# Gradients of functional connectivity in the mouse cortex reflect neocortical evolution

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## Abstract

Understanding cortical organization is a fundamental goal of neuroscience that requires comparisons across species and modalities. Large-scale connectivity gradients have recently been introduced as a data-driven representation of the intrinsic organization of the cortex. We studied resting-state functional connectivity gradients in the mouse cortex and found robust spatial patterns across four independent data sets. The principal gradient of functional connectivity shows a striking overlap with an axis of neocortical evolution from two primordial origins. Additional gradients reflect sensory specialization and aspects of a sensory-to-transmodal hierarchy, and are associated with transcriptomic and cytoarchitectonic features. While some of these gradients strongly resemble observations in the human cortex, the overall pattern in the mouse cortex emphasizes the specialization of sensory areas over a global functional hierarchy.

## Introduction

A crucial task in neuroscience is to compare and connect findings across species. This approach enables us to learn how different cortices – including our own – have evolved and acquired their unique capacities. A major challenge for the translation of findings from animal studies to the human brain is that most techniques used in neuroscience research are too invasive to be applied in humans. A notable exception is magnetic resonance imaging (MRI), which can safely be used to investigate human brain structure and function, and is increasingly applied in small animals. MRI has the potential to bridge between studies in humans and those in established experimental species, where complementary, highly specific measures are available.

Large-scale cortical gradients were recently put forward as an analytic tool to capture the intrinsic dimensions of cortical organization (Haak et al., 2017; Huntenburg et al., 2018; Margulies et al., 2016). In contrast to parcellation approaches, which emphasize the discreteness of cortical regions or networks (Glasser et al., 2016; Yeo et al., 2011), cortical gradients focus on the significance of overarching spatial patterns in cortical features. The gradient approach was initially applied to functional connectivity data derived from resting state functional magnetic resonance imaging (rsfMRI) in the human cortex. Human functional connectivity gradients have been shown to reflect functional specialization (Haak et al., 2017; Margulies et al., 2016; Peer et al., 2019; Wang et al., 2020), align with proxies of microstructural differentiation (Huntenburg et al., 2017; Larivière et al.,

2019) and predict behavioral symptoms in individuals with autism spectrum disorder (Hong et al., 2019).

Beyond being simply another approach to analyze rsfMRI data, the relevance of cortical gradients derives from their roots in classic theories of cortical organization and evolution. Early neuroanatomical studies described gradients in cortical microstructure and connectivity across species, and postulated that they represent the course of cortical evolution from two primordial origins (Abbie, 1942, 1940; Dart, 1934; reviewed in Pandya et al., 2015; Sanides, 1970, 1962). Based on this legacy, one core aim of contemporary work on cortical gradients is to use modern methods to describe and compare gradients across species. Which fundamental aspects of cortical organization can be found across cortices that widely vary in size and shape? To what extent do gradients represent species-specific organization?

Recent studies in non-human species have described cortical gradients based on tract-tracing connectivity data in the mouse, cat, marmoset and macaque monkey (Buckner and Margulies, 2019; Fulcher et al., 2019; Goulas et al., 2019b, 2019a; Margulies et al., 2016). Many of these studies included additional cortical features such as cytoarchitectonic differentiation or gene expression patterns. Their findings are compelling as they illustrate both similarities and differences across species, and highlight the potential cellular and molecular basis of cortical gradients. One potential limitation of this line of work is that it relies on post-mortem data, likely making it less sensitive to organizational features that reflect different functional modes. In addition, the data is typically averaged across predefined cortical parcels which prevents the detection of spatial patterns that do not adhere to predefined areal boundaries.

In the present study, we complement previous work on gradients in the mouse cortex with an analysis of functional connectivity gradients in vivo. We use four independent rsfMRI data sets, acquired in mice under an optimized anesthesia protocol, and present reproducible cortical gradients. We inspect these functional connectivity gradients with respect to their potential functional significance and their relationship to previously described gradients in structural features.

## Material and methods

### *Animal preparation for imaging*

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee (A*STAR Biological Resource Centre, Singapore, IACUC #161134/171203). A total of 10 female C57Bl6 mice were used. Animal preparation was performed as described in Grandjean et al. (2014). Anesthesia was induced with 4% isoflurane; subsequently, the animals were endotracheally intubated, placed on a MRI-compatible cradle and artificially ventilated (90 breaths/minute; Kent Scientific Corporation, Torrington, Connecticut, USA). A bolus with a mixture of medetomidine (Dormitor, Elanco, Greenfield, Indiana, USA) and Pancuronium Bromide (muscle relaxant, Sigma-Aldrich Pte Ltd, Singapore) was administered subcutaneously (0.05 mg/kg), followed by a maintenance infusion (0.1 mg/kg/h)

while isoflurane was simultaneously reduced and kept to .5%. Care was taken to maintain the animal temperature at 37°C.

