

1 **Sex differences in intrinsic functional cortical organization reflect differences**
2 **in network topology rather than cortical morphometry**

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23 **ABSTRACT**

24 Brain size robustly differs between sexes. However, the consequences of this anatomical
25 dimorphism on sex differences in intrinsic brain function remain unclear. We investigated the extent
26 to which sex differences in intrinsic cortical functional organization may be explained by
27 differences in cortical morphometry, namely brain size, microstructure, and the geodesic distances
28 of connectivity profiles. For this, we computed a low dimensional representation of functional
29 cortical organization, the sensory-association axis, and identified widespread sex differences.
30 Contrary to our expectations, observed sex differences in functional organization were not
31 fundamentally associated with differences in brain size, microstructural organization, or geodesic
32 distances, despite these morphometric properties being *per se* associated with functional
33 organization and differing between sexes. Instead, functional sex differences in the sensory-
34 association axis were associated with differences in functional connectivity profiles and network
35 topology. Collectively, our findings suggest that sex differences in functional cortical organization
36 extend beyond sex differences in cortical morphometry.

37

38 **Teaser**

39 Investigating sex differences in functional cortical organization and their association to differences
40 in cortical morphometry.

41 INTRODUCTION

42 Sex differences in human brain size are robust and widely acknowledged [1-7], but the
43 downstream functional consequences of this anatomical dimorphism are not well understood.
44 Indeed, sex differences in intrinsic brain function are sometimes deemed small or negligible beyond
45 differences attributed to brain size [2]. Nevertheless, diverging patterns of functional connectivity
46 between males and females have been reported even when controlling for differences in brain size
47 and most consistently in sensory and association regions [5, 8, 9]. These regions in fact represent
48 the two anchors of a key principle of hierarchical functional organisation, the sensory-association
49 (S-A) axis, differentiating localized primary sensory/motor areas from a more distributed set of
50 transmodal association regions, including regions belonging to the frontoparietal and default mode
51 networks (DMN) [10, 11]. However, the extent to which sex differences in intrinsic functional
52 cortical organization may be explained by neuroanatomical differences relating to brain size
53 remains unclear.

54
55 Brain size and its variability may have important consequences for the spatial distribution
56 of sensory and association areas across the cortical mantle, as illustrated by clear scaling patterns
57 over evolution and development. In fact, over the past 4 million years, hominin evolution has not
58 only shown a general trend of increasing body mass, but also an even more important relative
59 increase in brain size [12]. According to the tethering hypothesis, the brain's sensory systems,
60 acting as anchors, may have constrained the growth of the developing ancestral mammalian cortex
61 [13]. In this way, evolutionary cortical expansion may have led to the emergence of the S-A axis,
62 with association cortices distributed across the cortical mantle and untethered from sensory
63 hierarchies. Patterns of expansion across cortical regions along the S-A axis are also observed
64 across human development, with a more markedly distributed areal expansion across frontoparietal
65 association regions relative to limbic and sensorimotor areas [14]. Through the increase of overall
66 brain size, the differential expansion of sensory and association areas could thus be an important
67 product of mammalian evolution and development. It is however unclear whether brain expansion
68 and associated re-organization along the S-A axis may also extend to sex differences in cortical
69 morphometry (i.e., cortical shape and size), and thus result in different functional organization of
70 sensory and association regions in males and females.

71
72 Morphometric differences between male and female brains have been extensively reported,
73 with males showing a greater absolute brain volume by 8-13% on average [6]. It must be noted that
74 within-group variance in cortical morphometry –which is typically greater in males– is larger than

75 between-group mean effects, meaning that individual differences within sex are larger than group-
76 differences between sexes [15]. Nevertheless, contrary to the belief that males may have larger
77 brains as a sole consequence of their larger bodies [2], it has been repeatedly reported that sex
78 differences in brain size cannot be fully explained by differences in body dimensions, as quantified
79 by height and/or weight [1, 4, 5, 7]. Although individual differences in total brain size seem to
80 account for most differences in relative regional volumes [3], some sex differences still remain
81 statistically significant when the variance explained by total brain size is taken into account [7].
82 Therefore, there may be sex differences in the scaling of regional brain volume that go beyond
83 linear associations with overall brain and body size. In fact, sex differences in cortical morphometry
84 are partly located at the anchors of the S-A axis [6, 16]. Developmental trajectories of anatomical
85 change also appear regionally heterogeneous, with higher rates of global cortical thickness change
86 found in fronto-temporal association regions and lower rates found in sensory regions [17].
87 Morphometric cortical properties therefore seem to not only follow patterns of variation along the
88 S-A axis, but also differ between the sexes. Yet, how exactly sex-specific differences in cortical
89 morphometry may be relevant to differences in intrinsic brain function has not been directly
90 explored.

91
92 Consistent with patterns of morphometric variation and sex differences, robust evidence
93 points to sex differences in intrinsic functional connectivity (FC) at the poles of the S-A axis [5, 8,
94 9]. In fact, despite generally controversial findings on sex differences in brain function, findings of
95 stronger FC in females within the DMN [18-20] and in males within sensorimotor areas [19, 21]
96 are consistent and robust. Overlapping morphometric and functional patterns of sex differences
97 along the S-A axis thus suggest that differentiation in functional cortical organization may be
98 somewhat orchestrated by the cortical mantle's morphometric properties. Indeed, the structure, size,
99 and shape of the cortex not only physically support functional connections, but also determine their
100 length. Short- and long-range connections, as measured by geodesic distance (the distance
101 separating two regions along the cortical mantle) have in fact been found in sensory and association
102 regions respectively [22], thus also displaying patterns of variation along the S-A axis. With
103 increasing distance between regions, cortical function also appears to change more rapidly in
104 association regions relative to sensorimotor regions [23]. These patterns further mirror patterns of
105 microstructural cortical variability identified by post-mortem histology [22] and myelin-sensitive
106 in vivo magnetic resonance imaging (MRI) [22, 23]. As such, intrinsic functional activity, showing
107 variability between the sexes and along the S-A axis, seems to be embedded within the cortical
108 mantle and its microstructural organization. Accumulating evidence further supports the important

109 role played by cortical geometric properties, including size and shape, in sculpting functional
110 architecture. Established findings from graph theory suggest that a cortical functional network's
111 properties are largely determined by its spatial embedding, namely by the length of its connections
112 [24]. Peaks of DMN clusters on the S-A axis also appear to be equidistantly distributed relative to
113 primary areas [10], in line with the hypothesised untethering of association cortices from sensory
114 hierarchies during evolutionary expansion [13]. Furthermore, recent findings suggest that the
115 spatial organization of intrinsic cortical functional activity is dominated by long wave-lengths of
116 geometric eigenmodes [25]. This research builds on notions from neural field theory positing that
117 brain shape physically constrains brain-wide functional dynamics by imposing boundaries on
118 emerging functional signals [26, 27]. In the context of sex differences in functional cortical
119 organization, brain size also explains some –although not all– sex-specific variance in FC [28].
120 Together, these findings point to possible morphometric properties that may not only underpin
121 cortical functional architecture, but also be at the root of sex differences in functional organization.
122

123 In the current work, we therefore investigated the extent to which sex differences in intrinsic
124 functional cortical organization may be explained by differences in cortical morphometry, namely
125 brain size, microstructure, and the geodesic distances of connectivity profiles. To this end, we used
126 multimodal imaging data (including resting state functional MRI and structural T1 and T2 images)
127 of the Human Connectome Project (HCP) S1200 release [29], consisting of healthy young adults.
128 We began by computing the S-A axis as our measure of functional organization, given its relevance
129 to cortical morphometry and sexual dimorphisms, and tested for sex differences along this low
130 dimensional hierarchical organizational axis. Then, we identified the cortical morphometric
131 properties potentially constraining the S-A axis, including brain size, microstructural organization
132 (a low dimensional microstructure profile covariance (MPC) axis), and the mean geodesic distance
133 of connectivity profiles. Next, we probed associations between patterns of sex differences in
134 cortical morphometry and patterns of sex differences in the S-A axis. Contrary to our expectations,
135 we did not find evidence supporting a morphometric explanation of sex differences in functional
136 organization. As such, we further probed potential functional features that may intrinsically
137 underpin sex differences on the S-A axis, and our findings suggest that differences in FC profiles
138 and network topology may be a more plausible explanation of sex differences in functional
139 organization.

