How does evolution build a complex brain?

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Abstract. To understand how complex brains evolve one can examine a variety of the products of the evolutionary process and then infer the mechanisms that generate the differences observed. We address this issue using a number of techniques. We combine neurophysiological recording techniques with neuroanatomical tracing techniques and histochemical methods in an effort to accurately determine the functional subdivisions of the neocortex in a variety of mammals. By using these techniques we can determine common features of neocortical organization, or common cortical areas, which are considered homologous. We can observe modifications to patterns of cortical organization, or to cortical fields specifically, that are independently evolved and generally related to morphological and behavioural specializations. Comparative studies have led us to consider the development of the neocortex and the specific changes in developmental mechanisms that might account for the observed changes in extant adults. Both comparative studies and developmental studies allow us to formulate hypotheses regarding how the neocortex is constructed in the life of an individual, and in a lineage over time.

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Comparative work on a number of different mammals indicates that changes in the size of the cerebral cortex and in the number of its functional subdivisions are perhaps the most dramatic alterations to the mammalian brain in evolution (Stephan et al 1988, Krubitzer 1995). Indeed, there is over a 3000-fold difference in the size of the brain of the smallest mammals, some shrews and mice, and that of some cetaceans, such as dolphins and whales (see Manger et al 1998 for review; Fig. 1). Although the precise relationships between structure, function and behaviour are often difficult to understand, an increase in cortical surface area and number of interconnected cortical fields is generally associated with an increase in sensory, perceptual, cognitive and behavioural complexity. An obvious question is: how are more cortical fields added in evolution?

Our laboratory has addressed this question by comparing the brains of a variety of mammals that represent major branches of evolution (Fig. 2) using a number of techniques to subdivide the neocortex. These techniques include multiunit

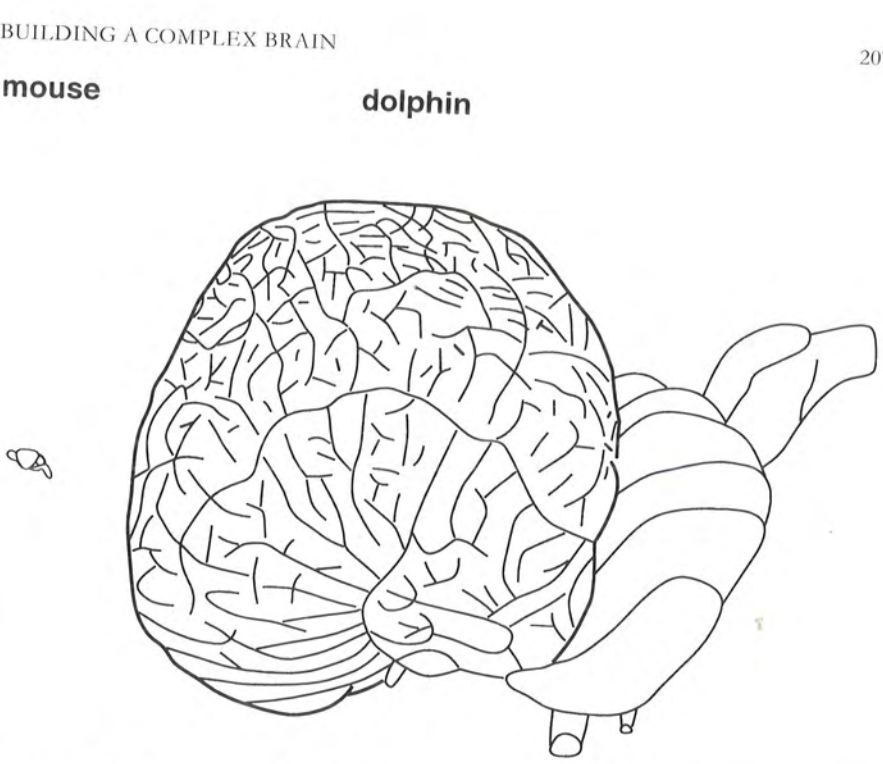


FIG. 1. A mouse brain and dolphin brain drawn to scale. There is not only a large difference in the size of the neocortex, but also in the number of functional subdivisions that reside therein. In this and the following figures, rostral is to the left and dorsal is to the top.

electrophysiological recordings that allow us to sample a large extent of the neocortex, and to assign different modalities to different regions of the cortex. In conjunction with this, the architecture of the cortex is examined in the same animals and physiological boundaries are correlated with architectonic distinctions. Finally, the corticocortical, interhemispheric and subcortical connections of individual fields are examined to determine the unique pattern of interconnections that each field possesses.

While we cannot study evolution directly, by examining the products of the evolutionary process we can ascertain which aspects of cortical organization are common to all mammals, which features are unique to certain species and what types of modifications to the brain are made. In this way, we can make inferences about the evolutionary process, and the constraints placed on evolving brains. Because the evolution of the neocortex is actually the evolution of developmental programmes that generate the adult form, upon which selection operates, a second approach to understanding cortical field evolution is to study the developmental mechanisms that contribute to area specification (Killackey 1990).

