmetry

Older mothers showed a pattern of increased hemispheric lateralisation with increasing number of children parented. Regions with high degree (number of edges; Table 2) consistent with a reduced HAROLD effect, include the bilateral frontal pole, bilateral inferior parietal lobule and bilateral posterior cingulate. Seventy percent of all observed edges (33/47) are between-hemisphere edges (left-right, or right-left connections); which is 1.4 times more edges between hemispheres than expected by chance ($p<.001$; Figure 3). This pattern of hemispheric lateralisation is in the opposite direction to that predicted by the HAROLD effect of age-related changes in fMRI activity.

Hypothesis 2: Reduced posterior to anterior shift

Older mothers showed a pattern of decreased frontal lobe integration with increasing number of children parented. Specifically, between the bilateral frontal pole, superior and middle frontal gyri and inferior frontal gyrus pars orbitalis, and posterior brain regions. Fifty eight percent of the observed edges (27/47) are between nodes in the prefrontal cortex and posterior brain areas; overall 1.7 times

more than expected by chance (p=.001; Figure 3). This pattern is in the opposite direction to that predicted by the PASA model on age-related functional brain activity.

Hypothesis 3: Increased network segregation

As the number of children increased, mothers had increased network segregation (p=.002), with the majority of the negatively-weighted edges *between* networks (45 edges; 96%) compared to *within* networks (2 edges; 4%;). Network segregation was not evenly distributed across the brain. There were nine times more edges between the fronto-parietal network and dorsal attention network, seven times more edges between the fronto-parietal network and visual network, and four to five times more edges between the default mode network and visual network in the default mode network compared to what would be expected in a randomly distributed network of the same size (Figure 4).

Discussion

Our results show a relationship between number of children parented and functional connectivity in the late-life parental brain. In mothers, there was a significant component with 47 negatively-weighted edges, indicating decreased functional connectivity with increased number of children. No significant component was found between number of children and functional connectivity in fathers, therefore the discussion focuses on motherhood and the influence of parenting multiple children on brain function in the late-life maternal brain.

Previous studies have reported neuroprotective effects of parenthood on brain structure later in life in mothers (de Lange et al., 2019; Ning et al., 2020; Orchard et al., 2019), and fathers (Ning et al., 2020). Therefore, we compared resting-state connectivity in older parents to three patterns of age-related functional activity, to assess whether the number of children a woman has parented is related to patterns of resilient brain function in late-life. Here we show patterns of functional connectivity in older mothers that show: i) increased hemispheric lateralisation, ii) decreased frontal lobe integration, and iii) increased network segregation. These patterns are in the opposite direction to that predicted by three patterns of functional brain changes in ageing, the HAROLD and PASA models, and the network integration/segregation effect. That is, as the number of children a woman has mothered increased, the

less similar her resting state brain activity is to patterns of age-related functional brain change. These results suggest that motherhood confers functional neuroprotection for the ageing maternal brain.

The first pattern of age-related functional changes that we investigated was the Hemispheric Asymmetry Reductions in Older Adults (HAROLD; Cabeza, 2002) effect, which represents a pattern of reduced functional lateralisation in older adults compared to younger adults. This age-related increase in bilaterality has been replicated many times across different tasks, and is believed to reflect functional compensation (Cabeza, 2002). In resting-state connectivity, we defined the HAROLD effect as increased functional connectivity between lateral hemispheres, which would be evident in the connectivity matrix as positively-weighted edges between nodes in the left and right hemispheres. We found a pattern of brain connectivity in the opposite direction to that predicted by the HAROLD model: mothers showed decreased connectivity between the left and right hemispheres with increasing number of children. In other words, older mothers with more children have retained hemispheric asymmetry, consistent with a resilient pattern of brain ageing, suggesting a protective effect of reproductive experience on the function of the ageing maternal brain.

The second pattern of age-related functional changes that we studied was the posterior-to-anterior shift in ageing (PASA) effect (Davis et al., 2008). In task-related fMRI, the PASA effect is represented as increased compensatory activation of the prefrontal cortex in response to task demands. Here, we defined a PASA effect in resting-state functional connectivity as increased connectivity between anterior and posterior brain regions, evident as positively-weighted edges between nodes of the frontal lobes and posterior brain regions in the connectivity matrix. Consistent with the overall pattern of results in this study, we found that the PASA effect reduced with increasing number of children a woman had parented, again suggesting that older mothers display patterns of resilient brain function in late life.

