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We are studying the genes that are expressed in specific areas of the neocortex in order to understand the principle for the formation of the primate brain.

I. Genes expressed in specific areas and layers of the neocortex

The neocortex emerged in mammals and evolved most remarkably in the primate. To understand the underlying mechanisms, we studied gene expression patterns within different areas of the neocortex. Over the last ten years
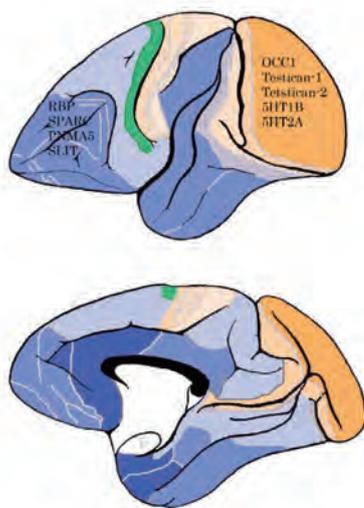
Figure 1. The expression of visual area specific genes (orange color) and association area specific genes (blue) and gdf7 (green) are schematically illustrated. Top and bottom views are medial and lateral surfaces, respectively. (cited from Yamamori & Rockland, Neurosci. Res., 55, 11-27, 2006)

we have reported the findings that are schematically illustrated in Figure 1.

In short, using differential display methods, we found three area-specific expression genes in the primate neocortex. Firstly, *occ1* is specifically expressed in the occipital cortex in the primate brain. Secondly, the other gene that showed marked difference within the neocortex is *gdf7*, a member of BMP/TGF-β family, which is specifically expressed in the motor cortex of the African green monkey (Watakabe *et al.*, J. Neurochem., 76, 1455-1464, 2001). Thirdly, *Rbp* (retinol-binding protein) is preferentially expressed in association and higher areas in the neocortex (Komatsu *et al.*, Cerebral Cortex, 15, 96-108, 2005).

To further screen area-specific molecules systematically in the monkey neocortex, we carried out another round of screening using the RLCS method (Suzuki et al. 1996; Shintani et al. 2004). In this analysis, mRNAs were purified from 4 distinct cortical areas, converted to cDNA by reverse transcription and digested with a pair of restriction enzymes for 2-dimensional analysis. Using the RLCS method we isolated genes that showed marked differences among four areas (area 46, primary motor area, TE and V1) and characterized the expression patterns. Examples of such genes we have previously reported are Testican-1, -2 (OCC1 related family genes), 5HT1B and 5HT2A (primary visual area enriched), and SPARC (an OCC1 related gene). This year, we reported another gene (PNMA5), whose expression is similar to RBP (an association area enriched gene) as shown in Figure 1.

II. Paraneoplastic Antigen-Like 5 Gene (PNMA5) is preferentially expressed in the association areas in a primate specific manner

As mentioned above, this year we reported enriched expression of the paraneoplastic antigen-like 5 gene (PNMA5) in prefrontal and sensory association areas but not in primary sensory areas, with the lowest expression level in the primary visual cortex. In situ hybridization in the primary sensory areas revealed PNMA5 mRNA expression is restricted to layer II: That is, along the ventral visual pathway, the expression gradually increased in the excitatory neurons from the primary to higher visual areas. This differential expression pattern was very similar to that of retinol-binding protein (RBP) mRNA, another association-area-enriched gene which we reported previously (Figure 2). Additional expression analysis for comparison of other genes in the PNMA gene family, PNMA1, PNMA2, PNMA3, and MOAP1 (PNMA4), showed that they were widely expressed across areas and layers, but without the differentiated pattern of PNMA5. In mouse brains, PNMA1 was only faintly expressed and PNMA5 was not detected. Sequence analysis showed divergence of PNMA5 sequences among mammals. These findings suggest that PNMA5 acquired a certain specialized role in the association areas of the neocortex during primate evolution (Takaji et al., Cereb Cortex. 2009, 19: 2865 - 2879).

