time of infection was unknown (Table 1) (*12*)]; **patient 4,** week 12, 0.017 (0.000–0.142); **patient 5,** week 10, 0.000 (0.000–0.106); **patient 6,** week 5, 0.000 (0.000–0.068); **patient 7,** week 11, 0.085 (0.033–0.318); **patient 8,** week 18, 0.422 (0.422–0.581); **patient 9,** week 9, 0.000 (0.000–0.308); **patient 10,** week 13, 0.265 (0.000–0.469); **patient 11,** week 16, 0.094 (0.000–0.094); **patient 12,** week 18, 0.033 (0.000–0.070). There was a trend toward an increase in the branch length from fulminant (patients 1 to 3) to acute resolving (patients 4 to 6) to progressing hepatitis (patients 7 to 12). One exception was represented by the median of patient 9, although the same case had a branch length maximum consistent with the trend observed in progressing hepatitis.

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Regulation of Area Identity in the Mammalian Neocortex by *Emx2* and *Pax6*

Kathie M. Bishop,[1] Guy Goudreau,[2] Dennis D. M. O'Leary[1]*

The contribution of extrinsic and genetic mechanisms in determining areas of the mammalian neocortex has been a contested issue. This study analyzes the roles of the regulatory genes *Emx2* and *Pax6*, which are expressed in opposing gradients in the neocortical ventricular zone, in specifying areas. Changes in the patterning of molecular markers and area-specific connections between the cortex and thalamus suggest that arealization of the neocortex is disproportionately altered in *Emx2* and *Pax6* mutant mice in opposing manners predicted from their countergradients of expression: rostral areas expand and caudal areas contract in *Emx2* mutants, whereas the opposite effect is seen in *Pax6* mutants. These findings suggest that *Emx2* and *Pax6* cooperate to regulate arealization of the neocortex and to confer area identity to cortical cells.

The mature mammalian neocortex is tangentially organized into distinct areas that serve specialized functions, such as sensory processing and motor control. These areas can be distinguished by differences in their input and output connections and architecture. It is assumed that the specification and differentiation of neocortical areas are controlled by an interplay between epigenetic (extrinsic) and genetic (intrinsic) mechanisms. Numerous studies suggest that diverse parts of the neocortex initially share potentials to develop features unique to a specific area (*1*) and have implicated thalamocortical input as a major epigenetic influence in this process (*2, 3*). Evidence for genetic regulation of arealization is limited to descriptions of graded or restricted patterns of gene expression in the embryonic neocortex (*4–6*) that are established independent of thalamocortical input (*5, 6*). Remaining unidentified, though, are genes that regulate arealization, presumably by conferring area identities to cortical cells and controlling the differential expression of downstream genes that govern, among other things, the area-specific targeting of thalamocortical afferents.

The regulatory genes that control neocortical arealization are assumed to be expressed in graded or restricted patterns in the embryonic cortex (*3*). Two genes that meet this criterion are the homeodomain transcription factor *Emx2* and the paired-box–containing transcription factor *Pax6*. Both genes are expressed in the dorsal telencephalic neuroepithelium, the proliferative zone that gives rise to cortical neurons (*7–9*). *Emx2* is expressed in low rostral to high caudal and low lateral to high medial gradients (*7, 8*), whereas *Pax6* is expressed in low caudal to high rostral and low medial to high lateral gradients (*8, 9*). On the basis of these expression patterns, we hypothesized that *Emx2* preferentially imparts caudal and medial area identities to cortical neurons, whereas *Pax6* preferentially imparts rostral and lateral identities (Fig. 1).

We have used molecular and connectional markers of area identity to test these hypotheses in mutant mice lacking either *Emx2* (*10*) or *Pax6* [*Small eye* (*Sey*)] (*11*) functional alleles. We predicted that, in the *Emx2* mutant neocortex, caudal and medial identities would be lost, resulting in a caudal and medial shift in rostral and lateral area identities (Fig. 1B). Conversely, we predicted that, in the *Pax6* mutant neocortex, rostral and lateral identities would be lost, resulting in a rostral and lateral shift in caudal and medial area identities (Fig. 1C). *Emx2* and *Pax6* (*Sey/Sey*) mutant mice die within hours after birth (*10, 11*), limiting our analysis to the late embryonic period, before the mice die but after some features of arealization have become evident.

The type II classical cadherins, *Cadherin6* (*Cad6*) and *Cadherin8* (*Cad8*), are expressed in areal patterns in the late embryonic mouse neocortex: *Cad8* has been reported to mark the rostrally located motor area, and *Cad6* marks the somatosensory area, located immediately caudal to the motor area, and the auditory area, located in the caudolateral neocortex (*12*). Because the cortex is reduced in size in both *Emx2* and *Pax6* mutants compared to wild type (Tables 1 through 5), we determined the proportion of the cortical surface covered by domains of cadherin expression as well as their absolute size (Fig. 2, B and D). No change in proportional sizes of cadherin expression domains would indicate that arealization per se has not been affected, suggesting that the full range of putative area identities is present in the smaller cortex and that all areas are uniformly reduced in size. A change in proportional sizes would indicate that areas are disproportionately affected in the mutant neocortex and therefore that *Emx2* and/or *Pax6* has a role in regulating arealization of the neocortex. This finding and interpretation would be most strongly supported by a change in absolute sizes of cadherin expression domains.