For the third pattern of age-related functional changes, we examined patterns of network integration/segregation. In healthy ageing, functional brain networks become less segregated and more integrated, apparent as weaker connectivity within networks, and greater connectivity between networks (Chan et al., 2014). Network segregation is considered beneficial for brain function, as the

networks are more functionally specialised, more easily adaptable to task demands, and more resilient (Wig, 2017). Here, we found that mothers with more children showed increased network segregation, with almost all of the negatively-weighted edges *between* networks, rather than *within* networks. This suggests that as the number of children an older woman has parented increases, the more similarly her brain functions to that of a younger person's brain. These results are consistent with a more flexible and resilient late-life maternal brain.

We found segregation between the frontoparietal network, the default mode network, the dorsal attention network, and the visual network. The frontoparietal network plays a central role in executive control and adaptive behaviour via flexible top-down modulation of other brain regions (Dixon et al., 2018). Compared to younger adults, older adults typically show a compensatory over-recruitment of the frontoparietal network during task performance (Campbell, Grady, Ng, & Hasher, 2012), and increased frontoparietal integration (and so, decreased segregation) (Chan et al., 2014; Chen et al., 2017). Here we found increased frontoparietal segregation between nodes of the frontoparietal network and nodes of the dorsal attention, default mode and visual network, again suggesting that mothers show "younger" patterns of brain activity with increasing number of children parented. Both the frontoparietal and default mode networks show increased connectivity with the visual network in older adults, compared to younger adults (Chen et al., 2017). In contrast, here we show *decreased* visual-frontoparietal and visual-default mode connectivity in mothers with more children. The frontoparietal network is flexibly co-activated with the default mode network and dorsal attention network, modulating introspection and self-referential processing (supported by the default mode network) or perceptual attention (supported by the dorsal attention network), depending on task demands (Dixon et al., 2018; Spreng, Sepulcre, Turner, Stevens, & Schacter, 2013). The task-negative default mode network and the task-positive dorsal attention network typically show anticorrelated activity in both resting-state and task-based functional connectivity, and this competitive relationship impacts behaviour and cognitive function. However anticorrelation between the default mode and dorsal attention networks is significantly reduced in older adults with and without mild cognitive impairment, compared to young adults (Esposito et al., 2018). Specifically, the anticorrelation found between the

posterior cingulate cortex of the default mode network, and the inferior parietal lobule of the dorsal attention network is significantly decreased in older adults with mild cognitive impairment, compared to older adults with healthy ageing (Esposito et al., 2018). We found decreasing functional connectivity (increased anticorrelation) between both the left posterior cingulate and right inferior parietal lobule, and between the right posterior cingulate and left inferior parietal lobule. The posterior cingulate and inferior parietal lobule are key regions in the present study, each showing a high number of connections. Taken together, the overall pattern of network segregation, and the specific networks that are segregated, are consistent with resilient patterns of brain activity in older mothers.

Results in the default mode network, specifically in the posterior cingulate, are also consistent with those previously found in the early postpartum period. Zheng et al. (2020) found reduced resting state functional connectivity within the default mode network in mothers at 3months postpartum, compared to non-mothers, using the posterior cingulate cortex as a seed. The posterior cingulate is involved in outward, preventative aspects of self-relevant thought, including duties and responsibilities to others (Johnson et al., 2006), as well as theory of mind abilities (Mars et al., 2012). The behaviours of early motherhood may be reflected by this functional adaptation, as motherhood requires a heightened focus on infant-related responsibilities, and more in tune with the thoughts, feelings and needs of their children during rest (Chase et al., 2014). Disruptions to this network are implicated in postpartum depression, with depressed mothers showing attenuated connectivity between the posterior cingulate and amygdala (Chase et al., 2014). Therefore, default mode network segregation may be a motherhood-related functional brain change that is altered in early parenthood and endures across the life span. However, the study of resting state functional connectivity in early parenthood remains sparse. Further studies are needed to characterise the resting state parental brain across the lifespan to determine the longevity of these effects.