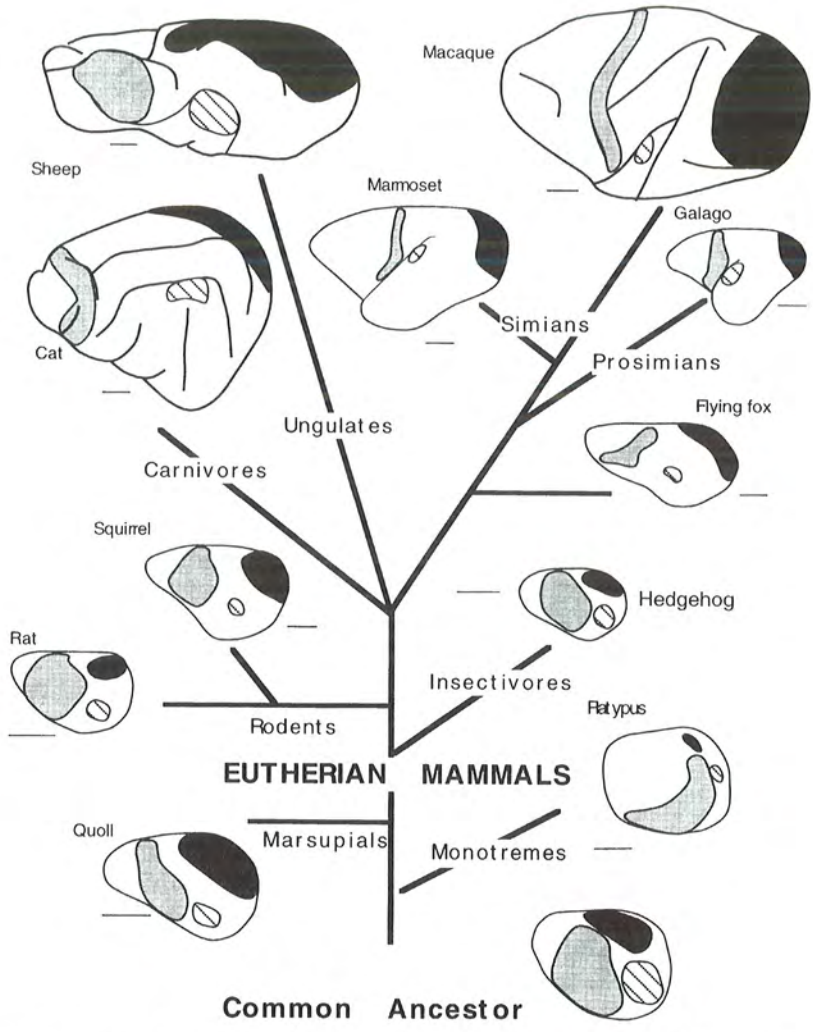


FIG. 2. A simplified evolutionary tree that depicts the major orders of mammals and the representative brain organization of common fields found in some of the species within each order. By comparing the brain organization across species and determining the common patterns of cortical field organization, it is possible to infer the organization of the common ancestor of all mammals. Once this is established, hypotheses regarding the changes in cortical organization that have occurred across lineages is possible. The black region denotes the primary visual cortex (V1), the striped region denotes the primary auditory cortex (A1) and the shaded region denotes the primary somatosensory cortex (S1). Note that the relative locations of these fields with respect to each other have shifted. Also, the relative amount of cortex assumed by these fields is often different for different animals. Finally, in animals with a greatly expanded neocortex (e.g. macaque monkeys), S1, A1 and V1 have moved far apart and new fields have been interspersed between these old fields.

This chapter outlines the cortical organization in a variety of different species, illustrates the features of organization that have been retained from the common ancestor and discusses the modifications to cortical organization that have occurred in different lineages. These observations allow us to evaluate the viability of current hypotheses regarding the development of the neocortex, and to propose the types of changes that might have occurred in developing brains during evolution to account for species differences.

Monotremes and marsupials

Electrophysiological recordings, coupled with architectonic analysis and studies of connections in two of the three species of extant monotremes, demonstrate that these animals have a constellation of cortical fields that represent the visual, auditory, somatosensory and, in the case of the platypus, the electrosensory epithelium (Krubitzer et al 1995). In both species, a primary somatosensory area, S1, can be readily identified with the foot represented most medially, followed by representations of the hindlimb, lower trunk, upper trunk, forelimb and face in a mediolateral progression (Fig. 3). S1 is coextensive with a myelin dark and cytochrome oxidase-dense field that receives inputs from the ventral posterior nucleus of the thalamus. Two additional representations of the somatosensory epithelium have also been observed and are termed the rostral field (R) and the parietal ventral area (PV). R contains a complete representation of deep receptors, while PV contains a complete representation of cutaneous receptors. Both species also contain at least one visual area, hypothesized to be the primary visual area (V1), and one auditory area, possibly A1 (Krubitzer 1998).

The most striking aspects of cortical organization in monotremes are the geographic arrangement of cortical fields and the cortical magnification of highly specialized peripheral body parts. For instance, V1 is just medial to the foot representation in S1, and auditory cortex is almost completely embedded in somatosensory cortex. In the platypus, the size of the bill representation in the neocortex is enormous. Indeed, across all of the representations examined, the representation of the bill assumes approximately 75% of the entire neocortex (Fig. 3).

Marsupials represent another early branch of evolution, since their ancestors diverged either with or slightly after the ancestors of extant monotremes (Westerman & Edwards 1992, Kirsch & Mayer 1998; Fig. 2). Our laboratory (Huffman et al 1999), as well as other laboratories (e.g. Sousa et al 1978, Beck et al 1996, see Johnson 1990, Rowe 1990 for review), demonstrate that like extant monotremes, marsupials possess a constellation of cortical areas that includes S1, S2/PV, R, V1, A1 and M (motor cortex). These fields have similar architecture, as well as similar patterns of connections from other cortical fields and from the

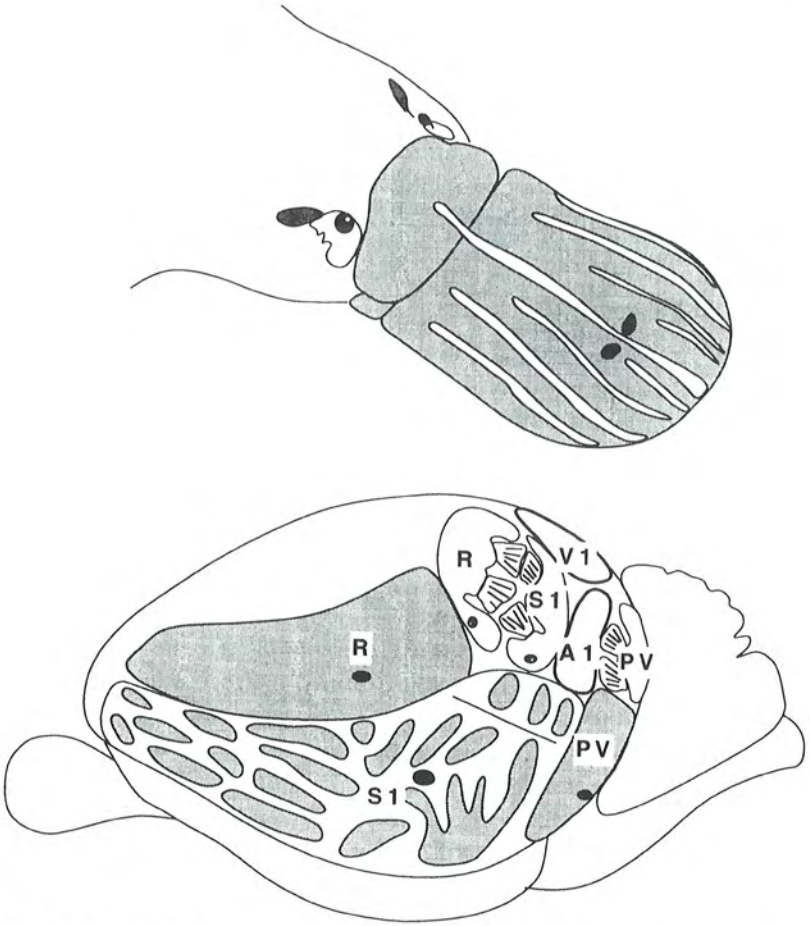


FIG. 3. The bill of the platypus (top) is highly specialized and contains both mechanosensory receptors (shaded) and electrosensory receptors (white) that are aligned in rostrocaudal stripes on the bill. The representations of the bill in the neocortex in the three somatosensory fields identified (bottom) is large and assumes most of the entire extent of the cortical sheet. In the primary somatosensory cortex (S1), representations of mechanosensory (shaded) and mechanosensory + electrosensory (white) are interdigitated with one another. In the rostral field (R), only mechanosensory and deep receptors are represented (shaded). In the parietal ventral area (PV) only mechanosensory receptors are represented (shaded). The other body part representations occupy far less of the neocortex. Primary visual cortex (V1) and auditory cortex (A1) are in locations that are unusual compared to other mammals.

thalamus (see Krubitzer 1995 for review). Like monotremes, the differences in cortical organization are, to a large extent, a reflection of morphological specialization. For instance, the dunnart is a small carnivorous marsupial that has a well-developed visual system which dominates the central nervous system. In

addition to large eyes, relative to the size of the head, the dunnart has a large area 17 (V1) that occupies approximately one-third of the entire neocortex.