The areal pattern of cadherin expression in the *Emx2* homozygous mutant cortex (*13*) is substantially different than that in wild type (Fig. 2). The domain of *Cad8* expression is expanded caudally (Fig. 2A). This expansion appears to be greater along the medial edge of the cortex than farther laterally, suggesting that *Cad8* expression may also be expanded medially. The domain of *Cad6* expression is shifted caudally and medially, as seen on the dorsal and

[1]Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. [2]Max-Planck-Institute for Biophysical Chemistry, Department of Molecular Cell Biology, Am Fassberg 11, 37077 Göttingen, Germany.

*To whom correspondence should be addressed. E-mail: doleary@salk.edu

lateral surfaces of the cortex (Fig. 2A). No changes in the cortical patterns of cadherin expression are observed in *Emx2* heterozygous mice (Tables 1 through 3). The domain of *Cad8* expression is significantly larger in the *Emx2* homozygous mutant neocortex, in both proportional and absolute area, as well as in its proportional and absolute linear extent across the cortical surface (Table 1 and Fig. 2B). In fact, the absolute area of the *Cad8* expression domain in *Emx2* homozygous mutants is almost double that in wild type, even though the surface area of the dorsal cortex is reduced by one-third. In *Emx2* homozygous mutants, the domain of *Cad6* expression on the dorsal cortical surface is significantly increased in proportional area and in both proportional and absolute width, compared to that in wild type (Table 2). In contrast, the area and length of the *Cad6* expression domain on the lateral cortical surface of *Emx2* homozygous mutants each exhibit both proportional and absolute reductions compared to that in wild type (Table 3 and Fig. 2B). These results suggest that areas located in rostral and lateral parts of the neocortex are expanded and shifted caudally and medially in the *Emx2* mutant neocortex.

Because *Pax6* is expressed in a countergradient to *Emx2*, we predicted that changes in domains of cadherin expression in *Pax6* mutant mice (*Sey/Sey* mutants) would be in the opposite direction of those observed in *Emx2* mutants (*14*). The domain of *Cad8* expression is contracted rostrally in the *Sey/Sey* cortex compared to that in the wild type (Fig. 2C). The *Cad6* expression domain is contracted both laterally and rostrally in the *Sey/Sey* cortex (Fig. 2C). The domains of *Cad8* and *Cad6* expression on the dorsal surface of the *Sey/Sey* cortex, which is about three-quarters the size of that in the wild type, show both proportional and absolute reductions in area (Tables 4 and 5 and Fig. 2D). Heterozygous mice (*Sey/+*) show no changes in cortical cadherin expression compared to wild-type mice (Tables 4 and 5). These results suggest that areas located in rostral and lateral parts of the neocortex contract rostrally and laterally in *Sey/Sey* mutants (*15*).

Changes in the expression domains of *Cad6* and *Cad8* in *Emx2* and *Pax6* mutants suggest corresponding changes in neocortical arealization. The primary neocortical areas (motor, visual, somatosensory, and auditory) receive area-specific inputs from the principal motor and sensory thalamic nuclei [ventrolateral (VL), dor-

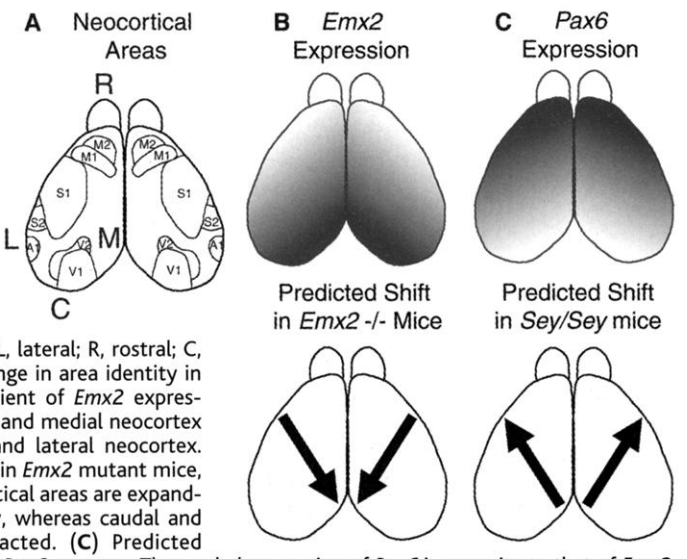

Fig. 1. Predicted changes in neocortical arealization in *Emx2* and *Pax6* mutant mice. Dorsal views of the surface of mouse neocortex. (**A**) Organization of the mouse neocortex, showing the auditory area (A1), the motor areas (M1 and M2), the somatosensory areas (S1 and S2), and the visual areas (V1 and V2). M, medial; L, lateral; R, rostral; C, caudal. (**B**) Predicted change in area identity in *Emx2* mutants. The gradient of *Emx2* expression is high in the caudal and medial neocortex and low in the rostral and lateral neocortex. Thus, it is predicted that, in *Emx2* mutant mice, rostral and lateral neocortical areas are expanded caudally and medially, whereas caudal and medial areas are contracted. (**C**) Predicted change in area identity in *Pax6* mutants. The graded expression of *Pax6* is opposite to that of *Emx2*, being high in rostral and lateral neocortex and low in caudal and medial neocortex. Thus, it is predicted that, in *Pax6* mutant mice, caudal and medial neocortical areas are expanded rostrally and laterally, whereas rostral and lateral areas are contracted.