RESULTS

Sex differences in the S-A axis of functional cortical organization (Figure 1)

We computed the S-A axis at the individual level as our measure of functional organization in subjects of the HCP S1200 release [29]. For this, we applied a non-linear dimensionality reduction algorithm on functional connectivity (FC) Fisher r-to-z transformed matrices. We only considered the top 10% of the row-wise z-values, representing each seed region's top 10% of maximally functionally connected regions [30, 31]. We thus found the well-replicated low dimensional axis of functional brain organization explaining the most variance in the data (21.86%) –spanning from unimodal (sensory, here particularly visual) regions to heteromodal (association) regions [10]– and defined it as the S-A axis (Fig. 1A). Then, to test for regional effects of sex on S-A axis loadings (Fig. 1B), we fitted a linear mixed effects model (LMM) including fixed effects of sex, age, and total SA, and random nested effects of family relatedness and sibling status (see Methods for more information on the nested structure of the HCP data and the statistical modelling). We identified sex differences in the S-A axis that were distributed across the seven intrinsic functional Yeo networks [32] (Fig. 1C). Positive *t*-values, depicted in blue, represent higher loadings in males relative to females on the S-A axis, whereas negative *t*-values, depicted in red, represent higher loadings in females relative to males. In Supplementary Figure S1, we also show that patterns of within-sex variability in S-A axis loadings are similar between males and females, with only a few regions showing statistically significant sex differences in variance.

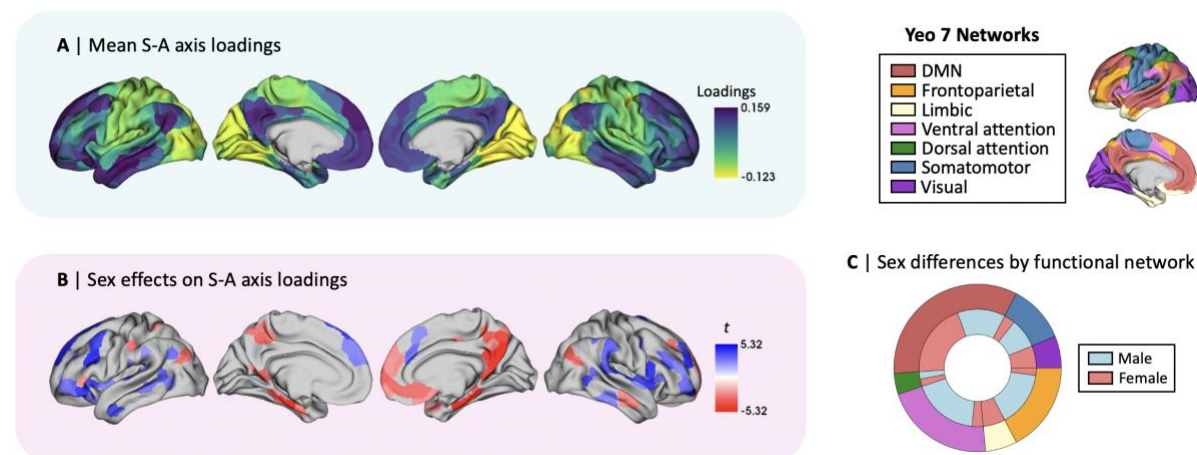


Figure 1. The sensory-association (S-A) axis of functional cortical organization and its sex differences. **A** | Mean S-A axis loadings (spanning from visual to DMN regions) across sexes; **B** | Thresholded *t*-map of linear mixed effect model (LMM) results showing false discovery rate (FDR)-corrected ($q < 0.05$) statistically significant effects of sex on the S-A axis, where blue represents higher male loadings and red represents higher female loadings; **C** | Functional network breakdown of parcels showing statistically significant sex differences in S-A axis loadings. The outer ring displays absolute proportions of statistically significant parcels by functional Yeo network, the inner ring displays absolute proportions by directionality of effects, where blue represents higher male loadings and red represents higher female loadings.

169 **Morphometric correlates of the S-A axis (Figure 2)**

170 We then investigated potential morphometric constrains of functional organization by probing
171 associations between the S-A axis and brain size, microstructural organization, and the mean
172 geodesic distance of connectivity profiles.

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174 First, we tested for associations between the S-A axis loadings and three measures of brain size
175 commonly used in the literature, namely intracranial volume (ICV), total brain volume (TBV), and
176 total surface area (SA). More specifically, ICV represents the entire volume encapsulated by the
177 cranium (i.e., including cerebrospinal fluid), TBV represents the total volume of grey and white
178 matter structures within the neocortex (excluding subcortical structures), and total SA represents
179 the entire SA of the neocortical mantle (see Methods for the exact computation of these measures).
180 Sex differences in brain size and other anthropometric measurements (height, weight, and body
181 mass index) are further reported in Supplementary Table S1. For each measure of brain size, we
182 fitted an LMM to test for regional effects of brain size on S-A axis loadings, and we found total SA
183 to have the most widespread effects amongst the three tested brain size measures (Fig. 2B; Fig. S2).

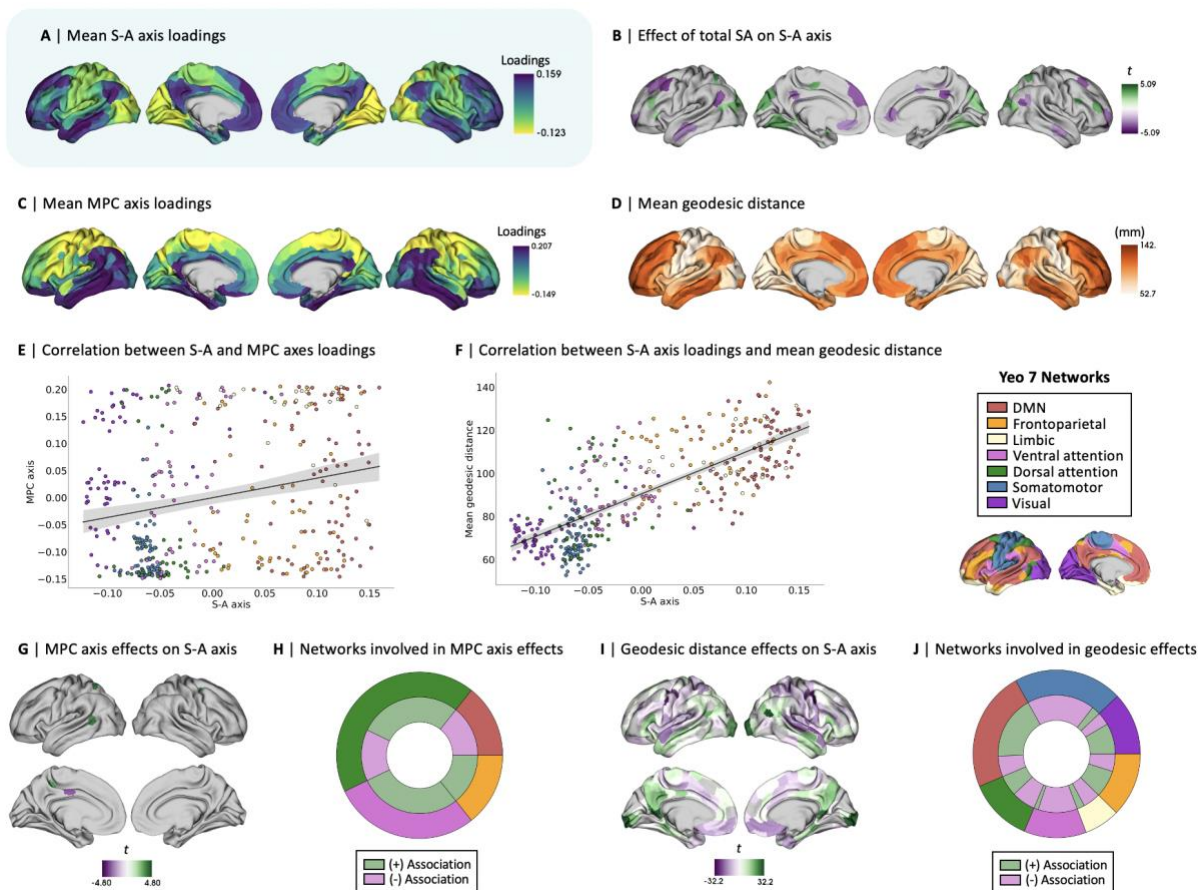
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185 Second, we computed a MPC axis of organization at the individual level, representing a low
186 dimensional representation of the similarity of T1-weighted (T1w) over T2-weighted (T2w) tissue
187 intensity across cortical regions and layers [33-35]. We computed the MPC axis by again
188 conducting non-linear dimensionality reduction on MPC matrices [30, 31], which were obtained by
189 sampling and correlating the intracortical microstructural intensity of 12 equivolumetric depth
190 profiles (see Methods). Following the same approach used for computing the S-A axis, we selected
191 the axis explaining the most variance in the data (25.97%) –spanning from sensory to paralimbic
192 regions– defining it as the MPC axis (Fig. 2C). We specifically selected this low-dimensional
193 representation of microstructural organization as it has been previously shown to covary with the
194 low-dimensional representation of functional organization (i.e., the S-A axis) [34]. To test for
195 whole-brain associations between the S-A and MPC axes, we correlated the spatial maps of the
196 axes' mean loadings (Fig. 2A and 2C) across all subjects (Fig. 2E; $r = 0.23$, $p_{spin} = .024$). We further
197 fitted an LMM to test for regional effects of MPC axis loadings on S-A axis loadings at the parcel
198 level (Fig. 2G and 2H), and found small and localized associations between the S-A and MPC axes.