The current functional connectivity results are in line with previous reports of structural brain changes in the ageing parental brain. In our previous study using the same sample, we found that the number of children an older woman mothered was associated with thicker cortical grey matter in the parahippocampal gyrus (Orchard et al., 2019). The parahippocampal gyrus is involved in memory

consolidation and cohesion (Köhler et al., 1998), and atrophy in the parahippocampal gyrus plays a key role in age-related memory decline (Burgmans et al., 2011). Furthermore, we found that verbal memory performance improved with increasing number of children mothered. A positive association between number of children parented and cortical thickness in a region associated with memory suggests that motherhood confers a cognitive benefit into late-life. Consistent with this argument, middle-aged women from the UK Biobank show "younger" brain-age with increasing number of children (de Lange et al., 2019; Ning et al., 2020). Taken together, the results of the current and previous studies of the effects of parenthood on the ageing brain are consistent with a neuroprotective effect of motherhood. That is, the results suggest that the effects of motherhood on the structure and function of the brain are both beneficial and long-lasting, persisting beyond the first few postpartum years. The emerging picture of neuroprotective effects of motherhood on the ageing brain is consistent with evidence from studies in rodents, where reproductive experience benefits neural, cognitive and behavioural abilities across the life-span (Gatewood et al., 2005; Kinsley et al., 1999; Macbeth & Luine, 2010).

While the current pattern of results are compatible with previous studies of the effects of parenthood on the structure of the late-life brain, the pattern of results obtained in the immediate post-partum period are less clear. Some studies show that early motherhood is associated with reduced grey matter in pregnancy (Hoekzema et al., 2017), whereas others show that the postpartum period is associated with increases in grey matter (Kim, Dufford, & Tribble, 2018; Kim et al., 2010; Luders et al., 2020). Regardless of the direction of the effect, interpreting changes in cortical thickness and its relationship to improved or impaired functional outcomes is not straightforward. In the cognitive ageing field, reduced cortical thickness is usually interpreted as a negative consequence of ageing; with thinner cortex associated with poorer functional outcomes (e.g. Fjell et al., 2006; Storsve et al., 2014). In contrast, in the parenthood field, both reduced (e.g. Hoekzema et al., 2017), and increased grey matter (e.g. Kim et al., 2018; Kim et al., 2010; Luders et al., 2020) has been interpreted as a positive outcome. By comparison, patterns of resting-state functional connectivity associated with parenthood are more easily interpretable. Our results indicate that the functional brain activity of older mothers with more children resembles "younger looking" patterns of brain activity.

We argue that late-life functional brain differences associated with parenthood are related to the environmental complexity of parenthood (Orchard et al., 2019). While the dramatic hormonal shifts of pregnancy are likely to play an initial role in maternal brain plasticity, hormone levels return to a pre-pregnancy state after birth. Many hormonal and biological changes are resolved in the first year, i.e., uterus size, lactation, etc. (Henry et al., 2016). In our sample, the hormonal exposure of pregnancy occurred three or more decades prior to testing. In contrast, the environmental complexity of parenthood endures throughout the entire parenting experience, possibly throughout the life-span of a parent, compounding with increasing number of children. This environmental complexity is consistent with the conceptualisation of caregiving as a dynamic, learning experience in humans, and as a period of plasticity (De Carli et al., 2019; Parsons et al., 2017). Motherhood may therefore contribute to cognitive reserve, by increasing cognitive and social demands.

Here, the functional brain changes associated with parenthood were only evident in mothers. This result may reflect obvious biological differences between mothers and fathers (e.g. pregnancy, birth, breast-feeding), or differences in care-giving responsibilities between the sexes. Given the age demographics of our sample, it is likely that our sample were predominantly in 'traditional' care-giving arrangements, with primary care-giving mothers and 'bread-winning' fathers (Broomhill & Sharp, 2005) and that this may have limited the cumulative impact of parenthood for the males in this cohort. Although we did not obtain a relationship between parenthood and functional connectivity in fathers, this null result should be interpreted with care, particularly as there are unknown factors (e.g. attachment, time spent in care) that are relevant for interpreting differing effects of parenthood on mothers and fathers, and as effects of fatherhood on late-life brain structure have been found in other large studies (Ning et al., 2020; Orchard et al., 2019).

The data used in this study were acquired as part of a larger study with broader aims. Detailed demographic information related to parenthood was not obtained from the participants, and so information about the parenting experiences is not available. Many of these unknown factors, such as birth and feeding methods, hormone levels, and parental sensitivity, potentially impact maternal