Table 1. *Cad8* Quantification in the *Emx2* cortex [mean ± SD (in parentheses)].

Genotype	Cortical area (cm^2)	Cortical length (cm)	*Cad8* area (cm^2)	*Cad8* length (cm)	Proportional area	Proportional length	*n*
+/+	0.069 (0.013)	0.405 (0.037)	0.008 (0.002)	0.073 (0.013)	0.114 (0.017)	0.180 (0.028)	8
+/−	0.076 (0.010)	0.424 (0.018)	0.009 (0.002)	0.085 (0.015)	0.122 (0.027)	0.200 (0.035)	12
−/−	0.046 (0.011)*	0.317 (0.031)*	0.014 (0.005)*	0.122 (0.026)*	0.298 (0.066)*	0.383 (0.050)*	10

*$P < 0.001$ (two-tailed *t* test); mutant value is significantly different from wild type and heterozygote combined.

Table 2. *Cad6* dorsal cortex quantification in the *Emx2* cortex [mean ± SD (in parentheses)].

Genotype	Cortical area (cm^2)	Cortical width (cm)	*Cad6* area (cm^2)	*Cad6* width (cm)	Proportional area	Proportional width	*n*
+/+	0.070 (0.012)	0.247 (0.024)	0.022 (0.004)	0.008 (0.016)	0.314 (0.063)	0.353 (0.038)	8
+/−	0.077 (0.009)	0.265 (0.014)	0.023 (0.004)	0.105 (0.015)	0.305 (0.056)	0.397 (0.044)	8
−/−	0.049 (0.013)*	0.211 (0.025)*	0.023 (0.005)	0.130 (0.020)*	0.491 (0.084)*	0.614 (0.044)*	9

*$P < 0.001$ (two-tailed *t* test); mutant value is significantly different from wild type and heterozygote combined.

Table 3. *Cad6* lateral cortex quantification in the *Emx2* cortex [mean ± SD (in parentheses)].

Genotype	Cortical area (cm^2)	Cortical length (cm)	*Cad6* area (cm^2)	*Cad6* length (cm)	Proportional area	Proportional length	*n*
+/+	0.067 (0.007)	0.414 (0.011)	0.032 (0.004)	0.370 (0.030)	0.482 (0.089)	0.896 (0.074)	5
+/−	0.060 (0.007)	0.392 (0.020)	0.035 (0.005)	0.356 (0.026)	0.582 (0.042)	0.907 (0.040)	8
−/−	0.039 (0.007)*	0.328 (0.036)*	0.012 (0.004)*	0.202 (0.015)*	0.293 (0.068)*	0.618 (0.047)*	6

*$P < 0.001$ (two-tailed *t* test); mutant value is significantly different from wild type and heterozygote combined.

sal lateral geniculate (dLG), ventroposterior (VP), and medial geniculate (MG), respectively]. During normal development, thalamocortical axons target and invade their neocortical areas in a precise manner (*16*). Therefore, as an additional assay for changes in area identity in the *Emx2* mutant neocortex, we used retrograde and anterograde axon tracing to map thalamocortical projections (*17*). This analysis was not done in *Sey/Sey* mutants because thalamic axons do not reach the cortex in these mice (*18*).

Retrograde labeling from the cortex of *Emx2* homozygous mutants indicates a caudal shift in the border between the somatosensory and visual areas compared to that of the wild type (Fig. 3A). Injections confined to the cortical plate of the occipital cortex, the location of the primary visual area, normally backlabel neurons in the dLG nucleus. However, in *Emx2* mutants, similarly placed injections label cells in the VP nucleus, which normally projects to the primary somatosensory area located rostral to the visual area (Fig. 3A). Deeper injections made into the subplate of the occipital neocortex in *Emx2* mutants backlabel neurons in both dLG and VP nuclei, indicating that dLG thalamic axons extend through the subplate below the occipital cortex but fail to invade the overlying cortical plate (*19*). Retrograde tracing from anterior and posterior portions of the occipital cortex reveals the expected topography in wild-type mice but again indicates a caudal shift in the border between the somatosensory and visual areas in *Emx2* mutants (Fig. 3B). In wild-type mice, injections into the anterior occipital cortex backlabel cells in the posterior dLG nucleus, and injections into the posterior occipital cortex backlabel cells in the anterior dLG nucleus (Fig. 3B). In contrast, in *Emx2* mutants, injections into the anterior occipital cortex do not label cells in the dLG nucleus but instead label cells in the VP nucleus; injections in the very posterior occipital cortex do label cells in the dLG nucleus, but their number is significantly reduced compared to the wild type (wild type = 319 ± 89, $n = 3$; mutant = 35 ± 29, $n = 3$; $P < 0.05$) (Fig. 3B). These findings suggest that the visual area in *Emx2* mutants is contracted and restricted to the extreme caudal part of the occipital cortex.