199

200 Third, we computed the mean geodesic distance of connectivity profiles at the individual level. The
201 mean geodesic distance of connectivity profiles is the mean distance along the cortical mantle
202 between each region and its top 10% maximally functionally connected regions. Group-level

203 patterns (i.e., averaged across all subjects; Fig. 2D) revealed shorter distances in visual and
 204 somatomotor (sensory) regions, and longer distances in frontoparietal and DMN (association)
 205 regions. We also tested for whole-brain associations between the S-A axis and patterns of mean
 206 geodesic distance of connectivity profiles by correlating their spatial maps (Fig. 2A and 2D)
 207 averaged across all subjects (Fig. 2F; $r = 0.76$, $p_{\text{spin}} < .001$). We again also fitted an LMM to test
 208 for regional effects of mean geodesic distance on S-A axis loadings at the parcel level (Fig. 2I and
 209 2J) and found strong and widespread associations between the S-A axis and patterns of mean
 210 geodesic distance.
 211



212
 213 **Figure 2. Morphometric correlates of the sensory-association (S-A) axis of functional cortical organization across**
 214 **sexes.** **A** | Mean S-A axis loadings (spanning from visual to DMN regions) across sexes; **B** | Statistically significant
 215 effects following false discovery rate (FDR) correction ($q < 0.05$) of linear mixed effect model (LMM) results showing
 216 total surface area (SA) effects on the S-A axis; **C** | Mean microstructural profile covariance (MPC) axis loadings
 217 (spanning from sensory to paralimbic regions) across sexes; **D** | Mean geodesic distance of connectivity profiles across
 218 sexes; **E** | Spatial correlation between mean patterns of S-A axis loadings and mean patterns of MPC axis loadings
 219 (color-coded by yeo network), $r = 0.23$, $p_{\text{spin}} = .024$; **F** | Spatial correlation between mean patterns of S-A axis loadings
 220 and mean patterns of mean geodesic distance (color-coded by yeo network), $r = 0.76$, $p_{\text{spin}} < .001$; **G** | Thresholded t -
 221 map of LMM results showing FDR-corrected statistically significant effects of MPC axis loadings on the S-A axis; **H**
 222 | Functional network breakdown of parcels showing statistically significant MPC axis effects on S-A axis. The outer
 223 ring displays absolute proportions by functional Yeo network, the inner ring displays absolute proportions by
 224 directionality of effects; **I** | Thresholded t -map of LMM results showing FDR-corrected statistically significant effects
 225 of mean geodesic distance on the S-A axis; **J** | Functional network breakdown of parcels showing statistically significant
 226 geodesic distance effects on the S-A axis.

227 **Morphometric correlates do not explain sex differences in the S-A axis (Figure 3)**

228 After establishing the morphometric correlates of the S-A axis, we probed whether sex differences
229 in cortical morphometry may explain sex differences in the S-A axis. First, we tested whether sex
230 differences in S-A axis loadings were moderated by total SA. For this, we modeled an interaction
231 term of sex by total SA on the S-A axis loadings within the original LMM (Fig. 3B) and found no
232 statistically significant effects across regions. In Supplementary Figure S3A-C, we further show
233 that this interaction effect when including height as a covariate to the LMM yields virtually the
234 same t -values as when height is not included as a covariate ($r = 0.99$, $p_{\text{spin}} < .001$). This suggests
235 that height –being an anthropometric feature that systematically differs between the sexes– does
236 not explain variance in the moderation of sex effects by total SA on the S-A axis loadings either.
237 We also plotted within-sex effects of total SA on S-A axis loadings, showing similar although
238 slightly diverging patterns of effects between males and females ($r = 0.65$, $p_{\text{spin}} = .001$; Fig. S3D-
239 F). However, the divergence of patterns between sexes may not be strong or systematic enough to
240 be interpreted as meaningful, as underlined by the lack of evidence of a statistically significant sex
241 by total SA interaction effect on the S-A axis. Second, we tested for regional sex effects on the MPC
242 axis loadings (Fig. 3C) and correlated this spatial t -map with the t -map depicting regional sex
243 effects on the S-A axis loadings (Fig. 3A). Here, we found no statistically significant association
244 between these two patterns of sex differences (Fig. 3E; $r = 0.03$, $p_{\text{spin}} = .388$). Third, we tested for
245 regional sex effects on the mean geodesic distance of connectivity profiles (Fig. 3D) and again
246 correlated this spatial t -map with the t -map depicting sex effects in the S-A axis loadings (Fig. 3A).
247 Again, we found no statistically significant association between these two patterns of sex
248 differences (Fig. 3F; $r = 0.04$, $p_{\text{spin}} = .395$). These results together suggest that sex differences in
249 the S-A axis are overall not fundamentally moderated by –or associated with– sex differences in
250 cortical morphometry.

251
252 As an additional sensitivity analysis, we found that including the MPC axis and the mean geodesic
253 distances as covariates in our LMM testing for sex effects on the S-A axis yields highly similar
254 regional sex effects to those reported in Figure 1A (for which the original LMM only included total
255 SA as a morphometric covariate, to control for brain size), as shown by the strong correlation of t -
256 maps ($r = 0.95$, $p_{\text{spin}} < .001$, Supplementary Fig. S4A). Similarly, the association between sex effects
257 when including all morphometric covariates versus not including any (i.e., also excluding total SA)
258 remains high despite a small decrease in correlation strength ($r = 0.81$, $p_{\text{spin}} < .001$, Supplementary
259 Fig S4B). These findings further suggest that sex differences in brain size only explain a minor
260 amount of variance in sex differences in the S-A axis.