*Data acquisition*

Data for the main data set were acquired on an 11.75 T (Bruker BioSpin MRI, Ettlingen, Germany) equipped with a BGA-S gradient system, a 72mm linear volume resonator coil for transmission and a cryoprobe 2x2 phased-array surface coil. Images were acquired using Paravision 6.0.1 software. An anatomical reference scan was acquired using a spin-echo Turbo-RARE sequence: field of view (FOV) = 17 × 9 $mm^2$, FOV saturation slice masking non-brain regions, number of slices = 28, slice thickness = .35, slice gap = .05 mm, matrix dimension (MD) = 200 × 100, repetition time (TR) = 2742 ms, echo time (TE) = 30 ms, RARE factor = 8, number of averages = 2. fMRI was acquired using a gradient-echo echo-planar imaging (GE-EPI) sequence with the same geometry as the anatomical: MD = 90 x 60, in-plane resolution = .19 x .15 $mm^2$, TR = 1000 ms, TE = 11.7 ms, flip angle = 50°, volumes = 180, bandwidth = 119047 Hz. Field inhomogeneity was corrected using MAPSHIM protocol. The animals were imaged over two sessions, each session consisting of three consecutive GE-EPI runs, resulting in 60 runs in total. The imaging parameters for additional data sets used to assess the robustness of the analyses can be found in the respective repositories and publications (see *Data and code availability).*

*Data preprocessing*

A study-specific template was created from the individual anatomical images using the *buildtemplateparallel.sh* script from the Advanced Normalization Tools (ANTs, 20150828 builds, Avants et al., 2011). The study template was subsequently registered to the reference template of the Allen Mouse Brain Atlas (Lein et al., 2007; Oh et al., 2014) common coordinate framework v3 (ccfv3) obtained through the Allen Software Development Kit (Allen SDK, https://github.com/AllenInstitute/AllenSDK). Individual anatomical images were corrected for b1 field inhomogeneity (*N4BiasFieldCorrection*), denoised (*DenoiseImage*), intensity thresholded (*ImageMath*), brain masked (*antsBrainExtraction.sh*), and registered to the study template, resampled to a .2 x .2 x .2 $mm^3$ resolution, using a SyN diffeomorphic image registration protocol (*antsRegistration*). The functional EPI time series were despiked (*3dDespike*) and motion-corrected (*3dvolreg*) using the Analysis of Functional NeuroImages software (AFNI_16.1.15, Cox, 1996). A temporal average was estimated using *fslmaths* (FMRIB Software Library 5.0.9, Jenkinson et al., 2012), corrected for the b1 field (*N4BiasFieldCorrection*), brain masked (*antsBrainExtraction.sh*), and registered to the anatomical image (*antsRegistration*). The fMRI times series were bandpass filtered (*3dBandpass*) to a .01 to .25 Hz band. An independent component analysis (*melodic*) was performed and used as a basis to train a *FIX* classifier (Salimi-Khorshidi et al., 2014) in 15 randomly selected runs. The classifier was applied to identify nuisance-associated components that were regressed from the fMRI time series. The EPI to anatomical, anatomical to study template, and study template to Allen Mouse Brain Atlas ccfv3 registrations were combined into one set of forward and backward transforms (*ComposeMultiTransform*). The combined transforms were applied to the denoised fMRI data.

*Dimensionality reduction of rsfMRI data*

Resting state time series of each rsfMRI run were masked to the isocortex using a downsampled mask from the Allen Mouse Brain Atlas. Data were smoothed within that mask with a Gaussian kernel (FWHM = .45 mm) using Nilearn (Abraham et al., 2014). A functional connectivity matrix was derived by computing the Pearson product-moment correlation between the time series of each pair of isocortical voxels. The individual matrices from all runs were Fisher r-to-z-transformed, averaged across all runs, and back-transformed to Pearson's r values. The average functional connectivity matrix was transformed into a positive similarity matrix which was decomposed through nonlinear dimensionality reduction using diffusion maps (Coifman and Lafon, 2006, implemented in https://github.com/satra/mapalign). In the diffusion map approach, the similarity matrix is first transformed into a Markov chain. We chose a diffusion operator of .5 to approximate transition in the Markov chain as Fokker–Planck diffusion, and render the method less sensitive to nonuniform sampling of the data on the underlying manifold. The eigenvectors of the Markov transition matrix represent the one-dimensional gradients of functional connectivity that are the focus of this study. The gradients are ordered by decreasing eigenvalue, i.e. they capture a decreasing amount of variance in the Markov transition matrix. Following the observation of an elbow in the variance plot we decided to focus our analysis on the first 6 gradients. These gradients were projected onto the isocortical voxels and sampled on a surface mesh representation of the Allen Mouse Brain Atlas for visualization.

*Geodesic distance*

In order to investigate the physical embedding of the functional connectivity gradients in the spatial layout of the cortex, we calculated the geodesic distance from areas of interest. Geodesic distance measures the length of the shortest line between two points on a surface, such that the line lies on the surface. We calculated the geodesic distance based on the surface mesh representation of the Allen Mouse Brain Atlas using the *gdist* package provided by the Virtual Brain Project (Leon et al., 2013).

For the dual origin analysis, we used volumetric masks of the piriform area and the hippocampal region from the Allen Mouse Brain atlas as source regions, and sampled these masks onto the surface mesh. We then calculated the distance of each isocortical surface node to both origins and created a combined distance map by subtracting the distance to hippocampus from the distance to the piriform area for each node (Fig. 1E). We also divided the cortex into two zones, where each surface node was assigned to the archicortical or paleocortical zone, depending on whether it had a shorter distance to the hippocampus or piriform area, respectively. The difference between the distributions of gradient 1 values in the two zones was computed as the statistical energy distance, implemented in Scipy. Significance was assessed using permutation testing with 1000 permutations. To compute the geodesic distance to primary sensory regions, we projected masks of the primary visual, auditory and somatosensory areas onto the surface mesh. We then found the center of each primary sensory region and used all centers as a single source to calculate the geodesic distance to all isocortical surface nodes. The spatial correlation between all distance maps and the functional connectivity gradients was calculated as the Spearman's rank correlation coefficient as implemented in Scipy (Virtanen et al., 2020, v1.3.3). Family-wise error correction for multiple comparisons was performed with a family-wise error rate (FWER)

of .01 using the Benjamini-Hochberg method as implemented in Statsmodels (Seabold and Perktold, 2010 v0.8.0).