neuroplasticity (Kim, Strathearn, & Swain, 2016). We were also unable to control for pregnancies that did not result in live birth and/or parenthood experience (miscarriage, termination, still-birth, infant loss, or adoption). Therefore, we are not able to confirm that some of our participants didn't experience additional exposure to pregnancy hormones. Notably, all existing studies of the influence of parenthood on the late life brain are limited by the sparse information on parenting in their sample (de Lange et al., 2019; Ning et al., 2020; Orchard et al., 2019). This limitation reflects the fact that existing studies have reported the effects of parenthood on brain structure and function using large datasets acquired for other purposes (here, to examine the effects of aspirin exposure on brain structure; de Lange et al. (2019) and Ning et al. (2020) used data from the UK BioBank). Furthermore, it is important to note that reproductive experience is related to other traits which impact brain health in human ageing, including exposure to sex steroid hormones. Some of these traits are heritable (e.g. age at menarche and menopause). In the absence of these data, we cannot rule out the possibility that the observed relationships between functional connectivity and number of children parented are explained by some of these uncollected variables. Future studies should control for these factors, as well as examine the potential effects of grandparenthood and other opportunities for interaction with children during later life, with more richly-phenotyped parenting data.

Taken together, the resting state functional connectivity patterns shown in older mothers were in the opposite direction to three separate patterns of age-related functional changes, consistent with a neuroprotective effect of motherhood on the ageing human maternal brain. This finding shows patterns of resilient brain function in the ageing maternal brain across hemispheres, lobes, functional networks, and specific regions. This interpretation is in line with previous literature in humans and rodents, and paints a consistent picture of motherhood-related resilience in human ageing, and suggests functional effects in the human brain that are both life-long and beneficial.

Acknowledgements

ERO, PGDW, SDJ, & GFE are supported by the ARC Centre of Excellence for Integrative Brain Function (CE140100007). SDJ is supported by an ARC Discovery Early Career Researcher

Award (DE150100406) and National Health and Medical Research Council Fellowship (APP1174164). The authors acknowledge the assistance of Thomas Close, Linden Parkes and Francesco Sforrazini for their analytical support. The Corresponding Author is Sharna D Jamadar, 770 Blackburn Rd, Melbourne Vic, 3800, Australia.

The data in this manuscript were used under license from the ASPREE and ASPREE-Neuro studies (https://aspree.org). ASPREE-Neuro was supported by an Australian National Health and Medical Research Council Project Grant (APP1086188). ASPREE was supported by the National Institutes of Health (grant number U01AG029824); the National Health and Medical Research Council of Australia (grant numbers 334047, 1127060); Monash University (Australia) and the Victorian Cancer Agency (Australia). The Principal ASPREE study is registered with the International Standardized Randomized Controlled Trials Register, ASPirin in Reducing Events in the Elderly, Number: ISRCTN83772183 and clinicaltrials.gov number NCT01038583. ASPREE-Neuro trial is registered with Australian New Zealand Clinical Trials Registry ACTRN12613001313729.

Competing Interests

The authors declare that there are no conflicts of interest.

Author Contributions

ERO, SDJ & PGDW designed the research question; ERO, SC, PGDW conducted the analysis, ERO wrote the first draft of the paper, with supervision provided by SDJ, PGDW & GFE. ERO & SDJ obtained approval for use of ASPREE-Neuro data. ES & GFE sourced funding for this work. All authors have contributed to and approved this work.

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Tables and Figures:

Table 1*: Number of participants with 1-6 children for males and females.*

	Mothers	Fathers
1 child	19	16
2 children	81	106
3 children	71	88
4 children	36	26
5 children	8	11
6 children	5	5
Total	**220**	**252**

Table 2: *Degree (number of significant edges) for each node in the motherhood network. N.B. The motherhood network consists of 47 edges, but in calculating degree, each edge is counted twice (once for each node in the edge), so total degree is 94.*

Lobe	Region (Brodmann Areas)	L	R	**Total**
Prefrontal	Frontal pole (10)	7	2	**9**
	Superior frontal gyrus (6,8,9)	1	2	**3**
	Rostral middle frontal gyrus (46, 10)	4	2	**6**
	Caudal middle frontal gyrus (9, 46)	2	1	**3**
	Pars orbitalis (47)	1	3	**4**
	Lateral orbitofrontal gyrus (11)		1	**1**
	Caudal anterior cingulate cortex (24)		1	**1**
Parietal	Postcentral gyrus (1, 2, 3)		1	**1**
	Superior parietal lobe (7)	2	2	**4**
	Inferior parietal lobule (40)	3	5	**8**
	Posterior cingulate cortex (23)	9	2	**11**
	Isthmus cingulate (26, 29)	2	1	**3**
Subcortical	Thalamus	3		**3**
	Caudate	1	1	**2**
Temporal	Temporal pole (38)		5	**5**
	Middle temporal gyrus (21)		1	**1**
	Inferior temporal gyrus (20)		3	**3**
	Bank of the superior temporal sulcus (21)		2	**2**
	Amygdala		1	**1**
Occipital	Lateral occipital cortex (17, 18, 19)		1	**1**
	Fusiform gyrus (37)		1	**1**
	Cuneus (18)	7	4	**11**
	Lingual (19)	7	2	**9**
Total		49	45	**94**