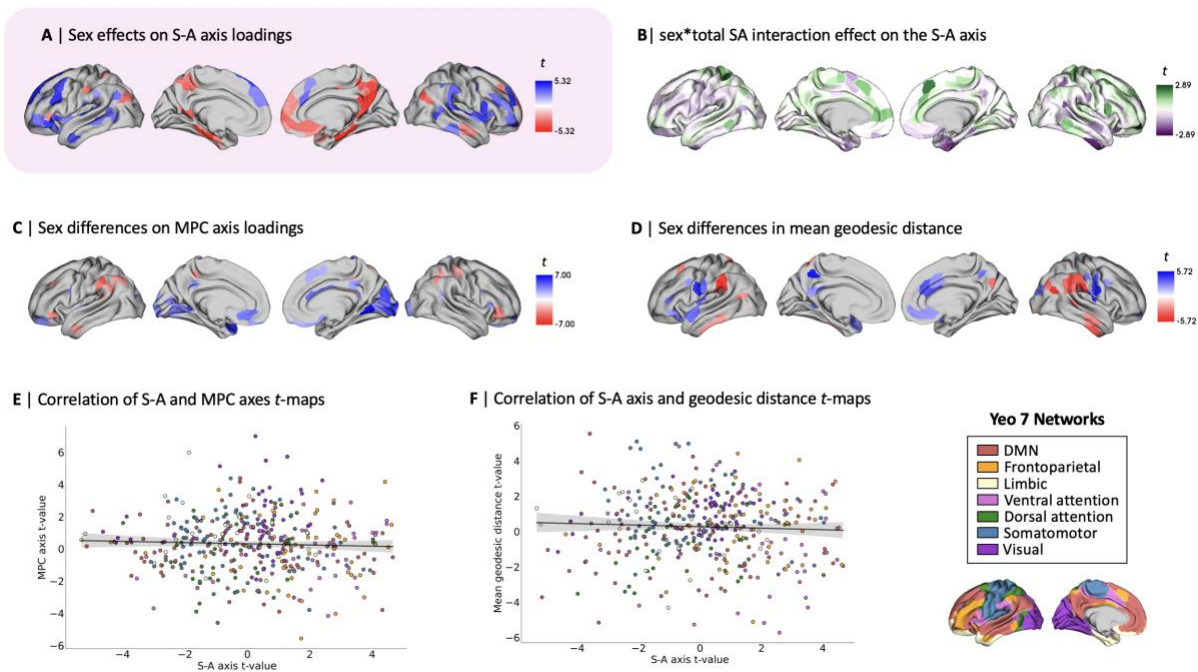


Figure 3. Morphometric correlates of sex differences in the sensory-association (S-A) axis. **A** | Thresholded t -map of linear mixed effect model (LMM) results showing false discovery rate (FDR)-corrected ($q < 0.05$) statistically significant effects of sex on the S-A axis, where blue represents higher male loadings and red represents higher female loadings; **B** | Unthresholded t -map of LMM testing for sex by total surface area (SA) interaction effects on S-A axis (there were no statistically significant sex effects after FDR correction); **C** | Thresholded t -map of LMM results showing FDR-corrected statistically significant effects of sex on the microstructure profile covariance (MPC) axis; **D** | Thresholded t -map of LMM results showing FDR-corrected statistically significant effects of sex on the mean geodesic distance of connectivity profiles; **E** | Scatterplot displaying the spatial correlation between patterns of sex differences (t -maps) in S-A axis loadings and in MPC axis loadings (color-coded by yeo network), $r = 0.03$, $p_{spin} = .388$; **F** | Scatterplot displaying the spatial correlation between patterns of sex differences (t -maps) in S-A axis loadings and in the mean geodesic distance of connectivity profiles (color-coded by yeo network), $r = -0.04$, $p_{spin} = .395$.

Intrinsic functional underpinnings of differences in the S-A axis (Figure 4)

Given that sex differences in the morphometric correlates of the S-A axis did not appear to explain sex differences in the S-A axis, we probed potential intrinsic functional underpinnings of sex differences on the S-A axis. We thus tested for associations between sex differences in the S-A axis loadings and sex differences in mean FC strength, FC profiles, and network topology.

First, we computed mean FC strength at the individual level from FC matrices, representing –for each parcel– the mean row-wise z-values of a given seed region’s top 10% maximally functionally connected regions. We then fitted an LMM to test for local effects at the parcel level of sex on mean FC strength (Fig. 4C and 4E), which revealed –amongst other sex differences– higher intrinsic FC in females in DMN regions and in males in somatomotor regions. To test associations between patterns of sex differences in the S-A axis loadings and in FC strength, we spatially correlated the t -maps (Fig. 4A and 4C) of the respective sex differences and did not detect a statistically significant

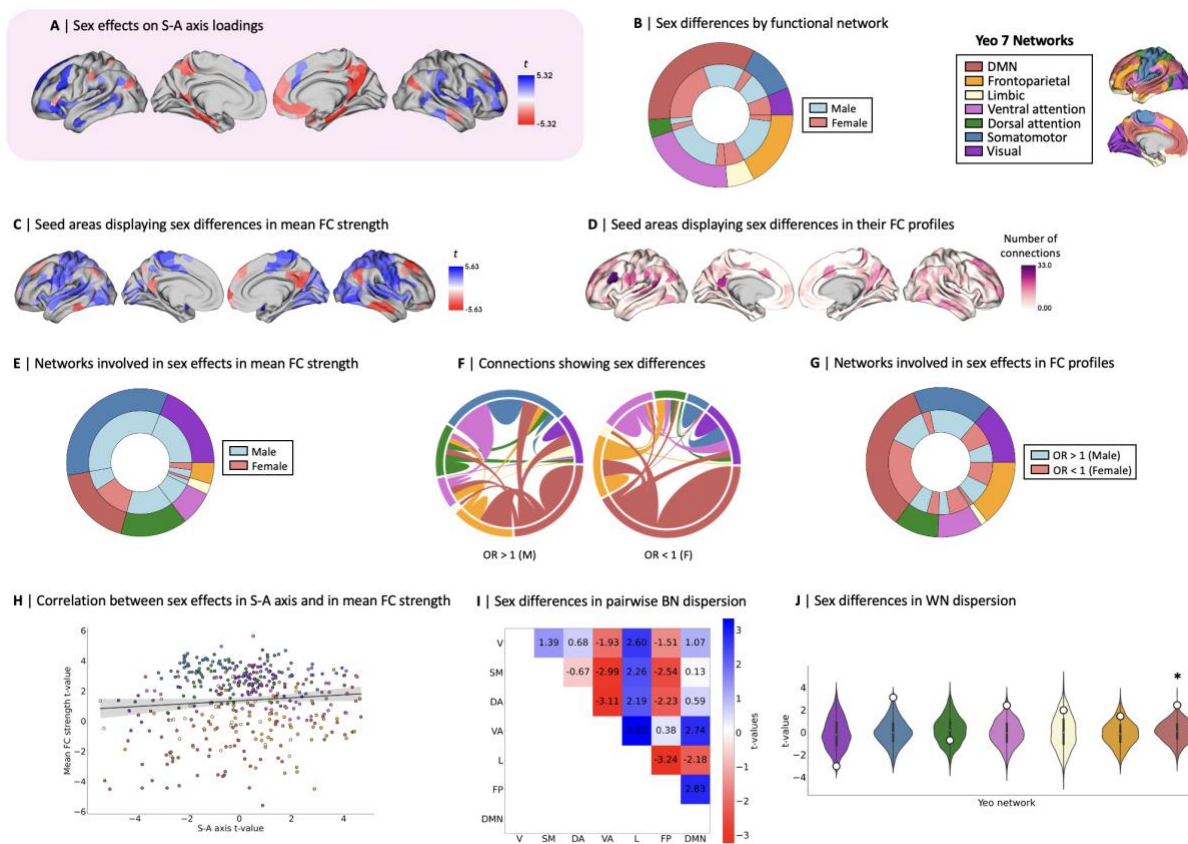
287 association between sex differences in the S-A axis and sex differences in FC strength (Fig. 4H; r
288 = 0.02, $p_{\text{spin}} = .380$).

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290 Second, we defined FC profiles at the individual level, for which we identified the top 10% of
291 maximally functionally connected regions. Using the Chi-square (χ^2) test of independence, we
292 assessed –for each possible pairwise connection along the 400x400 matrix– sex differences in a
293 given target region’s odds of belonging to the top 10% of maximally functionally connected regions
294 of a given seed region. We identified the direction of these sex effects with the odds ratio (OR),
295 where $OR > 1$ indicates a given region’s greater male odds and $OR < 1$ indicates a given region’s
296 greater female odds. Out of the 160000 tested functional connections, 2004 connections
297 (corresponding to 1.25% of all connections) displayed statistically significant sex differences in
298 their odds of constituting a seed’s top 10% connections after FDR correction (Fig. 4F), suggesting
299 that sex differences in S-A axis loadings may in part stem from differences in FC profiles, namely
300 differences in which functional connections are the strongest. For illustrative purposes, we
301 summarized spatial patterns of sex differences in FC profiles as the sum of connections showing
302 sex differences per seed region (Fig. 4D), as well as the overall networks involved in sex differences
303 in FC profiles (Fig. 4G).

