Cortical Field Complexity: Comparative Analysis of Cortical Field Sizes in Relation to Ethology and Sensory Specialization across 6 Clades of Mammals

By

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Abstract

Comparative studies provide important insights into the evolution of general features of brain organization as well as features that are unique across species. These unique differences in brain organization are thought to reflect specialized adaptations in body morphology, behavior and lifestyle. While there is a great diversity of mammalian behavioral and morphological specializations, we know little about evolutionary changes in the neocortical structures that support these differences. Using histological collections from the Krubitzer lab, a comparative dataset was created in order to measure the sizes of cortical fields across evolution. In this analysis we focus on 4 cortical fields, the primary somatosensory (S1), visual (V1) and auditory (A1) areas, as well as the motor cortex (M1). These areas were examined in 18 species representing 6 different mammalian lineages: 7 species of Rodentia (rodents), 5 species of Marsupialia (marsupials), 2 species of Chiropotera (bats), 2 species of Eulipotyphla (water shrews and hedgehogs), 1 from Scandentia (tree shrews) and 1 from Afrosoricida (tenrecs). We quantify the lineage-specific changes and general properties of the sizes of primary motor and primary sensory cortical fields using allometric analysis, and discuss several outliers from the trends. We found that larger brains with a larger neocortex devote less space to M1, A1 and S1, whereas V1 becomes exceptionally larger, or alternatively exceptionally smaller in mammals with a small neocortex. We also found several examples of mosaic evolution in S1 and one example in A1. The neocortex is the underlying substrate that supports adaptive sensorimotor specializations, yet this varies across species. We found that not all convergent sensorimotor specializations share the same underlying expansions and contractions of cortical fields, suggesting that there are multiple ways the neocortex can organize to “solve” for the same specialization in different species.

Introduction

How might a complex neocortex have emerged in evolution, and can we find homologous and/or convergent neocortical structures that support different theories of evolutionary change as we examine different phylogenetic lineages? The neocortex is a uniquely mammalian structure that is partitioned into multiple sub-regions, termed cortical areas or cortical fields. It is considered crucial for faculties like cognition, learning, and memory, as it facilitates parallel distributed processing as well as inference and generalization, having a direct role in our capacity for language, memory recall and extracting latent semantic structures from experiences (McClelland and O'Reilly 1995; Tomasello et al., 2017). Cortical fields, subregions of the neocortex, were originally defined by early anatomists such as Brodmann using architectonics (e.g. myelin stain, Nissl stain; Brodmann, 1909). Subsequently, in the middle of the 20th century, electrophysiological recording studies revealed that these architectonically defined cortical areas were also functionally distinct and topographically organized (Woolsey et al., 1952). Studies led by the Kaas lab further refined the definition of a cortical field suggesting that these functional and architectonically distinct regions of the neocortex also had unique patterns of connectivity (Kaas, 1995 for a review). Properties of these fields, such as cell types and densities, developmental trajectories and connectional networks vary within each cortical area as well as across evolutionary lineages.

The expansion of the neocortical sheet has arisen independently in multiple lineages (Krubitzer, 2007; Kaas, 2011; Krienen, 2020). While there are representatives of expanded neocortical sheets across clades, the types of these expansions, their

locations, sizes and the processes of development that generate them can be unique to each species- even within closely related species such as non-human primates and humans (Halley and Krubitzer, 2019). In the primate order there are differentially expanded regions of cortex such as posterior parietal cortex (PPC), inferotemporal cortex (IT) and frontal cortex, which all support different higher-order functions (Van Essen and Dierker, 2007; Reardon et al., 2018; Chaplin, 2013). For example, in humans a greater percentage of neocortical space exists between primary cortical fields (which includes the areas listed above; Krienen et al., 2020). Therefore, measures of cortical field extent, particularly the relative size of primary areas compared to non-primary areas, have the potential to reveal general principles of areal scaling as functions of environmental factors, behavioral dynamics, and sensory system adaptation across evolution.

Important structural and functional properties of *primary* neocortical fields include: A close degree of connection to sensory periphery, topographic structure and ubiquity across the mammal lineages. Further computational properties include: influence over information flow into other parts of the neocortex, dynamic scaling in size, response to the activity of sensory structures, specialization for information that is behaviorally relevant and dynamic changes in what information is being represented based on differences in development and adult experience (cross-modal plasticity and cortical “remapping”). Primary cortical fields are dynamic across long-term evolutionarily time spans and within the short-term lifespans of individuals (Braun et al., 2001; Ejaz et al., 2015; Kolasinski et al., 2016). Primary cortical fields also typically have very dense connections with thalamic nuclei that receive direct or indirect inputs from the sensory

periphery, in contrast with secondary and multimodal fields. For example, the primary visual cortex receives sensory information from the lateral geniculate nucleus of the thalamus which receives direct input from retinal ganglion cells. Primary cortical fields also have a topographical organization in which adjacent portions of the sensory receptor array are represented on adjacent portions of the cortical sheet. For example, the primary auditory area (A1) preserves a topographic structure in tonotopy from the cochlea, the primary somatosensory area (S1) represents adjacent portions of skin in somatotopy (although the somatotopic organization is sometimes discontinuous), and the primary visual area (V1) represents the contralateral visual hemifield in visuotopy. The primary motor area (M1) also preserves somatotopy, although the topography is fractured (Halley et al., 2022).

In many computational models of neocortical organization, primary cortical fields serve as the basis for the formation of more complex, multimodal, “higher-order” fields. How novel, higher-order neocortical fields emerge over evolutionary and developmental time remains a fundamental question in neuroscience. The self-organization of new fields can be modeled as the topographic structure of primary visual cortex propagating outwards to form secondary visual cortical fields during development (Imam and Finlay, 2020). Others have theorized that novel, more derived fields emerge between overlapping topographically organized structures radiating outwards from two or more fields (Rosa, 2002), and that these should be more variable across species since they are activity-dependent, less genetically determined and tend to arise later in development (Reiner Schulz and Reggia, 2005). Some have debated whether topographical structure is anything more than a spandrel resulting from an optimization

of wiring lengths (Wilson and Bednar, 2015), though still others imply that topographic relations must themselves be functional because they are preserved and dynamically routed into higher-order fields (Olshausen et al., 1993). Reversible inactivation studies (Girard et al., 1989) have shown that in primates there is a distributed hierarchy of activations across fields, with primary fields at the root of the tree, feeding activations forward, even though there are both feedforward and feed-backward cortico-cortical connections between primary and secondary fields (Felleman et al., 1991; Girard et al., 2001; Anderson et al., 2009,). This module aggregation hypothesis suggests that novel or more derived fields come from smaller modules responding to new inputs that arise along existing cortical field boundaries. These new modules eventually aggregate over time into a new cortical area, in concert with evolutionary selective pressures shaping an animal's ability to collect particular sensory information (Krubitzer, 1995). Essentially, it is theorized that higher-order areas and their formation depend on input from primary field allocation and connections.

Primary fields represent one essential feature with which to make meaningful, rigorous comparisons across species in terms of homology and convergence/divergence, not only because of the distinct structural properties outlined so far, but also because of their ubiquity across the mammalian lineages. Higher-order areas across species are often difficult to compare due to their variability, specificity and lack of shared structural properties (such as in connections, myelination patterns and types of neurons). By quantifying how much change occurs in primary fields we may be able to make inferences about how many of these more activity-dependent regions arise and how they differ across lineages.

Although their presence is ubiquitous across species and structurally distinguishable, primary fields are nonetheless variable in size and shape, over evolutionary lineages. This phenotypic plasticity of primary fields suggests that there is selection for the cortical fields (and the corresponding sensory system) to represent the specific features of sensory input. Parts of primary cortical fields can magnify or contract, as well as specialize for specific parameters of information from a given sensory structure. In many cases these changes appear to support behaviorally relevant features of the ethology of the particular species. For example, in certain species of echo-locating bats (*Pteronotus parnellii*, *Pteronotus quadridens*, *Carollia perspicillata* and *Rhinolophus rouxi)*, the dorsal auditory cortex has echo-delay topography called “chronotopy” because the temporal length of echo-delays are represented topographically (Hechavarría et al., 2013). This organization supports strong activations for short-delay responses that indicate relevant events such as potential collisions and prey capture. In contrast, mice, which are known to use frequency modulated vocalizations in the ultrasonic range, have a cortical region arguably within or near to the primary auditory cortex (region UF) which is not tonotopic (Honma et al., 2013). It seems that for certain parameters of sensory information to become highly organized in the primary auditory cortex, multiple behaviorally *crucial* factors may need to depend on this parameter (e.g. catching prey and navigating away from immediate collisions versus social communication, though this can be crucial in some cases as well). On the other hand, mice, a species that relies heavily on whisking for navigation, have highly specialized whisker-sensitive modules in the somatosensory cortex, known as barrels, which encode complex neighboring inter-whisker contacts

(Brecht et al., 2003; Fox et al.). The duck-billed platypus has an expanded S1 representation that supports mechanoreception and electroreception (Krubitzer et al., 1995), which is crucial to hunting for prey underwater, mating and navigation (Pettigrew, 1999). These specializations indicate that primary neocortical field organization is not strictly anatomically mapped from sensory periphery, but also, by computational means, organizes and specializes in such a way as to represent the functionally most relevant parameters from a set of possible inputs out of any given anatomically based sensory periphery.