*Gene expression*

We were interested whether the intrinsic functional organization of the mouse cortex, as revealed by functional connectivity gradients, was rooted in dominant spatial patterns of gene expression. To address this question we obtained gene expression data provided as part of the Allen Mouse Brain atlas using the Allen SDK interface. The data comprises 3D reconstructed data from RNA in-situ hybridization experiments, targeting 4117 genes expressed in the brain (Ng et al., 2009). We downloaded all available gene expression energy volumes. Since many of the energy maps do not cover all of isocortex, we created a mask containing only isocortical voxels with missing data in less than half of all maps, and only considered data withing that mask. We then averaged maps of experiments probing the same gene, and normalized the data using a sigmoid transfer function (Fulcher and Fornito, 2016) and standard scaling. A principal component analysis was performed on the standardized data and four components were kept, based on the elbow in the variance distribution. We projected these four principal components back into the voxel space and correlated them with the six functional connectivity gradients using Spearman's rank correlation and multiple comparison correction as described in *Geodesic distance*. Components of interest were sampled onto the surface mesh for visualization.

*Cytoarchitectonic differentiation*

To assess the relationship of functional connectivity gradients to cytoarchitectonic differentiation we adopted an ordinal scale of *cortical types* from Goulas et al. (2016). This scale is based on expert annotation of isocortical areas of the Allen Mouse Brain Atlas according to the cytoarchitectonic features of these areas in high-resolution Nissl stained sections (Van De Werd and Uylings, 2014). The cortical type ranges from 1 in agranular areas with a rather undifferentiated laminar pattern, to 4 in highly differentiated eulaminate areas with a pronounced layer IV (see Goulas et al., 2016 for more details). In order to relate this measure to the continuous functional connectivity gradients, we averaged the values for each gradient in each cortical area and computed Kendall rank correlations between gradient values and cortical type using Kendall's tau-b implementation in Scipy. Correction for multiple comparisons was performed as described in *Geodesic Distance*.

*Data and code availability*

The unprocessed mouse rsfMRI data are openly available via the openneuro.org and central.xnat.org repositories. We used the *MEDISO* subjects of the Mouse_rest_awake data set (doi: 10.18112/openneuro.ds001653.v1.0.1, Grandjean et al., 2014) for the main analysis and control animals from the following data sets to assess the robustness of our results: Mouse_rest_3xTG (doi: 10.18112/openneuro.ds001890.v1.0.1, Mandino et al., 2019); fMRI_AD_mouse2 (https://central.xnat.org/data/projects/fMRI_AD_mouse2, Grandjean et al., 2016b) and CSD_MRI_MOUSE (https://central.xnat.org/data/projects/CSD_MRI_MOUSE, Grandjean et al., 2016a). All code used for this manuscript is available on Github at https://github.com/grandjeanlab/MouseMRIPrep (preprocessing) and https://github.com/juhuntenburg/mouse_gradients (analysis and visualization). Standard

templates, parcellations and gene expression data are openly available as part of the Allen Mouse Brain Atlas (https://mouse.brain-map.org/).

## Results

To investigate the intrinsic functional organization of the mouse cortex, high quality rsfMRI data were obtained in ventilated, lightly-anesthetized mice (Grandjean et al., 2014). After careful preprocessing (Fig. S1), a functional connectivity matrix was calculated and decomposed into a set of one-dimensional gradients, each capturing part of the variance in functional connectivity patterns across the cortex. Each gradient can be thought of as a spectrum of functional connectivity similarity: Cortical locations that have similar gradient *x* values resemble each other in the aspect of functional connectivity that is captured in gradient *x*. Cortical locations positioned on opposite ends of gradient *x* differ maximally in this aspect of functional connectivity. When plotting the variance captured by each individual gradient, the first clear “elbow” is visible at gradient 6 (Fig. S2). We therefore focus on gradient 1-6 for the remainder of this study.

### *The principal gradient of functional connectivity recapitulates the dual evolutionary origin of the neocortex*

According to the dual origin theory, the neocortex (or isocortex) evolved in successive waves emanating from two allocortical origins: the archicortex (hippocampus) and the paleocortex (piriform cortex)(Abbie, 1942, 1940; Dart, 1934; Sanides, 1970, 1969, 1962). This process resulted in two trends of progressive microstructural differentiation. The two trends are paralleled by connectivity patterns as cortico-cortical connections preferentially occur within trends or between areas that occupy a similar position along their respective trend (Barbas and Pandya, 1989; reviewed in Pandya et al., 2015; Pandya and Sanides, 1973; Pandya and Yeterian, 1985). The dual origin concept implies a spatial organization where evolutionary older areas are pushed further away from the two origins as new areas emerge, forming a spatial gradient of neocortical evolution. We operationalized this gradient as the combined geodesic distance from the hippocampus and piriform cortex, and investigated whether it is reflected in the first six functional connectivity gradients. Strikingly, gradient 1, which captures the highest individual variance in the functional connectivity data, shows a strong spatial correlation to the distance from the two cortical origins ($r = 0.81$, $P < 0.01$, FWER = 0.01, Fig. 1B and E). When dividing the cortex into two zones based on the minimal distance to either origin, gradient 1 values between the paleocortical and archicortical zone differ significantly (statistical energy distance = 0.25, $P < 0.01$, permutation tests, Fig. 1D). These results provide evidence for a fundamental link between the intrinsic functional organization of the mouse cortex and the course of its evolution from two primordial origins.