304

305 Finally, we investigated network topology, namely the organization of networks along the S-A axis.
306 We computed between-network dispersion for each subject, quantifying the pairwise distance
307 between two given networks along the S-A axis, where a higher value indicates higher segregation
308 of the given pair of networks (21 pairs of Yeo networks in total) [36]. We also computed within-
309 network dispersion for each subject for the seven intrinsic networks under study [32], quantifying
310 the spread of regions within each network along the S-A axis, where a higher value indicates higher
311 segregation of the given network’s regions. LMMs did not show any statistically significant sex
312 differences in between-network dispersion for any of the network pairs (Fig. 4I). However, we
313 found greater male within-network dispersion in the DMN, $t = 2.41$, $p_{\text{spin}} = 0.001$ (Fig. 4J), revealing
314 a greater spread of regions belonging to the DMN along the S-A axis in males. The full statistical
315 results for the analysis of sex differences in network dispersion are summarized in Supplementary
316 Table S2.



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Figure 4. Intrinsic functional underpinnings of differences in the sensory-association (S-A) axis. **A** | Thresholded t -map of linear mixed effect model (LMM) results showing false discovery rate (FDR)-corrected ($q < 0.05$) statistically significant effects of sex on the S-A axis, where blue represents higher male loadings and red represents higher female loadings; **B** | Functional network breakdown of parcels showing statistically significant sex differences in S-A axis loadings. The outer ring displays absolute proportions of statistically significant parcels by functional Yeo network, the inner ring displays absolute proportions by directionality of effects, where blue represents higher male loadings and red represents higher female loadings; **C** | Thresholded t -map of LMM results showing FDR-corrected statistically significant effects of sex on mean functional connectivity (FC) strength; **D** | Number of connections (per seed region) showing statistically significant FDR-corrected sex differences in their odds of belonging to the given seed's top 10% of connections; **E** | Functional network breakdown of connections showing statistically significant FDR-corrected sex differences in mean FC strength; **F** | Connections between seed and target regions showing statistically significant FDR-corrected sex differences in FC profiles (OR > 1 meaning that males have higher odds than females of having a target region belong to a seed region's top 10% connections, where OR < 1 means that females have higher odds than males of having a target region belong to a seed region's top 10% connections; connections are color coded by network and weighed by number of connections between the network pairs); **G** | Functional network breakdown of connections showing statistically significant FDR-corrected sex effects in their odds of belonging to the given seed's top 10% of connections; **H** | Spatial correlation between patterns of sex effects in S-A axis loadings and patterns of sex effects in mean FC strength (color-coded by yeo network), $r = 0.02$, $p_{\text{spin}} = .380$; **I** | t -values for the sex contrast in between-network (BN) dispersion for each pairwise Yeo network comparison, where blue represents higher male BN dispersion and red represents higher female BN dispersion (no statistically significant sex effects after spin permutation and Bonferroni correction; $p_{\text{spin}} < .001$); **J** | t -values for the sex contrast in within-network (WN) dispersion for each Yeo network (displayed as white dots), plotted on null distributions of t statistics derived from 1000 spin permutations, where positive t -values represent higher male WN dispersion and negative t -values represents higher female WN dispersion, * indicates Bonferroni-corrected ($p_{\text{spin}} < .004$) statistical significance of the sex contrast. V, visual, SM, somatomotor, DA, dorsal attention, VA, ventral attention, L, limbic, FP, frontoparietal, DMN, default mode network.

343 **DISCUSSION**

344 In the current work, we investigated the extent to which sex differences in functional cortical
345 organization may be explained by differences in cortical morphometry, namely brain size,
346 microstructure, and the geodesic distances of connectivity profiles. We identified widespread sex
347 differences in adult functional cortical organization as defined by the S-A axis, which however did
348 not appear fundamentally associated with sex differences in brain size, microstructural
349 organization, nor the mean geodesic distance of connectivity profiles. This finding is particularly
350 striking given that the morphometric properties under study were all *per se* associated with the S-
351 A axis and differed between sexes. Instead, we observed that sex differences in the S-A axis were
352 related to differences in FC profiles and network topology, namely greater male dispersion within
353 the DMN. Collectively, our findings suggest that sex differences in functional cortical organization
354 go beyond neuroanatomical sex differences pertaining to cortical morphometry.

355
356 Different measures of brain size are commonly used in the literature, including ICV, TBV, and total
357 SA. Although these measures highly covary and are often used interchangeably, they quantify
358 different morphometric features of the brain, with sex differences in “brain size” ranging from 8%
359 to 13% depending on the selected measure [6]. The size and direction of sex effects also vary by
360 neuroanatomical property, such as different tissue types, brain regions, and features (including
361 cortical thickness, gyrification, and SA) [37]. Furthermore, morphometric features vary differently
362 as a function of age, whereby for example TBV but not ICV is affected by atrophy [6]. These
363 findings highlight the complex heterogeneity of neuroanatomical properties constituting brain size.
364 The potential for introducing non-linear bias in the detection of sex effects should therefore not be
365 overlooked, particularly when statistically controlling for brain size in the detection of sex effects
366 on brain structure and function [28, 38-41]. We addressed this issue by testing the effects of different
367 measures of brain size, namely ICV, TBV, and total SA, on the S-A axis. Here, given that total SA
368 had the most widespread effects on functional organization, we deemed it the most appropriate
369 measure of brain size, which we further included as a covariate in our models throughout our
370 analyses. The relevance of total SA is also supported by the theoretical assumptions motivating our
371 study, namely the relevance of cortical shape and geometry in constraining brain wide functional
372 dynamics [25-27] and thus sex differences in these features potentially underpinning sex differences
373 in the S-A axis. Our findings therefore highlight the diverging effects of different measures of brain
374 size and depict total SA as having the most substantial theoretical and statistical associations to a
375 low dimensional representation of functional cortical organization. As such, future research on sex

376 differences should also carefully select the measure of brain size that is most conceptually and
377 empirically pertinent to the research question under study in order to minimize bias.

378

379 By establishing morphometric correlates of the S-A axis in addition to brain size, namely a
380 microstructural axis of cortical organization [34, 35, 42] and the mean geodesic distance of
381 connectivity profiles [10, 42], our findings align with previous work and argue for the rooting of
382 functional cortical organization in cortical structure and shape. We show a particularly strong
383 association between the mean geodesic distance of connectivity profiles and the S-A axis,
384 supporting the relevance of the cortical mantle's shape in sculpting functional organization. This
385 may be a product of the cortical mantle's evolutionary expansion, where association regions are
386 untethered from sensory hierarchies [13], and long-range connections preserve the overall
387 connectedness of cortical networks by facilitating the communication between distant areas [24].
388 Furthermore, as indexed by the MPC axis, microstructural organization appears to mildly covary
389 with the S-A axis, supporting to some degree the well-established idea of structural constraints on
390 brain function [34, 35, 43]. In our study, we obtained intensity profiles via the ratio of T1w over
391 T2w imaging sequences, and although it is commonly used to measure myelin [34, 35, 44], the
392 T1w/T2w ratio has been described as an acceptable *qualitative* proxy for myelin in grey but not
393 white matter [45]. It is indeed thought to capture unique features of microstructural tissue that
394 appear largely independent of diffusion-based metrics, thus portraying a mix of neuroanatomical
395 features beyond pure myelin [46]. We therefore consider the T1w/T2w ratio –and the resulting MPC
396 axis– as a general measure of tissue microstructure, which may serve as a scaffold for functional
397 organization.

398

399 After establishing morphometric correlates of the S-A axis, we addressed our primary aim of
400 probing the extent to which sex differences in functional cortical organization may be explained by
401 sex differences in cortical morphometry. We observed slightly diverging results when including –
402 as opposed to excluding– total SA as a covariate in our model testing for sex differences in S-A axis
403 loadings, suggesting that sex differences in total SA explained some variance in sex differences in
404 functional organization. This finding is consistent with the systematic practice of controlling for
405 brain size when testing for structural and functional sex differences [28, 38-41]. Nevertheless, our
406 findings overall suggest that morphometric differences between the sexes are altogether not
407 substantial contributors of sex differences in the S-A axis of functional organization. We did not
408 find sex differences in S-A axis loadings to be moderated by total SA, nor any associations between
409 patterns of sex differences in the S-A axis and patterns of sex differences in the MPC axis or in the

410 mean geodesic distance of connectivity profiles. The negligible relevance of cortical morphometry
411 to sex differences in the S-A axis is striking given that morphometric properties appear *per se* to be
412 associated with the S-A axis and to differ between sexes. The mechanisms underpinning different
413 patterns of morphometric and functional sex differences may thus be independent from one another,
414 suggesting that sex differences in functional cortical organization may extend beyond the
415 connectome's supporting shape and structure.