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2009. The former title is indicated by an asterisk (*). Members with # belong to SRPBS (Strategic Research Program for Brain Science), NIPS.
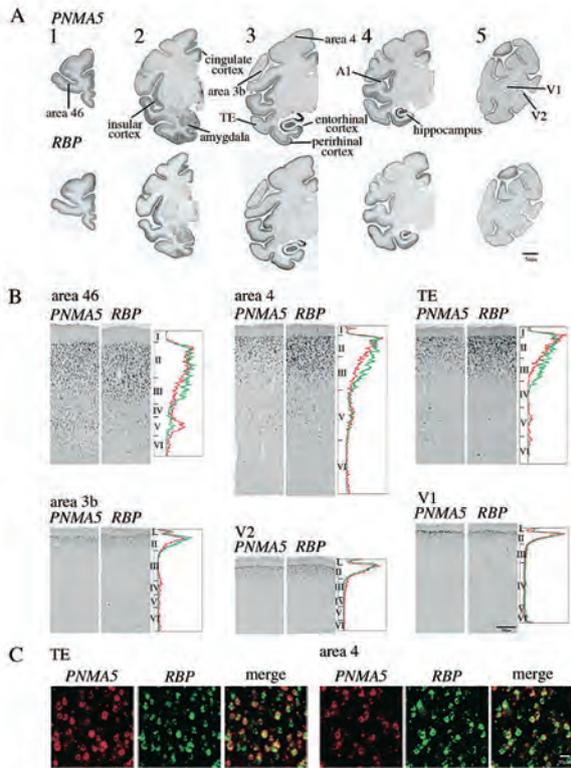
Figure 2. Comparison of expression patterns of PNMA5 and RBP mRNAs in macaque brains. (A) Coronal sections at positions A, C, D, E, F in Figure 2 are shown. (B) Higher magnification views of several areas. The expression patterns of PNMA5 and RBP mRNAs are shown, together with the laminar profiles of ISH signals quantified by measuring the optical density (red, PNMA5 mRNA; green, RBP mRNA). These profiles show the averages of the normalized values of two or three different macaques. The cortical layers were identified in reference to the Nissl staining of the nearby section. (C) Double ISH of PNMA5 and RBP mRNAs were performed to examine the coexpression of these mRNAs. Note that PNMA5 mRNA (left, red) is expressed in a large population of RBP-mRNA-positive cells (middle, green) in layer II. (cited from Takaji et al. Cereb Cortex. 19: 2865 - 2879)

III. Expression of immediate-early genes represents anatomical compartments within ocular dominance columns after brief monocular deprivation. Neocortical areas revealed by layer specific gene expression in rats

The primary visual cortex (V1) of primates is subdivided into compartments reflecting different neural circuits. Visual inputs from the two eyes activate alternating bands of cortex, thereby forming the well-studied ocular dominance columns (ODCs). In addition, the enzymatic reactivity of cytochrome oxidase (CO) reveals "blob" structures within the supragranular layers of ODCs. This year, we presented evidence for compartments within ODCs which have not previously been clearly defined (Takahata et al., 2009, 106:12151-12155). These compartments are revealed by the activity-dependent mRNA expression of immediate-early genes (IEGs), zif268 and c-fos, after brief periods of monocular deprivation (MD). After a 1-3 hr period of MD

produced by an injection of tetrodotoxin, IEGs were expressed in a patchy pattern that included infragranular layers, as well as supragranular layers, where they corresponded to the CO blobs. In addition, the expressions of IEGs in layer 4C were especially high in narrow zones along boundaries of ODCs, termed here, the "border strips" of the ODCs. After longer periods of MD (> 5 hr), the border strips were no longer apparent. When the MD was produced by a brief period of monocular eyelid suture, changes in IEG expressions were not evident in layer 4, however, the patchy pattern of the expression that was obvious in infragranular and supragranular layers remained. These changes of IEG expression after MD indicate that cortical circuits involving the CO blobs of the supragranular layers include aligned groups of neurons in the infragranular layers, and that the border strip neurons of layer 4C are highly active for 3 hr after MD (Figure 3).
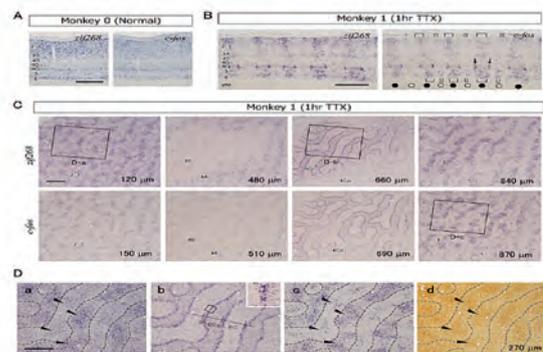
Figure 3. A, B: Coronal sections of ISH for IEGs, zif268 and c-fos, in V1 of Monkey 0 (normal; A) and Monkey 1 (1 hr TTX; B). IEG mRNA expression decreased in the deprived columns (open circles in B) after MD. Note that the widths of strong IEG mRNA expression (black brackets) are thinner than the actual widths of nondeprived columns (filled circles), and that the IEG mRNA expression remains in the center of deprived columns (open brackets). In addition, strong IEG mRNA signals were observed around the border of ODCs in layer 4Cβ (black arrows). Scale bar = 500 μm (A), and 1 mm (B), C: Tangential sections of ISH for IEGs in V1 of Monkey 1. The depth of the section from the pial surface is indicated on each panel. Sections are arranged by increasing depth. Scale bar = 1 mm, D: High magnification views of rectangle windows in C (a-c) and their adjacent section of superficial layer (270 μm away from the pia) stained for CO reactivity (d). Broken lines in a, c and d indicate the boundary of ODCs with reference to the patterns in b. Black arrowheads on the photomicrographs indicate the locations of CO blobs. Compartments of border strips (BSs), dark columns (DCs) and pale columns (PCs) are indicated in b. Insert in b represents a magnified view of the cortex within the rectangle area, at a single cell resolution. Scale bar = 500 μm (cited from Takahata et al., Proc Natl Acad Sci U S A. 2009, 106:12151-12155)