### *Gradients reflecting functional hierarchies between sensory and transmodal cortex*

Functional processing hierarchies are an established concept for the sensory cortex (Felleman and Van Essen, 1991) and have long been discussed as a more global concept extending to transmodal regions (Mesulam, 1998). Indeed, recent studies on human cortical organization have consistently found a global gradient that ranges from sensorimotor cortex, through heteromodal, to transmodal areas. This gradient describes a functional hierarchy of

increasing abstraction in the temporal (Baldassano et al., 2017; Hasson et al., 2008; Lerner et al., 2011), semantic (Huth et al., 2016) and spatial domain (Peer et al., 2019) and in a meta-analysis of a multitude of task concepts (Margulies et al., 2016). It also aligns with proxies of decreasing microstructural differentiation (Burt et al., 2018; Huntenburg et al., 2017). We explored the current data set for similar global patterns in the mouse cortex and found two functional connectivity gradients, gradient 2 and 5, that capture two aspects of a sensory-to-transmodal cortical hierarchy (Fig. 2A). Similar to the principal gradient in humans (Margulies et al., 2016), gradient 2 and 5 are correlated to the geodesic distance from centroids in primary somatosensory, visual and auditory areas ($r_{Gradient2} = 0.43$ and $r_{Gradient5} = 0.60$, $P < 0.01$, FWER = 0.01, Fig. 2B).

The transmodal extreme end of gradient 2 lies in medial and prefrontal regions such as the anterior cingulate cortex, retrosplenial, prelimbic and infralimbic areas, and the frontal pole. The sensory extreme on the other end comprises trunk and lower limb areas of primary somatosensory cortex as well as primary visual, auditory and motor cortex. Lateral regions such as the agranular insula, ecto- and perirhinal area fall in between these extremes. Of note, parts of the mouth, nose and barrel field primary somatosensory areas also cluster in the middle or even towards the “transmodal” end of gradient 2, somewhat defying the simple sensorimotor-to-transmodal description. Along gradient 5, the sensory regions cluster more clearly towards one end of the gradient. The transmodal extreme here includes prefrontal and lateral regions, while the medial retrosplenial areas are shifted more towards the sensory regions. Another clear difference to gradient 2 is that the motor regions fall on the “transmodal” end of gradient 5. Similar to the sensorimotor-to-transmodal gradient in the human cortex (Burt et al., 2018), gradient 5 is strongly correlated to the second principal component of cortical gene expression patterns ($r = 0.65$, $P < 0.01$, FWER = 0.01, Fig. 2C). Note that the first principal component of gene expression in our analysis reflected layer-specific patterns. The second principal component therefore captures the largest amount of transcriptomic variance parallel to the cortical sheet.

To assess their relationship to microstructural differentiation, we compared the six functional connectivity gradients to the ordinal scale of cytoarchitectonic type from Goulas et al. (2016) (Fig. 2D), and found a moderate association for gradient 2 and 5 ($\tau_{Gradient2} = -0.40$ and $\tau_{Gradient5} = -0.36$, $P < 0.01$, FWER = 0.01). Collectively, these results suggest that a global functional processing hierarchy from sensory to transmodal areas is an important feature of functional organization in the mouse cortex. In contrast to similar studies in humans, this general pattern is represented in two gradients that emphasize either medial or lateral aspects of transmodal cortex. Both gradients are associated with cytoarchitectonic differentiation, and one of them matches a dominant transcriptomic pattern.

*Gradients highlighting the sensory specialization of the mouse cortex*

In rodents like the mouse, extensive parts of the cortex are dedicated to sensory processing, and their most specialized sensory systems are most prominently represented. For example, mice heavily rely on their whiskers for spatial exploration and, correspondingly, the relevant barrel field area represents 19 % of their primary somatosensory cortex. The dominance of sensory areas is in contrast to the cortex of primates, especially humans, where hetero- and transmodal regions have massively expanded (Buckner and Krienen, 2013; Diamond and Hall, 1969). In line with the importance of sensory processing in mice, we found that the

remainder of the six considered functional connectivity gradients reflect a high level of specialization within sensory cortex.

Gradient 3 separates somatosensory (and motor) areas at one extreme end from visual and auditory regions at the other (Fig. 3A). This organization is remarkably similar to the second gradient found in the human cortex (Margulies et al., 2016) and the first gradient found in the macaque and marmoset cortex (Buckner and Margulies, 2019; Margulies et al., 2016). In the mouse cortex, this gradient also shows a substantial association with the third principal component of cortical gene expression patterns ($r = 0.61$, $P < 0.01$, FWER = 0.01, Fig. 3B). In figure 3E we show how gradient 3 and 5 represent two separate dimensions of organization in the mouse cortex, which capture previously established cortical clusters (here adapted from Harris et al., 2019) without imposing discrete boundaries. These two dimensions are reminiscent of the two major gradients described in the human cortex (Huntenburg et al., 2018). Gradient 4 and 6 capture variance associated with an even higher level of sensory specialization and emphasize some of the most important sensory inputs for the mouse. Within primary somatosensory cortex, gradient 4 separates the barrel-field and nose areas from the trunk and lower limb areas (Fig. 3C & F). Gradient 6 singles out the auditory cortex versus all other sensory regions (Fig. 3D & G). Together, these three gradients point to a strong dissociation of different sensory dimensions in the mouse cortex. They highlight the importance of somatosensory areas, particularly those representing the barrel field and nose, and of the auditory cortex.