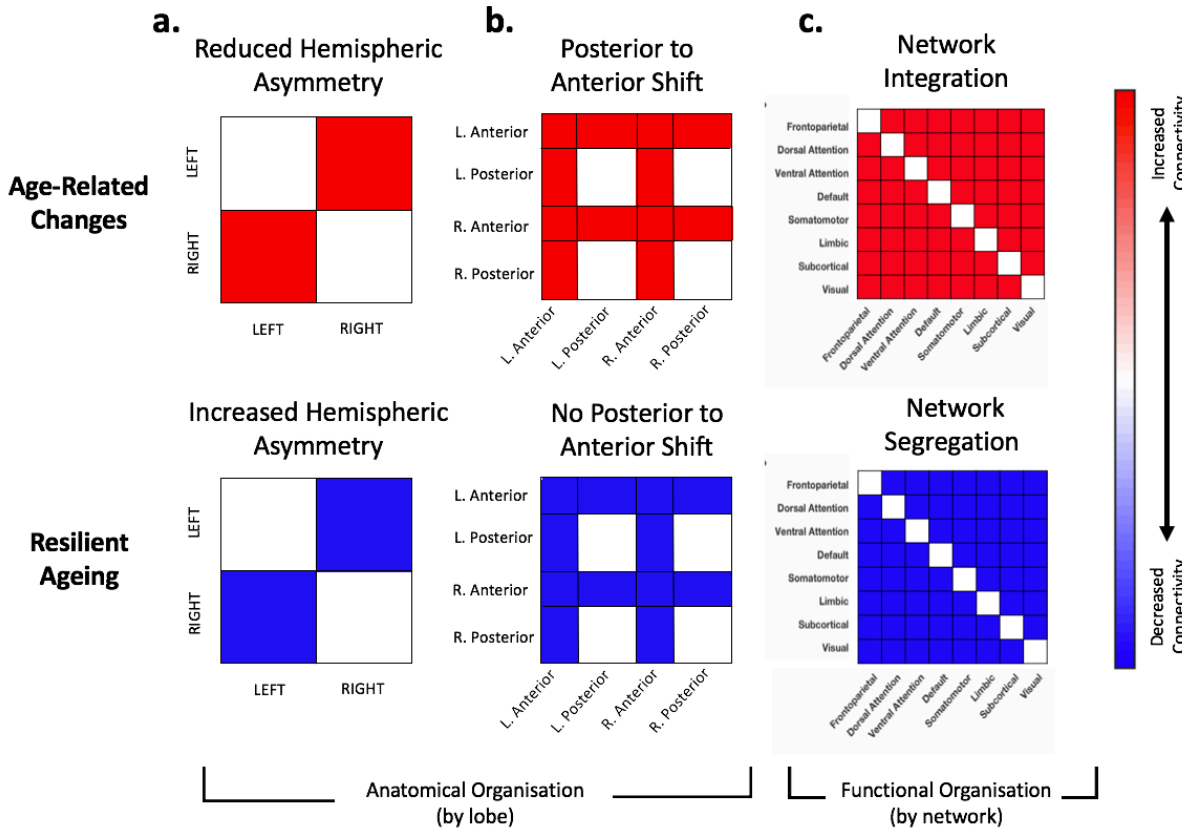

Figure 1: Cartoon schematics for three patterns of age-related functional brain changes in the late life parental brain. Red denotes increased resting state functional connectivity, and blue denotes decreased resting state functional connectivity. In order to assess hemispheric asymmetry and frontal compensation, regions are ordered according to anatomy (hemispheres and lobe assignment), whereas, assessing network integration/segregations requires a functional organisation (networks). **A. Hemispheric Asymmetry:** Cartoon schematic of the HAROLD effect (Hemispheric Asymmetry Reductions in Older Adults; (Cabeza, 2002)). Ageing is associated with reduced functional lateralisation. Therefore, results consistent with ageing in the HAROLD pattern would show *increased* functional connectivity between hemispheres (left-right/ right-left), shown in red. Decreased functional connectivity between hemispheres would be consistent with a reduced HAROLD effect (increased hemispheric asymmetry), indicative of resilient ageing, shown in blue. **B. Posterior to Anterior Shift:** Cartoon schematic of the PASA pattern (posterior-anterior shift in ageing; (Davis et al., 2008)). Ageing is associated with increased connectivity between anterior (prefrontal) regions and other areas (shown in red), consistent with a shift from posterior to anterior processing. Decreased functional connectivity between nodes of the frontal lobe and other regions would be consistent with a reduced PASA