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The evolution of the neocortex in mammals: intrinsic and extrinsic contributions to the cortical phenotype

Sarah J. Karlen and Leah Krubitzer[1]

Department of Psychology, 174G Young Hall, University of California, One Shields Avenue, Davis, CA 95616, USA

Abstract. The neocortex is that portion of the brain that is involved in volitional motor control, perception, cognition and a number of other complex behaviours exhibited by mammals, including humans. Indeed, the increase in the size of the cortical sheet and cortical field number is one of the hallmarks of human brain evolution. Fossil records and comparative studies of the neocortex indicate that early mammalian neocortices were composed of only a few parts or cortical fields, and that in some lineages such as primates, the neocortex expanded dramatically. More significantly, the number of cortical fields increased and the connectivity between cortical fields became more complex. While we do not know the exact transformation between this type of increase in cortical field number and connectivity, and the emergence of complex behaviours like those mentioned above, we know that species that have large neocorticies with multiple parts generally have more complex behaviours, both overt and covert. Although a number of inroads have been made into understanding how neurons in the neocortex respond to a variety of stimuli, the micro and macro circuitry of particular neocortical fields, and the molecular developmental events that construct current organization, very little is known about how more cortical fields are added in evolution. In particular, we do not know the rules of change, nor the constraints imposed on evolving nervous systems that dictate the particular phenotype that will ultimately emerge. One reason why these issues are unresolved is that the brain is a compromise between existing genetic constraints and the need to adapt. Thus, the functions that the brain generates are absolutely imperfect, although functionally optimized. This makes it very difficult to determine the rules of construction, to generate viable computational models of brain evolution, and to predict the direction of changes that may occur over time. Despite these obstacles, it is still possible to study the evolution of the neocortex. One way is to study the products of the evolutionary process—extant mammal brains—and to make inferences about the process. The second way to study brain evolution is to examine the developmental mechanisms that give rise to complex brains. We have begun to test our theories regarding cortical evolution, generated from comparative studies, by 'tweaking' in a developing nervous system what we believe is naturally being modified in evolution. Our goals are to identify the constraints imposed on the evolving neocortex, to disentangle the genetic and activity dependent mechanisms that give rise to complex brains, and ultimately to produce a cortical phenotype that is consistent with what would naturally occur in evolution.

[1]This paper was presented at the symposium by Leah Krubitzer, to whom correspondence should be addressed.

2005 Percept, decision, action: bridging the gaps. Wiley, Chichester (Novartis Foundation Symposium 270) p 146–163

Throughout evolution, one of the most dramatic changes to the mammalian brain has been an increase in the size of the neocortex and a change in the number of cortical fields. A cortical field can be defined using a variety of criteria including architectonic appearance, neuronal response properties, and cortical and subcortical connections (Kaas 1982, 1983, Krubitzer 1995). While all mammals have cortical fields that are uniquely interconnected to form processing networks, different species have different numbers of cortical areas, and this variability is thought to generate the behavioural diversity exhibited by various mammals. In general, mammals with larger neocortices and a greater number of cortical fields appear to exhibit more complex behaviours and to possess a greater number of more flexible behavioural repertoires. Although the exact transformation between the addition of cortical fields and the observed changes in sensory, perceptual and cognitive behaviours is not known, the addition of cortical fields may act to enhance particular stimulus features, generate probabilities based on sensory experience, and construct a species-specific interpretation of the environment based on the physical parameters that a particular animal can detect (Krubitzer & Kahn 2003).

The goal of our laboratory is to understand how changes in brain size and complexity are generated in different lineages and, once generated, how these changes are translated into complex behaviours, such as perception and cognition. Specifically, we are interested in how functional areas are specified, how cortical fields are added, and how connections between fields are modified in different lineages.

Unfortunately, understanding the process of brain evolution is hindered by two major obstacles. First, changes that occur in the neocortex accumulate slowly over many generations in different lineages. As a result, cortical evolution cannot be studied directly and is not particularly amenable to laboratory experimentation. Second, unlike portions of the skeleton, soft tissue, such as the brain, is not preserved in the fossil record; therefore information regarding changes that occur in the brain is derived from endocasts of fossil skulls (Jerison 1973, see Kaas 2005 for review), which can only provide information about the size and shape of the brains of our mammalian ancestors. Because of these problems associated with studying evolution directly, one can only make inferences about the evolutionary processes. This can be done by examining the brains of living mammals and performing a comparative analysis, and by utilizing a developmental approach to examine the mechanisms that may have been altered in evolution to account for the neocortical modifications observed in different lineages. The latter approach is a viable means for understanding evolution because the evolution of the neocortex is, in essence, the evolution of developmental mechanisms that recreate brain phe-

notypes in successive generations and that give rise to brain changes within and across lineages over time.

What have we learned about cortical evolution using the comparative approach?

