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#: SRPBS (Strategic Research Program for Brain Sciences), NIPS

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* (an *OCC1* related gene) and *PNMA5* whose expressions are similar to RBP (an association area enriched gene) as shown in Figure 1.

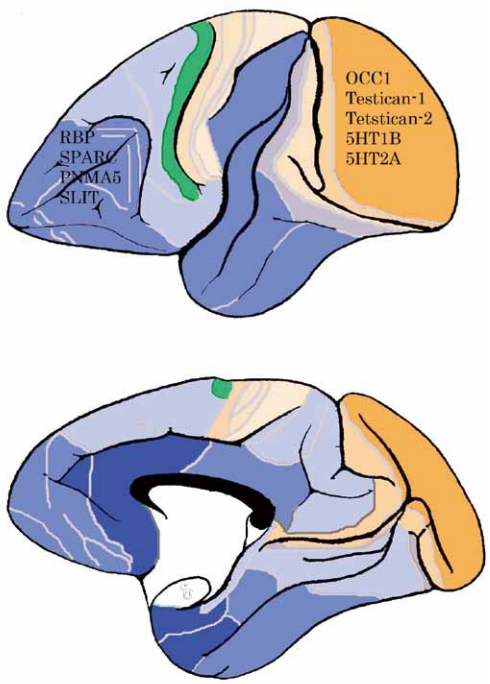

Figure 1. The expression of visual area specific genes (orange color) and association area specific genes (blue) and gdf7 (green) are schematically illustrated. Top and bottom views are medial and lateral surfaces, respectively. (cited from Yamamori & Rockland, Neurosci. Res., 55, 11-27, 2006)

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,

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2010. The former title is indicated by an asterisk (*).

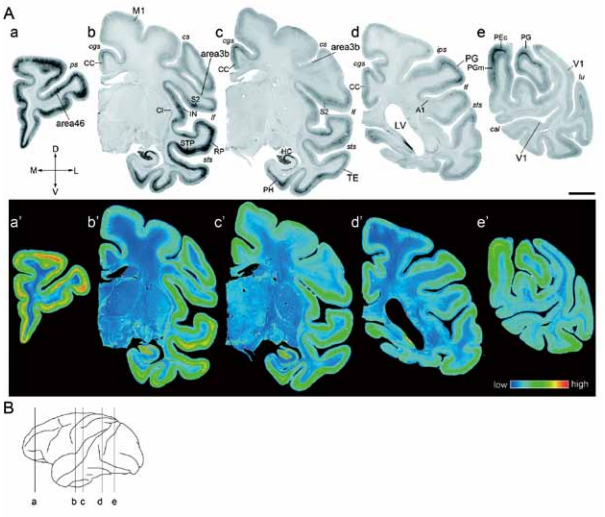

Figure 2. ISH Analysis of SLIT1 Gene in Macaque Brain.
(A) Coronal sections of a macaque monkey brain were obtained from the positions corresponding to a-e in the brain diagram shown in B. The representative six cortical areas (area 46, TE, PG, M1, area 3b and V1) are magnified in Figure 3. Pseudocolor representations of the same sections in a-e are shown in a'-e'. SLIT1 mRNA expression was observed in the entire cerebral cortex at variable levels. Note that the most intense signal was observed in the frontal pole section (a and a'). A1, primary auditory area (core region); M1, primary motor area; V1, primary visual area; CC, cingulate cortex; Cl, claustrum; HC, hippocampus; IN, insular cortex; LV, lateral ventricle; PEc, PE caudal part; PGm, PG medial part; PH, parahippocampal area; RP, rostral parabelt area; STP, superior temporal polysensory area; S2, secondary somatosensory area; cal, calcarine sulcus; cgs, cingulate sulcus; cs, central sulcus; ips, inferior parietal sulcus; lf, lateral fissure; lu, lunate sulcus; ps, principal sulcus; sts, superior temporal sulcus. Orientation of each section is indicated: D, dorsal; V, ventral; L, lateral; M, medial. Scale bar=5 mm. (B) Lateral view of the macaque neocortex. The lines indicate the planes sliced for the sections shown in A. (Cited from Sasaki et al., Cereb Cortex. 20, 2496-2510, 2010)

