DIVISION OF BRAIN BIOLOGY

Professor
YAMAMORI, Tetsuo

Associate Professor
WATAKABE, Akiya

Assistant Professor:	*KOMINE, Yuriko*
	SADAKANE, Osamu
Specially Appointed Assistant Professor:	
	KOMATSU, Yusuke#
NIBB Research Fellow:	*OHTSUKA, Masanari*
Technical Staff:	*OHSAWA, Sonoko*
Postdoctoral Fellow:	*TAKAJI, Masafumi#*
	*OHTSUKA, Masanari**
	HATA, Katsusuke#
	NAKAGAMI, Yuki
	TAKEDA, Yuta#
Visiting Scientist:	*HIRAKAWA, Reiko*
SOKENDAI Graduate Student:	*SHUKLA, Rammohan*
Technical Assistant:	*NAKAMURA, Tohru*
	MORITA, Junko
	IMAI, Akiko
	KOTANI, Keiko
	KON, Yayoi
	IWASWE, Etsuko
	KAJITANI, Tomoki#
	TAKAHASHI, Yoichi#
	HIRAYAMA, Yuka#

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

This year we have reported three findings. One is methylation of genes and their controls selectively expressed in the association area by methyl-binding proteins in macaque monkeys. The other is that we confirmed the existence of ocular dominance columns in the new world monkey marmosets. The third is the primate visual area selective gene expression, which pattern was different from that previously reported.

I. DNA methylation and methyl-binding proteins control differential gene expression in distinct cortical areas of macaque monkey

The neocortex, which is present only in mammals and is enlarged in primates, consists of anatomically and functionally distinct areas that form different sensory modalities and functions. Studies have been done to address the underlying mechanisms that control the formation of the cortical layers and primary sensory areas. However, the molecular mechanisms that form and maintain these areas remain to be elucidated. The different regional specializations relate to the different gene expression profiles and resultant distinctive histochemical phenotypes seen in different cortical regions. Recent analyses of gene expression patterns in rodents reveal four patterning centers that control graded transcription in the neocortex. It can be expected that primates, with their much greater arealization, will have other molecular specifications besides that found in rodents.

We have isolated genes that are specifically expressed in the neocortical areas in primates, and reported two groups of genes that are differentially expressed either in the macaque primary visual cortex or in the occipital lobe (Tochitani *et al*., 2001; Takahata *et al*., 2009; Watakabe *et al*., 2009), or in the association areas of cortex including the frontal cortex (Komatsu *et al*., 2005; Takaji *et al*., 2009; Sasaki *et al*., 2010). Pronounced area-selectivity and activity-dependency of these genes occur in primates, but not in the rodents, lagomorphs or carnivores we examined. We thus suggest that the mechanisms underlying this gene expression may be an important clue to the evolution of the primate cerebral cortex (Takahata *et al*., 2011; Yamamori, 2011). We previously clarified the functions of two of the V1-selective genes, HTR1B and HTR2A, in the macaque primary visual cortex (Watakabe *et al*., 2009), and functional studies are currently underway for other molecules. Identifying mechanisms underlying these unique area-selective genes is important for understanding the development, evolution and function of the primate neocortex.

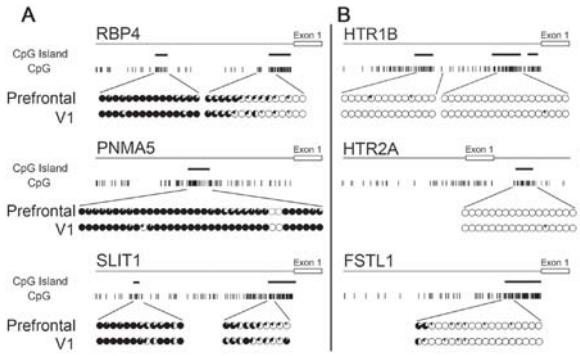

Figure 1. Methylation of association area- and V1-selective gene promoters.
Map of CpG base motifs in each AA- (A) and V1-selective gene (B) and their methylation status in the vicinity of the promoter region for tissues from the prefrontal cortex of area 46 (upper rows) and V1 (lower rows). The sites of CpG islands (CpGI) are represented by thick bars. The fraction of methylated DNA at each CpG site is shown by the proportion of the black area in each circle, with a full black circle indicating complete methylation. The left and right panels show the AA- and V1-selective gene promoters, respectively. The CGI in the promoter region of the AA-selective genes was hypermethylated, whereas that of the V1-selective genes was almost completely unmethylated. The same pattern was observed for genomic DNA obtained from both adult macaque PFC and V1 tissues (Cited from Hata *et al*., J. Neurosci. 2013 33(50):19704-19714).