Primary cortical field sizes are not only variable across species due to both mosaic and concerted evolution, but they are also highly dynamic within the lifetime of a single individual, known as cortical plasticity. The neocortex is rapidly modulated by activity-dependent and attentional processes, called “remapping,” that can occur over days (Braun et al., 2001; Ejaz et al., 2015; Kolasinski et al., 2016) to lifetimes. While in humans it is known that hands and face representations are magnified relative to the trunk (Penfield and Boldrey, 1939), this homuncular map is not static across individuals and can be altered in proportion to the amount of experience spent with a given activity (e.g. learning to play a stringed instrument can alter the representation of the hand; Elbert et al., 1995). Skilled learning processes in adult owl monkeys can generate reorganization of the hand representation, specifically in area 3b of the primary somatosensory cortex following differential tactile stimulus of the hands (Jenkins et al., 1990; Recanzone et al., 1992). Notably, portions of the hand that were trained on a tactile discrimination task had expanded representation in the somatosensory cortex. Also, imposed digital syndactyly and selective deafferentation can change the

representation of the hand (Kaas et al., 1983; Allard et al., 1991). Essentially, cortical fields are dynamic and can reorganize in such a way as to optimally reflect most recent stimulus driven activity, representing those features of the statistics in experiences that are most necessary to an individual in a given context.

In addition to magnification and contraction of representations within them, functional properties can also be repurposed (a process termed cross-modal plasticity). For example, in the blind mole rat (*Spalax ehrenbergi*), the primary visual cortex has been co-opted by the auditory system (Doron et al., 1994; Bavelier and Neville, 2002). In short-tailed opossums (*Monodelphis domestica*) that have been experimentally bilaterally enucleated prior to the formation of thalamocortical connections, V1 is reduced in size but is still present, and is functionally co-opted by the auditory and somatosensory system (Kahn and Krubitzer, 2002). In congenitally blind humans, a visual area in the occipital lobe responsible for navigation in sighted humans (area OPA), is recruited to support echo-acoustic spatial navigation in echo-locating blind individuals, a skill which can be spontaneously discovered or learned through training (Norman and Thaler, 2023). Studies and debates around the fusiform face area (FFA), a higher order area that represents a complex topographical space of faces along 3 axes in the ventral visual stream, has revealed that even in humans who are blind since birth, haptic experience with faces will activate FFA, meaning that visual experience is not necessary for face-selectivity but rather the FFA will use any kind of available sensory inputs for identification of an individual's face (Ratan-Murty and Kanwisher, 2020).

Although there are only a few large data sets that have compared the brains of a wide range of mammals, these studies have informed us of foundational aspects of

brain evolution. Relationships investigated thus far include: the relationship between complex behavioral repertoires and sizes of neural structures (Jerrison, 1974); the scaling relationships of cortical fields in 30 species of mammals (Kaskan, 2005); the changes in neurodevelopmental schedules related to size changes (Finlay et al., 2001); and the relationship between cortical expansion and the size of the thalamus (Halley and Krubitzer, 2019). In nearly all species examined we can find variation in the scaling of primary fields that correspond to ethology, such that behavioral relevance seems to drive variation in neocortical partitioning, supporting the concept of mosaic evolution (changes in scaling that are independent of overall changes in brain size). However, among large datasets it is also evident that overall brain size is a strong predictor of the size of other brain structures (concerted evolution), and can even predict social group size in some mammals (primates, carnivores and some Eulipotyphla, see Dunbar and Bever, 1998). Thus, when analyzing sizes of brain structures, concerted evolution is also fundamental (Stephan et al., 1981; Finlay & Darlington 1995; Finlay et al., 2001; Strideter, 2005; Kaskan et al., 2005; Charvet et al., 2013; Halley and Krubitzer, 2019).

Despite these handful of important studies, fundamental questions about the evolutionary dynamics producing neocortical fields remain. Novel techniques in emerging fields of neuroscience such as neuroepigenetics, single-cell transcriptomics and proteomics, use the molecular signatures of a given area to compare across species and are beginning to investigate a broad extent of the evolutionary tree (for an example see Laurent et al., 2019). We hope that databases on molecular signatures of neocortical fields can eventually be correlated with cytoarchitectonic measures, such as the one presented here. Other questions remain: for example, how does within lifetime

neocortical reorganization and cross-modal plasticity interact with epigenetic factors and how do these changes become incorporated into the genome (Figure 1) (Maya-Vetencourt and Pizzorusso, 2013; Krubitzer and Stolzenberg, 2014)? Similarly, how and why more activity-dependent plasticity might be present in some species and in some areas of the brain rather than others, remains unknown. More or less plasticity in cortical fields might emerge based on a given species's exposure to a particular environmental factor such as more environmental variability through enrichment (Praag et al, 2000), or environmental uncertainty and stress- which is known to increase mutation rates (Badyaev, 2005; Jablonka, 2017). Computational models have predicted interactions of learning capacity, phenotypic plasticity and trajectories through evolutionary state spaces, named the "Baldwin effect." (Baldwin, 1896; Hinton and Nowlan, 1987; Bateson, 2004; Mery and Kawecki 2003; Wilson and Prescott, 2022). It is known that specialized sensorimotor behaviors such as echolocation and manual dexterity have independently evolved in different lineages of mammals (Krubitzer and Padberg, 2007; Madsen and Surlykke, 2013; Liu et al., 2014) but it is not clear if cases

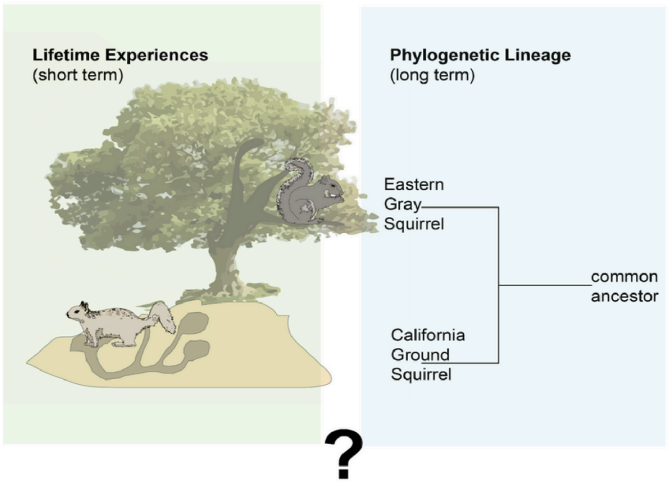


Figure 1: Timescales contributing of change in cortical organization. Cortical fields can be highly dynamic, adapting to unique body morphologies, behavioral specialization and ecological niches, even with the lifetime of an individual. We include closely related species that have very different ethology: the California ground squirrel(Otospermophilus Beecheyi) nests in underground burrows and eastern gray squirrel(Sciurus Carolinensis) nest arboreally. Remarkably both species show an expanded visual cortex which is evidence of a phylogenetically conserved trait in their lineage, and diverges from other rodents. Questions remain as to how these two timescales of change influence each other.

of convergent evolution in behaviors are always supported by the same or similar convergent neurobiological structures.

To address these questions and more we have created a new comparative dataset on cortical field size, relative to the total cortical sheet for the primary motor area (M1), and primary somatosensory (S1), auditory (A1), and visual (V1) areas in a sample of 18 diverse mammalian species, representing 6 different lineages. Each of the samples of extant species in our datasets represents an evolutionary “snapshot” across a dynamic evolutionary history (Krubitzer, 2009) as well as a dynamic lifetime experience “snapshot,” since we have chosen to include some of the same species raised in radically different environment conditions (e.g. wild caught vs laboratory reared, see Table 1). By analyzing size

Order	Species	Common Name	Sample Size
Afrosoricida	*Echinops telfairi*	Lesser Hedgehog Tenrec	2
Chiroptera	*Pteropus poliocephalus*	Gray Headed Flying Fox	3
Chiroptera	*Rousettus aegyptiacus*	Egyptian Fruit Bat	3
Eulipotyphla	*Neomys anomalus milleri*	Mediterranian Water Shrew	3
Eulipotyphla	*Erinaceus europaeus*	European HedgeHog	3
Marsupialia	*Dactylopsila Trivirgata*	Striped Possum	2
Marsupialia	*Dasyurus hallucatus*	Northern quoll	3
Marsupialia	*Monodelphis Domesticus*	Gray short tailed opossum	3
Marsupialia	*Sminthopsis Murina*	Slender Tailed Dunnart	1
Marsupialia	*Trichosurus Vulpecula*	BrushTail Possum	3
Rodentia	*Otospermophilus Beecheyi*	Ground Squirrel	3
Rodentia	*Sciurus Carolinensis*	Eastern Gray Squirrel	1
Rodentia	*Microtus Ochrogaster*	Prarie Vole	3
Rodentia	*Peromyscus Californicus*	CA Deermouse	3
Rodentia	*Arvicanthis Niloticus*	Nile Grass Rat	3
Rodentia	*Mus Musculus*	Lab Mouse	3
Rodentia	*Mus Musculus*	Wild Mouse	3
Rodentia	*Rattus Norvegicus*	Wild Norway Rat	3
Rodentia	*Rattus norvegicus * Albino WISTAR Rat*	Lab Rat Long Evans	3
Scandentia	*Tupaia Belangeri*	Northern Tree Shrew	3

Table 1: A summary table of all the species measured for the study, their respective order, species, common name and the number of individuals analyzed for each species. Note that we include both wild mice and laboratory reared mice, as well as wild rats and laboratory reared rats, but are not considered in the current analysis. Also note that monotremes are included in the supplementary material but are not considered in this analysis.

changes and correlating these changes with ecological and ethological data we can make inferences about the role of the neocortex in behavioral specialization. We use this dataset to address two important questions: 1) What are the conserved, lineage-specific trends in mammalian cortical field sizes in proportion to the total neocortical organization? 2) Are convergent sensory-cortical relationships part of shared differences in related phylogenetic lineage or specific to those species and thus based on other potential ecological and behavioral factors? In the process of addressing these questions we have also standardized archival data material for the public's use as part of a research modernization process, so that this information may be incorporated into other interdisciplinary datasets and used to potentially model processes that contribute to the organization of the neocortex across mammalian evolution.