The striped possum is an arboreal marsupial that dwells in the tropical rain forests of Northern Queensland (Australia). This animal has an elongated fourth digit that is used to extract insects from holes in the tree bark, and specialized flaps that cover the lateral and frontal base of the incisors (J. E. Nelson, personal observation 1997). The cortex of the striped possum differs dramatically from that of the dunnart in that substantially less of the total amount of the neocortex is devoted to area 17, and the somatosensory cortex has expanded representations of the fourth digit, gums and tongue (Huffman et al 1999).

The most notable difference in the organization of marsupial neocortex compared to monotreme neocortex is the arrangement of cortical fields with respect to each other (Fig. 2). For instance, the primary visual area (Rosa et al 1999) is at the caudal pole of the cortex, and auditory cortex resides lateral to somatosensory cortex (Gates & Aitkin 1982, Aitkin et al 1986). In marsupials that have an expanded neocortex relative to their body size (e.g. the striped possum), more sensory fields have been observed, and more cortex is interposed between areas of the retained constellation (S1, A, V1, S2/PV, R, M).

Eutherian mammals

Although there is a dramatic variation in the size of eutherian mammal brains compared to metatherian (marsupials) and prototherian (monotremes) brains (Fig. 1), a number of components of organization are similar, despite over 150 million years of independent evolution. For instance, all eutherian mammals examined have S1, V1 and A1, as well as S2/PV, R and M. The general rostrocaudal organization of cortical fields is the same, with V1 residing most caudally, A1 located lateral and rostral to V1 and S1 located rostral to both (Fig. 2). The internal topographical organization of the retained cortical fields is also similar to that described in monotremes and marsupials. Like non-eutherian mammals, cortical fields in eutherians differ dramatically in the magnification of different portions of their sensory epithelium. For instance, most primates have a large magnification of the fovea in V1; this is particularly dramatic in a number of Old World monkeys. In monkeys that have a high degree of manual dexterity and tactile discriminatory abilities, such as those with glabrous hands with opposable thumbs, the representation of the glabrous hand assumes a large portion of all anterior parietal fields, including S1 (Nelson et al 1980, Kaas 1983). A similar cortical magnification of the glabrous hand representation in S1 has independently evolved in racoons (Welker & Seidenstein 1959, see Johnson 1990 for review). In racoons, there is a remarkable similarity in the structure of the hand compared to that of primates. The racoon's hand, unlike cats, is used extensively in

fine tactile discriminations necessary for prey capture. Finally, like the mammals described previously, the amount of cortex devoted to a particular sensory system can vary dramatically, depending on the peripheral morphology of the animal, especially the size and density of receptor epithelium and the degree to which any specialized structure is used.

Two of the largest differences in neocortex of eutherian mammals compared to other mammals is an increased neocortical size in several lineages, and an increased cortical field number. For instance, a number of primates and cetaceans have a large cortical sheet that can be subdivided into a number of different cortical fields. In macaque monkeys, the number of visual fields alone has been estimated to be over 30 (Kaas 1997, Rosa 1997). The mechanisms by which cortical fields are added is not understood, and theories of cortical field addition are incomplete (e.g. Krubitzer 1995). However, it is clear that new cortical fields are not added hierarchically; rather, new fields are interspersed between the constellation of cortical fields that are present in all mammals, and therefore presumed to be evolutionarily older and retained from the common ancestor. Thus, with the addition of a new field to the retained plan, new interactions between afferent populations are established, and old connectional patterns while retained, are likely to have changed their relationships.

For instance, the ancestral mammal was likely to have possessed a network that consisted of connections between the lateral geniculate nucleus and V1, and between area V1 and extrastriate cortex immediately adjacent to it (V2). This basic network has been identified in a variety of species. Thus, marsupials and Old World monkeys share this component of visual cortical organization. However, new cortical fields have been added in primates, such as MT (the middle temporal visual area) and DL (the dorsolateral visual area). These new fields are interconnected with V1 as well as V2, and other extrastriate cortical fields and associated thalamic nuclei. Although the V1 to V2 connection has been maintained, the addition of new fields (and connections) to this old network is likely to have changed the existing network. While cortical fields and their connections may be homologous, it is unlikely that they are strictly analogous.

Similarities and differences

A survey of a wide variety of mammals indicates that there are common patterns of organization across species, which include a constellation of rostrocaudally and mediolaterally distributed cortical fields such as V1, S1, A1, R, M, PV/S2. While these fields appear to undergo large shifts in geographic location, and the amount of cortex allotted to any particular sensory system can vary dramatically, the general rostrocaudal organization is maintained, and thalamocortical and corticocortical connections share common patterns. There are several types of

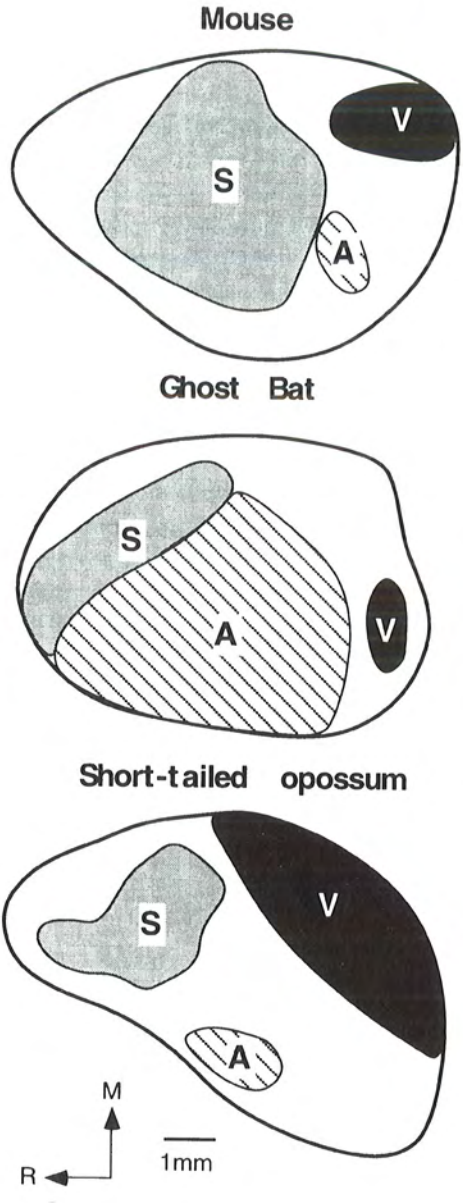
modifications that have been made to brains in evolution. Many of these changes take the same form, despite the fact that they have often evolved independently. Some of the modifications include: a change in size of the cortical sheet; a change in amount of cortex allotted to different sensory systems (Fig. 4); a change in the internal organization of a cortical field; shifts in cortical field location relative to other cortical fields; changes in connections of cortical fields; and module formation.