Anterograde tracing of thalamocortical projections is consistent with the retrograde tracing results. Injections into the dLG nucleus of *Emx2* mutants label axons in the subplate beneath the caudal occipital cortex, but in comparison to the wild type, few invade the cortical plate (Fig. 3C). Injections into the VP nucleus of *Emx2* mutants label axons that extend farther caudally than in the wild type and aberrantly invade the cortical plate of occipital cortex, whereas in the wild type, VP axons invade the cortical plate of the more rostrally located parietal cortex (the location of the primary somatosensory area) (Fig. 3D). Thalamocortical projections in heterozygous *Emx2* mutants resemble those in the wild type (*19*). Overall, anterograde and retrograde tracing of thalamocortical projections in *Emx2* mutants provides evidence for a contraction of the visual area and a caudal shift in the border between the somatosensory and visual areas.

Area-specific connections between thalamic nuclei and neocortical areas are reciprocal. Injections into thalamic nuclei backlabel cortical neurons in wild-type mice and *Emx2* mutants (Fig. 3, C and D). Injections into the dLG nucleus backlabel significantly fewer cells in the occipital cortex of *Emx2* mutants compared to the wild type (wild type = 139 ± 4, $n = 3$; mutant = 19 ± 13, $n = 3$; $P < 0.05$). The few labeled cells in *Emx2* mutants are restricted to the very caudal cortex (Fig. 3C). Injections into the VP nucleus in wild-type mice backlabel cells in the parietal cortex but backlabel none in the occipital cortex (Fig. 3D). In contrast, VP injections in *Emx2* mutants backlabel a substantial number of cells in the occipital cortex (Fig. 3D). These findings suggest that, in *Emx2* mutants, corticothalamic neurons located in the occipital cortex have acquired a somatosensory area identity instead of their usual visual area identity. These changes in area-specific corticothalamic projections in *Emx2* mutants are consistent with the changes observed in area-specific thalamocortical projections. Together, they suggest that the primary visual area is substantially reduced and restricted to the very caudal part of the neocortex, with a corresponding caudal shift in the border between visual and somatosensory areas.

Emx2 is reported to be expressed in a small patch of neuroepithelium in the ventral-most