From these findings, we propose a model of the functional architecture of macaque V1 (Takahata et al., Proc Natl Acad Sci U S A. 2009, 106:12151-12155; Fig 4A). The functional unit that is seen in CO preparation in the supragranular layers extends to layer 6, with the exception of layer 4C. In addition, border strips reside in the vicinity of the boundaries of ODCs in layer 4C. As for the ODCs, the normally cryptic border strips are revealed by IEG mRNA expression after MD with TTX injection. Border strips of the left and right

eye column adjoin each other with the boundary of ODCs between them. Previously, Horton described CO blobs that included layers 5/6 in macaques (Horton & Hubel, 1981), and the existence of dim CO blobs in infragranular layers has been suggested for squirrel monkeys and prosimian galagos (Carroll & Wong-Riley, 1984; Condo & Casagrande, 1990)). Horton and his colleagues have also suggested the existence of border strips along ODCs in layer 4C in terms of a gap between anterograde tracer signals from the open eye and CO enzymatic activity after long-term MD by eye enucleation (Horton & Hocking, 1998), and pale stripes in layer 4C in CO staining in the strabismus monkeys (Horton, Hocking and Adams, 1999). Although there has been no

subsequent evidence for this architecture, our data strongly supports their model except for their suggested absence of a blob structure in layer 4A, as our data indicates that the blob structure also includes layer 4A.

Publication List

〔**Original papers**〕

● Moroni, R.F., Inverardi, F., Regondi, M.C., Watakabe, A., Yamamori, T., Spreafico, R., and Frassoni, C. (2009). Expression of layer-specific markers in the adult neocortex of BCNU-Treated rat, a model of cortical dysplasia. Neuroscienc. *159*, 682-691.

● Takahata, T., Higo, N., Kaas, J.H., and Yamamori, T. (2009). Expression of immediate-early genes reveals functional compartments within ocular dominance columns after brief monocular inactivation. Proc. Natl. Acad. Sci. USA *106*, 12151-12155.

● Takaji, M., Komatsu, Y., Watakabe, A., Hashikawa, T., and Yamamori, T. (2009). Paraneoplastic Antigen-Like 5 Gene (PNMA5) Is Preferentially Expressed in the Association Areas in a Primate Specific Manner. Cereb. Cortex. *19*, 2865 - 2879

● Watakabe, A. (2009). Comparative molecular neuroanatomy of mammalian neocortex: What can gene expression tell us about areas and layers? Dev. Growth Differ. *51*, 343-354.
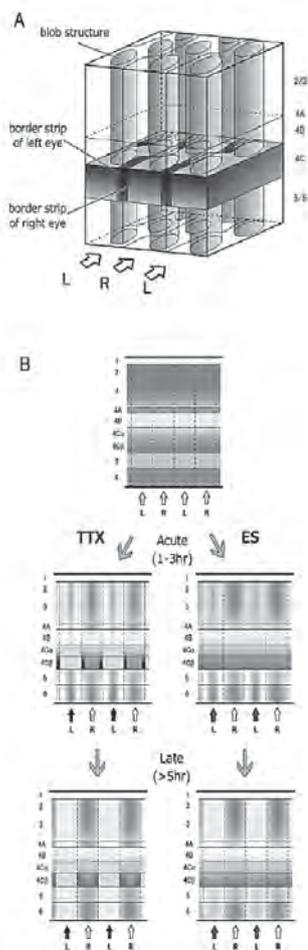
Figure 4. A: Our schematic model of V1 architecture. Blob structure extends from layer 2 to layer 6, with the exception of layer 4C. In addition, there is a border strip structure in the vicinity of the boundaries of ODCs in layer 4C. Although we did not detect strong IEG mRNA signals in layers 4B and 4Cα, previous reports can also be referred for the structure (12, 27). B: Our schematic view of changes of IEG expression, showing the distribution of somas of active neurons after MD (this case, deprivation of the left eye) by TTX or ES. In MD by TTX injection, IEG mRNA expression decreases in interblob regions in both columns, especially in deprived columns, and increases in border strips in nondeprived columns. After a period of time, IEG mRNA expression eventually levels out within each column. This sequential pattern is mostly similar in MD by ES, except that IEG mRNA expression hardly changes in layer 4C. L/projection from left eye, R/projection from right eye (cited from Takahata et al., Proc Natl Acad Sci U S A. 2009, 106:12151-12155).