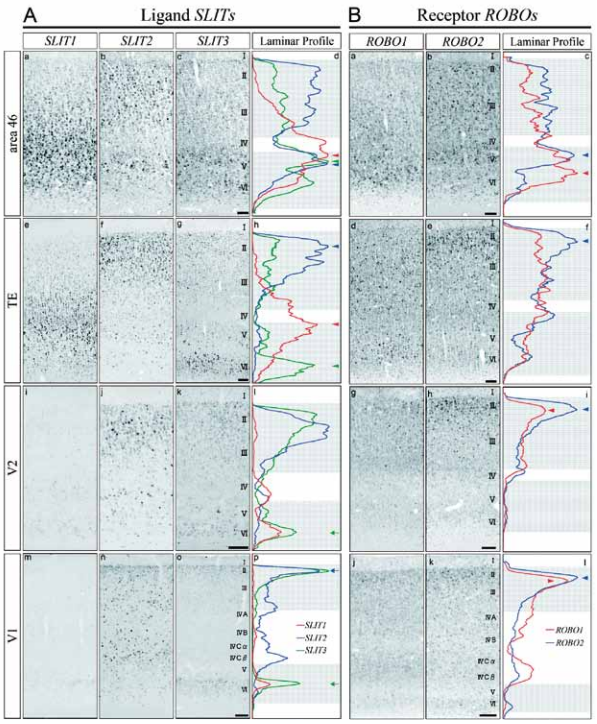

Figure 3. Differential Laminar Patterns of SLIT and ROBO genes in Macaque Cortex.
(A) ISH of SLIT1, SLIT2 and SLIT3 mRNAs in area 46 (a - c), TE (e - g), V2 (i - k) and V1 (m – o), respectively. The laminar profiles of the ISH signals are also shown. Red, blue and green lines indicate the profiles of SLIT1, SLIT2 and SLIT3 mRNAs respectively (d, h, l and p). The ISH signals of SLIT mRNAs were strongest in area 46 and weakest in V1. Their laminar patterns showed complementarity in TE. Arrowheads indicate the peaks of ISH signals for SLIT mRNAs in the association areas. Arrows indicate the peaks of the signals in the lower sensory areas (see in more detail in text) (B) ISH of ROBO1 and ROBO2 mRNAs in area 46 (a, b), TE (d, e), V2 (g, h) and V1 (j, k). The laminar profiles of the ISH signals are also shown. Red and blue lines indicate the profiles of ROBO1 and ROBO2 mRNAs (c, f, i and l), respectively. Arrowheads indicate the peaks of ISH signals for ROBO mRNAs. Scale bar=100 μm. (Cited from Sasaki et al., Cereb Cortex. 20, 2496-2510, 2010)

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, which is predominantly observed in GABAergic interneurons in the cortex, was restricted to excitatory projection neurons in the dLGN. 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

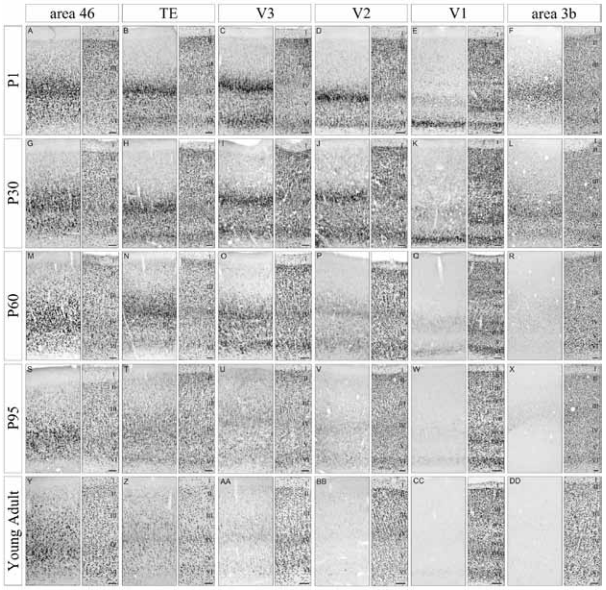

Figure 4. Postnatal Alteration of SLIT1 mRNA Expression in Various Cortical Areas.
The expression of SLIT1 mRNA in six cortical areas (area 3b, V1, V2, V3, TE and area 46) at five postnatal ages are shown. Coronal sections for ISH of SLIT1 mRNA (left panels) and the adjacent sections for cresyl violet staining (right panels) of the macaque neocortex 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)