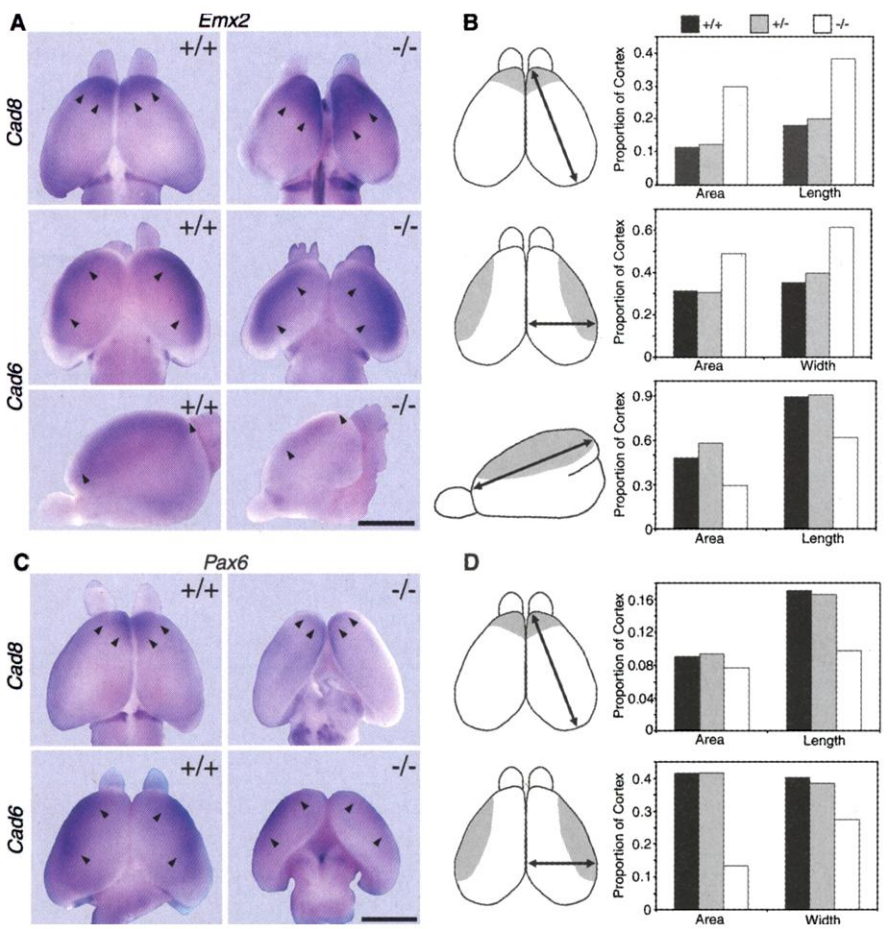

Fig. 2. Areal patterns of cadherin expression are altered in opposing manners in *Emx2* and *Pax6* mutants. Dorsal and lateral surfaces of the cortex of E18.5 *Emx2* and *Pax6* wild-type (+/+) and mutant (−/−) mice. (**A**) *Cad8* expression is expanded caudally, and *Cad6* expression is expanded caudally and medially in the *Emx2* mutant neocortex. Arrowheads indicate borders of the cadherin expression domains. (**B**) Quantification of cadherin expression in the *Emx2* wild-type, heterozygote, and mutant cortices. Arrows show the axis for the width and length measurements of cadherin expression domains presented in graphs and in Tables 1 through 5. In proportion to the size of the cortex, *Cad8* area and length are increased, whereas *Cad6* area and width are increased on the dorsal surface and decreased in area and length on the lateral surface of the *Emx2* mutant cortex. (**C**) In *Pax6* mutants, cortical *Cad8* expression is contracted rostrally, and *Cad6* expression is contracted rostrally and laterally. Arrowheads indicate borders of the cadherin expression domains. (**D**) Quantification of cadherin expression in the *Pax6* wild-type, heterozygote, and mutant cortices indicates that, in proportion to overall cortical size, *Cad6* and *Cad8* expression are decreased in area, length, and width in *Pax6* mutants. Scale bars in (A) and (C), 2.0 mm.

part of the dorsal thalamus (*20*). Although this part does not generate cells of the principal sensory and motor thalamic nuclei (*21*), we used several markers to confirm the normal development and organization of the dorsal thalamus in *Emx2* mutants (*22*). Patterns of acetylcholinesterase (AChE) staining and *Gbx2* expression and general morphology revealed by nuclear 4′,6-diamidino-2-phenylindole staining are all normal in the dorsal thalamus of *Emx2* mutants (Fig. 4, A and B) (*19*). Thus, alterations in thalamocortical and corticothalamic projections in *Emx2* mutants can be presumed to be due to changes intrinsic to the neocortex.

Our findings implicate *Emx2* and *Pax6* in the genetic control of neocortical arealization. They cannot be explained by a potential delay in neocortical development (*23*), nor are the expansions and contractions of the cadherin expression domains secondary to a loss of thalamocortical input (*15*). In addition, the observed changes in cadherin expression domains are not simply a by-product of the reduced overall size of the cerebral cortex in *Emx2* and *Pax6* mutants, because the changes are disproportionate in each mutant and opposing in the two mutants as predicted from the countergradients of *Emx2* and *Pax6* expression. Similarly, the observed changes in thalamocortical and corticothalamic projections in the *Emx2* mutants are not due simply to a caudal truncation of the neocortex with an associated loss of the visual area. Arguing against this possibility are the changes in cadherin expression in *Emx2* mutants, especially the expansion of the *Cad8* expression domain in the frontal cortex (the motor area), in both proportional and absolute size. Instead, the findings presented here indicate a disproportional, but orderly, arealization of the *Emx2* mutant neocortex reflected by an expansion of rostral areas and a contraction of caudal areas, and an opposite effect on arealization in the *Pax6* mutant neocortex.

Changes in the areal expression patterns of the cadherins and the area-specific distribution of corticothalamic neurons in the mutants suggest that *Emx2* and *Pax6* confer area identities to cortical cells, including projection neurons. The changes in cadherin expression and, presumably, receptors for axon guidance molecules that control corticothalamic axon targeting may