The comparative approach is a method that allows us to deduce general characteristics of the nervous system, the types of brain changes that are possible, and the constraints that direct the course of evolution (Bullock 1984, Krubitzer 2002). Using this approach, we and others (Campos & Welker 1976, Catania 2002, Johnson et al 1994, Kaas 2005, Krubitzer 1995, Krubitzer & Kahn 2003, Levitt & Eagleson 2000, Reep et al 1989) have examined a variety of species using the criteria described above and have come to some firm conclusions regarding homologous cortical areas and general features of cortical organization that all mammals share. For example, all mammals have a similar constellation of specifically interconnected cortical fields (Krubitzer 1995, Krubitzer & Huffman 2000). As shown in Figure 1, the primary auditory area, A1 (Ehret 1997), the primary somatosensory area, S1 (Johnson 1990, Kaas 1983), and the primary visual area, V1 (Rosa & Krubitzer 1999), have been identified in all, or nearly all, mammals examined (Krubitzer 1995, Krubitzer & Kahn 2003 for review; Fig. 1 phylogeny based on Murphy et al 2004). These primary areas contain a complete representation of the sensory epithelium that is coextensive with a unique architectonic appearance and pattern of connectivity. While the second auditory area (A2), the second somatosensory area/parietal ventral area (S2/PV), a rostral deep field (R), the second visual area (V2), and primary motor area (M1) appear to be common to all mammals as well, other cortical areas that have been described appear to be derived and limited to particular lineages, such as the extrastriate visual areas in primates. Regardless of the morphological and behavioural specializations of many mammals (e.g. Catania 2000, Henry et al 2005, Krubitzer 1995), the conserved constellation of fields shown in Figure 1 is always present, even in the absence of apparent use (Bronchti et al 2002, Cooper et al 1993, Heil et al 1991). The ubiquity of these fields, aspects of their corticocortical and thalamocortical connectivity, and their general geographic arrangement across species indicate that they were present in the common ancestor, cannot be eliminated under most or all circumstances, and reflect the constraints imposed upon the evolving neocortex.

Despite the high degree of similarities between species, comparative studies also indicate that there are large degrees of freedom for phenotypic change. For example, the amount of cortex allotted to different sensory systems, termed sensory domains, varies across mammals and is related to the sensory receptor arrays and the senses that are most behaviourally relevant to the animal (Krubitzer & Kahn 2003). Furthermore, within a particular sensory domain, the relative size of primary

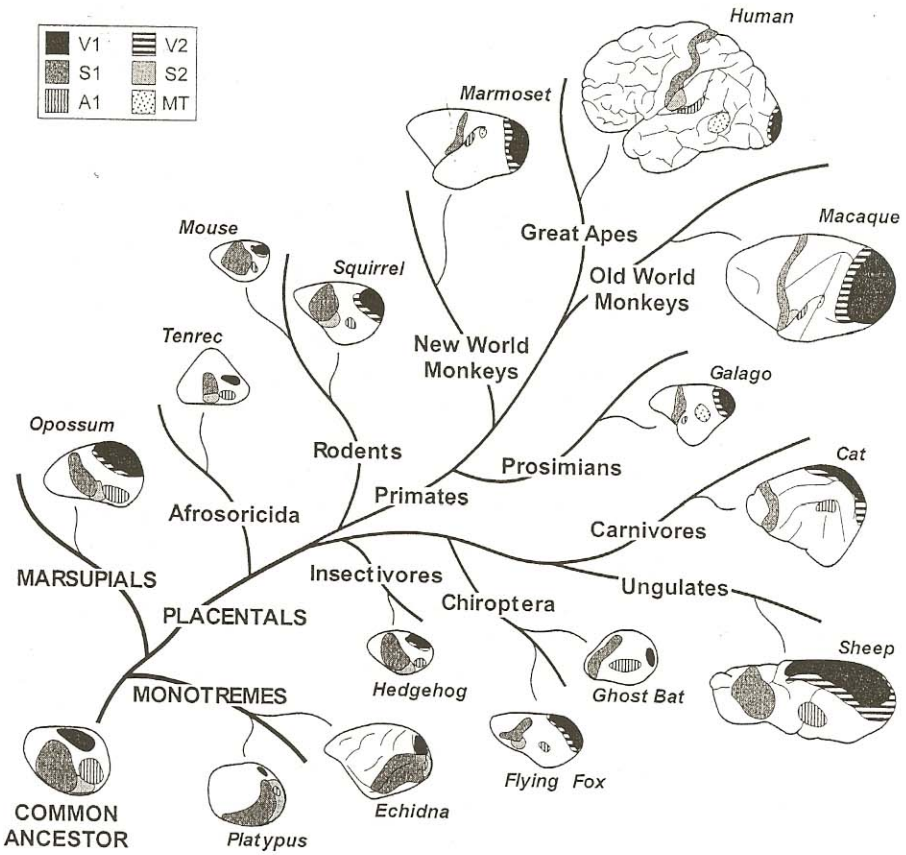

FIG. 1. An evolutionary tree depicting the phylogenetic relationship of some of the major mammalian orders and the cortical organization of some of the sensory fields that have been described in particular species. All of the mammals shown have a similar constellation of cortical fields, including A1, S1, S2, V1 and V2, as defined by architectonic appearance, neuronal response properties, and cortical and subcortical connections. The ubiquity of these fields suggests that they are most likely homologous areas that arose from a common ancestor. Other areas, such as MT, which has only been observed in the primate order, are derived and limited to particular lineages. A1, primary auditory area; S1, primary somatosensory area; S2, secondary somatosensory area; V1, primary visual area; V2, secondary visual area; MT, mediotemporal area. Phylogenetic relationships based on Murphy et al (2004). Rostral is left, medial is up.