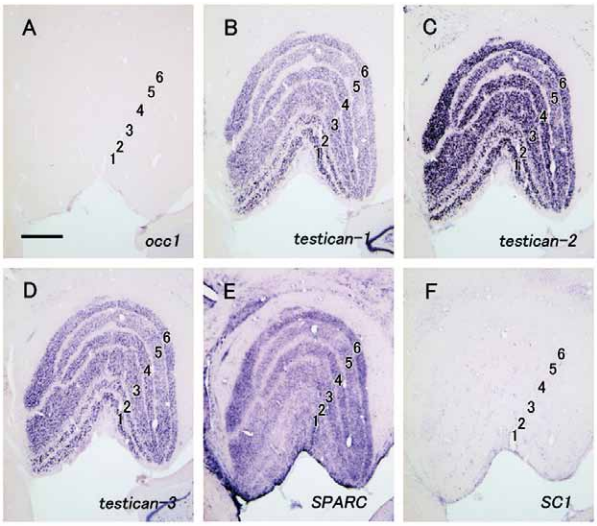

Figure 5. Normal expression patterns of occ1-related genes (A: occ1, B: testican-1, C: testican-2, D: testican-3, E: SPARC, F: SC1) in coronal sections of normal adult macaque dLGN. Scale bar = 1 mm. (Cited from Takahata et al., J Chem Neuroanat. 40, 112-122, 2010).

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.

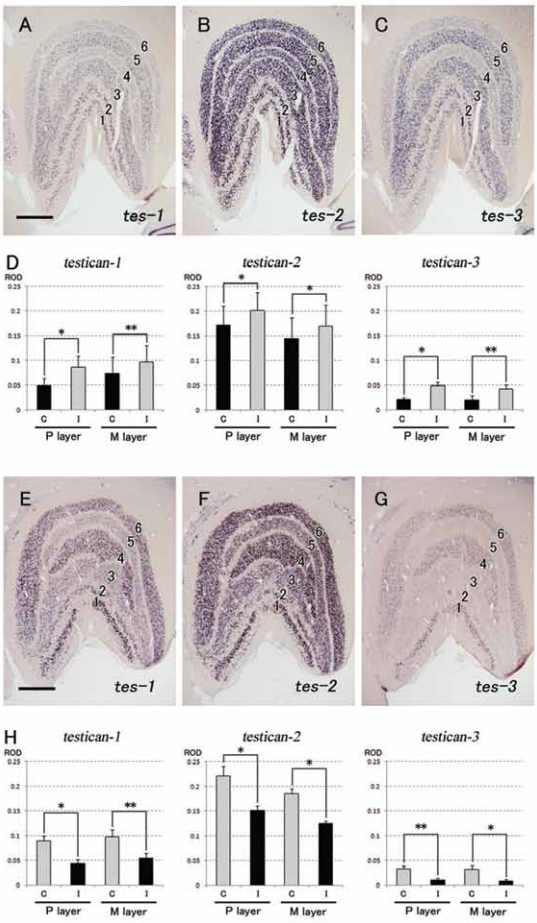

Figure 6. Significant decreases in transcripts of the three testican genes were observed after monocular inactivation in the dLGN. A-C, E-G: Coronal sections of ISH for testican-1 (A, E), testican-2 (B, F) and testican-3 (C, G) in the contralateral (A-C) or ipsilateral (E-F) dLGN to the inactivated eye. 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 = 1 mm. (Cited from Takahata et al., J Chem Neuroanat. 40, 112-122, 2010).

Publication List

〔Original papers〕

- Watakabe, A., Komatsu, Y., Ohsawa, S., and Yamamori, T. (2010). Fluorescent in situ hybridization technique for cell type identification and characterization in the central nervous system. Methods *52*, 367-374.
- Takahata, T., Hashikawa, T., Tochitani, S., and Yamamori, T. (2010). Differential expression patterns of OCC1-related, extracellular matrix proteins in the lateral geniculate nucleus of macaque monkeys. J. Chem. Neuroanat. *40*, 112-122.
- Puig, M.V., Watakabe, A., Ushimaru, M., Yamamori, T., and Kawaguchi, Y. (2010). Serotonin modulates fast-spiking interneuron and synchronous activity in the rat prefrontal cortex through 5-HT1A and 5-HT2A receptors. J. Neurosci. *30*, 2211-2222.
- Sasaki, T., Komatsu, Y., Watakabe, A., Sawada, K., and Yamamori, T. (2010). Prefrontal-enriched SLIT1 expression in Old World monkey cortex established during the postnatal development. Cereb. Cortex *20*, 2496-2510.