*Robustness of functional connectivity gradients across different data sets*

In order to assess the robustness of our results, we performed the same preprocessing and dimensionality reduction in three additional rsfMRI data sets, comprising 70, 32 and 20 functional runs, respectively. We found that all data sets showed a clear dominance of gradient 1 in terms of variance represented (Fig. S2). This principal gradient was strikingly robust in its spatial pattern, reflecting the dual origin of the neocortex in all four data sets (Fig. S3). Two of the three test data sets showed an elbow in individual variance at gradient 6, exactly like the main data set, the third test data set had an earlier elbow at gradient 4 (Fig. S2). Gradients 2-6 had a somewhat higher variability across data sets. However, we made the following observations: The *sensory specialization* gradients (3,4 and 6 in our main analysis) were found in all test data sets, only their order differed across data sets (Fig. S4). We also found one or two *sensorimotor-to-transmodal* gradients (2 and 5 in our main analysis) in all three test data sets. These gradients showed some diverging spatial patterns compared to our main data set so that there was no clear one-to-one mapping between data sets. Taken together, these results demonstrate that the general aspects of the gradient organization described above are present in all four analyzed data sets. The principal gradient is highly reproducible across all data sets. The sensory specialization gradients are also stably represented across data sets while the sensorimotor-to-transmodal gradients show more variance.

## Discussion

We investigated the intrinsic functional organization of the mouse cortex through resting-state functional connectivity gradients. The most robust organizational feature was found to be a

prominent gradient reflecting the spatial progression of cortical evolution from archi- and paleocortical origins (Fig. 1 & S3). Several stable gradients represent highly specialized sensory modalities in the mouse cortex (Fig. 3 & S4). Other spatial features indicate a global functional hierarchy of increasing abstraction from sensory input, and decreasing cytoarchitectonic differentiation (Fig. 2 ).

*Intrinsic functional organization reflects the dual origin of the mouse cortex*

We found that the principal gradient of functional connectivity reflects the dual origin of the mouse neocortex. This finding resonates with a recent study demonstrating a fundamental division of the mouse cortex into an archicortical and a paleocortical zone based on tract-tracing data (Goulas et al., 2019b). These authors also showed, that the dual organization was reflected in physical distance and gene expression data. Here, we used rsfMRI data acquired in vivo, and did not apply a cortical parcellation. This approach enabled us to complement the aforementioned findings with a gradient of functional connectivity, that captures the continuous progression from the two cortical origins, rather than dividing the cortex into discrete zones. This principal gradient was reproduced across four independent data sets (Fig. S2 & S3) and provides robust evidence that the intrinsic functional organization of the mouse cortex in vivo is strongly influenced by the course of neocortical evolution. Interestingly, studies on the intrinsic functional organization of the human cortex so far have not found a gradient clearly representing the dual origin axis. One possible reason is that it is harder to operationalize such a gradient based on simple geodesic distance from allo- and paleocortex, given the complex shape and folding of the human cortex. Another explanation could be that the human cortex is evolutionary further removed from its allocortical origins. It has been proposed that the massive expansion of transmodal areas in humans has *untethered* these areas from the influence of molecular gradients that constrain the organization of sensory regions (Buckner and Krienen, 2013). According to this hypothesis, the large transmodal regions of the human cortex are governed by different mechanisms and constraints than the sensory areas dominating the rodent cortex. Thus, while the distance from archicortex and paleocortex appears to be a constitutional feature of the rodent cortex, it might have been superseded by other organizational aspects in the human cortex.

*Global functional hierarchies and sensory specialization*

A core organizational aspect in the human cortex is a progression from sensorimotor to transmodal areas that reflects a global functional processing hierarchy (Glasser et al., 2016; Huntenburg et al., 2018; Margulies et al., 2016). We found aspects of a similar pattern represented in two separate gradients in the mouse cortex (Fig. 2). Gradient 5 in particular, overlaps with the previously described spatial distribution of T1w:T2w, a proxy for intracortical myelination (Fulcher et al., 2019). Similar to our findings, Fulcher et al. (2019) show that the sensory-to-transmodal progression is associated with decreasing cytoarchitectonic differentiation, and with a dominant axis of variance in gene expression. However, the sensory-to-transmodal pattern in the intrinsic functional organization of the mouse differs in important ways from the respective findings in humans. We found that the gradients representing a sensory-to-transmodal progression in the mouse capture relatively less of the variance, and are more variable in their exact layout, than is the case for the human cortex.

Instead of a single robust sensorimotor-to-transmodal gradient, the variance associated with this type of organization is spread across at least two gradients (in the main analysis gradient 2 and 5, Fig. 2A) – one emphasizing medial and one lateral transmodal regions, one clustering motor areas with the sensory, the other with the transmodal extreme. In turn, several gradients and their associated variance in the mouse cortex are robustly associated with the dissociation and specialization of different sensory modalities (gradient 3,4,6, Fig. 3 & S4). As pointed out in the previous section, these differences might reflect the extent and importance of transmodal versus sensory regions in the two species. The behavior of mice is strongly influenced by direct input from their environment. Presumably, this translates to an intrinsic functional organization of their cortex that is dominated by sensory processing, driven by thalamic inputs. Humans, in contrast, have evolved to heavily rely on cognitive capacities that require abstraction from direct sensory input and are typically associated with computations in transmodal regions. It is plausible that such processing requires a strong emphasis on a sensory-to-transmodal organization, defined by cortico-cortical connections.