The evolution of cortical field development

These observations in extant mammals beg the question: are theories of cortical field specification within the life of the developing individual consistent with changes that are occurring in cortical fields in different lineages over time? Indeed, because a series of small changes to the developing nervous system are likely to account for much of the phenotypic variability in brain organization observed in extant mammals, theories of cortical development should accommodate the observations outlined throughout this chapter. Likewise, in order to understand the mechanisms of change, comparative neurobiologists must incorporate findings from developmental neurobiology in any theory of cortical field evolution. Thus, comparative studies combined with molecular techniques used to study the developing nervous system will allow us to answer several important questions regarding brain evolution. These include:

(1) How are rostrocaudal and mediolateral relationships between cortical fields maintained across species?
(2) What accounts for the large sensory domain shifts in cortical territory when cortical size is held constant?
(3) How does the cortical sheet increase in size and in the number of functional subdivisions?

The maintenance of thalamocortical afferent relationships with rostrocaudal coordinates of the cortex in all species examined suggests that there is likely to be some early specification in the developing cerebral cortex that helps to align incoming thalamocortical afferents (Fig. 5). A number of studies indicate that intrinsic patterning mechanisms within the telencephalon regulate regionalization of major subdivisions of the brain, independent of afferent input (e.g. Cohen-Tannoudji et al 1994, Levitt et al 1997, see Rubenstein et al 1999, Rubenstein 2000, this volume for review). For the neocortex in particular, one proposal is that the differential expression of regulatory genes such as *Emx2* and *Pax6* sets up a general rostrocaudal molecular gradient which controls the ordered growth of thalamic afferents to appropriate cortical locations (Gulisano et al 1996,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see Chenn et al 1997 for review). Such a mechanism could account for the stability of organization of homologous cortical fields observed in extant adult mammals. If such gradients exist to maintain relative thalamocortical relationships, how might they change in different lineages to induce differential occupation of sensory representations on the cortical sheet?

While it is possible that there may be some shift in gradients, particularly with changes in cortical sheet size (Fig. 5C, D), it is likely that other factors contribute to changes in the allotment of cortical territory in different species which have a similar sized neocortex, but have striking differences in peripheral morphology (e.g. mouse, ghost bat, short-tailed opossum; Fig. 4). The large shifts in afferent distribution observed in different lineages (Figs 4 and 6), in relation to changes in receptor density, distribution and type, suggest that major sensory domains are set up by the thalamus (O'Leary 1989, Schlaggar & O'Leary 1991), which reflects differences in the periphery (Kaas 2000, this volume). If cortical size is held constant, a genetically mediated change in the peripheral morphology can have resounding consequences for the organization of the entire neuroaxis (see Kaas 2000, this volume). Thus, while thalamocortical topographical relationships may be specified early via differential gene expression, precise visuotopic, somatotopic and cochleotopic distributions are likely to be driven by changes in the periphery, which in turn affects the organization of afferent target structures such as the brainstem and thalamus (Figs 5 and 6).

The final question of how the neocortex increases in size and number of cortical fields is not well understood. There are several recent suggestions about how the cortical sheet increases in size. One possibility is that decreased apoptosis (Kuida et al 1998, Rakic 2000, this volume) leads to an increase in the size of the cortical sheet. A second possibility is that an increase in the time over which cells proliferate in the ventricular zone can increase the size of the resulting cortical sheet exponentially (Rakic 1995, Kornack & Rakic 1998). It is more difficult to determine the possible mechanisms that contribute to

FIG. 4. The organization of major sensory domains in distantly related species with a similar size neocortex. These species differ in their peripheral morphology and specialized behaviours associated with their expanded sensory systems. For instance, in the mouse, a large portion of the neocortex is devoted to processing somatic inputs, particularly from the vibrissae. The amount of cortex occupied by auditory and visual inputs is much smaller. The ghost bat is an echolocating microchiropteran bat that has devoted a substantial portion of its neocortex to processing auditory inputs. The visual system in the short-tailed opossum is well developed and occupies a large extent of the neocortex. The most notable feature of these brains is that if cortical sheet size is held constant, the cortical territory occupied by major sensory domains can change dramatically, depending on the sensory (peripheral) specialization of the animal. This suggests that specification of major sensory domains occurs later in cortical development and is dependant on activity from the periphery (see Kaas 2000, this volume). A, auditory cortex; M, motor cortex; R, rostral field; S, somatosensory cortex; V, visual cortex.

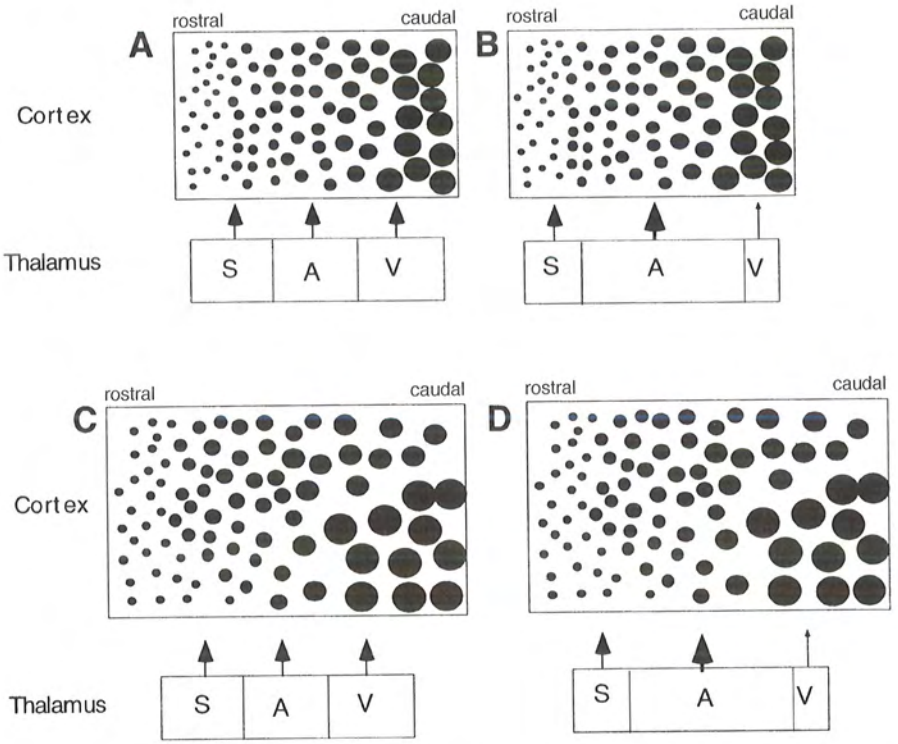

FIG. 5. Several possible ways in which sensory domain shifts might work in conjunction with molecular gradients to assign static thalamic afferent relationships in different mammals. (A) A high to low molecular gradient (e.g. *Emx2*) might work to align visual, auditory and somatosensory portions of the thalamus in an appropriate rostrocaudal fashion on the cortical sheet. Changes in major sensory representation may occur in the absence of changes to the molecular gradients that define rostrocaudal thalamocortical afferentation (B). Rather, a change in the size of the thalamic nuclei associated with a particular sensory system can result in a large change in the amount of cortex allotted to a particular sensory system (see Fig. 4). Thalamic changes are related to changes in the sensory epithelium. In a number of species, the size of the neocortex has increased. This may result in a small re-alignment of molecular gradients (C) in the absence of any dramatic changes to the thalamus. Finally, changes in both the cortical surface area and thalamic nuclei may account for differences in cortical allotment of different sensory systems (D), but this need not happen simultaneously in a particular lineage. It can be staggered in time. A, auditory inputs; S, somatosensory inputs; V, visual inputs. Large filled circles represent high concentrations and progressively smaller circles represent smaller concentrations of some molecule (taken in part from O'Leary et al 1999).