Methods

We developed a standardized method to quantify the sizes of cortical fields from histological data and measured the sizes of many cortical fields that were histologically distinct in our preparation. Here we focus our analysis on the primary motor cortex (M1) as well as primary somatosensory (S1), auditory (A1) and visual (V1) cortex in a total of 18 mammalian species (Figure 4). We examined 2-3 individuals per species (with 2 exceptions) across 6 clades, for a total of 49 cases. For each species we endeavored to include 3 individuals, however for some species there was only one sample available (see Table 1 for summary). For all cases adults were used, but for wild caught animals the exact age of the adult animal is not known.

Archived slides containing tissue from over 30 years of previous experiments in the Krubitzer lab were located, and for most cases histological records were obtained, and this data was digitized. For all species examined, the neocortex was peeled from the brainstem and thalamus, manually flattened between glass slides, and sectioned tangentially on a freezing microtome at 30 –50 µm (depending on the size of the brain) (Figure 2, A - C). Alternate sections were stained for myelin (Gallyas, 1979) or cytochrome oxidase (Wong-Riley, 1979). Tissue was mounted onto glass slides and then cover-slipped.

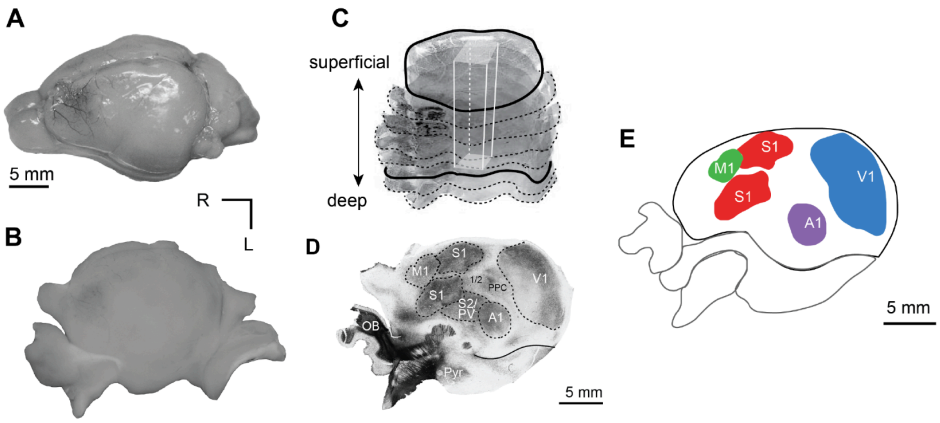


Figure 2: Quantifying Cortical Fields.A. Brains were extracted from the skull and cortical hemispheres were peeled from the brainstem and thalamus. **B.** The cortex was flattened under a glass slide. **C.** The tissue was cut tangentially and stained for myelin, and the series of sections were registered to one another. **D.** The boundaries of cortical fields were determined from differences in myelin staining across a series under magnification with a Zeiss projector. Final cortical field boundaries were determined from the series of sections. **E.** These tracings are then scanned, and the cortical fields were assigned a unique color identifier. The surface area of each field is measured as pixels and converted into millimeters squared relative to a scale bar. In most species 2-3 cases were analyzed and the averages of all measurements were used in the analysis. This example was taken from our studies in Egyptian fruit bats (Halley et al., 2022)

When drawing architectonic boundaries, the entire series of sections were used. In some cases, the cortex on the medial wall was pulled out and flattened and in other cases, this was not done. This was considered in our analysis, and all cases were analyzed without the medial wall to standardize across cases. Each section from the entire series of sections was then placed under a Zeiss projector for magnification. Cortical field boundaries were drawn by the same two experienced investigators for all cases included in this study. A scale bar was included in each drawing. Cortical fields were traced by hand, getting a visual average over multiple slices in the series (Figure 2. C., D.). All distinguishable cortical field boundaries were included in our drawing (see Supplementary Figure 1), but we limit our analysis to M1, S1, A1 and V1. Where

possible, and for most cases, we also included the overall size of the piriform cortex (PYR) and the olfactory bulb (see Supplementary Figure 1). For all cases, when measuring the overall size of the cortical sheet we used the largest section in the series.

Once the drawings were finalized, they were scanned and imported into Adobe Illustrator where each tracing was converted into a vector path and uniquely color coded (see Figure 2, E.). Using Python, the vector paths were converted into color differentiated paths and the square area of the pixels was calculated and converted in square millimeters. The conversion was done by getting a square that is 1 millimeter by 1 millimeter for each magnification setting based on a scale bar, then dividing the pixel amount of this square by the total pixel areas.

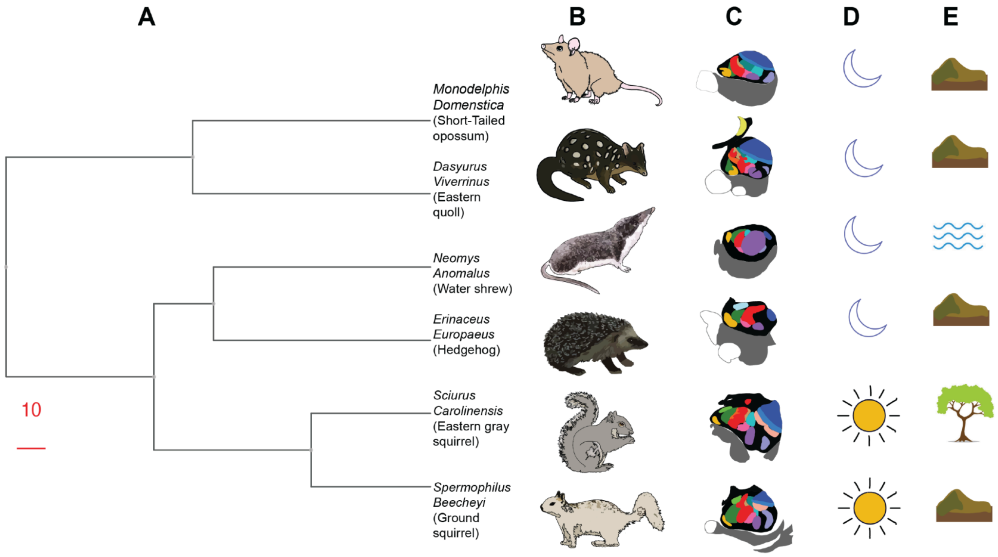


Figure 3: Creation of a complex database. A. Phylogenetic lineage information with a tree scale bar representing evolutionary distance, corresponding to the horizontal branch lengths of the tree (using phytools, Yu, 2022). **B.**Unique traits such as morphological specializations. **C.** All cortical field area measurements traced in mm2. **D.** an example of other behaviorally relevant ethological information such as diel pattern and **E.**major habitat preference, ground-dwelling, amphibious (water and land) and arboreal represented as simplified icons here.

In addition to any data located in the histological records for each case, we collected information on ethological aspects of the life of each species in multiple categories: diel patterns, diet, habitat preferences, habitat breadth, mating types, social structure and morphological or behavioral specializations (e.g. specialized digits, wings, whiskers, echolocation, electroreception) (Figure 3, A., B., D., E.). This data is used to inform our understanding of the context in which cortical field organization might change and will also be applied to correlational analysis with our results on cortical field sizes in

a separate study. The total dataset contains the ethological information along with the area sizes in millimeters squared, area sizes as a percentage of the total cortex, the Krubitzer lab's case number, National Center for Biotechnology Information (NCBI) taxonomic identity number of each species, and, when possible, age, weight, gender, hemisphere (e.g. left side or right side), perfusion procedure.

While there are many potential statistical analyses that may be applied to this data, we focus our preliminary analysis here on allometry, a technique which allows us to measure scaling relationships among anatomical sizes and other morphological or behavioral traits, while taking into account growth factors and proportionality in biological forms (Gould, 1966). To quantify the relative size of the cortical fields compared to the total neocortex size, we first logarithmically transformed all data (log10), then ran a linear regression on the the sizes of each cortical field in millimeters squared as predicted by the total size of the neocortex (Figure 10), taking phylogenetic relationships into account (Figure 4). Next, we separated this data by phylogenetic clade and ran the same analysis per group, obtaining regression line slopes and coefficients for each of the 4 fields (Figures 5-9). Note that model bounds are reported as the Beta or slope followed by a set of brackets containing the lower 95% confidence bound and upper 95% confidence bound (e.g., 1.2 [0.25, 1.25]). For an allometric analysis in the context of this dataset, a slope of 1 indicates that as the neocortex size increases, the cortical field size changes proportionally, as predicted by the concerted evolution model. A slope of less than 1 indicates that as overall neocortex size increases, the size of the cortical field decreases in size, and a slope of greater than 1 indicates that as the overall neocortex size increases, the cortical field size increases a greater amount than proportionally to the neocortex size increase. Slopes that are less than or greater than 1 are evidence of mosaic evolution since the variation in cortical field size cannot be simply predicted by the size change of the overall neocortex.