It has been proposed that the sensorimotor-to-transmodal functional hierarchy is rooted in the variation of microstructural differentiation across the cortex, chiefly the thickness and architecture of supgranular versus infragranular layers or “externopyramidization” (Goulas et al., 2019a, 2018; Sanides, 1962). While the phenomenon of externopyramidization is pronounced in the human cortex, the mouse cortex is comparatively homogeneous in this aspect, further speaking to a less crucial role of the sensory-to-transmodal axis. The degree of externopyramidization increases progressively along the phylogenetic tree from rodents to marmosets, to macaque monkeys, to humans (Goulas et al., 2019a). Interestingly, the emphasis on a sensorimotor-to-transmodal gradient appears to follow the same trend: While we found no singular clear-cut sensorimotor-to-transmodal gradient in the mouse cortex, such gradients have been described in the marmoset (Buckner and Margulies, 2019) and macaque monkey (Margulies et al., 2016) based on connectivity data from tract-tracing experiments. However, in contrast to the human cortex, the sensorimotor-to-transmodal pattern was represented in the secondary gradient in those species, while the prominent principal gradient reflected the dissociation of different sensory systems. In line with the idea that ontogenetic development reproduces evolution, the sensorimotor-to-transmodal gradient also becomes increasingly important and pronounced during human development (Larivière et al., 2019; Paquola et al., 2019).

In summary, the intrinsic functional organization of the mouse cortex is strongly influenced by the specialization of sensory areas. This is in contrast to the human cortex which is more strongly governed by a sensorimotor-to-transmodal axis. This axis is present, yet less pronounced in the mouse cortex. Findings in different species and across development suggest that the sensorimotor-to-transmodal differentiation becomes increasingly important with the extension of the transmodal cortex and a growing reliance on information integration and abstraction.

*A new perspective on the functional organization in animal studies*

What is the advantage of the gradient approach for studying cortical organization? We have shown here that gradients can reveal organizational patterns that do not respect areal boundaries (most strikingly the principle gradient) and might be missed when imposing classic parcellation schemes. Without doubt, parcellations and the assignment of specific

functions to discrete areas can be an extremely useful tool. And after all, we base much of the interpretation of the different gradient patterns on the known function of different areas. However, the functional assignment approach can also encourage oversimplified one-to-one mappings. This is particularly true in animal research, where highly controlled behavioral tasks and specialized measurement techniques sometimes prompt a mapping of individual task variables to small populations of cells. Gradient-based analyses, instead, produce superimposed representations that capture dominant patterns while also preserving information about other, coexisting functional dimensions. We apply this approach to resting-state functional connectivity data here, but believe that the general framework can be useful for many types of contexts and data. An excellent example is a recent study recording single cell responses in the posterior cortex of mice performing a visuomotor navigation task (Minderer et al., 2019). Using two-photon imaging, Minderer et al. show that task-relevant features are encoded in wide-spread gradients, rather than confined areas. Differences across the cortex are expressed in the weights of feature combinations and show, for example, that multimodal integration is most pronounced in regions where gradients encoding visual and motor aspects of the task overlap. The authors emphasize that we might miss much of the information in our data when confining our analyses to overly simplistic functional mapping, in particular when studying complex tasks or parts of the cortex that we know little about. We agree with this perspective and believe that gradient-based analyses are one important way of extracting more information from the increasingly complex data being acquired.

*Limitations and open questions*

We used rsfMRI data to study the intrinsic functional organization of the mouse cortex in vivo. In contrast to post-mortem studies, this approach allows us to obtain data from the entire, intact and functioning cortex of each animal. However, imaging living animals comes with its own challenges. Physiological processes can introduce artifacts such as magnetic field distortions caused by air filled sinuses or movements associated with heart beat and breathing, which can only partly be removed during preprocessing. Furthermore, high-quality fMRI in rodents requires the animals to be lightly anesthetized. Here we used an optimized anesthesia strategy, aided by mechanical ventilation (Grandjean et al., 2014). While this protocol provides stable anesthesia and minimizes effects on spontaneous cortical activity, the anesthetic agents inevitably affect the physiological and brain state of the mice. It is possible that this modulation underlies some of the observed differences to findings from human rsfMRI performed in the awake state. To reduce the chance that our results reflect phenomena specific to our data, rather than meaningful organizational principles, we cross-reference the rsfMRI findings with independent measures and previous studies, and reproduce them across four independent data sets.

Another point that warrants reflection is the interpretation of the gradients presented here. Although our analyses are largely data-driven and exploratory, our interpretations are obviously influenced by our assumptions and the literature we consulted. Alternative interpretations might be equally plausible. For example, the association of gradient 1 with distance from paleocortex (piriform cortex) could also reflect the role of piriform cortex as the primary cortex of the olfactory system, one of the dominant senses in the mouse. Which of

several possible interpretations will turn out to be most convincing, can only be settled by accumulating more evidence in future studies.

## Conclusion

We studied the intrinsic functional organization of the mouse cortex through gradients of functional connectivity. Our work complements previous studies on cortical organization in the mouse by focusing on superimposed, continuous gradient patterns extracted from in vivo rsfMRI data. We interpreted our findings in the context of the dual origin theory and cortical gradients described in other species, and conclude that a shift in gradient patterns occurs along the phylogenetic tree. Robust and dominant patterns in the mouse cortex display a strong link to the evolutionary origin of the neocortex, and highlight sensory specialization. In humans the emphasis has shifted to a core sensorimotor-to-transmodal pattern underlying a global hierarchy of information integration.