416
417 Given that sex differences in morphometric correlates of the S-A axis did not seem to explain sex
418 differences on the S-A axis, we probed and found potential intrinsic functional underpinnings of
419 sex differences on the S-A axis. Firstly, the sex differences we observed in the S-A axis loadings
420 were distributed across functional networks, and notably in the DMN, frontoparietal and ventral
421 attention networks. This is consistent with previous findings of greater individual variability in the
422 functional topography of these association networks relative to lower-order sensory networks,
423 which have also been shown to contribute the most to sex classification in youth [8]. We also
424 observed sex differences in intrinsic FC strength, replicating previous widely established findings
425 of greater FC in females within DMN regions [18-20] and in males within somatomotor regions
426 [19, 21]. However, these patterns did not spatially overlap with patterns of sex differences in the S-
427 A axis, suggesting that FC strength is not a feature of intrinsic FC that is captured by sex differences
428 in our low dimensional representation of functional organization. Instead, we found that sex
429 differences in the S-A axis were related to differences in FC profiles, which also presented
430 qualitative sex differences in the proportional breakdown of networks involved. Females seemed to
431 make more top connections involving the DMN relative to males, whereas males displayed more
432 top connections involving the somatomotor networks relative to females. These sex differences in
433 the configuration of connections may not only underly the recurrence of sex differences in these
434 networks [18-21], but may also explain sex differences in network topology.

435
436 We in fact observed greater male dispersion (i.e., decreased similarity on the S-A axis) within the
437 DMN (and the somatomotor network barely not surviving the Bonferroni correction), which is also
438 consistent with previous findings of generally more segregated male networks [47]. These network-
439 specific topological sex differences may be related to greater female odds of connections within the
440 DMN, and greater male odds of somatomotor connections with other networks. Concretely,
441 network topology, which represents the organization of functional communities within and between
442 functional networks [36], may reflect brain states [48]. Network topology has also been associated
443 with different cognitive features including arousal [49], awareness and consciousness [50],

444 behavior and task performance [51], and cognitive flexibility [52]. The balance between integration
445 and segregation is complex, dynamic, and necessary to maintain the brain's metastability [53] by
446 reaching a point of equilibrium between global organization and local specialization [43]. The brain
447 is a highly interconnected and metabolically expensive organ, and its organization is required to
448 dynamically balance topological efficiency and energy utilization in response to transient cognitive
449 and physiological demands [54]. Our findings of sex differences in network topology may therefore
450 pertain to intricate sex differences not only in brain states at rest, which may underpin cognitive
451 differences, but also in energy expenditure, which would reflect physiological differences.

452
453 Despite the novel insights gained through our study, some limitations must be acknowledged.
454 Firstly, by only considering biological sex, we neglected possible effects of gender on functional
455 organization and its morphometric correlates. Findings may indeed appear more nuanced if we
456 moved beyond the unrealistic assumption of a clear-cut sexual dimorphism of brain structure and
457 function [55], as the relevance of considering transgender individuals in the study of sex differences
458 is being increasingly recognized [56]. Nevertheless, we intentionally focused on the biological and
459 dichotomous variable of sex assigned at birth given that our study aimed to study biological
460 mechanisms relating to cortical morphometry. We did not venture in the intricacies of gender as
461 they require an additional careful consideration of complex social and environmental influences,
462 which go beyond the scope of our study. Secondly, we focused on neocortical functional
463 organization, excluding subcortical structures and the cerebellum despite their substantial
464 contributions to whole brain organization through their notable structural integration with the cortex
465 [57]. In fact, the amygdala and hippocampus are hypothesized to be at the origin of mammalian
466 cortical evolution [58] and have also repeatedly shown both structural [6] and functional [59] sex
467 differences. Nevertheless, our exclusive focus on the neocortex was motivated by the relevance of
468 using the S-A axis as our measure of functional organization, which is obtained by reducing the
469 dimensionality of FC matrices of cortical data [10]. By using the S-A axis, our work identified sex
470 differences embedded in a key macroscale organizational principle that is closely tied to
471 evolutionary expansion and cortical morphometry, going beyond previous research on functional
472 differences between the sexes that solely focus on intrinsic brain function. Thirdly, the
473 morphometric properties considered in our study are not exhaustive, overlooking the contributions
474 of other morphometric measures such as local volumes of grey matter. The inclusion of the MPC
475 axis [34, 35] and the mean geodesic distance of connectivity profiles [10, 42] was however
476 supported by their theoretical and empirical relevance to functional cortical organization,
477 particularly its low dimensional embedding.

478 All in all, our study opens a new set of questions pertaining to the mechanisms underpinning sex
479 differences in functional cortical organization, given that they do not appear to be fundamentally
480 rooted in cortical morphometric differences. Our findings instead suggest that sex differences in the
481 S-A axis are to some extent intrinsically related to differences in FC profiles and network topology.
482 Therefore, future research should explore factors driving males and females to form distinct
483 functional connections and to adopt divergent system-level organization of functional networks.
484 Recognizing the human body as a complex system of systems, future work should investigate other
485 biological factors that may contribute to functional sex differences such as genes located on sex
486 chromosomes [16] and sex hormones [60, 61]. Environmental factors should equally be considered,
487 for example stress, which has also been found to contribute to sex differences via epigenetic
488 mechanisms [62]. Investigating the mechanisms underpinning sex differences in functional
489 organization is crucial to gain a deeper understanding of discrepancies in predisposition to
490 psychiatric disorders, for example greater female vulnerability to affective disorders throughout the
491 lifespan [63, 64] and particularly during hormone transition periods such as puberty, pregnancy,
492 and menopause [65].

493 **MATERIALS AND METHODS**

494 **Participants and Experimental Design**

495 Our analyses were conducted on the publicly available data of healthy young adults from the Human
496 Connectome Project (HCP) S1200 release (<http://www.humanconnectome.org/>) [29]. We selected
497 subjects with available functional, T1, and T2 data, resulting in a final sample of 1000 individuals
498 (536 females) with a mean age of 28.73 ± 3.71 years, including 284 monozygotic twins (MZ), 184
499 dizygotic twins (DZ), 443 non-twin siblings, and 89 unrelated individuals. Subjects were all born
500 in Missouri but recruited in an attempt to broadly reflect the racial and ethnic composition of the
501 United States population. Recruitment efforts aimed to yield a subject pool capturing a wide range
502 of variability—in socioeconomic and behavioral terms—in order to be representative of the general
503 healthy population. The term “healthy” was thus broadly defined. Individuals with documented
504 neurodevelopmental and psychiatric disorders, or reporting physiological illnesses such as high
505 blood pressure or diabetes were excluded, but not individuals who reported smoking, being
506 overweight, or a history of recreational drug use or heavy drinking (if they had not experienced
507 severe symptoms). Informed consent was obtained for all study subjects. More detailed information
508 about the HCP study design and recruitment procedure is available elsewhere [29, 66].

509

510 **Structural MRI acquisition and preprocessing**

511 The HCP’s MRI data was acquired on a customized 3T Siemens Skyra ConnectomeScanner with a
512 32-channel head coil at Washington University across four scanning sessions held over two days.
513 Structural MRI images were acquired on the same day via high resolution T1-weighted (T1w) and
514 T2-weighted (T2w) sequences. Two separate T1w images were acquired and averaged, with
515 identical scanning parameters using a 3D MPRAGE sequence (0.7 mm isovoxels, FOV = 224 mm,
516 matrix = 320×320 mm, 256 sagittal slices; TR = 2400 ms, TE = 2.14 ms, TI = 1000 ms, flip
517 angle = 8° , BW = 210 Hz per pixel, ES = 7.6 ms). Two separate T2w images were acquired and
518 averaged, with identical scanning parameters using a variable flip angle turbo spin-echo (3D T2-
519 SPACE) sequence, with the same isotropic resolution, matrix, FOV, and slices as for the T1w
520 sequence (TR = 3200 ms, TE = 565 ms, BW = 744 Hz per pixel, total turbo factor = 314). The
521 preprocessing steps included co-registering the T1w and T2w images, bias field (B1) correction,
522 registration to MNI space, segmentation, and surface reconstruction. See [29, 66, 67] for more detail
523 on the HCP’s MRI protocols and the FreeSurfer segmentation pipeline.