Lastly, we obtained percentages for each field relative to the total neocortex and similarly grouped these by phylogenetic clade (Figures 5-9). We also report general statistical information: medians and median absolute deviations were used instead of means and standard deviations for cases of non-normal distributions.

Results

Grouped by clade, we first present general findings on the total area of the cortical sheet, which includes all cortical fields, and the total amount of cortex not occupied by S1, A1, V1 and M1, which we refer to here as "other" cortex. We then show the general findings on the four main primary cortical fields (M1, S1, A1, V1), the medians and median absolute deviations (MAD) for each of the 4 four fields per clade. We then report results of the allometric analysis, and how the cortical fields change in proportion to the total cortical sheet. A representative case from each of the species examined, with the four fields focused on in our analysis, is shown in Figure 4. Phylogenetic

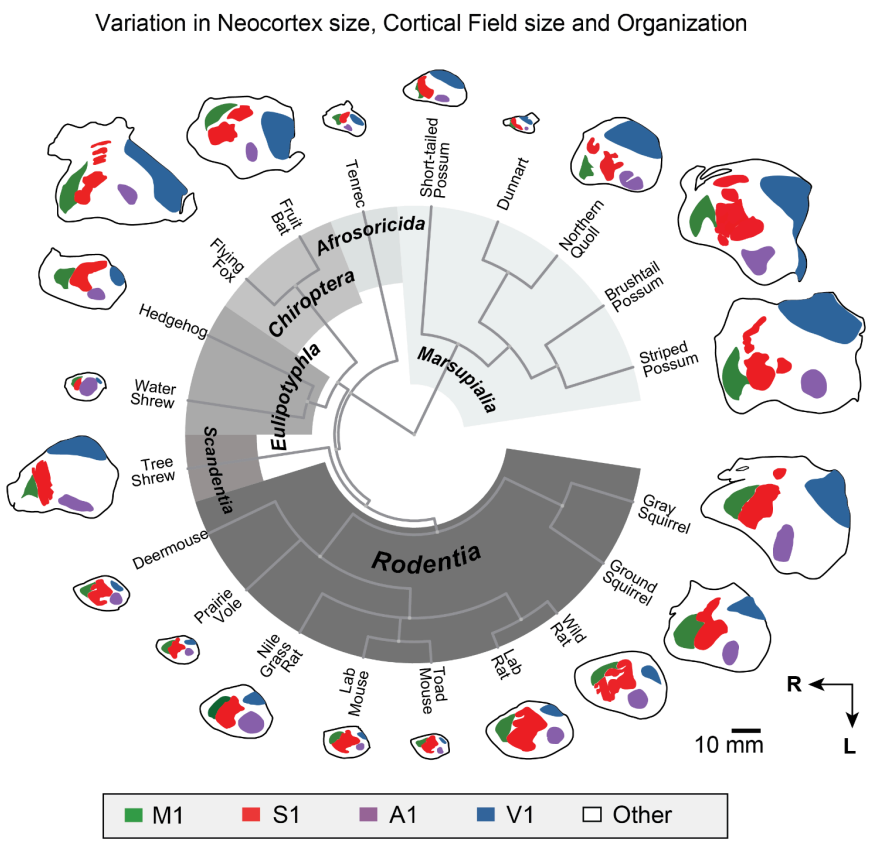


Figure 4: Cortical Field Variation. A phylogenetic tree showing the relationships of the different species examined, illustrating the size of the neocortex and the four cortical fields examined in this study. We examined 18 species of mammals (7 Rodentia, 5 Marsupiala, 2 Chiroptera, 2 Euliopotphlya, 1 Scandentia, 1 Afrosoricida). Illustrations above are drawn to scale and depict representative cases; In most species, surface area measurements are averages of 2-3 cases, depending on availability. In the selection of cases we measured, emphasis was placed not only on phylogenetic diversity, but also closely related species that exhibit differences in lifestyle and/or environmental conditions over their lifetime (e.g. tree squirrels and ground squirrels, lab rats and wild rats). "Other" refers to the unassociated cortex, and is here represented as white-colored areas. Note that while all species have the constellation of cortical fields examined, there are large differences in the overall size of the cortical sheet, and differences in the shape and relative size of some cortical fields in some species.

relationships are depicted by an evolutionary tree, with clades highlighted in shades of gray. The allometric results shown in Figures 5-9 represent the conserved, lineage-specific trends of these four fields across the clades examined. We also include ethological behaviors of interest for each clade, highlighting behavioral/anatomical specializations. Lastly, in Figure 10, we contrast the same general information and allometry across all the clades in the sample. Note that for all analysis only the dorsolateral neocortical surface was considered (see Methods).

Within **Marsupials** we see a large diversity in the total size of the neocortex ranging from the striped possum (*Dactylopsila trivirgata*) whose average neocortex size is 566.68mm^2 to the slender tailed dunnart (*Sminthopsis murina*) whose average neocortex size is 10.99 mm^2, a difference of 98.06%. In marsupials, the average percentage of cortex not occupied by the four fields we measured ("other" cortex) is 26.7% with a median absolute deviation of 6.8 (Figure 9, #1-5). The striped possum (*Dactylopsila trivirgata*) has the largest percent (32.8%) of "other" cortex, as well as the largest total neocortex size in our sample and the Northern quoll (*Dasyurus hallucatus*) has the smallest percentage of "other" cortex (20.6%) which notably, is *not* the smallest total neocortex of the Marsupial clade(Figure 9, #1-5). The average (median) size of M1 is 8mm^2 with a median absolute deviation (MAD) of 11.06 mm^2. Note that median is used as the average, and MAD is used as a deviation in cases in which the distribution is highly skewed, since mean and standard deviation would not be representative of central tendency. Average S1 is 17 mm^2 (MAD = 22.46 mm^2), A1 is 9.9mm^2 (MAD = 14.1 mm^2) and V1 is 36.5mm^2 (MAD = 41.39 mm^2). Our allometric analysis for all of the cortical fields (with some exceptions) aligns with the principle of concerted evolution, the smallest fields are in the smallest overall brains (slender tailed dunnart) and the largest are in the largest brains (striped possum). The allometric coefficients, or slopes, in marsupials are all close to 1 (M1 = 1.1 [0.79, 1.40], S1 = 0.93[0.7, 1.17], A1 = 1.1[0.92,

1.30], V1 = 0.97[0.73, 1.20]), indicating all fields are changing in proportion to the size of overall cortex. Both V1 and S1 have higher intercepts than M1 and A1 (see Figures 5-8, #1-5), indicating that in general, V1 and S1 are proportionally larger than the other fields. In contrast, M1 and A1 are proportionally smaller. One divergence from allometric predictions is in S1: the smallest S1 is in the slender tailed dunnart at 1.90 mm^2 (17% of its neocortex) and the largest S1 is in the brushtail possum (*Trichosurus vulpecula*) at 66.3 mm^2 (16% of its neocortex), but this is not the largest overall neocortex in the sample. However, the brushtail possum's total cortex size is only slightly under the largest sample (the striped possum) at 404 mm^2, and is the second largest cortex in the sample of marsupials. In consideration of marsupial ethology, all species are nocturnal, solitary in social structure and ground dwelling, except the striped possum and brushtail possum who are arboreal.

Within **Rodents** there is also a large diversity of total neocortex size in our sample, which ranges from the largest, the Eastern gray squirrel (*Sciurus carolinensis*) whose average size of neocortex is 320.56 mm^2 to the (wild-caught) house mouse (*Mus musculus*) whose average size 20.14 mm^2, which is a difference in size of 93.71%. In Rodents, the average percentage of "other" cortex is 35.7%, (MAD=4.83%) (Figure 9, #12-20). The wild brown rat (*Rattus norvegicus*) has the largest amount of "other" cortex (40.7%) and the (Laboratory reared) domestic house mouse (*Mus musculus*) has the smallest (27.7%). The average size of M1 is 3.38 mm^2 (MAD= 2.28 mm^2), S1 is 9.97 mm^2 (MAD = 6.53 mm^2), A1 is 4.43 mm^2 (MAD = 4.08 mm^2) and V1 is 3.31 mm^2 (MAD = 2.59). The allometric slope for M1 (0.88[0.73, 1.02]) is low in rodents, indicating M1 decreases as the size of the cortex increases, but confidence intervals suggest this is still close enough to 1 to be considered isometry (proportional scaling). The allometric slope for S1 (0.69[0.52, 0.86]) is low. This is likely because smaller sized rodent brains have a proportionally expanded S1 (Figure 6, #12-20) and larger sized rodent cortices

have a smaller S1. This indicates that as Rodent neocortices increases in size, S1 gets proportionally *smaller*. Driven by the large expansion of V1 in squirrels the allometric slope is above isometry (1.2 [1.07, 1.42]), meaning that V1 is expanding proportionally more than the rate of overall neocortical expansion, known as hyperallometry (Figure 8, #12-20), this is still close to one, but confidence intervals indicate is above isometry. A1 scales proportionally with overall size of neocortex (0.99 [0.73, 1.25]). Differing from allometric predictions, the smallest S1 is in the prairie vole, at 5.45mm^2 and the largest is in the ground squirrel at 38.7 mm^2. General ethology of rodents suggests that the barrel cortex, which represents the whiskers, may be extremely important to smaller brained, smaller-sized rodents, who use it for navigation in primarily nocturnal and arboreal habitats (Muchlinski et al., 2020). Only squirrels and the Nile grass rats are diurnal in this sample, and squirrels have the largest percentage of V1 out of total cortex in the rodent clade. In the Eastern gray squirrel, V1 occupies 13% of the total cortex, and in the ground squirrel V1 occupies 8% (the average size of V1 in rodents is 10.4%). Interestingly, Nile grass rats have a relatively large V1 as well, the largest of the non-squirrel species, at 8.04% in addition to a relatively large A1 at 12.8% (the average percent of A1 in Rodents is 6.3%). Although we included wild caught and laboratory reared rats and mice in this study, they are not included in our current results but will be part of a broader study.