This year, we demonstrated striking differences of DNA methylation between the promoter regions of the genes selectively expressed in the association areas (AAs) and the primary visual cortex (V1) in macaque monkeys (figure 1). Although the methylation levels of promoters of each area-selective gene showed no regional difference, MBD4, among five known methyl-binding proteins, was enriched in the AAs (Figure 2). MBD4 was mainly observed in NeuN-positive cells, specifically bound to and activating the AA-selective genes both in culture and *in vivo* in the macaque monkey neocortex (Figure 3). Thus, our results provide evidence for the critical role of DNA methylation and methyl-binding proteins in the differential gene expression profiles in the primate neocortex (Published in Hata *et al*., J. Neurosci. 33(50):19704-19714, 2013)

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2013. The former title is indicated by an asterisk (*).

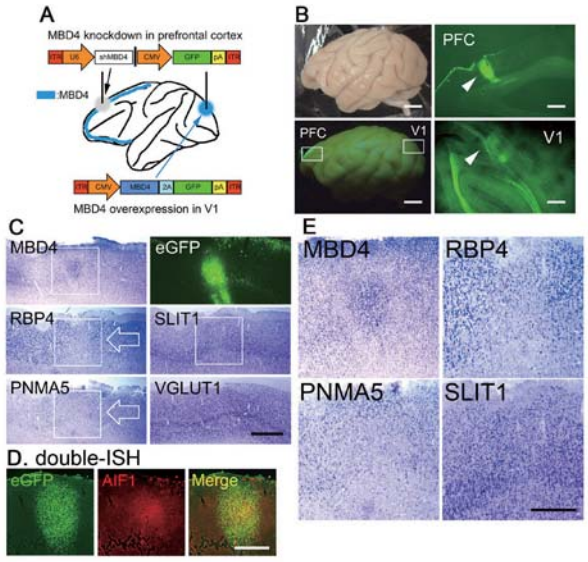

Figure 2. Expressions of the AA-selective RBP4, PNMA5 and SLIT1 in sections including PFC in AAV-shMBD4-injected monkey
A. An illustration of the AAV1-mediated gene transfer procedures in the macaque brain. AAV1-U6-shMBD4 was injected into PFC to examine the effects of loss of function of MBD4, whereas AAV1-CMV-MBD4 was injected into V1 to examine the gain of function of MBD4. B. Four weeks later, eGFP signals were observed in the injected sites in PFC and V1. Scale bar = 10 mm. C. The ISH signals of MBD4, RBP4, PNMA5, SLIT1 and VGLUT1 (an excitatory neuron specific marker gene) are shown. The white boxes of MBD4, RBP4 and PNMA5 indicate areas around the injection site, demonstrating decreased expression, but note that MBD4 ISH signals were enhanced at the injected site, possibly due to the damage-induced proliferation of glial cells or the migration of glial cells from uninjected regions. RBP4 and PNMA5 expression were restricted to neurons, and these signals were decreased around the injected site (white arrows). The MBD4 signals were only enhanced in the center region, with decrease observed in the signals just outside this focus, forming a donut-like appearance of MBD4 expression. Scale bar = 2 mm. D. Double-ISH of eGFP (green, left) and AIF1/Iba1 (red, middle), an activated microglial marker. A merged image (right) is also shown. Scale bar = 2 mm. E. Higher magnification photomicrographs of the white boxes shown for MBD4, RBP4, PNMA5 and SLIT1 in the upper panels, delineating clear boundaries of the decreased areas of expressions (except for SLIT1). Scale bar = 1 mm. Cited from Hata *et al*., J. Neurosci. 2013 33(50):19704-19714).

II. Dynamic changes of gene expression in the primary visual cortex revealed by monocular activation in adult marmosets

The primary visual cortex (V1) of primates is estimated to occupy more than 30% of the cerebral cortex. It has many characteristic features that enable highly complex information processing, e.g., formation of distinct functional columnar structures (ocular dominance and orientation columns, or color domains etc.), and parallel processing. We previously showed that there are a group of genes that are conserved in several species of primate but not in ferrets or mice (Takahata *et al*., 2008, 2012), suggesting that there are primate-specific mechanisms for expression of these genes. An important common feature of these genes was the activity-dependent expression in V1, which we showed by monocular inactivation of retinal activity using tetrodotoxin (TTX) (Tochitani *et al*., 2001; Takahata *et al*., 2009; Watakabe *et al*., 2009; Yamamori, 2011). Whereas this experiment revealed the requirement for retinal activity in gene expression in V1, it has not been clear how the incoming visual inputs induce the expression of these genes.