524 **Functional MRI (fMRI) acquisition and preprocessing**

525 The HCP's fMRI data was collected after the structural sequences and following the HCP's minimal
526 processing pipeline, as described above. A total of 1h of resting-state functional data was collected
527 across four identical 15min scanning sessions, equally split over two days (LR1, RL1, LR2, RL2),
528 with a gradient echo EPI sequence at a resolution of 2 mm isotropic (FOV = 208 × 180 mm, matrix
529 = 104 × 90 mm, 72 slices covering the whole brain, TR = 720 ms, TE = 33 ms, multiband factor of
530 8, FA = 52°). The multimodal surface matching algorithm (MSMAll) was used to co-register the
531 data to the HCP template 32 k_LR surface space, consisting of 32492 nodes per hemisphere (59412
532 nodes excluding the medial wall). A more detailed description of the resting state fMRI data
533 acquisition and analysis protocol is available elsewhere [67, 68].

534

535 **Functional connectivity (FC) and the sensory-association (S-A) axis of functional organization**

536 Throughout this work, we used the Schaefer 400 parcellation (clustered into 7 networks: visual,
537 somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, DMN [32]). This widely
538 used functionally-derived parcellation scheme was originally obtained via a gradient-weighted
539 Markov Random Field model integrating local gradient and global similarity approaches [69]. The
540 vertex-wise functional timeseries were therefore averaged within the Schaefer 400 cortical parcels.
541 FC matrices (400x400) were then computed at the individual level –per scanning session– by
542 correlating cortical timeseries in a pairwise manner using the Pearson product moment. We
543 normalized the correlation coefficients using Fisher's z-transformation. Final FC matrices were
544 obtained by averaging each subject's matrices across their four scanning sessions. From these FC
545 matrices and for each subject, we computed the S-A axis of functional organization, as described
546 below.

547

548 We conducted data reduction on the FC matrices to yield macroscale gradients of functional
549 organization [10]. For this, we used diffusion map embedding, a non-linear manifold learning
550 algorithm that reduces complex, high-dimensional structures of data (in our case affinity matrices)
551 to low-dimensional representations combining geometry with the probability distribution of data
552 points [30]. Thus, cortical parcels that are strongly interconnected are represented closer together
553 in the resulting low dimensional manifold of FC data, whereas parcels with low covariance are
554 represented farther apart, as indexed by the parcels' gradient loadings. To this end, we used the
555 BrainSpace Python toolbox [31] to compute 10 gradients with the following parameters: 90%
556 threshold (i.e., only considering the top 10% row-wise z-values of FC matrices, representing each
557 seed region's top 10% of maximally functionally connected regions), $\alpha = 0.5$ (α controls whether

558 the geometry of the set is reflected in the low-dimensional embedding – i.e., the influence of the
559 sampling points density on the manifold, where $\alpha = 0$ (maximal influence) and $\alpha = 1$ (no influence)),
560 and $t = 0$ (t controls the scale of eigenvalues). These parameters were selected for consistency with
561 previous studies [10, 34] and represent choices that are recommended to retain global relations
562 between datapoints in the embedded space whilst being relatively robust to noise. In order to
563 increase comparability for further between-subject analyses, Procrustes alignment was used to align
564 individual gradients to mean gradients, which were computed by applying diffusion map
565 embedding –with the same parameters listed above– to the mean FC matrix (i.e., FC matrices
566 averaged across all subjects). The computation of these FC gradients was carried out independently
567 per hemisphere (i.e., considering the top 10% row-wise z-values of only half of the FC matrices,
568 shaped 200x200) and the gradient loadings resulting from both hemispheres were subsequently
569 concatenated. This decision was made for consistency and comparability reasons within our study,
570 so that the top 10% functional connections selected for data reduction corresponded to those
571 considered in the calculation of the mean geodesic distance of connectivity profiles –which were
572 only computed per hemisphere– as described further below). We verified and confirmed the stability
573 FC gradients when computing them per hemisphere versus at the whole brain level, as shown by
574 the spatial correlation of mean gradient loadings ($r = 0.98$, $p_{\text{spin}} = .001$). Finally, we took the well-
575 replicated principal gradient explaining the most variance in the data and spanning from visual to
576 DMN regions [10], which we labeled the S-A axis and used to represent functional organization for
577 subsequent analyses.

578
579 We also computed, for each subject, mean FC strength at the parcel level in a seed-wise fashion, by
580 averaging the row-wise z-values of each seed region’s top 10% maximally functionally connected
581 regions –again per hemisphere– and subsequently concatenated the hemispheric mean FC strength
582 values to reconstruct whole brain data.

583 584 **Cortical microstructure and microstructural profile covariance (MPC)**

585 Microstructural properties –including myelin and cellular characteristics– show depth-dependent
586 variation along cortical columns, as reported by histology [34, 70, 71] as well as *in vivo* and *post*
587 *mortem* neuroimaging [33-35, 71], which illustrates cortical hierarchy [11]. Similar to previous
588 work [35], we quantified cortical microstructure, or “microstructural profile intensity” (MPI), using
589 the myelin-sensitive MRI contrast obtained from the T1w/T2w ratio from the HCP minimal
590 processing pipeline described above [67] (a reliability check is reported in the Supplementary
591 Methods, Fig. S5). The T1w/T2w ratio uses the T2w image to correct for inhomogeneities in the

592 T1w image [44]. Then, we followed the previously described protocol [33-35] to compute our
593 measurement of MPC, which reflects the variation of MPI, across cortical depths. In short, we
594 generated 14 equivolumetric surfaces within the inner and outer cortical surfaces, then excluded the
595 inner- and outer-most surfaces, thus remaining with 12 surfaces representing cortical layers. Surface
596 generation was based on a model compensating for cortical folding by altering the pairwise
597 Euclidean distance (ρ) of intracortical surfaces throughout the cortex and thus preserving fractional
598 volume between the surfaces. For each surface, ρ was calculated as defined in Eq. 1.

599

$$600 \quad \rho = \frac{1}{A_{out} - A_{in}} \cdot \left(-A_{in} + \sqrt{\alpha A_{out}^2 + (1 - \alpha) A_{in}^2} \right) \quad (1)$$

601

602 for which α denotes a fraction of the total volume of the segment that the surface accounts for,
603 while A_{out} and A_{in} respectively denote the surface areas of the outer and inner cortical surfaces.

604

605 Across the whole cortex and from the outer to the inner surfaces, we systematically sampled MPI
606 values layer-wise for each of the 64,984 vertices of the HCP template 32 k_LR surface space, which
607 we then averaged within each of the 400 Schaefer parcels, per layer. Following a previously
608 described protocol [33], we constructed subject level 400x400 matrices using pairwise Pearson
609 partial correlation on the MPI profiles of cortical parcels (i.e., correlating the MPI values across 12
610 layers between parcels), controlling for overall mean cortical MPI, followed by log transformation.
611 We then used these matrices to compute MPC gradients –here directly at the whole brain level
612 instead of independently within hemispheres– by following the same procedure and using the same
613 toolbox and parameters as for computing the FC gradients [30, 31], as described above and
614 previously done [33-35]. We also selected the principal gradient of MPC explaining the most
615 variance in the data, which we labeled the MPC axis and used to represent microstructural
616 organization in subsequent analyses.

617

618 **Measures of brain size**

619 In our analyses we included different measures of brain size typically used in the literature,
620 including intracranial volume (ICV), total brain volume (TBV), and total surface area (SA). For
621 ICV, we used the FreeSurfer output measure *IntraCranialVol*, which is an estimate of ICV based
622 on the Talairach transform. We computed our own measure of TBV by summing the volumes of the
623 following FreeSurfer output measures: *TotCort_GM_Vol*, *Tot_WM_Vol*, *3rdVent_Vol*,
624 *L/R_ThalamusProper_Vol*, *L/R_Caudate_Vol*, *L/R_Putamen_Vol*, *L/R_Pallidum_Vol*,

625 *L/R_Hippo_Vol*, *L/R_Amygdala_Vol*, *L/R_AccumbensArea_Vol*, *L/R_ChoroidPlexus_Vol*,
626 *L/R_LatVent_Vol*, *L/R_InfLatVent_Vol*. We chose to include volumes that are anatomically located
627 within the cortical sheath, which we considered relevant given our study's focus on cortical
628 functional organization (thus excluding the volumes of subcortical structures). We computed total
629 SA by using the FreeSurfer `mri_surf2surf` tool to resample cortical white matter surface for each
630 subject.