cortical areas also varies, depending on the importance of the sensory system in question. For instance, in the arboreal squirrel, a highly visual rodent, a large proportion of the neocortex is devoted to the visual system, and the relative size of V1 is large, as compared to other primary sensory areas (Fig. 2A, based on Kaas et al 1989, Krubitzer et al 1986, Luethke et al 1988). In the mouse, which relies more

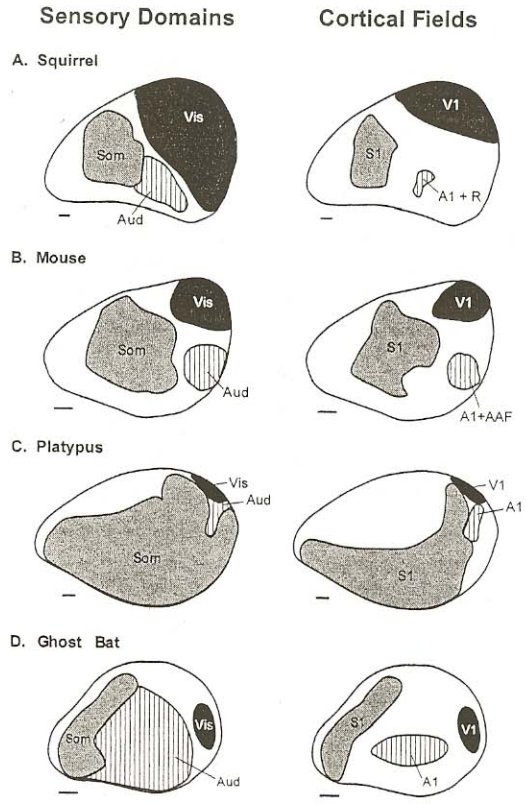

FIG. 2. The sensory domain allocation (left) and location and size of primary cortical fields (right) in the neocortex of four different mammals with different sensory specializations. The squirrel (A) is a highly visual rodent with much of its neocortex devoted to the visual system (A, left), and the relative size of V1, as compared to other primary sensory areas, is large (A, right). The mouse (B), which relies more on its somatosensory system, particularly its vibrissae, than its visual system, has a large portion of its neocortex devoted to somatosensory processing (B, left), and the relative size of S1 is larger than V1 or A1 (B, right). The duck-billed platypus (C) has an extremely well developed bill which it uses almost exclusively for feeding and navigating, and most of its neocortex is devoted to the somatosensory system (C, left). The relative size of S1, as compared to other primary fields, is quite large (C, right). The ghost bat (D) is an echolocating mammal that relies heavily on its auditory system. A large proportion of its neocortex is devoted to the auditory system (D, left), and the relative size of A1 is large compared to other primary fields (D, right). Scale bar = 1 mm. Squirrel: Kaas et al (1989), Krubitzer et al (1986), Luethke et al (1988). Mouse: Carvell & Simons (1986), Stiebler et al (1997), Wagor et al (1980), Woolsey (1967). Platypus: Krubitzer et al (1995). Ghost bat: Krubitzer (1995), Wise et al (1986).

on its vibrissae than its visual system, much of the neocortex is devoted to the somatosensory system, and in particular, to processing input from the vibrissae. The relative size of S1 is larger in the mouse than the size of either V1 or A1 (Fig. 2B, based on Carvell & Simons 1986, Stiebler et al 1997, Wagor et al 1980, Woolsey 1967). Similarly, the duck-billed platypus has an extremely well developed bill that is composed of densely packed mechanosensory and electrosensory receptors. The platypus uses its bill for most activities including navigating in water, prey capture, predator avoidance and mating. Most of the neocortex in the platypus is devoted to the somatosensory system, and the relative size of S1 compared to other primary fields is quite large; in fact, approximately two-thirds of the cortex is involved in processing input from the bill (Fig. 2C, based on Krubitzer et al 1995). Finally, the ghost bat is an echolocating mammal that relies on its auditory system for most vital behaviours. It is not surprising that a large proportion of its neocortex is devoted to the auditory system, and that the relative size of A1 is large compared to other primary fields (Fig. 2D, based on Krubitzer 1995, Wise et al 1986). In addition to these, other mammalian species show the same trend, namely, that the relative size of a sensory domain and the primary cortical area within that domain are related to the behavioural relevance of that sensory system. These and other types of modifications, such as the addition of cortical fields, changes in connectivity and the addition of modules, constitute a limited number of the systems level changes that have occurred in evolution. Presumably, these modifications account for the high degree of variability in sensory processing and related behaviours observed across mammals.