cortical field addition in different lineages. One possibility is that changes in activity patterns of incoming thalamic afferents generate new cortical fields (Krubitzer et al 1993, Krubitzer 1995). This might be accomplished simply by the addition of new cells to the developing thalamus (Fig. 6) which in turn

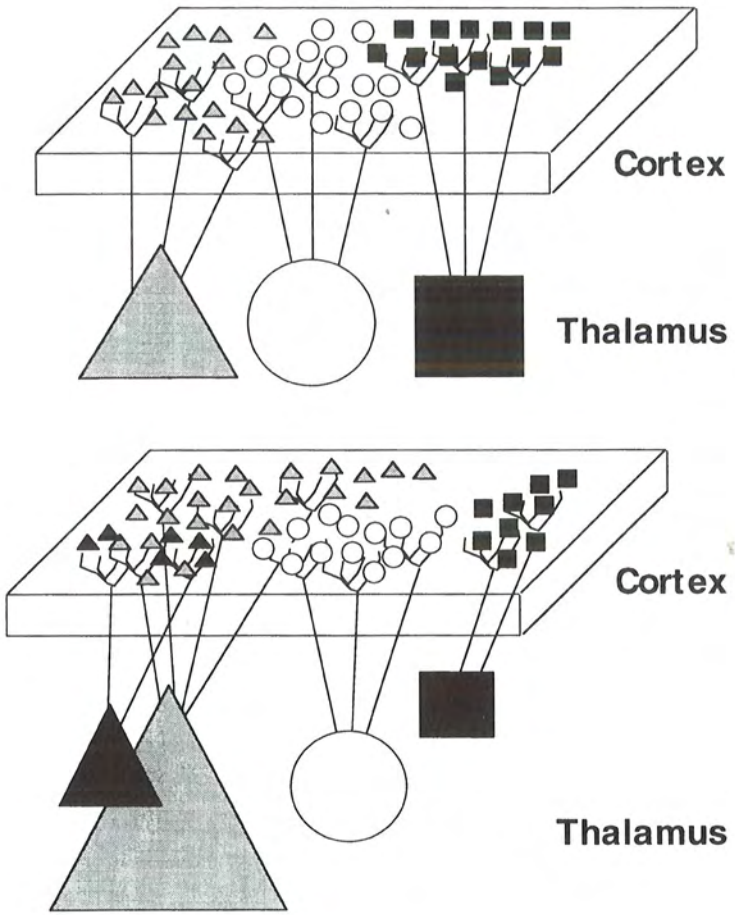

FIG. 6. A representation of shifting afferents on the cortical sheet in different lineages in evolution. The hypothesized ancestral state is depicted at the top. In this figure, thalamic inputs from different nuclei (large square, circle and triangle) are distributed in some fashion on the cortical sheet. These afferent distributions have a rostrocaudal relationship to each other. In different lineages, the afferent distribution of inputs from particular thalamic nuclei is clearly altered (bottom) so that the relationships between afferents can change. While the rostrocaudal relationships appear to be largely maintained, there is a clear change in the amount of cortex particular afferents capture, and the relative position of afferents with respect to each other. In some species, new thalamic nuclei evolve, or new receptors evolve and generate modular organization within existing thalamic nuclei (small, dark triangle). The inputs from new receptor types (e.g. electrosensory receptors) are interspersed between existing inputs on the cortical sheet and form modules within a field. Modular organization of the neocortex is a common feature found across sensory systems and across mammals (see Krubitzer 1995).

projects to the cortex. This might also be accomplished by discorrelating old inputs from retained thalamic nuclei and combining them in novel ways to generate new cortical fields (Krubitzer 1998).

In conclusion, it has been proposed at this symposium that a few changes are likely to account for much of the phenotypic variability observed in the neocortex of extant mammals. I have tried to outline here some of the possible changes that might be occurring. These include genetically mediated changes in the size of the cortical sheet, changes in peripheral morphology, changes in receptor density, distribution and type, and changes in the thalamus that lead to discorrelations in activity of incoming afferents and/or addition of cells. It is improbable that all of these genetic changes are occurring simultaneously in any given lineage. Rather they are likely to be staggered across time in different lineages to ultimately produce the types of organizations observed in extant species.

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References

Aitkin LM, Irvine DRF, Nelson JE, Merzenich MM, Clarey JC 1986 Frequency representation in the auditory midbrain and forebrain of a marsupial, the northern native cat (*Dasyurus hallucatus*). Brain Behav Evol 29:17–28

Beck PD, Pospichal MW, Kaas JH 1996 Topography, architecture, and connections of somatosensory cortex in opossums: evidence for five somatosensory areas. J Comp Neurol 366:109–133

Chenn A, Braisted JE, McConnell SK, O'Leary DDM 1997 Development of the cerebral cortex: mechanisms controlling cell fate, laminar and areal patterning, and axonal connectivity. In: Cowan WM, Jessell TM, Zipursky SL (eds) Molecular and cellular approaches to neural development. Oxford University Press, New York, p 440–473

Cohen-Tannoudji M, Babinet C, Wassef M 1994 Early determination of a mouse somatosensory cortex marker. Nature 31:460–463

Gates GR, Aitkin LM 1982 Auditory cortex in the marsupial possum *Trichosurus vulpecula*. Hear Res 7:1–11

Gulisano M, Broccoli V, Pardini C, Boncinelli E 1996 *Emx1* and *Emx2* show different patterns of expression during proliferation and differentiation of the developing cerebral cortex in the mouse. Eur J Neurosci 8:1037–1050

Huffman KJ, Nelson J, Clarey J, Krubitzer L 1999 The organization of somatosensory cortex in three species of marsupials, *Dasyurus hallucatus*, *Dactylopsila trivirgata*, and *Monodelphis domestica*: neural correlates of morphological specializations. J Comp Neurol 403:5–32

Johnson JI 1990 Comparative development of somatic sensory cortex. In: Jones EG, Peters A (eds) Cerebral cortex, vol 8B: Comparative structure and evolution of cerebral cortex. Plenum Press, New York, p 335–449

Kaas JH 1983 What if anything is S1? Organization of the first somatosensory area of cortex. Physiol Rev 63:206–230