Within **Chiroptera**, our sample size is limited to 2 species. The largest cortex size of the two is the Gray headed flying fox (*Pteropus poliocephalus*) at 286.76 mm^2 and the Egyptian fruit bat (*Rousettus aegyptiacus*) is 176.28 mm^2 which is a difference of 38.52%. In the flying fox the percentage of “other” cortex is 24%, and in the Egyptian fruit bat it is 31% (Figure 9, #9-10). In both bats S1 and A1 are occupy a similar amount of cortex; S1 occupies 11.6% of the neocortex in Egyptian fruit bats and 10.4% in the flying fox; A1 occupies 4.9% of the neocortex in Egyptian fruit bats and 3.12% in the

flying fox. In both bats, allometry shows that V1 is proportionally expanded as neocortex size increases, and this is mainly due to the flying fox, in which V1 occupies 20.6% of the total cortex (Figure 8, #9-10). Ethologically, both the Egyptian fruit bats and flying foxes are highly visual, fruit foragers that play a role in plant pollination and seed dispersal in their respective ecosystems. While they are primarily nocturnal, they have been observed to be active during the day as well (Connell et al., 2006). The Egyptian fruit bat is an interesting animal because it uses its visual system heavily in a nocturnal pollination context, as well as using echolocation for navigation. Recent studies have shown that Egyptian fruit bats actively echolocate during the day (Eitan et al., 2022).

Within **Eulipotyphla**, for which we have only two species, the European hedgehog (*Erinaceus europaeus*) has an average total cortex size of 109 mm^2 and the water shrew (*Neomys anomalus milleri*) has an average total cortex size of 26.23 mm^2, which is 75.83% smaller that the cortex of the hedgehog. Both species have a similar amount of “other” cortex; 47% in water shrews and 46.3% in hedgehogs (Figure 9, #7-8). Between these two species all cortical fields, except for A1, are larger in the species with larger neocortex size, a confirmation of concerted evolution. However, A1 is proportionally much more expanded in the water shrew at 7.4mm^2, which occupies 28% of the total neocortical sheet, as opposed to the hedgehog where A1 occupies 5%. M1 is 8.17mm^2 in the hedgehog, at 7.53%, S1 is 19.4mm^2 at 18%, A1 is 5.52 mm^2 at 5.2%, and V1 is 8.28 mm^2. In the water shrew: M1 is 1.18 mm^2 at 4.5 %, S1 is 19.4mm^2 at 17.9%, A1 is 7.37, at 28% and V1 is 8.20mm^2 at 2.35 %. Note that V2 is highly contracted in the water shrew, where A1 is expanded. Allometric lines reveal the same A1 and V1 outlier in the water shrew (Figure 7-8, #8, dashed line and in Figure 10). The major ethological factor that differs in these two species is that the water shrew is amphibious (semiaquatic), occupying streams and riverbanks between water and land, and the hedgehog is terrestrial. Studies of coronal sections in the specific water shrew

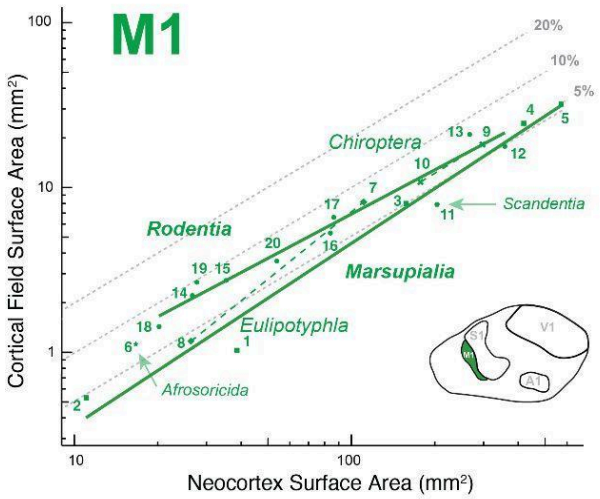
case analyzed show an expanded lateral geniculate, the visual nucleus of the thalamus that projects to V1, which confirms an expanded A1 as opposed to an expanded S1- which is linked with underwater whisking to catch prey in the American water shrew (*Sorex Palustris*, see Catania 2008). It has been suggested that underwater sound detection, including echo-location like behaviors may aid in catching prey underwater in various shrews of the same order (Tomasi, 1979; Thomas and Jalili, 2004), and recent molecular evidence (18 amino acid residues) support convergent echolocation in bats, dolphins and common shrews (*Sorex Araneus;* see Chai et al., 2020).

Within **Afrosoricida** we examined the tenrec (*Echinops telfairi*) which has an average cortical sheet size of 16.6 mm^2. In the tenrec 42.8% of cortical space is devoted to “other” cortex, which is close to half of all the neocortex and yet it is one of the smaller brains in our collection (Figure 9, #6). M1 has an average size of 1.14mm^2 which occupies 6.8% of the total cortex, S1 has an average size of 1.62mm^2 which occupies 9.7% of the total cortex, A1 has an average size of 0.76mm^2 which is 4.6% of the total cortex and V1 is the largest primary field at 1.88mm^2, which is 11.3% of the total cortex (Figures 5-8, #6). It is interesting that V1 is relatively large, and further investigation into closely related species is necessary to understand if this is due to concerted or mosaic evolution. Tenrecs are also known to rely on echolocation through two methods, first the standard larynx and mouth produced sounds, and second by stridulating quills on one side of its back (Gould, 1965; Endo et al., 2020). This complex behavior is of interest, as the tenrec is the only known mammal species to produce this insect-like method of sound generation (Endo et al., 2020). We note here that tenrecs dwell in semi-desert conditions (Stankowich and Stensrud, 2019) and also have a greatly expanded piriform cortex (region responsible for smell and taste) which is over twice the size of its neocortex, at 49.4 mm^2 (see Supplementary Figure 1: the gray-colored region represents piriform cortex).

Within **Scandentia** we include a single species, the Northern tree shrew (*Tupaia belangeri*), whose average total cortical sheet is 192.88 mm^2 and it is one of the medium-large brains in our collection. “Other” cortex in the tree shrew occupies 37.2% of the cortical sheet (Figure 9, #11). M1 is 7.90mm^2, which is 4.09% of the total cortex; S1 is 11.9mm^2 which is 6.17% of the total cortex; A1 is 10.3mm^2 which is 5.34% of the cortex; and V1 is 25.8mm^2 which is 13.4% of the cortex (Figures 5-8, #11). Again, in the tree shrews, which are diurnal, arboreal frugivores with a symbiotic relationship to pitcher plants in rainforests (Clarke et al., 2009), V1 occupies the largest amount of cortex of the primary fields. Tree shrews have a complex visual system with color vision and a secondary visual pathway (Shriver and Noback, 2019). Notably the tree shrew is the closest living relative to primates of all of the species in our study.

In **all of the species** examined the range of sizes of the total neocortical sheet is high; it is the largest in the striped possum (*Dactylopsila trivirgata*), a Marsupial with a total cortical area of 566.68mm^2, and the smallest is in the slender tailed dunnart (*Sminthopsis murina*) with a total area of neocortex of 10.99 mm^2. The average “other” cortex is 29.62mm^2 (MAD= 32.02mm^2), with a wide range of variation. The average size of M1 is 5.31mm^2 (MAD=5.7mm^2), occupying an average of 6.61% of the total cortex. Rodents have a slightly larger M1 than marsupials within our sample, especially in the smallest brains. The slope of M1 (0.97 [0.83, 1.09), see Figure 10) indicates that M1 scales in proportion to the size of the neocortex. The intercept indicates that M1 occupies a relatively *smaller* proportion of the entire neocortex. Marsupials account for most of the range in sizes of M1. The average size of S1 is 11.91mm^2 (MAD= 11.1mm^2), occupying an average 17% of the total neocortex. S1 has a low allometric slope (0.83 [0.65, 0.98]), which the confidence intervals suggest is significant. This is likely because smaller sized rodents have a proportionally larger S1 (see Figure 6 and Figure 10). Essentially, when looking at the entire set of clades together, the larger the

size of the neocortex, the *smaller* S1 will be. The average size of A1 is 6.2mm^2 (MAD = 6.34mm^2), occupying an average of 6.85% of the total neocortex. A1 occupies a relatively *smaller* proportion of the cortical sheet based on the intercept of the allometric line, but the allometric slope of A1 (0.92 [0.75, 1.12]) is close to isometry, meaning A1 scales roughly proportionally to the size of the neocortex. Marsupials account for most of the range in sizes of A1 (as well as M1). The average size of V1 is 4.70mm^2 (MAD=5.74mm^2) which is 11.25% of the total neocortex. Marsupials and Chiroptera (megabats) in our sample have a relatively large primary Visual cortex (V1) across a range of brain sizes, occupying between 15% and 25% of the entire neocortex. The smallest V1 is in the water shrew, for which the contracted V1 is potentially related to the much more expanded A1 (Figure 10 and Figure 7-8). The high slope of V1 (1.25 [1.0, 1.48]) means as the size or the neocortex increases, the size of V1 gets *larger*. The scaling of V1 across our sample is largely driven by the rodents (see Figure 8) with a smaller neocortex, which have a relatively small V1, that is then driven positively by the presence of a more expanded V1 in squirrels.