The comparative approach has yielded important insights into cortical field evolution. In particular, it has allowed us to identify a homologous constellation of cortical fields and their connections, and to appreciate the types of modifications that have been made to the brain throughout evolution. Furthermore, it has revealed that the number of modifications made to the neocortex appears to be constrained and that some of these modifications, such as cortical domain allocation and cortical field size, appear to be related to specialized morphology and use.

How is the developmental approach utilized to study cortical evolution?

A developmental approach can be used to determine how cortical domains and cortical field size have changed in relation to peripheral, morphological specializations and use. This approach can also be used to uncover the mechanisms that give rise to aspects of cortical field organization, as well as to understand how these mechanisms have been altered in different lineages to account for phenotypic variability. In general, studies of the developing nervous system that seek to understand how structures and areas emerge and how these areas become precisely interconnected fall into two main categories.

The first category includes studies that examine the intrinsic or genetic contribution to aspects of neocortical development. This group is varied and includes descriptions of spatial and temporal aspects of the normal developmental processes that are thought to be intrinsic to the neocortex (Donoghue & Rakic 1999, Rubenstein et al 1999), such as the assignment of the rostrocaudal axis (Fukuchi-Shimogori & Grove 2003, Muzio & Mallamaci 2003), the formation of thalamocortical connections (Bishop et al 2000, Inoue et al 1998) and the emergence of particular architectonic features (Fukuchi-Shimogori & Grove 2001, Hamasaki et al 2004). Most of these types of studies alter the genetic environment of a developing animal via mutations, over-expression, or ectopic placement of a gene or gene product. One problem with these types of studies is that they are often confounded, since a single gene or gene product is usually involved in several different processes at multiple stages of development. Furthermore, while genetic mutations are an integral part of evolution, many of the mutations that are studied in developmental experiments result in offspring that do not survive postnatally. Since these types of mutations would result in non-viable offspring, the evolutionary relevance of some genetic models is unclear. However, an extremely important strength of this approach is that it allows us to directly examine potential genetic mechanisms that give rise to cortical attributes in development and evolution. Another method used to examine the types of intrinsic changes that give rise to phenotypic variability is to physically alter some aspect of the developing neocortex (e.g. Huffman et al 1999, Schlaggar & O'Leary 1991) and determine whether the resulting changes that are observed in cortical organization are consistent with the types of changes that occur in evolution. The advantage of this approach is that an area of interest can be manipulated directly, without the confounds associated with global genetic changes. The goal of physical manipulations is to understand whether specific changes that are made in the developing cortex can induce the formation of cortical phenotypes that are similar to those observed in extant lineages. This approach does not test the mechanism that may naturally cause these types of manipulations; it only looks at the results of the manipulations. This is different from the genetic manipulation approach described above, which examines genetic mechanisms that cause the variation observed in extant lineages. Nonetheless, both approaches are aimed at understanding the intrinsic or genetic contribution to neocortical development.

The second category includes studies that examine the role of extrinsic factors in generating aspects of neocortical organization, such as sensory-driven activity. There are two main methods used to study the extrinsic contributions to the cortical phenotype. First, physical manipulations can be made to the developing sensory receptor array, such as enucleating an eye or removing vibrissae. As described above, the advantage of physical manipulations is that they can be made directly to specific structures, without the confounds of changing a global developmental factor.

Another method that can be used to examine the role of extrinsic factors in generating cortical phenotype is to change the external environment in which the animal develops. The advantage to this approach is that it does not cause any genetic or physical confounds with the animal's normal developmental process. Furthermore, this approach mimics natural processes and can provide some insights into how alterations in the nervous system and behaviour are shaped by the environment and, ultimately, incorporated into the genome via natural selection (see Krubitzer & Kaas 2005). Unfortunately, it is difficult, although not impossible, to generate experiments that can fully explain developmental mechanisms by only manipulating the environment.

Because all of the techniques that are used to examine the intrinsic and extrinsic contributions to the phenotype have limitations associated with them, it is best to use a combination of these approaches to understand how structures and areas emerge in the developing nervous system and how those areas evolve. Together, these types of studies have already uncovered a number of important developmental mechanisms that may be responsible for the types of modifications that have been made to the neocortex, such as cortical domain allocation, cortical field size determination and connectivity.

Intrinsic mechanisms that shape cortical field development and evolution

One way that aspects of neocortical organization, including geographic location, patterns of connections, relative cortical field size and module formation can be changed is by altering genes intrinsic to the neocortex. For example, transcription factors, such as *Emx2* and *Pax6,* appear to play an important role in assigning the geographic relationships between primary fields in the rostrocaudal axis and the patterning of thalamocortical connections (Bishop et al 2000, Muzio & Mallamaci 2003, see O'Leary & Nakagawa 2002). In mice lacking *Emx2*, thalamic afferents from the ventral posterior nucleus (VP), which normally innervate S1, are shifted far caudally, into cortex that would normally develop into visual cortex (Fig. 3A, Bishop et al 2000), demonstrating that changes in gene expression can play an important role in the patterning of thalamocortical connections. In terms of cortical field size and location, mice genetically engineered to overproduce nestin-*Emx2* have a larger V1 than wild-type animals and other primary fields, such as S1, shift rostrally on the cortical sheet (Fig. 3C, Hamasaki et al 2004). Finally, when the signalling protein FGF8 (fibroblast growth factor 8), which is involved in setting up anterior–posterior patterning via the regulation of *Emx2* expression (Fukuchi-Shimogori & Grove 2003) and is normally located in the rostral pole of the neocortex, is electroporated into an ectopic location caudal to S1, a duplicate cortical barrel field (defined histochemically) is observed just caudal to S1 (Fig. 3E, Fukuchi-Shimogori & Grove 2001). This suggests that altering patterns of gene expression

