We are studying genes that are expressed in specific areas of the neocortex in order to understand the principles governing 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 primates. To understand the underlying mechanisms of the primate brain we studied gene expression patterns within different areas of the neocortex.

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

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), which are preferentially expressed in the primary visual cortex, and *SPARC* (*OCC1* related gene) and *PNMA5* whose expressions are similar to RBP (an association area enriched gene) as shown in Figure 1.

II. Prefrontal-Enriched SLIT1 Gene Expression pattern in Old World Monkey Cortex that is established during Postnatal Development

This year, we reported enriched expression of the *SLIT1* gene in prefrontal and sensory association areas with the lowest expression level in the primary visual cortex. mRNA of *SLIT1*, an axon guidance molecule, was enriched in the higher-order association areas, but with developmentally related changes. *SLIT1* mRNA was mainly distributed in the middle layers of most cortical areas, abundantly in the prefrontal cortex and faintly in primary sensory areas. The lowest expression was in the primary visual area (V1) (Figure2). Analyses of other SLIT (*SLIT2* and *SLIT3*) mRNAs showed enriched expression in the higher-order association areas with a distinct laminar pattern. In contrast,
the receptor Roundabout (ROBO1 and ROBO2) mRNAs were widely distributed throughout the cortex (Figure 3). Perinatally, SLIT1 mRNA was abundantly expressed in the cortex with modest area specificity. Downregulation of expression initially occurred in lower-order sensory areas around postnatal day 60 and followed in the association areas (Figure 4). Thus, prefrontal-enriched SLIT1 mRNA expression results from a reduction in expression, specific for areas and layers. These results suggest that its role is altered during postnatal development, and that this is particularly important for prefrontal connectivity in the postnatal monkey cortex.

III. Differential expression patterns of occ1-related genes in adult monkey lateral geniculate nucleus

The extracellular matrix (ECM) plays important roles in the development and plasticity of the central nervous system. Last year, we reported that expression of OCC1, testican-1, testican-2, testican-3, SPARC and SC1 mRNAs, which encode for the ECM protein family, exhibit distinct patterns in the adult macaque visual cortex in an activity-dependent manner (Takahata et al., Cereb Cortex. 19, 1937-1951, 2009). This finding suggests that OCC1-related proteins play crucial roles in the visual processing pathway. We therefore examined the mRNA expression patterns of occ1-related genes in the dorsal lateral geniculate nucleus (dLGN) of adult monkeys. testican-1 and testican-2 mRNAs were strongly expressed in both excitatory projection neurons and GABAergic interneurons in the dLGN. testican-3 mRNA expression was predominantly observed in GABAergic interneurons in the cortex, while SPARC mRNA was strongly and exclusively expressed in nonneuronal cells in the dLGN. Interestingly, the neuronal SC1 mRNA expression was selectively observed in koniocellular layers of dLGN, while it is preferentially observed in blob regions of the primary visual area, suggesting a K-pathway preference of expression (Figure 5). Monocular inactivation experiments using tetrodotoxin injections demonstrated that the expression of testican-1, testican-2 and testican-3 mRNAs in
the dLGN are dependent on sensory activity (Figure 6). The differential expression patterns and activity dependence suggest that products of occ1-related genes may modulate visual processing and plasticity at the level of the dLGN, as well as V1.

Figure 6. Significant decreases in transcripts of the three testican genes were observed after monocular inactivation in the dLGN. A-C, E-G: Coronal sections of ISH for testican-1 (A, E), testican-2 (B, F) and testican-3 (C, G) in the contralateral (A-C) or ipsilateral (E-F) dLGN to the inactivated eye. D, H: Statistical analysis of ROD in all three genes for each layer in the contralateral (D) or ipsilateral (H) dLGN to the inactivated eye. C is contralateral receiving layers (layer 1 for P layers and layers 4 and 6 for M layers), and I is ipsilateral receiving layers (layer 2 for P layers and layers 3 and 5 for M layers). */P < 0.05, **/P < 0.01 in paired Students’ t-test (n = 4 each). Scale bar=100 μm. (Cited from Takahata et al., J Chem Neuroanat. 40, 112-122, 2010).

Figure 4. Postnatal Alteration of SLIT1 mRNA Expression in Various Cortical Areas. The expression of SLIT1 mRNA in six cortical areas (area 3b, V1, V2, V3, TE and area 46) at five postnatal ages are shown. (A-F) P1, (G-L) P30, (M-R) P60, (S-X) P95, (Y-DD) juveniles. Scale bar=100 μm. (Cited from Sasaki et al., Cereb Cortex. 20, 2496-2510, 2010).

Figure 5. Normal expression patterns of occ1-related genes (A: occ1, B: testican-1, C: testican-2, D: testican-3, E: SPARC, F: SCI) in coronal sections of normal adult macaque dLGN. Scale bar = 1 mm. (Cited from Takahata et al., J Chem Neuroanat. 40, 112-122, 2010).