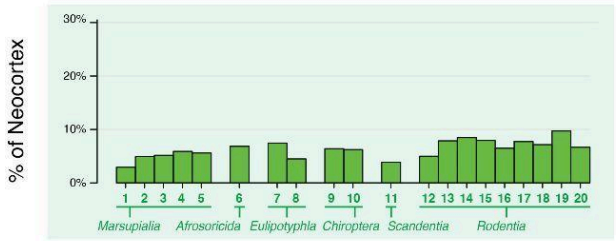
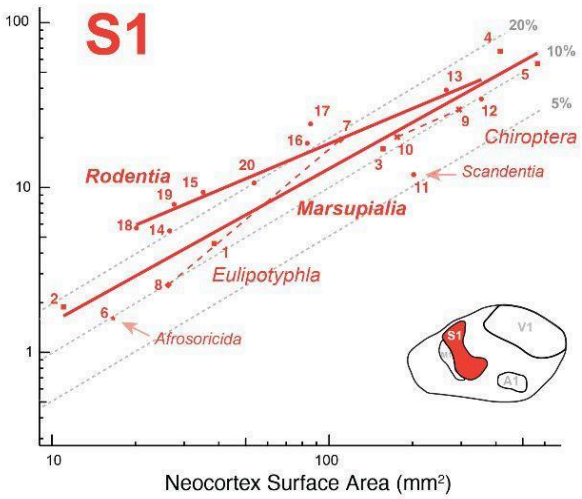


Figure 5: The relative size of M1. In all species, M1 occupies less than 10% of the whole neocortex. Rodents have a slightly larger M1 than marsupials within our sample, especially in the smallest brains. The low slope indicates that in larger brains, M1 occupies a smaller proportion of the entire neocortex.



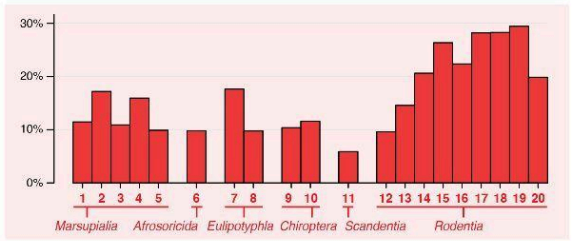
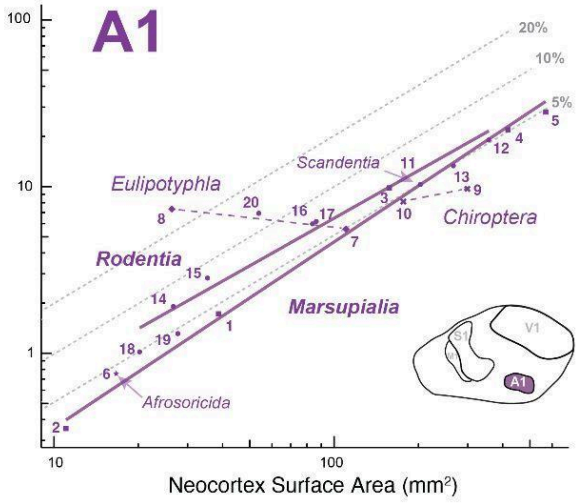


Figure 6: The relative size of S1. Primary somatosensory cortex occupies between 10% and 30% of the neocortex within our sample. Smaller rodents have exceptionally S1 proportions within the neocortex. Trend lines indicate that as brains increase in size, the relative size of S1 decreases.



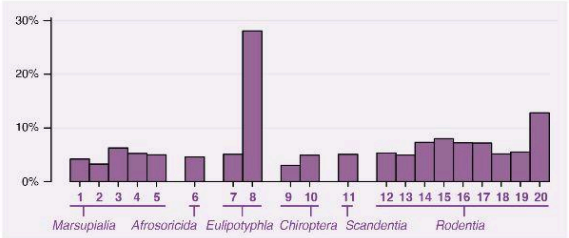
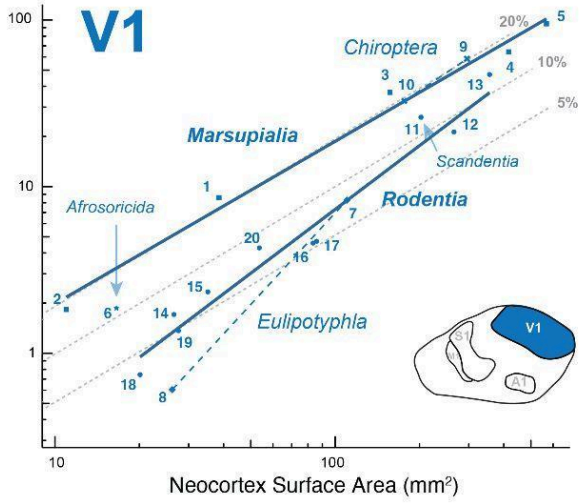


Figure 7. The relative size of A1. While the auditory cortex occupies a relatively small area of cortex in most of our sample, it is exceptionally large in the water shrew, which may be involved in echolocation, and to a lesser extent the Nile grass rat, which may be doing complex vocalization.



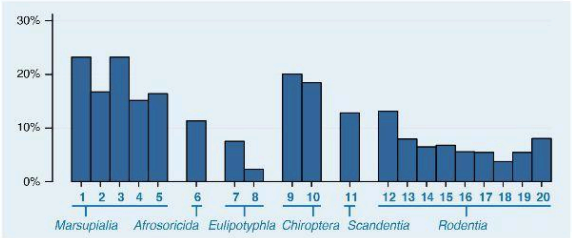


Figure 8. The relative size of V1. The high slope of V1 scaling across our sample (1.20; see Figure. 6) is largely driven by the smaller rodents, which have an exceptionally small V1. The smallest V1 proportion is in Water Shrews, which have an expanded A1 (Figure 6, Figure 9).

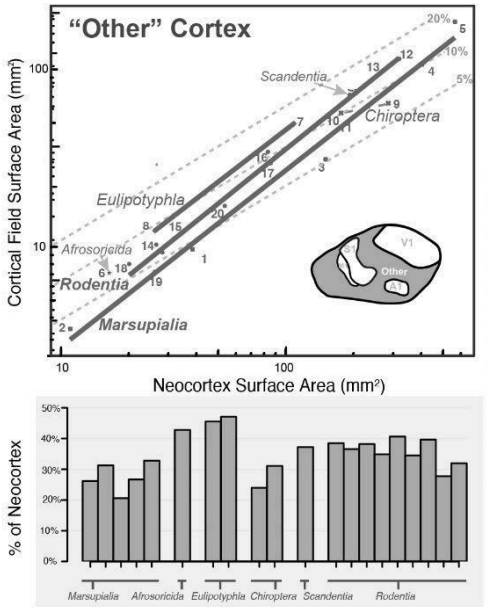


Figure 9. The relative size of the "Other" neocortex. Both Marsupials (0.99) and Rodents (1.02) have an allometric slope close to 1 (isometry), meaning that unassociated cortex scales proportionally to the size of the overall cortex.The northern quoll (#3) and striped possum (#5) are slight outliers. The clades with the highest percent of unassociated cortex are Eulipotyphla and