Kaas JH 1997 Theories of cortex organization in primates. In: Rockland KS, Kaas JH, Peters A (eds) Cerebral cortex, vol 12: Extrastriate cortex in primates. Plenum Press, New York, p 91–125

Kaas JH 2000 Organizing principles of sensory representations. In: Evolutionary developmental biology of the cerebral cortex. Wiley, Chichester (Novartis Found Symp 228) p 188–205

Killackey HP 1990 Neocortical expansion: an attempt toward relating phylogeny and ontogeny. J Cog Neurosci 2:1–17

Kirsch JAW, Mayer GC 1998 The platypus is not a rodent: DNA hybridization, amniote phylogeny and the palimpsest theory. Philos Trans R Soc Lond B Biol Sci 353:1221–1237

Kornack DR, Rakic P 1998 Changes in cell-cycle kinetics during the development and evolution of primate neocortex. Proc Natl Acad Sci USA 95:1242–1246

Krubitzer L 1995 The organization of neocortex in mammals: are species differences really so different? Trends Neurosci 18:408–417

Krubitzer L 1998 What can monotremes tell us about brain evolution? Philos Trans R Soc Lond B Biol Sci 353:1127–1146

Krubitzer L, Calford M, Schmidt L 1993 Connections of somatosensory cortex in megachiropeteran bats: the evolution of cortical fields in mammals. J Comp Neurol 327:473–506

Krubitzer L, Manger P, Pettigrew J, Calford M 1995 The organization of somatosensory cortex in monotremes: in search of the prototypical plan. J Comp Neurol 351:261–306

Kuida K, Haydar TF, Kuan C-Y et al 1998 Reduced apoptosis and cytochrome c-mediated activation in mice lacking caspase 9. Cell 94:325–337

Levitt P, Barbe MF, Eagleson KL 1997 Patterning and specification of the cerebral cortex. Annu Rev Neurosci 20:1–24

Manger P, Sum M, Szymanski M, Ridgway S, Krubitzer L 1998 Modular subdivisions of dolphin insular cortex: does evolutionary history repeat itself? J Cog Neurosci 10:153–156

Nelson RJ, Sur M, Felleman DJ, Kaas JH 1980 Representations of the body surface in postcentral parietal cortex of *Macaca fascicularis*. J Comp Neurol 192:611–643

O'Leary DDM 1989 Do cortical areas emerge from a protocortex? Trends Neurosci 12:401–406

O'Leary DDM, Yates PA, McLaughlin T 1999 Molecular development of sensory maps: representing sights and smells in the brain. Cell 96:255–269

Rakic P 1995 A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. Trends Neurosci 18:383–388

Rakic P 2000 Radial unit hypothesis of neocortical expansion. In: Evolutionary developmental biology of the cerebral cortex. Wiley, Chichester (Novartis Found Symp 228) p 30–45

Rosa MGP 1997 Visuotopic organization of primate extrastriate cortex. In: Rockland KS, Kaas JH, Peters A (eds) Cerebral cortex, vol 12: Extrastriate cortex in primates. Plenum Press, New York, p 127–203

Rosa MGP, Krubitzer L, Molnár Z, Nelson JE 1999 Organization of visual cortex in the northern quoll, *Dasyurus hallucatus*: evidence for a homologue of the second visual area in marsupials. Eur J Neurosci 11:907–915

Rowe M 1990 Organization of the cerebral cortex in monotremes and marsupials. In: Jones EG, Peters A (eds) Cerebral cortex, vol 8b: Comparative structure and evolution of the cerebral cortex. Plenum Press, New York, p 263–334

Rubenstein JLR 2000 Intrinsic and extrinsic control of cortical development. In: Evolutionary developmental biology of the cerebral cortex. Wiley, Chichester (Novartis Found Symp 228) p 67–82

Rubenstein JLR, Anderson S, Shi L, Miyashita-Lin E, Bulfone A, Hevner R 1999 Genetic control of cortical regionalization and connectivity. Cereb Cortex 9:524–532

Schlagger BL, O'Leary DDM 1991 Potential of visual cortex to develop an array of functional units unique to somatosensory cortex. Science 252:1556–1560

Sousa APB, Gattass R, Oswaldo-Cruz E 1978 The projection of the opossum's visual field on the cerebral cortex. J Comp Neurol 177:569–587

Stephan H, Baron G, Frahm HD 1988 Comparative size of brains and brain components. Comp Primate Biol 4:1–38

Welker WI, Seidenstein S 1959 Somatic sensory representation in the cerebral cortex of the racoon (*Procyon lotor*). J Comp Neurol 111:469–501

Westerman M, Edwards D 1992 DNA hybridization and the phylogeny of monotremes. In: Augee ML (ed) Platypus and echidna. The Royal Zoological Society of New South Wales, Mosman, p 28–34

DISCUSSION

Karten: What is the most important take-home message about your work?

Krubitzer: We need to re-evaluate what a cortical field is, and not just think of it as a homogenous structure. It is not as straightforward as this. A new cortical field is just a new pattern of connection interactions.

Purves: You have demonstrated that cortical fields often comprise iterated units of some sort, but is it fair to conclude that all of these so-called modules represent the same kind of entity? I'm thinking in particular about barrels and ocular dominance columns. Can barrels, which represent particular peripheral receptors and that arise in the cortex more or less independently of the function of the system, be compared with ocular dominance columns, which have no peripheral correlate and seem to be much more dependent on activity?

Krubitzer: The similarity between the two is that cortex is segregating inputs from the two eyes and from the separate barrels. In addition, if you look at the sizes of modules across animals with different sized brains, you find that even if the size of the cortex varies by a factor of a few thousand, the module size only varies by a factor of two. Therefore, the cortex is not only segregating inputs, but it is maintaining a constant module size. There must be a number of competing factors that account for this restricted size, and people have argued that there's a selection for minimal connection length.

Pettigrew: I would like to add that we have been working on the platypus, and it has stripes in its primary somatosensory cortex that are like the ocular dominance columns in monkey striate cortex. The barrel fields segregate inputs from whiskers, the ocular dominance columns segregate input from the two eyes, and in the case of the platypus there are mechanoreceptors that detect mechanical vibrations in the water and electroreceptors that detect electrical information in the water. It turns out that the two sets of stripes in the platypus do what I think (we can't prove it yet), they will carry out a sophisticated analysis comparing two different sensory arrays for small differences that will give distance, just as the ocular dominance columns do

in monkeys. The ocular dominance columns enable the monkey to interdigitate the two representations of the two eyes, so it can have binocular vision. However, there is a paradox because, although there is segregation of inputs into barrels, in the case of the barrels you don't want information from one whisker, you want all the information from all the whiskers, in order to obtain information on the shape of a bundle of food, for example. Therefore, at some level all this information has to be put together—a tension between segregation of inputs and fusion. Another example of this occurs in the auditory system of the owl, where the same afferent is split into two pathways, i.e. for time analysis and for intensity analysis. These give rise to two representations that deal with time and intensity information. The two, when finally put back together, give rise to a map of auditory space that could not have been generated by mixing the inputs early on. In the case of the platypus, the two sets of stripes analyse prey, e.g. if a shrimp flicks its tail, the electrical stimulus arrives instantaneously, whereas the mechanical stimulus takes much longer to travel through the water (Pettigrew et al 1998). Therefore, the neurons above layer IV, where these two stripes integrate, give rise to information about how far away the stimulus is. In the visual system, the advantage of mixing information from the two columns is that it gives rise to stereopsis. Therefore, we have to realize that there is segregation, but that it is also integrated in the layers above layer IV to give rise to a novel function.