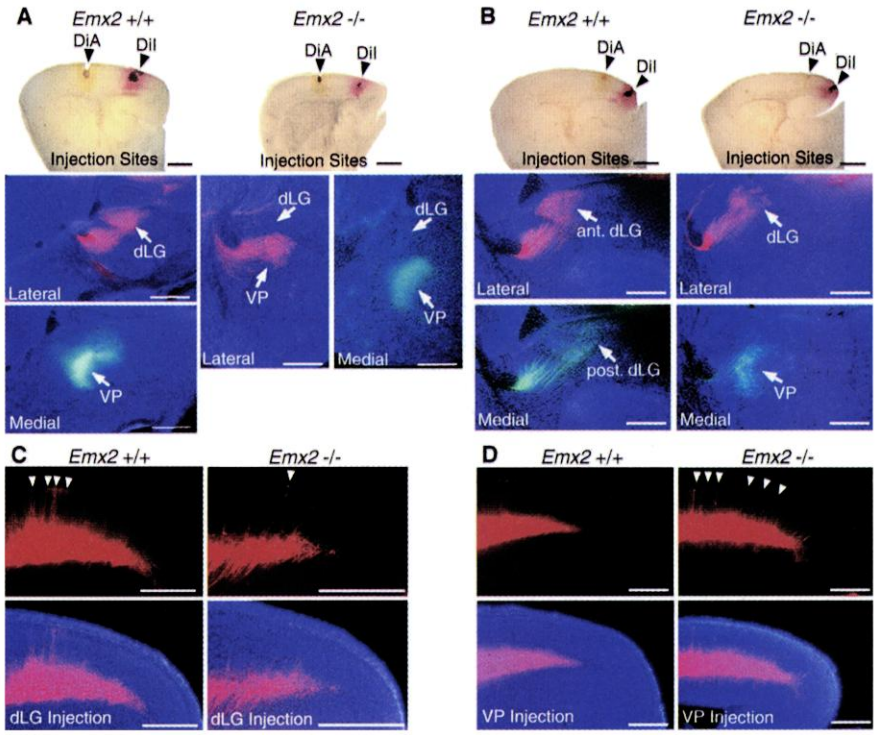

Fig. 3. Changes in area-specific thalamocortical and corticothalamic projections in *Emx2* mutants indicate a caudal shift of the somatosensory cortex and a contraction of the visual cortex. Sagittal sections through E18.5 *Emx2* wild-type and mutant brains show retrograde and anterograde DiI (red) and DiA (green) tracing and bisbenzimide counterstain (blue). Rostral is to the left in all panels. (**A**) Injections into the somatosensory cortex (DiA) backlabel VP thalamic neurons in both wild-type and mutant brains (medial sections). Injections into the occipital cortex at the normal location of visual cortex (DiI) label dLG neurons in *Emx2* wild types but label VP neurons in *Emx2* mutants (lateral sections). (**B**) In *Emx2* wild types, injections into anterior (DiA) and posterior (DiI) parts of the occipital cortex backlabel neurons in posterior (medial section) and anterior (lateral section) parts of dLG, respectively. However, in *Emx2* mutants, injections into the anterior occipital cortex (DiA) backlabel neurons in the VP nucleus (medial section), whereas injections into the posterior occipital cortex (DiI) backlabel neurons in the dLG nucleus (lateral section). (**C**) DiI injections into the dLG nucleus label axonal branching and backlabel cortical plate neurons (arrowheads) in the occipital cortex in *Emx2* wild types. In *Emx2* mutants, axons are found in the subplate below the occipital cortex and invade the cortical plate only sparsely. Backlabeled cells (arrowheads) are located more caudally than in wild types. (**D**) Axonal branches and backlabeled neurons (arrowheads) labeled by DiI injections into VP are shifted caudally in *Emx2* mutants compared to wild type. Scale bars, 0.4 mm.

Table 4. *Cad8* quantification in the *Pax6* cortex [mean ± SD (in parentheses)].

Genotype	Cortical area (cm^2)	Cortical length (cm)	*Cad8* area (cm^2)	*Cad8* length (cm)	Proportional area	Proportional length	*n*
+/+	0.087 (0.008)	0.455 (0.021)	0.008 (0.001)	0.078 (0.007)	0.092 (0.018)	0.173 (0.024)	8
+/−	0.077 (0.006)	0.433 (0.019)	0.008 (0.001)	0.072 (0.004)	0.098 (0.020)	0.166 (0.010)	6
−/−	0.063 (0.005)*	0.420 (0.017)	0.005 (0.001)*	0.041 (0.007)*	0.072 (0.009)†	0.098 (0.016)*	6

*$P < 0.001$. †$P < 0.05$ (two-tailed *t* test); mutant value is significantly different from wild type and heterozygote combined.

Table 5. *Cad6* quantification in the *Pax6* cortex [mean ± SD (in parentheses)].

Genotype	Cortical area (cm^2)	Cortical width (cm)	*Cad6* area (cm^2)	*Cad6* width (cm)	Proportional area	Proportional width	*n*
+/+	0.086 (0.009)	0.269 (0.016)	0.035 (0.004)	0.108 (0.010)	0.416 (0.045)	0.403 (0.029)	12
+/−	0.085 (0.004)	0.267 (0.011)	0.035 (0.005)	0.103 (0.011)	0.417 (0.049)	0.386 (0.032)	8
−/−	0.070 (0.006)*	0.213 (0.017)*	0.009 (0.003)*	0.058 (0.010)*	0.134 (0.051)*	0.274 (0.048)*	8

*$P < 0.001$ (two-tailed *t* test); mutant value is significantly different from wild type and heterozygote combined.

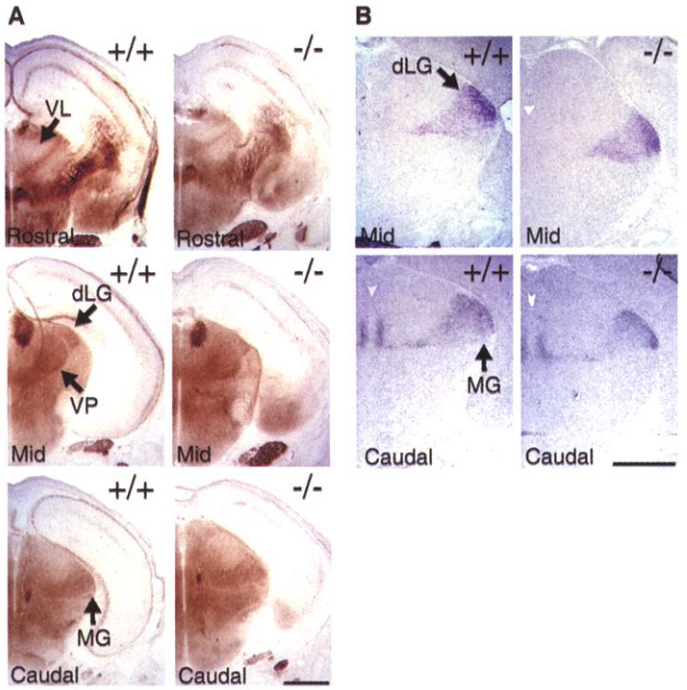

Fig. 4. The dorsal thalamus appears normal in *Emx2* mutants. Coronal sections through the E18.5 *Emx2* wild-type and mutant thalamus; medial is to the left. (**A**) AChE histochemical staining indicates that thalamic nuclei that project to primary motor and sensory cortical areas appear normal. (**B**) *Gbx2* expression in the dLG (mid section) and the MG (caudal section) thalamic nuclei is the same in *Emx2* mutants as compared to wild type. Scale bars, 0.5 mm.