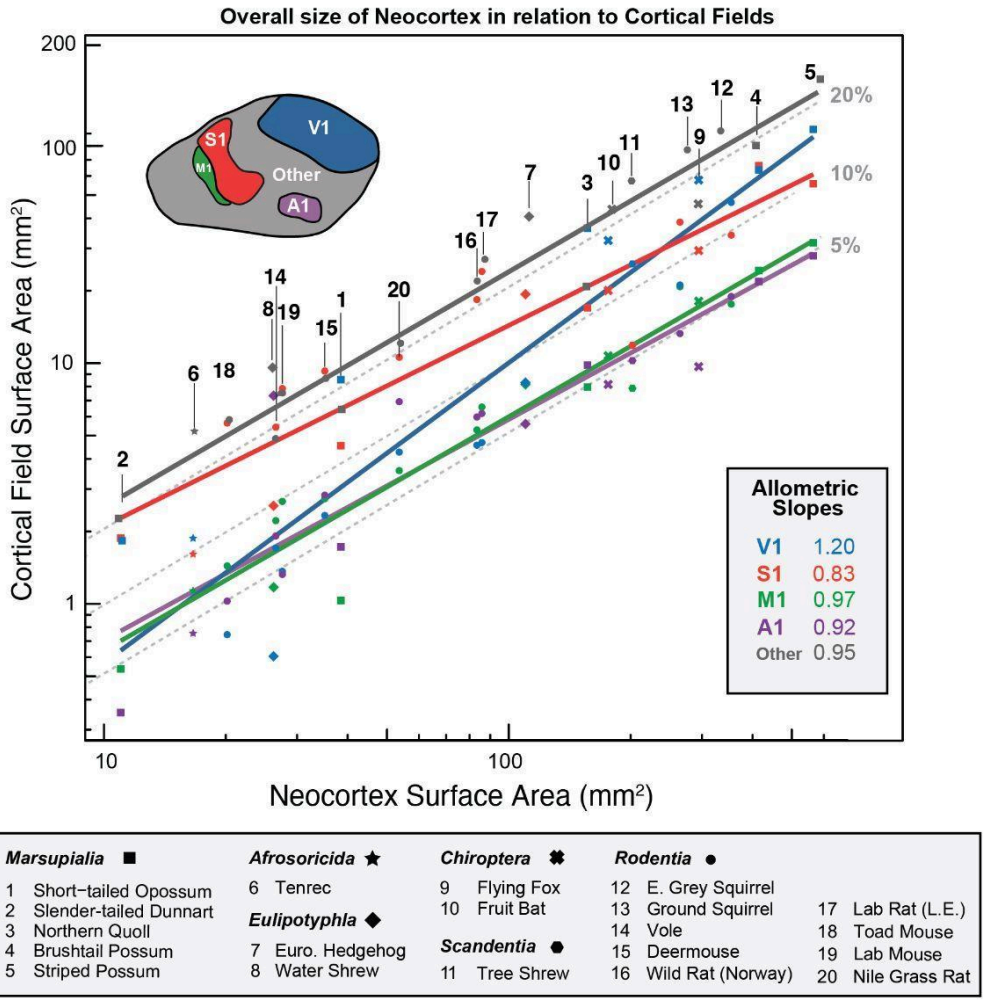


Figure 10: Scaling of M1, S1, A1, V1 and "Other" cortex, relative to the overall size of the cortical sheet across 18 species (20 samples total). In larger neocortices, M1, S1 and A1 occupy a relatively smaller amount of the cortical sheet, while V1 occupies a larger amount of cortex (but see Fig. 8 above). S1 has an exceptionally low allometric slope, driven by the fact that in the smallest brains, S1 occupies an exceptionally large proportion of cortex. Numbers indicate individual species data from the key. Isometric lines are indicated by dotted gray lines, positioned at percentage values shown to the right. Different point symbols are used for different clades, shown in the key.

Discussion

We will first discuss our results in terms of concerted (predicted by overall brain sizes) and mosaic evolution (some areas scale independently of the rest), focusing mostly on Rodents and Marsupials, for which we have the largest sample size. Second, we will speculate on why certain differences may have occurred in the context of ethology and evolutionary time, making note of outlier cases. Finally, we will discuss the measure of the "other" cortex and what this could mean.

In Rodents and Marsupials, there are examples of mosaic-like variation in the sizes of area S1. In Marsupials, S1 is relatively largest in the brushtail possum (16%), which is not the largest brain in our sample. The brushtail possum's total cortex size is only slightly smaller (404mm^2) than the striped possum (567mm^2), where S1 occupies 9.9%. Similarly, in rodents the smallest relative S1 is in the prairie vole, at 21% (5.45mm^2) and the relative largest is in the ground squirrel at 14.6% (38.7mm^2), neither of which are the smallest or largest brains in the clade. One thing that is clear from our large sample size in Rodents and Marsupials is that compared to other cortical fields, the primary somatosensory area (S1) follows principles of mosaic evolution. Further studies that include more species in other lineages are needed to elucidate whether S1 is overall more non-linearly variable than concerted allometry would predict across species.

Another important interaction is between S1 and V1 in Rodents. S1 is expanded and V1 is contracted proportionally. In rodents, diurnality appears to have strong influence on the size of V1, because it is only the diurnal species, which are rare across Rodents, that have a proportionally expanded V1. This suggests that diurnality is a more derived feature of Rodents, leading to more mosaic-like changes in cortical fields. When examining the Rodent phylogenetic tree (Campi and Krubitzer 2010), it is clear that the most common ancestor of all Rodents, and the majority of species in the

lineage, is nocturnal (Huchon et al., 2007; Steppan et al., 2004). Rodents diverged ~66 million years ago and squirrels, who are diurnal, diverged from the branch *Aplodontidae* about 40 million years ago. The Nile grass rat is also a relatively recently evolved rodent, having emerged from the *Muridae* branch ~ 2.4 to 1.5 million years ago (Ducroz et al., 1998). The expansion of V1 in rodents is more mosaic than concerted, and takes place in what might be considered more derived species.

Recent studies have shown that Egyptian fruit bats are highly visual, active during the day, and can echolocate during the day (Eitan et al., 2022). The fact that S1 and A1 are slightly larger in the Egyptian fruit bat than in the flying fox (which has the larger neocortex of the two) might suggest that there are mosaic adjustments in cortical organization for navigation by echolocation in the smaller bodied Egyptian fruit bat. However, echolocation does not always predict cortical field combinations. In the water shrew, A1, which occupies a relatively small area of the cortex in most species in our large sample, is exceptionally large, and could be associated with using echolocation to detect prey underwater. Given that the American water shrew has an expanded S1 and not A1 (Catania,2008), we also examined samples of the medial geniculate of the thalamus to confirm that A1, not S1, is expanded in our particular species of water shrew. However, neither the tenrec, nor the Egyptian fruit bat have an expanded A1, and both of these species are known echolocators (Gould, 1965; Endo et al., 2020; Eitan et al., 2022). The fact that the tenrec does not have an expanded A1 is a testament to concerted evolution in this species. Without more species in Afrosoricida, it is difficult to say whether this is a lineage based trend or a mosaic, adaptive expansion that might relate to the tenrec's unique capacities and conditions. Overall, this data on echolocation capacities in our sample implies that while there are convergent behaviors (echolocation), with known convergent molecular signatures (Chai, Simin, et al. 2020), the organization and scaling of cortical fields does not

necessarily converge but is rather more flexible; there are many cortical “solutions” to the same convergent behaviors (see Figure 11).

The interpretation of our results on the “other” cortex across species remains up for debate. At the very least this is an informative measure in terms of how many neocortical field spaces (both secondary, multimodal and higher order) we were able to resolve using our methods (see Supplementary Figure 1 of all areas resolved). At best, this is a measure of the amount of space in which the cortex is agnostic to a given sensory input and is “activity-dependent”, the precursors to high-order, multi-modal fields, across species. These areas could represent those spaces that are complex, and “built” during development. While the “other” cortex is close to allometry (Figure 10, gray line) there are a few cases where the “other” cortex does not scale with the overall size of the neocortical sheet, such as in the fruit bat which is not the largest of the two bats, and in the Northern quoll which has a small percentage “other” cortex but is not the smallest of the species. The amount of space of “other cortex” is variable, in part due to the limits of our knowledge of how these spaces are subdivided across species. Modeling the underlying rules for how primary fields eventually inform high-order fields is a next step that may allow us to make predictions about what these “other” neocortical areas might represent in specific species, and how this changes over evolution.

Echolocation & the size of auditory cortex

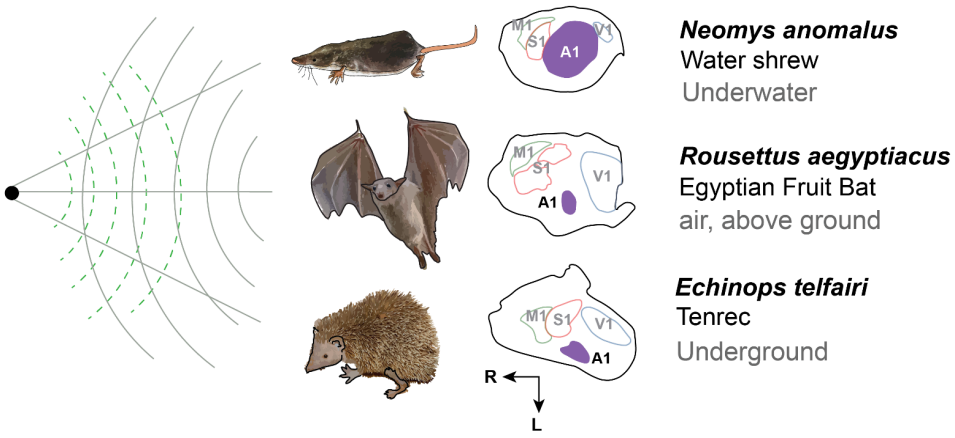


Figure 11: Convergent Specializations do not imply convergence of cortical field organization: The water shrew, Egyptian fruit bat and tenrec all have specializations that support echolocation. While all three species use their mouth to produce sonar, the tenrec also uses stridulating quills. Note that only the water shrew has an expanded A1 (Fig. 7, #8) and a contracted V1 (Fig. 8, #8). By contrast, in the Egyptian fruit bat - a highly visual bat that also echolocates - V1 is relatively large (Fig. 8, #10). This suggests that an expansion of A1 is only present in some echolocating species.