Rubenstein: Is the segregation of the mechanoreceptors and electroreceptors stripes present at birth?

Pettigrew: The trouble with studying the platypus is that it is 60% politics and 40% science, so the number of animals we can work on is small and our developmental data are limited. We do know that electroreceptors develop first. It's possible that the young are using these to find the mother's milk.

Rakic: The fundamental difference between ocular dominance columns and both mechanoreceptors and electroreceptors is in the ocular dominance columns there is segregation of inputs from two sides subserving the same function, whereas for mechanoreceptors and electroreceptors there is segregation for two different functions. A more comparable example would be the development of the M and P subsystems in the primate visual system. These two subsystems, which subserve different functions, develop early and independently by the molecular cues (Meissirel et al 1997). In contrast, ocular dominance columns are determined by competition between functionally identical inputs from the two sides (Rakic 1981).

Pettigrew: But in barrels we are talking about segregation or fusion of inputs from the same whisker, the same modality. There are two images of the outside world and the animal has to analyse those, as well as it can, before it mixes them.

If they are mixed too early, the fine information needed to make this integration possible is lost.

Hunt: You implied that it is necessary to keep representations separate to get the maximum amount of information. If you didn't do this would you get any information at all?

Pettigrew: Yes. There are some marsupials that bring the eyes together in the thalamus. They don't have stereopsis, but it does mean that they can increase the signal to noise ratio. But the point is that there are definite advantages of mixing the two representations.

O'Leary: Leah Krubitzer showed some beautiful examples where the entire cortex was the same size, but the size, and sometimes absolute location within the cortex, of relative primary sensory areas differed. To explain how area-specific thalamocortical targeting occurs during development to generate those types of patterns, I imagine there would need to be proportional changes in the diencephalon, perhaps in terms of the size of the various primary sensory nuclei as well as their relative locations. Have you looked at whether these correlations exist?

Krubitzer: Yes, In the ventroposterior nucleus of the platypus, trigeminal input dominates almost the entire thalamus.

O'Leary: What do you observe in the thalamus of the platypus where V1 is shifted more rostral and medial relative to S1, compared to a species such as a rat? Is there a correlation between the relationships of V1 and S1 and the relative positioning of the lateral geniculate nucleus and the ventroposterior nucleus?

Krubitzer: It is difficult to identify where the lateral geniculate nucleus is in the platypus. Regidor & Divac (1987) have placed it ventral to the ventroposterior nucleus, whereas Campbell & Hayhow (1971) and Ulinski (1984) have suggested that it is a small wedge at the lateral portion of the thalamus, However, it hasn't shifted dramatically. Even though there is a shift in these cortical fields, the visual cortex is not in front of somatosensory cortex, and in our developmental studies, there is not a complete reversal of the positions of visual and somatosensory cortex.

Levitt: Wouldn't this suggest that some sort of organizing centre is present?

Krubitzer: Yes. After listening to some of the presentations at this symposium, and seeing some of the borders, I have come to the conclusion that there must be.

Herrup: It would be worth tracing the tangentially migrating cells from the ganglionic eminence to find out if they play a role in parcelling out these fields. The cells of neocortex are generated in a strict order with respect to location and phenotype, and their fate is governed in part by when they were born. If there were similar temporal changes in the quality of cells coming out of the ganglionic eminence, you might conclude that their fates were similarly linked to their birth date.

Levitt: But the evidence suggests that there is lineage inheritance, so it's the glutaminergic projection neurons that are specified. The γ-aminobutyric acid (GABA)ergic neurons may not need specific field information, perhaps.

Herrup: If you did birth date studies of the GABAergic cells, you might find that all the early-born cells are posterior. The prediction would be, if you engineered a smaller cortex by taking out a section from the anterior region, and that operation blocked the migratory path, you would generate a dysfunctional cortex. The reason it works is that you take out a posterior portion and the cells are all going that way.

Karten: Another point that stands out is that what is reflecting is the changing periphery more than anything else.

Herrup: But how could you also change the balance?

Karten: The question is, does coding occur at one level or at all levels, e.g. the size of the nose, the size of the eye, the numbers of ganglion cells and the nuclei. Events don't evolve singularly, what we see is an end picture, so if you only focus on the brain without keeping in mind that these are all derivative factors, you may run the risk of falsely isolating one phenomenon.

Reiner: I was interested in Leah Krubitzer's data on map relocation and cortical ablations. What are the implications of these results for the specificity of handshake hypothesis? Certain cortical afferents have to shake hands with certain thalamic afferents in order for them to end up in the correct place. In this case the wrong ones shake hands, but an appropriate field still forms in more or less the appropriate place relative to other fields. This seems to argue for some kind of ordered ingrowth notion rather than for a specificity of handshake notion. It also seems that Leah Krubitzer's results have implications for the notion that gradients of molecules specify where particular afferents will go. Her results suggest that it is not the absolute level of the molecule that is critical, but rather the relative levels.

Molnár: There is evidence for plasticity after the actual handshake, once the afferents go through the internal capsule (see discussion after Molnár 2000, this volume). The key to explaining this, and the large amount of relocation of thalamocortical projection, is that early activity must be involved.

Levitt: When you make those lesions, there is a retrograde effect, but you don't know when this occurs. It must be impacting on the thalamus, and if you remove cortex, you also remove thalamus. During development, those neurons die quickly, and unless you follow this closely it's difficult to know what the initial relationship was between cortex and thalamus, and how soon the thalamus adapts to the lesion.

Molnár: Pat, you are referring to early experiments in newborn rats in which lesions are made in the visual cortex (Cunningham et al 1987). In these rats, the lateral geniculate nucleus degenerates after the cortical lesion. The stage we used

for our experiments in *Monodelphis*, however, corresponds to embryonic day (E) 11 in the rat (Molnár et al 1998), which may explain the difference. Perhaps at this early stage thalamus does not depend on cortical factors for its survival and this would provide enough time for the thalamocortical projections to distribute over the smaller cortex. When the dependency of the thalamic neurons sets in, the matching between thalamus and reduced cortex occurs on a general scale in *Monodelphis* rather than only in the nuclei originally destined to the lesioned cortical site. In our *Monodelphis* experiments, the thalamus reduces in size in proportion to the size of the cortical lesion, and the lateral geniculate nucleus remains present, although smaller. The comparison of the study of Cunningham et al (1987) and ours suggests that if lesions are made later, the projections are specified and they have no means of re-specifying themselves, but if the lesion is made at an age equivalent to E11, then they can still substantially rearrange and it's a completely different type of experiment paradigm.