be indicative of a direct role for *Emx2* and *Pax6* in their regulation, or they may be an indirect effect of the regulation of area identity. Similarly, the changes in area-specific thalamocortical projections suggest that *Emx2* and *Pax6* are involved either directly or indirectly in the regulation of axon guidance molecules within the cortex that control thalamocortical axon targeting. The restricted cortical expression of Eph receptor tyrosine kinases and their ligands, the ephrins, which act as axon guidance molecules in several systems, makes them candidates for controlling the development of area-specific projections between the thalamus and cortex (*4, 24*).

Emx2 and *Pax6* may act independently or in a combinatorial manner (possibly with other transcription factor genes) to specify neocortical areas. Because areas in the neocortex have sharp borders, it is likely (but not required) that the graded expression patterns of *Emx2* and *Pax6* are translated to regulate some downstream genes in restricted patterns with abrupt borders that relate to areas. Studies in *Drosophila* have shown that gradients of transcription factors can be translated through thresholding mechanisms and combinatorial actions into sharply bordered expression patterns of downstream genes. For example, a single regulatory protein, such as Dorsal, distributed in a graded manner across several tissues can establish sharply patterned, tissue-specific expression of downstream genes through a thresholding mechanism based on concentration-dependent differences in binding efficacy to promoter and repressor elements (*25*). Analysis of the regulation of the *even-skipped* gene has shown that multiple transcriptional activators and repressors expressed in graded patterns act combinatorially to generate sharp borders of *even-skipped* expression (*26*). Although the downstream targets of *Emx2* and *Pax6* in the cortex have yet to be identified, transcription factors such as *T-brain1* and *Id2* are expressed in the neocortex in discrete patterns with abrupt borders (*27*) that may be controlled by upstream regulatory genes expressed in gradients.

Emx2 and *Pax6* appear to be independently regulated (*28*). The opposing gradients of *Emx2* and *Pax6* may be induced by signals secreted from the poles of the cortex. Several secreted proteins are candidates for these inductive signals, including the BMP, WNT (2b, 3a, 5a, and 7a), and FGF8 proteins (*29*). In addition, cortical expression of the transcription factor *Gli3* is required for *Emx2* expression (*30*). Thus, combinations of inductive signals and upstream transcription factors may specify gradients of *Emx2* and *Pax6*. A better understanding of the roles of *Emx2* and *Pax6* in regulating neocortical arealization will require identifying the patterning mechanisms that establish their differential expression, identifying downstream targets, and defining the mechanisms by which they, in combination with other factors, intrinsic and extrinsic, control the process of arealization of the neocortex.

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13. Embryonic day 18.5 (E18.5) embryos were obtained from timed-pregnant matings of *Emx2* heterozygous mice maintained on a C57BL/6J background (E0.5 = noon of day of vaginal plug detection). Animal care was in accordance with institutional guidelines. Littermate sets of cortices from wild type, heterozygote, and homozygous mutants were analyzed to control for differences in developmental rate. Whole-mount in situ hybridization of cortices {modified from work by D. Henrique *et al.*, [*Nature* **375**, 787 (1995)]} used digoxygenin-labeled riboprobes encoding *Cad6* and *Cad8*. *Emx2* mice were genotyped by polymerase chain reaction on tail DNA. Whole-mount in situ hybridizations were quantified with the Image program from the National Institutes of Health. Quantification was done blinded to genotype.
14. Whole-mount in situ hybridization on E18.5 *Pax6* mutant brains was performed as in (*13*). Embryos were obtained from matings of heterozygous *Sey* mice maintained on a C57BL/6J × DBA/2J background. *Sey* mice were genotyped by examination of the eyes: homozygous mutants lack eyes, and heterozygotes have reduced external eye size and a pronounced size reduction of the developing lens at E18.5. Analysis was as in (*13*), done blinded to genotype.
15. Although *Sey/Sey* brains lack thalamic input to the cortex (*18*), this is not the cause of the observed changes in cortical *Cad6* and *Cad8* expression patterns. Normal expression of areal markers, including cadherins, has been observed in the neocortex of *Gbx2* and *Mash-1* mutant mice, which lack thalamocortical input (*5, 6*).
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23. A delay in overall development of the cortex cannot account for our findings. The mutant patterns of cadherin expression and thalamocortical projections do not resemble patterns observed at earlier stages in wild types. For example, the domains of cadherin expression do not exhibit significant proportional expansions or contractions during normal embryonic cortical development (*6*); axons from VP neurons do not transiently invade the visual area at early stages and the somatosensory area later, and vice versa for dLG axons; and the distribution of corticothalamic projection neurons is area specific, even at the earliest times that they can be labeled (*16*). *Emx2* and *Pax6* mutants also show opposite changes in cadherin expression patterns, which cannot be accounted for by a delay in cortical development in both mutants.
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28. Independent regulation of *Emx2* and *Pax6* is suggested by their temporal coexpression and the findings that *Emx2* is expressed in the *Pax6* mutant and that *Pax6* is expressed in the *Emx2* mutant in graded patterns in the developing neocortex similar to those in wild-type mice [A. Stoykova, R. Fritsch, C. Walther, P. Gruss, *Development* **122**, 3453 (1996); K. M. Bishop and D. D. M. O'Leary, unpublished observations; see Web fig. 2, available at www.sciencemag.org/feature/data/1045964.shl]. In addition, analysis of the *extra toes* mouse mutant (a null mutation of *Gli3*) shows that the loss of *Gli3* expression correlates with a loss of *Emx2* expression in the rostral forebrain but does not affect *Pax6* expression in the developing neocortex (*30*).
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Language Discrimination by Human Newborns and by Cotton-Top Tamarin Monkeys