effect (less frontal compensation), indicative of resilient ageing (shown in blue). **C. Network Integration/Segregation:** Cartoon schematic of integration and segregation. Within-network connections lie on the diagonal (e.g. visual-visual), and between-network connections lie on the off-diagonal (e.g. visual-default mode). Ageing is associated with increased integration and decreased segregation of functional networks. Results consistent with age-related changes would show increased functional connectivity between networks (integration), shown in red. Decreased functional connectivity between networks (segregation) would therefore indicate resilient ageing, shown in blue.

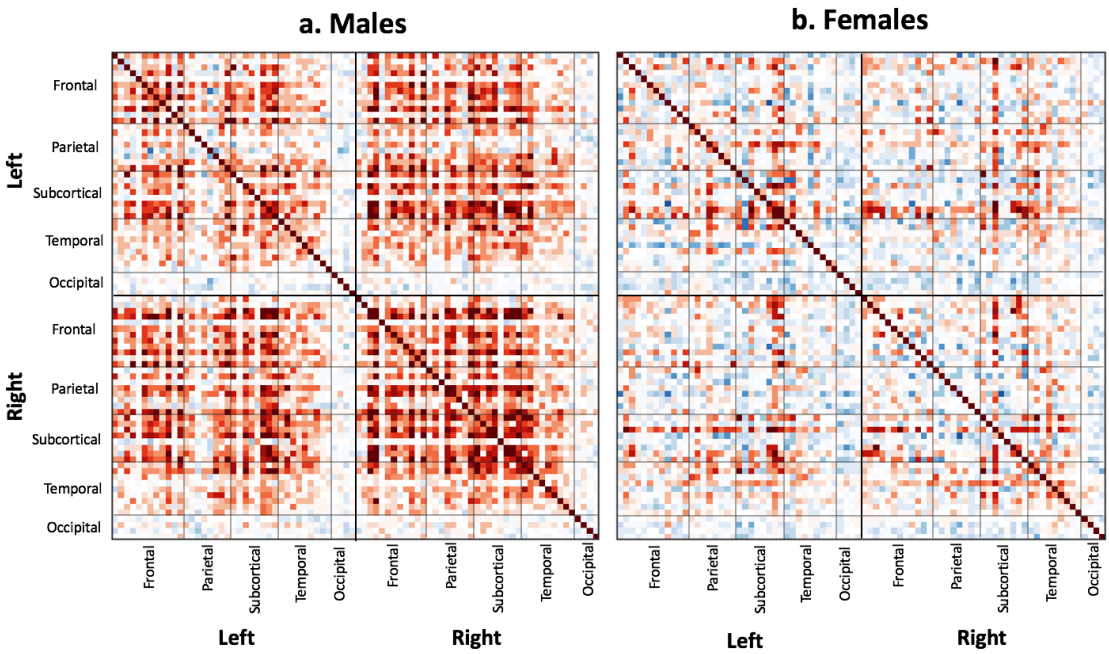

Figure 2: Mean functional connectivity for healthy older males (*N*=252) and females (*N*=220) who parented between one and six children. Each row and column of the 82x82 matrix represents a node (41 nodes in the left hemisphere and 41 in the right), organised by anatomical division (i.e., frontal, parietal, subcortical, temporal, occipital). Each cell represents a the functional connectivity between two nodes, after adjusting for age, education, haemoglobin and cortical thickness. Blue cells indicate negative edge weights and red cells indicate positive edge weights. The matrix has diagonal symmetry, such that the upper and lower triangles are mirrored. These matrices show the global functional connectivity for mothers and fathers, and do not reflect results of any significance tests.