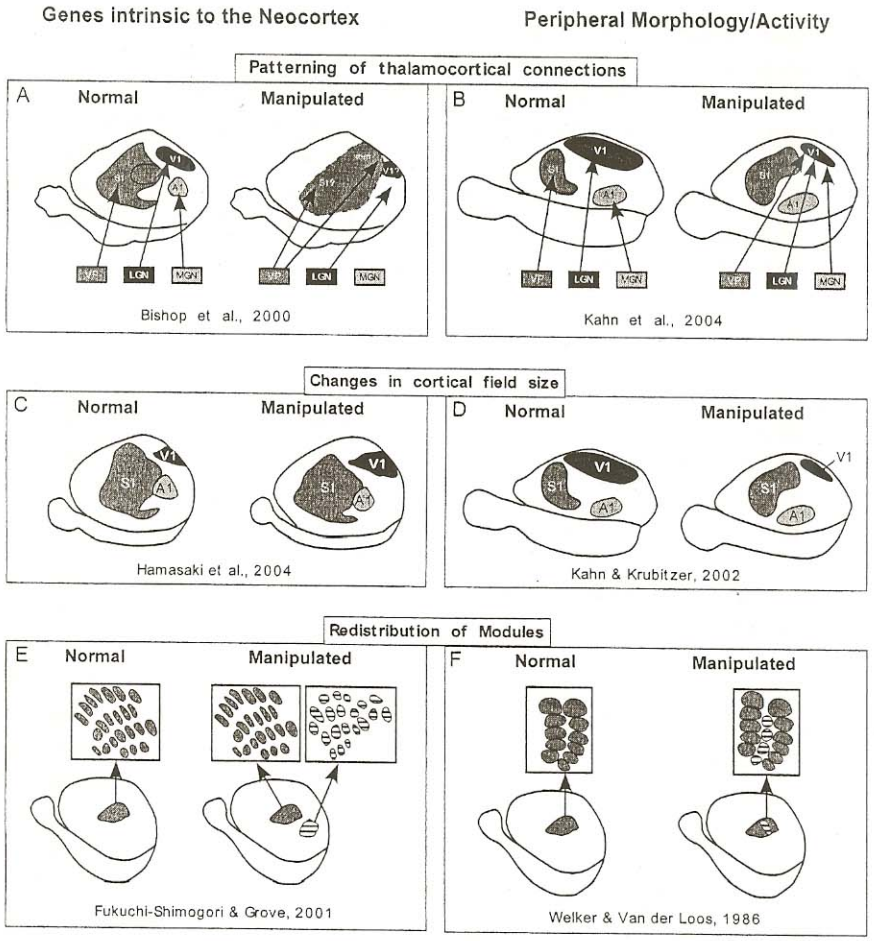

FIG. 3. Similar types of system level changes can be induced in cortical development through intrinsic genetic mechanisms or through extrinsic changes in peripheral morphology and activity. First, thalamocortical connections can be shifted caudally in transgenic mice by altering the expression of the *Emx2* gene (A). Alternatively, thalmocortical afferents can be shifted by changing peripheral morphology, such as bilaterally enucleating the eyes early in development (B). Another consistent modification made to the neocortex in different lineages has been a change in the size of a cortical field. The size of a cortical field can be changed by overexpressing genes, such as the overproduction of nestin-*Emx2* in transgenic mice that results in a larger V1 than wild-type animals (C). Alternatively, cortical field size can be decreased by altering peripheral morphology by bilaterally enucleating the eyes early in development of opossums (D). Although the direction of change is different, in the former study V1 increases and in the later it decreases, both types of manipulations result in changes in the size of V1. Finally, another type of modification that can be made to the neocortex is the addition of modules. This has been accomplished experimentally by electroporating the signalling molecule FGF8 into an ectopic location in cortex, caudal to the normal location of the barrel fields (E) or by selectively breeding mice to grow an extra row of whiskers (F). In both experiments, additional barrels were generated.

can restructure the formation of modules on the cortical sheet. Taken together, these studies in developing animals indicate that several of the ubiquitous features of cortical organization, such as thalamocortical and corticocortical connectivity, primary sensory field size and location, and modular organization, can be genetically regulated.