Limitations

A major limitation is that we cannot significantly measure within species variability, which would allow us to compare lineage-specific phenotypic plasticity, because our sample size (statistical power) is too low at 3 cases per species. Studies have looked at large samples of humans and compared allometric scaling across mammals, but very few have looked specifically at within species variation of cortical fields (Krubitzer and Seelke 2012; Charvet et al, 2013). However, we chose to cover breadth across the evolutionary tree rather than depth in this study.

In some cases, the neocortex on the medial wall was pulled out and flattened and in other cases, this was not done. Therefore, for our analysis we only measured the dorsolateral portions of the neocortex- meaning that some of the cortex is cut off for some species. This confounds the measurements of the size of V1 in some species since a relatively large proportion of V1 is on the medial wall (e.g. squirrels, bats). We

plan to do a separate analysis including only the cases in which the medial wall was pulled out.

For each case we only drew cortical field boundaries that we could consistently identify, and these included but were not limited to the primary and secondary visual areas (V1 and V2), the primary and second somatosensory areas/parietal ventral area (S1, S2/PV), auditory cortex, and motor cortex. Divisions of posterior parietal cortex and any other easily identifiable areas were also drawn and measurements and data analysis of these areas will be added to the supplementary material. We plan to do a more extensive analysis of all of the cortical fields that we could consistently identify (shown in Supplementary Figure 1).

Minor differences may be the result of phenotypic plasticity, especially when the number of species within the clade is low. Phenotypic plasticity may be the result of slight environmental differences in samples. Many of our species are wild caught. Samples of wild-caught species could have variation based on unknown environmental factors such as caloric resources and predation stressors of a particular environment during that temporal period. Our samples cannot represent the entire range of the conditions of the population for that species. For the most part specific ethological information of the individuals who were wild caught are not available. For example, mice may radically change their social patterns, from aggressive to commensural, depending on the spatial distribution of the habitat that a colony occupies (Gray et al. 1997; Frynta et al., 2005). Fine-grained specificities on naturalistic behaviors would increase our understanding of how cortical organization might interact with changing environmental factors, but this cannot be analyzed here as we have only general ethology compiled from literature searches and various sources of online biodiversity data (such as Animal Diversity Web and Encyclopedia of Life; Parr et. al, 2014). We plan to do a more

extensive analysis on wild caught rats and mice, comparing them to their laboratory counterparts.

Lastly our data is limited in the coarseness of the neocortical measurements, we cannot show subdivisions within a neocortical field, just the total area of a field on the neocortex. The data is also limited in sample size because in some cases we only have one sample. Our goal was to get as much breadth across the evolutionary tree as possible, even though we may have less than 3 samples. For this reason, complete statistical analysis of variance is not possible.

Other important caveats regarding neocortical fields is that there is often sampling bias and inconsistencies across species (Gaucher, 2020). Oftentimes we checked neocortical field boundaries with electrophysiological data but in some cases these were not available. However, one important strength of our study is that the same two investigators examined all of our samples and used similar criteria to define neocortical field boundaries.

Conclusion

Mammals vary in the size of the neocortex and the sizes of primary cortical fields that compose it. Our allometric analysis (Figures 5-10) suggests that in general, as the size of the neocortex increases, a relatively smaller proportion is occupied by these primary cortical fields (see slopes for S1, M1, A1 in Figure. 3) except for V1, which has a high allometric slope (Figure 8 and Figure 10), suggesting that larger neocortices have larger primary visual cortex proportionally speaking (aligns with Kaskan et al, 2005). This trend is driven by the exceptionally small V1 in the smaller rodents within our sample (Figure 8, #12-20). Larger brains with a larger neocortex devote less space to primary motor (M1) and auditory (A1) and somatosensory cortex (S1) whereas visual cortex (V1) is an exception. As the neocortex changes in size, V1 becomes

exceptionally larger or alternatively exceptionally smaller in mammals with a small neocortex. Overall mammals vary widely in the relative size of motor and sensory fields, and these variations suggest specific behaviorally relevant tasks that are often reflected by the ethological patterns of the species. The neocortex supports adaptive behavioral specializations, however there are examples of convergent behavioral specialization that do not share the same underlying neurobiological convergences in cortical organization, suggesting there are multiple diverse ways, even within a lineage, in which the neocortex can “solve” for the same convergent behavioral specializations. With the ongoing compilation of this interdisciplinary dataset on the sizes of neocortex, neocortical fields and ethological data across mammals, we hope that more questions and models of the underlying dynamics of the evolution of the neocortex can be investigated.

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Supplementary

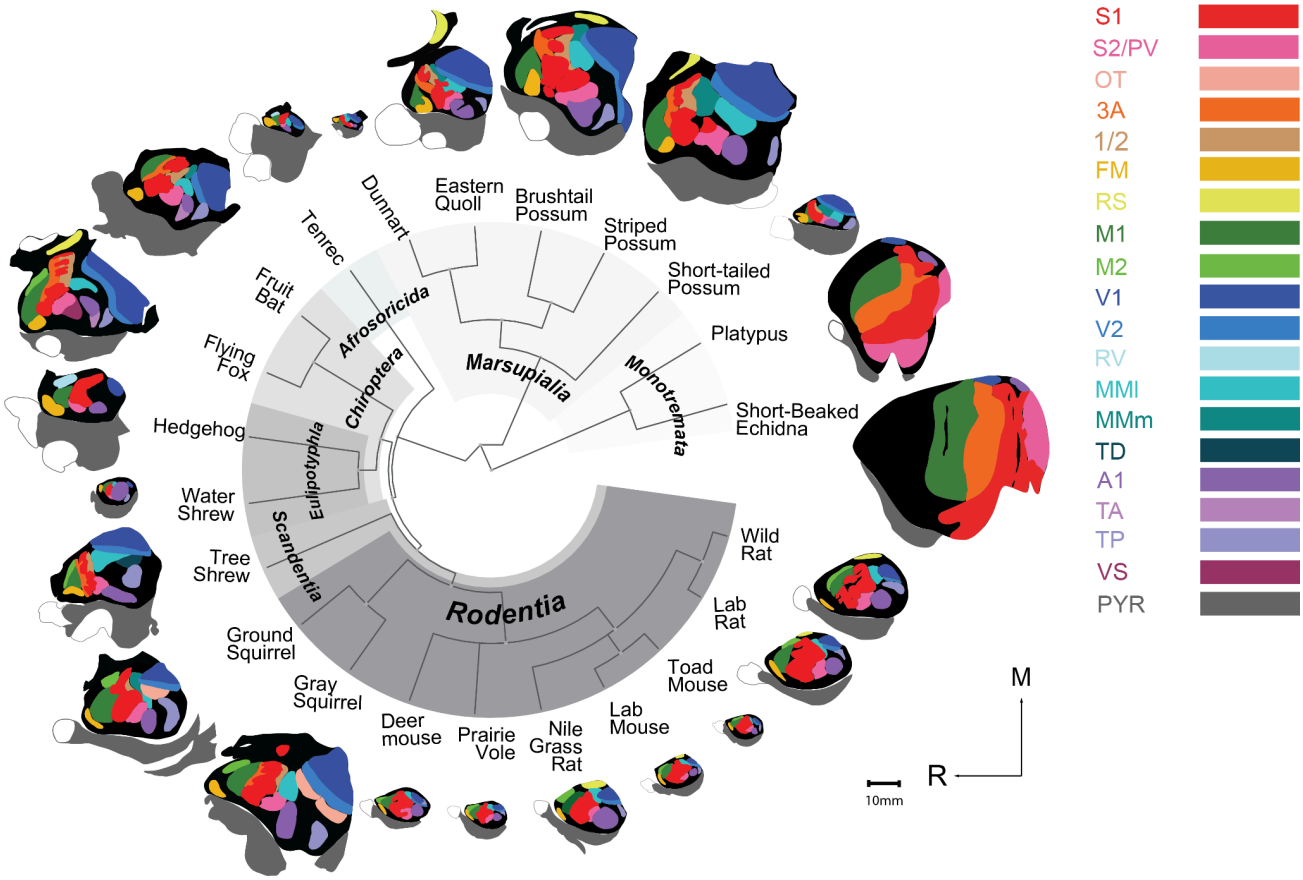

Figure 1: Cortical Field Variation. Diagrams of the neocortex and four primary cortical fields in 23 species of mammals (*9 Rodentia, 5 Marsupiala, 2 Chiroptera, 2 Euliopotphlya, 2 Monotremata, 1 Scandentia, 1 Afrosoricida*). Diagram above includes Monotremata, and shows all possible fields measured. The legend to the side shows color codes and abbreviations for each field (S1, primary somatosensory, S2/PV Secondary somatosensory, OT is Occipital temporal, 3A is Somatosensory area (deep), ½ is somatosensory area caudal to S1, FM is Frontal Myelinated field, RS is retrosplenial cortex, M1 is primary motor area, M2 is secondary motor area, V1 is primary visual area (striate cortex), V2 is secondary visual area, RV is rostral visual area, MMl is lateral multimodal area, MMm is medial multimodal area, TD is temporal dorsal area, A1 is primary auditory area, TA is Temporal anterior area, TP is Temporal posterior area, VS is ventral somatosensory area, PYR is Piriform cortex. In this figure, the black-colored areas are the “Other” cortex- the remaining cortex after subtracting all known fields.