Puelles: Another way to see constraint in the way the different fields have evolved, is that not only is the overall topology of different sensory, motor, limbic or associative areas maintained, but also the new areas for each modality are connected to the others, i.e. functionally related areas all remain close neighbours. This speaks of an invisible boundary isolating the local common modality from other modalities during development. Therefore, new visual fields may appear because the individual pre-existent fields allow new positions within the common boundary to be occupied by additional maps, rather than extending themselves (this assumes that differences in the number of cortical maps really exist and there is no problem with map detection procedures). This means to me that the new map-generating mechanism has a morphogenetic basis, i.e. it is only possible to have new visual field representations in the visual portion of the overall topology, but the total number of maps can increase possibly due either to overall increase in size of the cortex, or to miniaturization of the essential modules without accompanying size increase. All this implies parameter changes within the all-enclosing field and a resulting novel equilibrium state of the system. Apart from genetic constraint due to early specification of precursor populations in the diverse cell sources, there may also be some additional constraint imposed by the way the fibres come from the thalamus and fasciculate/defasciculate in the subcortical white matter.

Krubitzer: So you don't evolve a new visual field within the somatosensory cortex. New visual areas always appear adjacent to or intermingled with old visual areas. The way we tend to think about processing implies that cortical fields are added hierarchically, but this is not the case. They are inserted into existing networks, and may often evolve from primary fields, which makes primary fields difficult to identify as homologous. The geometric relationship of thalamocortical afferents to each other is important, and if a new type of visual

input evolves, it will stick close to things that it's correlated with in terms of activity patterns.

Goffinet: Several years ago Divac & Oberg (1990) reported that the echidna had a large thalamic MD and a large prefrontal cortex. Do you agree with this?

Krubitzer: The notion that echidnas have a large prefrontal cortex is incorrect, because when you make inferences about evolution you have to think of the most parsimonious explanation that accounts for a given structure. If this notion were correct, you would have to argue that there were many transformations in between monotremes and humans, and this is unlikely. What has probably occurred is that there has been an independent expansion of orbital frontal cortex, and the large frontal cortex in echidnas is probably related to the olfactory system. In terms of their MD size, it really isn't that large.

Goffinet: So, this frontal lobe would be olfactory?

Krubitzer: It probably is olfactory. If you visually inspect the brain of the animal you can understand a lot about it. This echidna's brain has a large olfactory bulb that has many fissures. When you flatten the cortex, you see that a large portion of the cortex is occupied by piriform cortex, compared to sensory cortex.

Puelles: What is the size of the claustrum in echidnas?

Krubitzer: We haven't looked at that.

Butler: Divac et al (1987) have reported that echidnas do not have a claustrum, so this structure may be unique to marsupial and placental mammals.

Karten: Does anyone know what the claustrum is doing physiologically?

Pettigrew: It may represent a link between behaviour and the re-jigging of visual cortical circuitry.

Karten: How different is the cytoarchitecture in the claustrum compared to the isocortex?

Puelles: It is rather homogeneous at any transverse section plane, although there are distinct areas and subnuclei along its considerable rostrocaudal extent (from olfactory nucleus and orbital cortex, anteriorly, to amygdala, caudally); these express different immunocytochemical markers and show different distributions of transmitter receptors or neuropeptide receptors. A large part of the claustrum is connected reciprocally point to point with the whole isocortex, which creates claustral areas with presumed predominant function related to vision, somesthesia, hearing, olfaction, etc. Thalamic afferents have been described from the posterior and intralaminar nuclear complexes in the dorsal thalamus.

Karten: Have any behavioural deficits been associated with lesions in the claustrum?

Puelles: The problem is that experiments placing tracer injections and making lesions in the claustrum are difficult to analyse, because you often cannot be sure whether the connections you see belong to the claustrum itself, as compared to the closely neighbouring insular or piriform cortexes, or the striatum, due to the

problem of fibres of passage. There are also few scientists who work specifically on the claustrum. However, mammals with scarce and relatively primitive isocortex, such as the echidna and platypus, are possibly of choice for investigating this daunting component of the pallium. I would be surprised if it is not present and rather massive in these animals. After all, it must have evolved from some earlier primordium in stem amniotes. Divergent evolution of the mammalian cortex from the reptilian cortex probably cannot be understood completely without explaining the joint origin of the cortex, the claustrum and the pallial parts of the amygdala (Striedter 1997, 1998, Puelles et al 2000). Our relative inconclusiveness (or lack of interest) about relevant field homologies (see discussion elsewhere in this book on the sauropsidian dorsal ventricular ridge) handicaps drawing uncontested conclusions from studies restricted to comparing isolated cell populations.

References

Campbell CB, Hayhow WR 1971 Primary optic pathways in the echidna, *Tachyglossus aculeatus*: an experimental degeneration study. J Comp Neurol 143:119–136

Cunningham TJ, Haum F, Chantler PD 1987 Diffusable proteins prolong survival of dorsal lateral geniculate neurons following occipital cortex lesions in newborn rats. Dev Brain Res 37:133–141

Divac I, Oberg RGE 1990 Prefrontal cortex: the name and the thing. In: Scherdtfeger WK, Germroth P (eds) The forebrain in nonmammals. Springer-Verlag, Berlin, p 213–220

Divac I, Holst M-C, Nelson J, McKenzie JS 1987 Afferents of the frontal cortex in the echidna (*Tachyglossus aculeatus*). Indication of an outstandingly large prefrontal area. Brain Behav Evol 30:303–320

Meissirel C, Wikler KC, Chalupa LM, Rakic P 1997 Early divergence of magnocellular and parvocellular functional subsystems in the embryonic primate visual system. Proc Natl Acad Sci USA 94:5900–5905

Molnár Z 2000 Conserved developmental algorithms during thalamocortical circuit formation in mammals and reptiles. In: Evolutionary developmental biology of the cerebral cortex. Wiley, Chichester (Novartis Found Symp 228) p 148–172

Molnár Z, Knott GW, Blakemore C, Saunders NR 1998 Development of thalamocortical projections in the South American grey short-tailed opossum (*Monodelphis domestica*). J Comp Neurol 398:491–514

Pettigrew JD, Manger P, Fine SLB 1998 The sensory world of the platypus. Philos Trans R Soc Lond B Biol Sci 353:1199–1210

Puelles L, Kuwana E, Puelles E et al 2000 Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon. submitted

Rakic P 1981 Development of visual centers in the primate brain depends on binocular competition before birth. Science 214:928–931

Regidor J, Divac I 1987 Architectonics of the thalamus in the echidna (*Tachyglossus aculeatus*): search for the mediodorsal nucleus. Brain Behav Evol 30:328–341

Striedter GF 1997 The telencephalon of tetrapods in evolution. Brain Behav Evol 49:179–213

Striedter GF 1998 Progress in the study of brain evolution: from speculative theories to testable hypotheses. Anat Rec 253:105–112

Ulinski PS 1984 Thalamic projections to the somatosensory cortex of the echidna, *Tachyglossus aculeatus*. J Comp Neurol 229:153–170