Franck Ramus,[1]*† Marc D. Hauser,[2] Cory Miller,[2] Dylan Morris,[2] Jacques Mehler[1]

Humans, but no other animal, make meaningful use of spoken language. What is unclear, however, is whether this capacity depends on a unique constellation of perceptual and neurobiological mechanisms or whether a subset of such mechanisms is shared with other organisms. To explore this problem, parallel experiments were conducted on human newborns and cotton-top tamarin monkeys to assess their ability to discriminate unfamiliar languages. A habituation-dishabituation procedure was used to show that human newborns and tamarins can discriminate sentences from Dutch and Japanese but not if the sentences are played backward. Moreover, the cues for discrimination are not present in backward speech. This suggests that the human newborns' tuning to certain properties of speech relies on general processes of the primate auditory system.

A fundamental question in the study of language evolution and acquisition is the extent to which humans are innately endowed with specialized capacities to comprehend and produce speech. Theoretical arguments have been used to argue that language acquisition must be based on an innately specified language faculty (*1*, *2*), but the precise nature and extent of this "language organ" is mainly an empirical matter, which notably requires studies of human newborns as well as nonhuman animals (*3*–*5*). With respect to studies of humans, we already know that newborns as young as 4 days old have the capacity to discriminate phonemes categorically (*6*) and perceive well-formed syllables as units (*7*–*9*); they are sensitive to the rhythm of speech, as shown in experiments in which newborns distinguish sentences from languages that have different rhythmic properties but not from languages that share the same rhythmic structure (*10*, *11*); however, newborns do not discriminate languages when speech is played backward (*10*), and neurophysiological studies suggest that both infants and adults process natural speech differently from backward speech (*12*, *13*). All these studies indicate that humans are born with capacities that facilitate language acquisition and that seem well attuned to the properties of speech. Studies of nonhuman animals, however, show that some of these capacities may predate our hominid origins. For example, insects, birds, nonprimate mammals, and primates process their own, species-typical sounds in a categorical manner, and some of these species perceive speech categorically (*14*–*18*).

Our aim here is to extend the comparative study of speech perception in three directions. First, using the same design and the same material, we have conducted joint experiments on human newborns and on monkeys. Second, whereas most studies of nonhuman animal speech perception involve extensive training before testing on a generalization task, our experimental approach—the habituation-dishabituation paradigm—involves no training and parallels the method used in studies of infant speech perception. Thus, conditions are met to appropriately compare the two populations. Third, most studies of speech processing in animals involve tests of phonemic perception. Here, we extend the analysis to sentence perception, thereby setting up a much broader range of perceptual problems.

Our experiments were run on human newborns and cotton-top tamarin monkeys (*Saguinus oedipus oedipus*). The stimuli consisted of 20 sentences in Japanese and 20 sentences in Dutch uttered by four female native speakers of each language. Conditions in which the two languages are pitted against one another were compared with conditions in which speakers of the same language are contrasted. In addition, sentences within a session were played either forward or backward. To more readily control for prosodic features of the signal, we reran all conditions with synthesized exemplars of the original sentences. Synthesized sentences were created with the MBROLA diphone synthesizer (*19*). Phoneme duration and fundamental frequency were preserved, whereas the phonetic inventory was narrowed to only one phoneme per manner of articulation: all fricatives were synthesized as /s/, vowels as /a/, liquids as /l/, plosives as /t/, nasals as /n/, and glides as /j/.

[1]Laboratoire de Sciences Cognitives et Psycholinguistique, l'Ecole des Hautes Etudes en Sciences Sociales/Centre National de la Recherche Scientifique, 54 Boulevard Raspail, 75006 Paris, France. [2]Department of Psychology, Harvard University, Cambridge, MA, 02138, USA.

*To whom correspondence should be addressed. E-mail: f.ramus@ucl.ac.uk

†Present address: Institute of Cognitive Neuroscience, 17 Queen Square, London WC1N 3AR, UK.