CRediT author statement:

JMH - Conceptualization, Methodology, Software, Validation, Formal Analysis, Writing - Original Draft, Visualization, Funding acquisition
LYY - Investigation
FM - Investigation
JG - Methodology, Software, Validation, Investigation, Resources, Data Curation, Writing - Review & Editing, Supervision, Funding acquisition

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## Figures

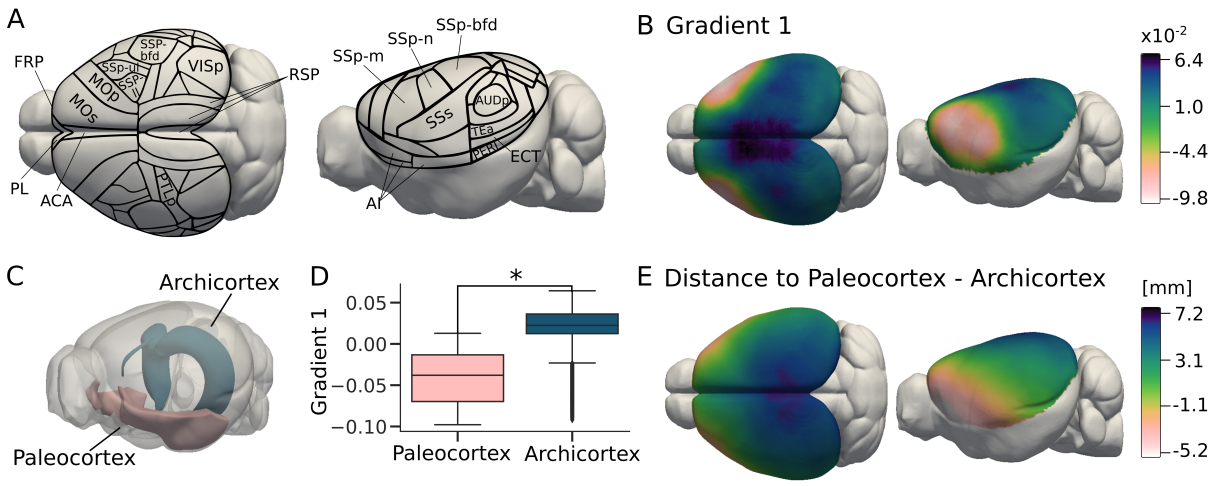


**Fig. 1.** The principal gradient and the two neocortical origins. A) Cortical parcellation based on the Allen Brain Mouse Atlas. B) The principal gradient of functional connectivity sampled on the surface mesh. Note that the gradients are abstract dimensions and have no units. C) Mesh representations of the hippocampal area (archicortex, *petrol*) and piriform area (paleocortex, *rose*), that were used as source regions to calculate geodesic distance to the two neocortical origins. D) The cortical surface was divided into two groups based on the minimal distance of each surface node to either paleocortex or archicortex. Shown are the gradient 1 values in either zone (* P<0.01, permutation tests). E) Map of the combined geodesic distance, obtained by subtracting distance to archicortex from distance to paleocortex. Negative values indicate that a surface node is closer to the paleocortex than to the archicortex and vice versa.