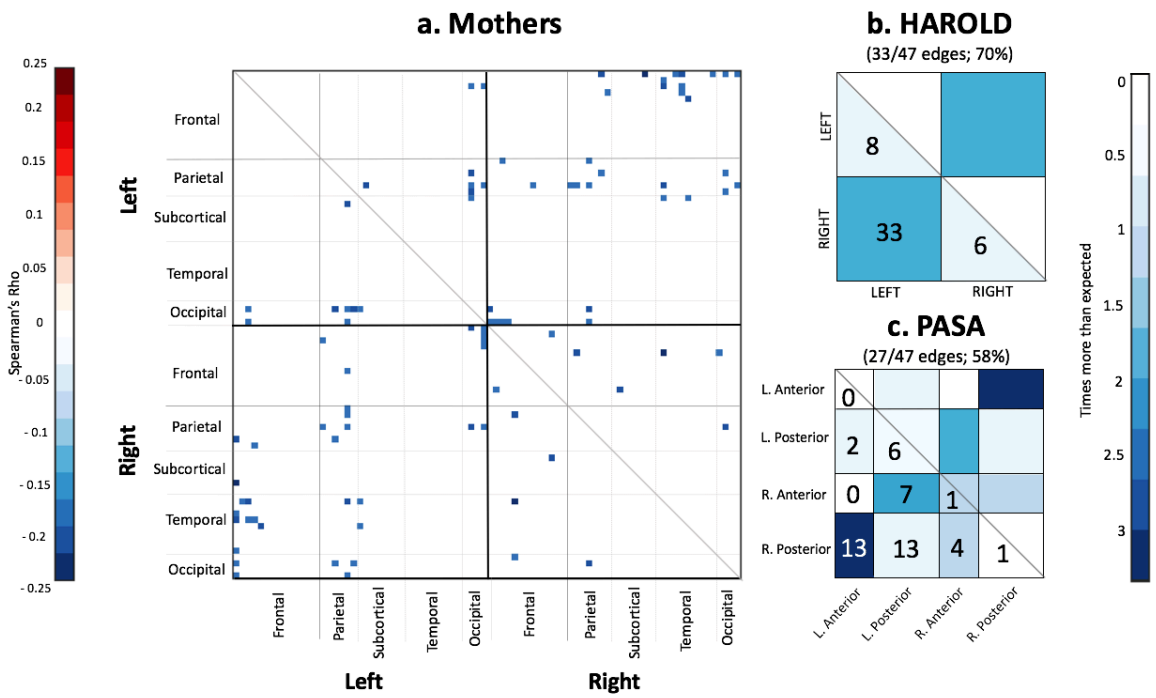

Figure 3: A. Resting State Functional Connectivity for Mothers: Adjacency matrix of pairwise correlations for the 47 edges associated with motherhood. The 82 ROIs are arranged by hemisphere then by anatomical division (frontal, parietal, subcortical, temporal, occipital) such that each row and column of the 82x82 matrix represents one node (41 nodes in the left hemisphere and 41 in the right). Each cell represents a significant edge between two nodes. The matrix has diagonal symmetry, such that the upper and lower triangles are mirrored, and each contains 47 edges. Blue cells signify negative edge weights and red cells signify positive edge weights. All edges related to motherhood are negatively weighted, showing decreasing functional connectivity between node pairs as number of children increases. **B. Schematic of results in relation to HAROLD (Hemispheric Asymmetry Reductions in Older Adults; (Cabeza, 2002)):** The blue quadrants highlight between-hemisphere connections (33 left-right and right-left edges), which is 1.4 times as many edges between hemispheres as would be expected by chance. The lighter coloured quadrants highlight within-hemisphere connections; left-left (8 edges; one third [0.34] as many edges as would be expected by chance) and right-right (6 edges; one quarter [0.25] of what would be expected by chance). Older mothers show decreased functional connectivity between hemispheres with increasing number of children. Seventy percent (33/47) of the observed edges are between-hemisphere connections. Decreasing functional connectivity between the left and right hemispheres indicates *increased* hemispheric asymmetry with increased number of children mothered. This pattern is consistent with a *reduced HAROLD effect*, indicative of resilient ageing in mothers. **C. Schematic of results in relation to PASA (Posterior to Anterior Shift in Ageing; (Davis et al., 2008)):** The blue bars highlight connections with the left and right prefrontal cortex, coloured to compare the observed edges with the number that would be expected by chance. For example, there are 3.2 times as many edges between left anterior (frontal) regions and right posterior regions than would be expected by chance. Sixty-four percent (30/47) of the observed edges show decreased functional connectivity between regions of the prefrontal cortex and other regions, indicating *less* frontal compensation with increasing number of children mothered. This pattern is consistent with a *reduced PASA effect*, indicative of resilient ageing.