Extrinsic mechanisms that shape cortical field development and evolution

Alternatively, altering peripheral morphology and sensory driven activity early in development can also have a dramatic affect on neocortical organization (Kahn et al 2004, Rakic et al 1991, Sur & Leamey 2001 for review). Alterations in peripheral morphology can influence domain allocation, the size of a cortical field, and the development of thalamocortical afferents. For example, animals that were bilaterally enucleated early in development, prior to thalamocortical innervation, had massive changes in cortical organization and architecture, in that there was a large change in sensory domain allocation. All of what would be visual cortex, including V1 was taken over by the somatosensory and auditory systems. Furthermore, the size of V1 was greatly reduced in enucleated animals (Fig. 3D, Kahn & Krubitzer 2002, Rakic et al 1991), and alterations in thalamocortical connections were observed (Kahn et al 2004). In experimental animals, primary visual area (V1), as defined by electrophysiology and myeloarchitecture and which is normally connected only with the lateral geniculate nucleus (LGN), formed connections with the LGN, the ventral posterior nucleus (VP), and the medial geniculate nucleus (MGN) (Fig. 3B). Interestingly, in this study in *Monodelphis domestica*, as well as in a previous study by Rakic et al (1991), it was observed that bilateral enucleation early in development leads to the emergence of a new architectonic area, called area X, that appears between areas 17 and 18. Area X has been shown to process auditory and somatic inputs, primarily from the head, vibrissae and snout (Kahn & Krubitzer 2002). These results suggest that early changes in peripheral morphology, such as bilateral enucleation, can have an enormous effect at multiple levels of the nervous system. While the fact that V1 is still present in enucleated animals indicates that some aspects of arealization are intrinsically regulated and do not depend on specific sensory inputs, alterations in domain allocation, cortical field size, connectivity and the emergence of a new cortical area indicate that extrinsic mechanisms play an equally important role in cortical field development and evolution.

In addition to determining the connections and relative size of primary cortical areas, changes in peripheral morphology can also influence modular organization. Early work by Welker & Van der Loos (1986) demonstrated that mice that were selectively bred to have an extra row of whiskers developed an additional row of barrels that were inserted within the barrel field of S1 (Fig. 3F, see also Catania & Kaas 1997). Finally, alterations in the type and amount of sensory stimulation that

the developing nervous system is exposed to can have a dramatic affect on the resulting phenotype. For example, rats reared in a chronically noisy environment had a large disruption in the development of A1, and those reared with exposure to pure tones developed frequency maps in A1 that showed a specific expansion of the pure tone frequency (Chang & Merzenich 2003, Zhang et al 2002, 2003). Taken together, these studies indicate that several features of cortical organization, sensory domain allocation, cortical field size, thalamocortical connectivity and modular organization can be regulated by peripheral morphology and sensory-derived activity.

Conclusions

Since the same types of modifications (e.g. changes in cortical field size) that occur naturally in the neocortex can be accomplished in more than one way, it is critical to examine the roles of both intrinsic genetic factors and extrinsic factors that contribute to different attributes of the developing nervous system, and ultimately, that generate phenotypic diversity in different lineages. For example, we can test the importance of intrinsic genetic factors by comparing patterns of gene expression in highly derived animals, such as an echolocating bat and a mouse. These animals have a similar size neocortex but different, highly derived peripheral morphologies which are specialized for processing different modalities of sensory information. Furthermore, the neocortex of these animals is markedly different in terms of sensory domain allocation and the relative size of primary fields (Fig. 2). Given that all mammals possess a common plan of organization, it seems reasonable to conclude that the presence of the primary cortical areas, their connections, their modular organization and their size are, in large part, genetically determined. The caveat to this is that the relationship between specific patterns of gene expression and cortical field development is still unclear. Nevertheless, if genes play a significant role in patterning the cortex, then we would expect animals with similar sized cortices and noticeably different sensory domains, such an echolocating bat (Fig. 2D) and a highly somatic animal like the mouse (Fig. 2B), to have the same genes regulating general features of cortical development, yet to have slightly different gradients of expression or, possibly, differences in temporal expression. In other words, the same genes most likely control cortical patterning in all mammals; the differences between species are probably derived from slight differences in how and when those genes are expressed. Therefore, by comparing the patterns of gene expression in highly derived animals, we can deduce which genes are actually involved in assigning cortical domains and understand how and when the expression patterns of those genes are altered during development in different lineages.

In conclusion, by combining the comparative approach with a developmental approach it is possible for us to study the roles of genes and sensory-driven activity derived from peripheral morphology on the formation of cortical fields in development, and to make accurate inferences about how these mechanisms contribute to neocortical evolution. Not surprisingly, changes in intrinsic patterns of gene expression or in the sensory receptor arrays in the periphery can have remarkably similar effects on cortical field formation, indicating that the same types of phenotypic modifications can be accomplished via different mechanisms. Only by using a multidisciplinary approach can we hope to unravel the mechanisms used in evolution, in different lineages, and to understand how these mechanisms are altered in nature to generate the wide range of diversity seen in mammalian species.