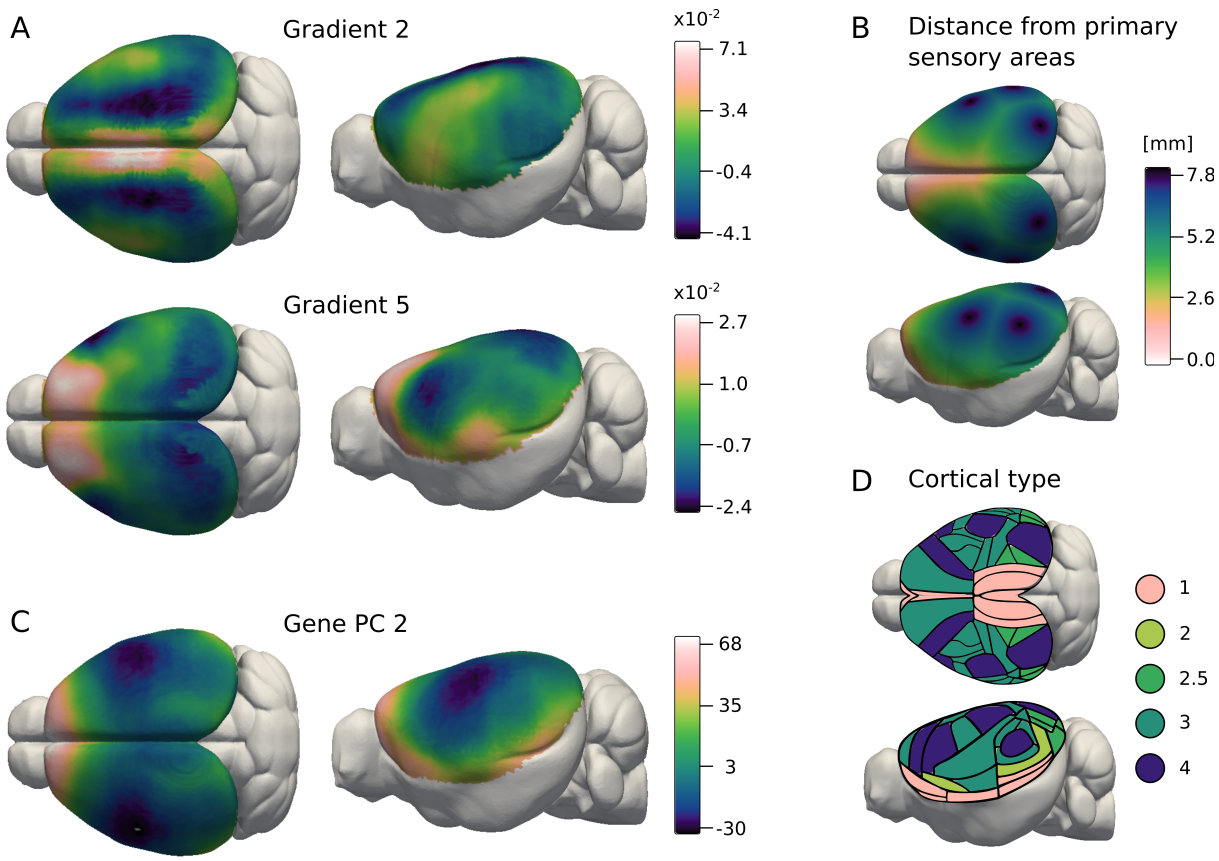


**Fig. 2.** Sensory-to-transmodal gradient patterns. Functional connectivity gradient 2 and 5 (A) show a general sensory-to-transmodal organization that is associated with the geodesic distance from primary sensory centers (B). The second principle component of gene expression patterns (C) shares prominent spatial features with gradient 5. A nominal scale of cytoarchitectonic differentiation (D) is negatively related to both sensory-to-transmodal gradients.

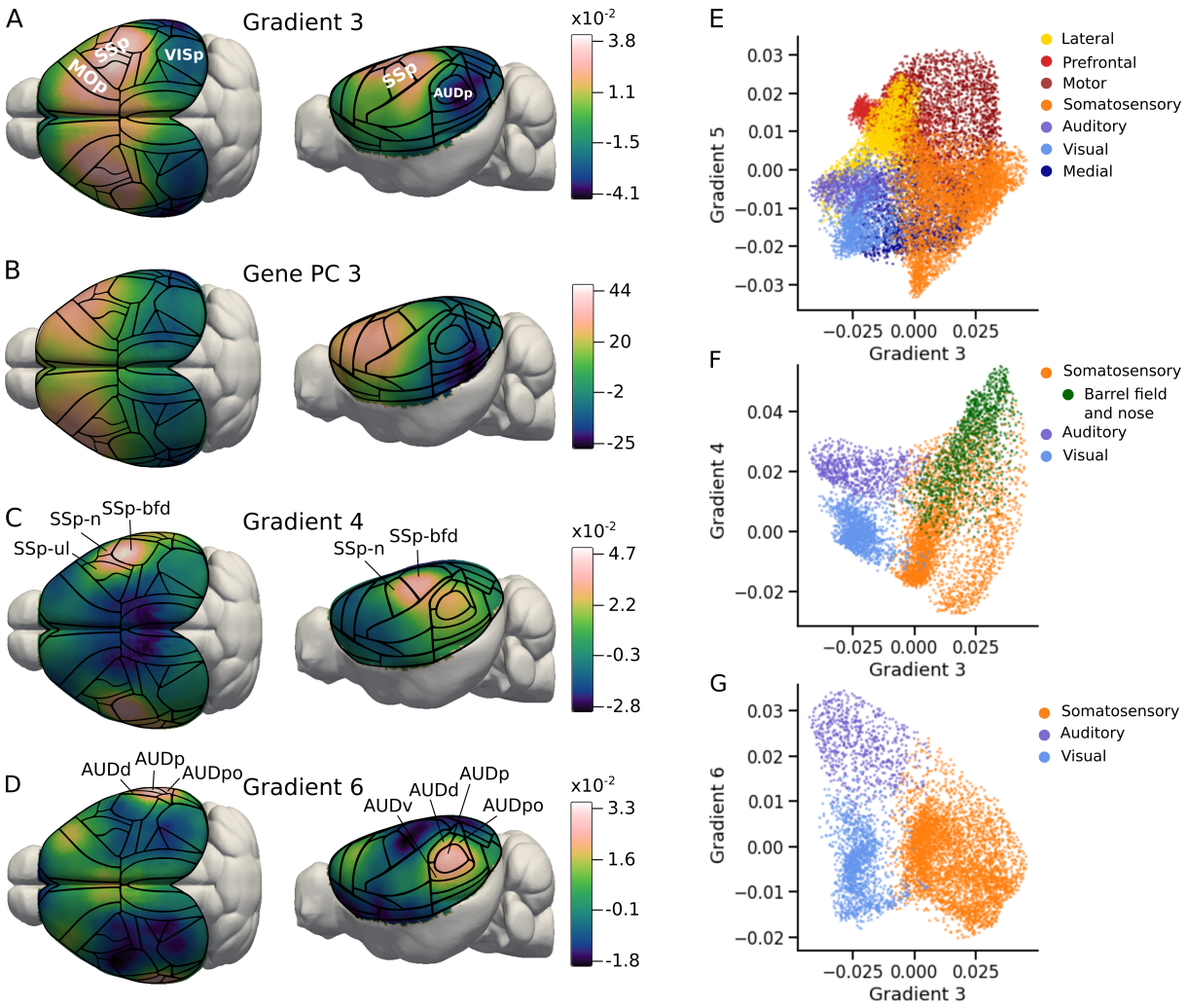


**Fig. 3.** Gradients of sensory specialization. A) Gradient 3 reflects a dissociation between somatomotor cortex and auditory/visual areas. This pattern strongly resembles the third principal component of gene expression (B). C) Gradient 4 highlights the barrel-field and nose area of the primary somatosensory cortex. D) Gradient 6 separates auditory areas from the rest of the cortex. E-G) Scatter plots of different gradient combinations help to visualize the aspects of functional organization captured in these gradients. The cluster assignment has been adapted from Harris et al. (2019). In order to highlight features of sensory specialization, only sensory clusters are plotted in F) and G).