631

632 **Geodesic distances of connectivity profiles**

633 Geodesic distances, representing the shortest distance between two vertices along the folded
634 cortical mantle's curvature, were computed using the Micapipe toolbox [72], and following the
635 previously described protocol [73]. In short, geodesic distance matrices were computed for each
636 subject along their native cortical midsurface. The first step consisted in defining a centroid vertex
637 for each cortical parcel, identified as the vertex having the shortest summed Euclidean distance
638 from all other vertices within the parcel. Then, Dijkstra's algorithm [74] was used to compute
639 geodesic distances between the centroid vertices and all other vertices on the on the native
640 midsurface mesh. The vertex-wise geodesic distance values were then averaged within each parcel
641 to form the geodesic distance matrices. From these individual matrices, we finally averaged –
642 parcel-wise– the geodesic distance values of each seed parcel's top 10% maximally functionally
643 connected regions per hemisphere, thus obtaining for each subject the mean geodesic distance of
644 functional connectivity profiles by region.

645

646 **Statistical Analysis**

647 Given that the HCP sample includes different levels of kinship, we used linear mixed effects models
648 (LMMs) to account for sibling status (MZ, DZ, non-twin siblings) and family relatedness. In fact,
649 all LMMs mentioned in this work consistently included sex, age, and total SA as covariates (unless
650 otherwise mentioned), and controlled for random nested effects of family relatedness and sibling
651 status. In addition, effects on cortical data obtained via LMMs underwent false discovery rate
652 (FDR) correction ($q < 0.05$), thus correcting for multiple comparisons across the 400 Schaefer
653 parcels. Throughout this work, we also tested for associations in brain-wide patterns displayed in
654 the form of cortical maps, for which we used Spearman-rank correlation followed by spin-
655 permutation tests to control for spatial autocorrelation [75].

656

657 After computing the S-A axis of functional brain organization, we tested for sex differences in the
658 S-A axis loadings with an LMM. Then, we investigated which measure of brain size (out of ICV,

659 TBV, and total SA) had the largest effect on the S-A axis parcel loadings using separate LMMs
660 (respectively only including ICV, TBV, or total SA as a covariate, in addition to sex, age and the
661 random nested effect of family relatedness and sibling status). The reason underlying our decision
662 to systematically include total SA as a covariate in all our LMMs (as the measure of brain size) is
663 that it showed the most widespread effects on the S-A axis loadings out of the three tested measures.
664 Then, we investigated associations between the S-A axis and cortical morphometry, namely the
665 MPC axis and the mean geodesic distance of connectivity profiles, using both LMMs and
666 Spearman-rank correlations of cortical maps.

667
668 To probe whether sex differences in cortical morphometry may explain sex differences in the S-A
669 axis, we tested whether sex differences in the S-A axis loadings were moderated by total SA by
670 modelling an additional interaction term of sex by total SA on the S-A axis loadings within the
671 original LMM. We also tested for sex differences in the MPC axis and in the mean geodesic distance
672 of connectivity profiles, and conducted Spearman-rank correlations of cortical *t*-maps for the sex
673 contrast in the S-A axis and in the morphometric measures. Finally, we conducted sensitivity
674 analyses to test for sex effects on the S-A axis yielded by an LMM including all morphometric
675 measures as covariates (i.e., including the MPC axis and the mean geodesic distance of connectivity
676 profiles, in addition to total SA), as well as an LMM not including any morphometric measures as
677 covariates (i.e., also excluding total SA). We then tested the similarity of both these sex effects with
678 the original sex effects on the S-A axis with a Spearman-rank correlation of the cortical *t*-maps.

679
680 In order to probe the potential intrinsic functional underpinnings of sex differences in the S-A axis,
681 we tested for sex differences in FC strength (also with an LMM), as well as sex differences in FC
682 profiles, i.e., the presence of sex differences in the top 10% of maximally functionally connected
683 regions used to compute the S-A axis. To this end, we built 400x400 binary matrices at the subject
684 level –based on the subjects’ individual FC matrix *z*-values– in which we marked in a seed-wise
685 fashion (along the matrix rows) whether the given parcel (along the matrix column) belongs to the
686 given seed’s 10% maximally functionally connected regions, where 1 = parcel belongs to the seed’s
687 top 10% maximally functionally connected regions and 0 = parcel does not belong to the seed’s top
688 10% of maximally functionally connected regions. We then summed the binary matrices separately
689 within sexes in order to fill 160000 contingency matrices –one for each cell (i.e., functional
690 connection) of the 400x400 FC matrix– as follows:

	Parcel belongs to the seed's top 10% of maximally functionally connected regions	Parcel does <u>not</u> belong to the seed's top 10% of maximally functionally connected regions
Males	Cm	NCm
Females	Cf	NCf

691

692 where Cm and Cf respectively denote the number of males and females for which the parcel
693 (corresponding to the matrix column) constitutes the given seed's (corresponding to the matrix row)
694 top 10% of maximally functionally connected regions, and where NCm and NCf respectively denote
695 the number of males and females for which the given parcel does not constitute the given seed's
696 top 10% of maximally functionally connected regions.

697

698 We then conducted the Chi-square (χ^2) test of independence (degrees of freedom = 1) on each
699 contingency table to test for sex differences in the odds of each parcel of belonging to the top 10%
700 of maximally functionally connected regions of each seed region. Given the large number of tests
701 conducted here ($400 \times 400 = 160000$), we controlled for multiple comparisons using FDR correction.
702 We quantified the size of these sex effects with the odds ratio (OR), calculated as defined in Eq. 2.:

703

$$704 \quad OR = \frac{Cm/NCm}{Cf/NCf} \quad (2)$$

705

706 where $OR > 1$ indicates greater male odds –and $OR < 1$ indicates greater female odds– of a given
707 region of belonging to a given seed's top 10% of maximally functionally connected regions.

708

709 We also tested for sex differences in network topology, i.e., how nodes are physically organized in
710 networks and how networks are physically organized along the S-A axis. For this, we computed
711 two measures of network dispersion: between-network and within-network dispersion. Between-
712 network dispersion is defined as the Euclidean distance between a pair of network centroids, where
713 a higher value indicates that networks are more segregated from one another along the S-A. Within-
714 network dispersion is defined as the sum squared Euclidean distance of network nodes (i.e., parcel
715 loadings) to the network centroid, where a higher value indicates wider distribution and segregation
716 of a given network's nodes along the S-A axis. At the individual level, we thus computed between-

717 network dispersion between all networks in a pairwise fashion (21 pairs), and within-network
718 dispersion for all 7 networks, by defining network centroids as the median of the S-A axis loadings
719 of all parcels belonging to a given network, following a previously described protocol [36]. Then,
720 we computed sex differences in each of the 21 between-network dispersion metrics and 7 within-
721 network dispersion metrics using LMMs. For each model, we computed a null distribution of t -
722 values for sex differences using 1000 spherical rotations of the Schaefer parcellation scheme in
723 order to shuffle the network labels [75], against which we computed our p -value to determine
724 statistical significance. We then assed p_{spin} -values against Bonferroni-corrected two-tailed α -levels
725 of 0.001 (0.025/21) and 0.004 (0.025/7) for between-network and within-network dispersion sex
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741 Input on analysis: MDH, GB, and SLV. Writing—original draft: BS. Writing—review and editing:
742 BS, MDH, LW, GB, JS, SW, SBE, SLV. Supervision: SLV.

743

744 **Competing interests:** Authors declare that they have no competing interests.

745

746 **Data and materials availability:** All data needed to evaluate the conclusions in the paper are
747 present in the paper and the Supplementary Materials. We obtained human data from the open-
748 access Human Connectome Project HCP S1200 young adult sample. Data are available upon
749 request at <http://www.humanconnectome.org/>. Analyses were conducted in Python and R: The code
750 used in this manuscript is available at https://github.com/biancaserio/sex_diff_gradients. The code
751 and tutorials for functional gradient decomposition and to generate geodesic distances can further
752 be found at <https://brainspace.readthedocs.io/en/latest/index.html> and
753 <https://micapipe.readthedocs.io/en/latest/> respectively.

754

755 **SUPPLEMENTARY MATERIALS**

756 Supplementary results and methods can be found in the Supplementary Materials.

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