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DISCUSSION

Diamond: There are far more species now extinct than those currently existing. We can assume that extinct species didn't survive because something didn't work as efficiently as it needed to work. Presumably, cortical evolution is interesting because what didn't work in extinct species might in many cases have been something about brain organization. Could you guess about some of the cortical organizations that extinct species might have had, but which didn't work?

Krubitzer: It would be dependent on the context in which that species was evolving. My guess is that it wasn't an experiment in brain organization but it had more to do with a rapid change in the environment. What I hope I have demonstrated is that phenotypic variability is in part genetically driven and in part activity dependent. The intracellular mechanisms that allow for plasticity are probably genetically determined, but the phenotypes that unravel aren't, which means they masquerade as evolution. As long as the environment in which that individual is unravelling is

constant, that phenotype is going to look the same. An important property of the mammalian neocortex is that it is plastic. If there are small changes in the environment, the cortex is going to be able to adapt fairly well. One thing we are now doing is looking at the behaviour of these animals, to see what an expanded visual cortex is doing in congenitally deaf mice. In terms of the extinctions, it is not so much that they have mucked around with changing cortical organization, but the environment has changed dramatically. Even if there is some normal distribution of phenotypes that could be generated under some normal distribution of environmental conditions, if the environmental fluctuations become extreme and fall outside of the distribution of potential changes that could be made to neural and non-neural tissue, then extinction occurs. When I am talking about activity or external influences on phenotype, I am talking about sensory inputs and so on, but even passive changes such as in pH, body temperature or amount of food present can factor into extinction.

Logothetis: Can you replay your theory in slow motion? I missed most of it because you were talking so fast. You said that we have some kind of organization and then there is some kind of input coming in. In the beginning it is sparse and the input is reaching existing areas, and these are cells among other cells. Is this what you are showing?

Krubitzer: No. What I'm showing is there is a given pattern of thalamic activity, from the ventral posterior nucleus or LGN, for example, imposed on the cortical sheet in a particular animal. Let's say that within some lineage some change occurs in peripheral morphology. For example, I develop a highly sensitive vibrissal system. This change promotes changes in thalamic organization, and in turn, I develop a modular organization or some segregation of those inputs in the neocortex. It may be beneficial to keep those inputs separate (e.g. it helps me make finer discriminations of roughness and so on). This sort of change is constantly occurring. Perhaps I have an evolution of a new receptor type, for instance a new ganglion cell type. This will have an influence of the next structure, in this case the LGN, which in turn is going to change cortical organization. For the somatosensory system, a very small change in a small piece of tissue such as the dorsal column nucleus is going to have a large cortical signature. I don't think you need big tweaks in peripheral morphology to generate these changes in the cortex.

Logothetis: I am still not clear about what your theory is. You are showing small targets interspersed in the cortex, and then at some point these may aggregate in the cortex on the basis of similarity.

Krubitzer: Let me try to illustrate this. If we look at something like V2, people have argued that it is not a single representation but is actually three representations interdigitated. Each of the bands in V2 obey all the rules of a cortical field. In some species the segregation may continue because it works out.

Diamond: Maybe it will help if you define what an alternative theory might be.

Krubitzer: An alternative theory might be that there is something inherent about V1: it is absolutely genetically specified and there is some change in spatial expression of genes or the temporal pattern of gene expression that leads to a new cortical field.

Logothetis: What you have just proposed as an alternative doesn't contradict your theory.

Krubitzer: My theory assumes that cortical fields are not generated simply by mechanisms intrinsic to the cortex. Instead, connections and activity are involved in generating new cortical fields or new patterns of organization. If you take a strictly genetic approach, you would have to argue that some change in the spatial and/or temporal pattern of expression of some gene, or the addition or deletion of an allele is solely responsible for some aspect of cortical organization and the emergence of cortical fields in development.

Logothetis: You are saying that the genetic mechanisms determine the range.

Krubitzer: Yes. I was pointing out that there are large genetic constraints which means that you can't eliminate particular fields, such as V1, their location (in caudomedial cortex) is for the most part invariant, and aspects of thalamocortical and corticocortical connectivity are similar in all mammals.

Logothetis: You seem to be suggesting that at some point the cells decide they have a greater advantage if they are closer to each other and share connectivity than if they are dispersed.

Krubitzer: Exactly. At some point I would rather be mapped next to the representation of koniocellular cells, than next to the representation of M cells that share a similar portion of retinal space. It is a compromise that depends on what will work for that particular animal. All of this is inference.

Derdikman: Can we gain insights about sensorimotor function from looking at the evolution of the sensorimotor areas and comparing them to behaviour within species?

Krubitzer: Yes. Although some fields may be homologous, such as V1 and M1, it doesn't mean that they are analogous (i.e. have the same function). Most people at this meeting have been discussing single units and looking at behaviour. They then correlate activity with some aspect of behaviour or type of sensory stimulation. That unit sits within a cortical field which sits within a network. In the platypus, there is a small V1, no apparent V2, a small undifferentiated LGN, and a micro ophthalmic eye. In monkeys, V1 is present as well as V2 and several other extrastriate areas to which V1 projects. The LGN is laminated and highly differentiated, and the eye is highly developed. V1 projects to and receives feedback from several extrastriate cortical areas; this is not the case for the platypus. Is V1 doing the same thing in a platypus as it is in a monkey? I doubt it.