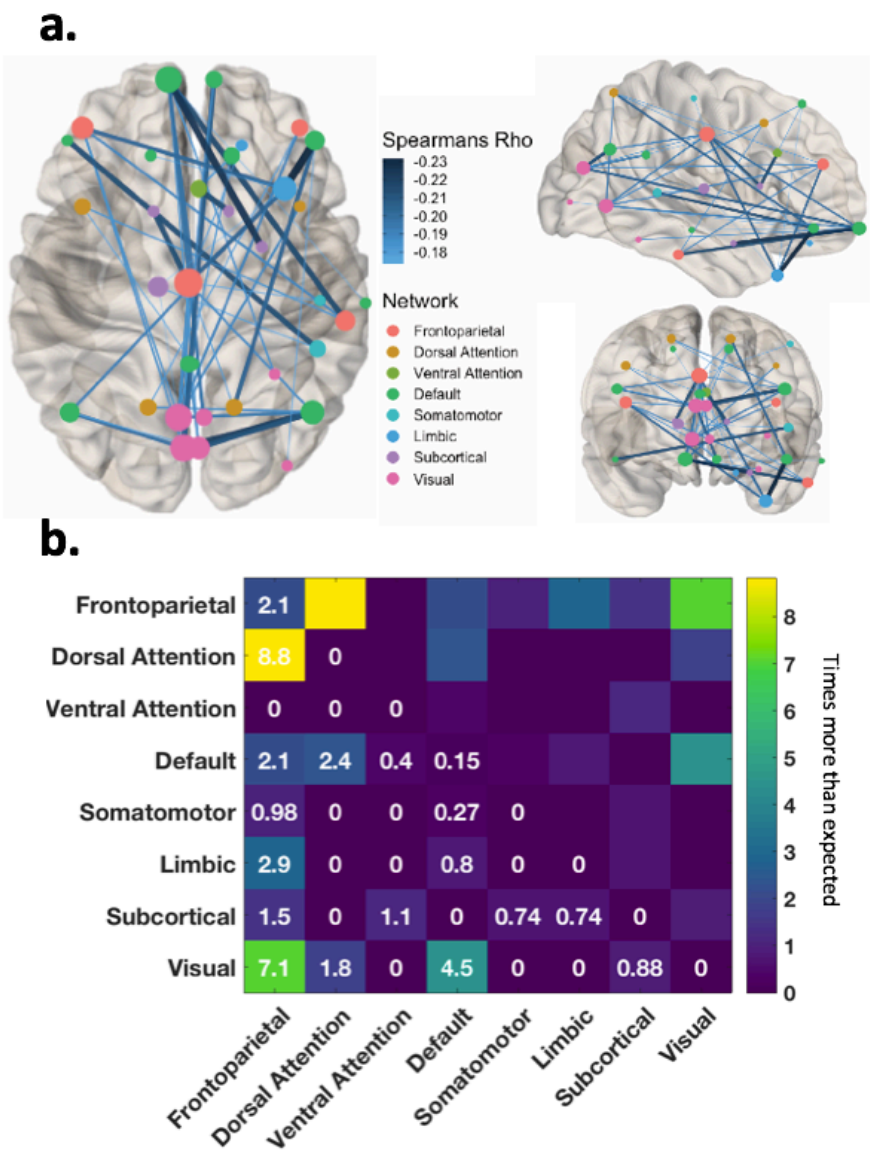

Figure 4: Functional connectivity within and between brain networks (i.e. frontoparietal, dorsal attention, ventral attention, default mode, somatomotor, limbic, subcortical and visual; as classified by Yeo et al. (Thomas Yeo et al., 2011)). **A**: MNI-space network graph of the edges associated with motherhood (left: axial; top: coronal; bottom: sagittal). The nodes are weighted by degree, such that nodes with a higher number of connections are larger. Edges are weighted by the strength of Spearman's Rho, such that edges with stronger connectivity are darker and thicker **B**: **Segregation and Integration:** Functional connectivity within and between networks. Within-network connections lie on the diagonal (e.g. visual-visual), and between-network connections lie on the off-diagonal (e.g. visual-default mode). The numbers denote how many more edges are observed between networks, compared to what we would expect by chance, after adjusting for the capacity of the network, e.g we observed 7.1 times more edges between the frontoparietal network and the visual network than we would expect by chance. All edges related to motherhood are negatively weighted, therefore decreasing connectivity between networks (off-diagonal) indicates increased network segregation with increasing number of children mothered.