Treves: I am trying to think of something that is *not* in your theory. You showed us a lot of evidence about cortical flexibility or adaptation, and some nice evidence

about thalamic flexibility. Can you contrast this cortico-thalamic readiness for change with subcortical structures that are more genetically hardwired?

Krubitzer: There aren't a lot of data on this. If you look across a lot of mammals, the cortex has changed most dramatically. The thalamus has changed quite a bit as well. But brainstem structures haven't changed as much, because they are doing things that are important for survival such as breathing and regulating heartbeat. Those genes involved in specification for parts of the brain that are necessary for life function, are also necessary for non-life sustaining functions, such as aspects of cortical arealization. This leads to serious functional integration, and places huge constraints on changing particular aspects of the non-life sustaining features of organization (i.e. cortical organization) that this gene encodes. Thus, the brain is a compromise.

Brecht: In your theory there are modules that are then sorted out to areas. This is not what we feel when we look at the rodent work. There the modules appear to be something very different from the maps. You can have modules or barrels, or you don't have them, but you always have the map. More importantly you can genetically make a map. You put on FGF8 and then you have second barrel cortex: this is a genetic mechanism that is being read by the afferent axons. Do you know of any instance where there is evidence for modules that then get sorted out into two maps?

Krubitzer: No, but it would be difficult to actually observe such a thing given the large time scales of evolution. The point I was making earlier about the barrel business and whisking versus not whisking is that the barrels are in some sense epiphenomenal. They are not needed to do certain things. Jonathan Horton recently showed in squirrel monkeys half of them had ocular dominance columns (OCDs) and half of them didn't, which suggests that OCDs are not necessary for particular aspects of visual processing.

Brecht: Doesn't the whole developmental work suggest maps are something very different from modules?

Krubitzer: You can de-correlate modules with function and you can also de-correlate architectonically defined cortical areas such as S1 or V1 with function. In most mammals, primary cortical field function and architecture are highly correlated.

Haggard: I'd like to encourage you to speculate. You have been talking mainly about primary cortical areas. Particularly in the context of decision making, what will these tell us about secondary cortical areas? Traditionally these have been thought of as more abstract, and didn't seem to have this close allegiance to the periphery.

Krubitzer: That's a good point. I talked almost exclusively about primary areas. Developmental neurobiologists do the same because they are really easy to identify. My gut tells me that there is something different about primary fields versus secondary cortical areas. Primary fields may be more genetically regulated or constrained than other fields. If we look at most non-primary cortical areas architectonically, they aren't particularly distinct. I think these fields are more susceptible to environmental influences during development than in adults. It is not a coincidence that as we examine extrastriate cortex in macaque monkeys, everyone argues about what the boundaries of cortical fields are. This may be because in the experiments in which we are trying to figure out what particular cortical fields are doing, and how cortex is subdivided, by training the animal to perform some experimenter driven task, we are actually changing the cortex. In developing animals I think this cortex is extremely plastic. We did an experiment many years ago where we mapped somatosensory cortex in a one day old monkey. We found that S1 was in place, but we couldn't find area 1 or area 2. This indicates to me that the environment (i.e. patterns of sensory receptor array activity) play a larger role in directing the organization of these fields than it does for primary fields.

Haggard: You have described a mechanism for the module to break away, aggregate and make a new area. Would this allow secondary areas to arise from primary ones? Wouldn't it just give you more primary ones. The evolution of secondary areas might need a different mechanism.

Krubitzer: Part of me thinks that primary fields such as S1 and V1 are the 'off-ramp' for change, in that all new fields may originate in primary fields and segregate or move out of them. I agree that the mechanisms I describe for primary cortex may not be the same for association cortex.

Sparks: You talked about anatomical controls to show that the inputs are the same, but I didn't hear you talking about anatomical experiments to show that the outputs of areas were the same. This relates to a general concern I have about 'remapping' and adaptive remapping. Is it really adaptive? As the cortical area is invaded and formerly visual cells are now activated by auditory stimuli, is the sensation evoked still vision or is it auditory? Is the attribution of the activity changed or is only the stimulus that induces the activity changed? By looking at output mappings you could get a handle on this.

Krubitzer: That's a good idea. One other thing we have been doing is looking at behaviour in a more global sense: is it adaptive? Are these animals better at performing some tasks with this expanded cortex? One of the things we have also started to do is to map the reorganized areas more closely. For example, in the bilaterally enucleated animals almost all of reorganized cortex contains representations of the vibrissae of the head and face, which overlaps with auditory inputs. To me this suggests that there is some functional dependence between the vibrissae of the face and the auditory system for localization that doesn't exist in normal animals. Your question will be difficult to answer even if we look at outputs. The best way we can get at this is to look at humans who have lost a sensory system. Unfortunately I don't think their cortex gets invaded to the same extent unless their loss is congenital.