Synaptic transmission triggers the expression of a group of genes, which play roles in neural plasticity, differentiation, proliferation etc. Immediate early genes (IEGs) are classic activity-dependent genes, which are expressed within minutes of stimulation without the requirement for de novo protein synthesis. IEGs, such as c-Fos and Zif268 (also known as early growth response 1, Egr-1), are often used as the marker of neural activities. Previous studies in rodents demonstrated that visual stimulation induces the expression of Zif268 and c-Fos proteins at the peak level within one hour from stimulus onset, suggesting that input-driven gene activation in V1 reaches the maximum level within a short period of time. Compared with rodents, primates have far more developed visual systems. To our knowledge, however, there has been no information about visually evoked transcription in primate V1 within one hour. We designed a series of monocular visual stimulation experiments using adult marmosets, in order to understand the transcriptional regulation of the activity-dependent genes in primates.

We selected common marmosets (*Callithrix jacchus*), a New World monkey, because of its size, ease of handling, and transgenic (Sasaki *et al*., 2009) and gene manipulation potentials (e.g., Watakabe *et al*., 2012). One debated issue in marmoset vision research is whether ocular dominance columns (ODCs) exist or not. With particular relevance to our study, Markstahler *et al*. (1998) reported columnar ZIF268 immunostaining in layer IVCβ two hours after

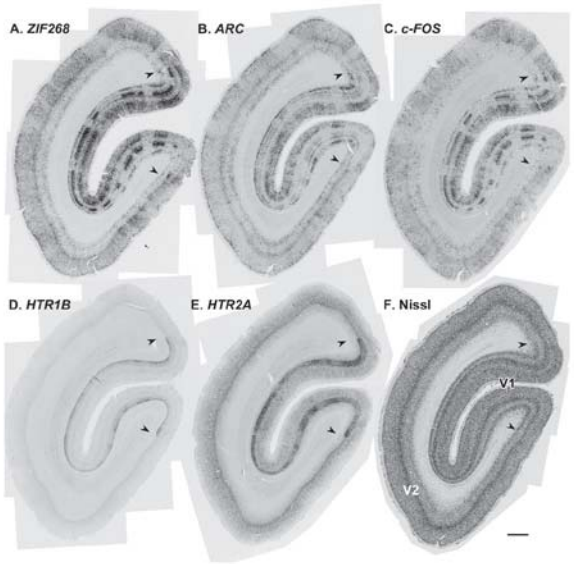

Figure 3. mRNA expression patterns of five stimulation events in visual cortices of adult marmosets. (A-E) mRNA expression patterns for activity-dependent genes we examined in the visual cortex 24 min after light stimulation: (A) ZIF268, (B) ARC, (C) c-FOS, (D) HTR1B, (E) HTR2A. (F) Cortical section stained for Nissl substance. Adjacent sections were analyzed. Arrowheads indicate V1/V2 boundaries. The columnar signal patterns within V1 indicate ocular dominance columns. Scale bar: 1 mm. visual cortex (Cited from Nakagami *et al*., Front. Neural Circuits. 7:43, 2013)

monocular visual stimulation following transient (24 h) monocular TTX injection, which they called "physiological ODCs". To investigate the visually evoked gene expression in primates, marmoset V1 is potentially a very good model.

We modified Markstahler's method to examine the mRNA expression of a set of activity-dependent genes in adult marmoset V1. We showed that these genes were expressed in a columnar fashion in V1 of all the monocularly visual stimulated marmosets, which shows strong evidence for the segregation of right and left eye inputs in the marmoset activity-dependent genes induced by monocular light.

Using this experimental system, we investigated the detailed time course of expression for (1) HTR1B and HTR2A mRNAs that represent the primate-specific V1-enriched and activity-dependent genes that we have previously reported (Watakabe *et al*., 2009; Takahata *et al*., 2012), and (2) IEGs of c-FOS, ARC (also known as Arg3.1), and ZIF268. Each of these activity-dependent genes revealed complex and characteristic time courses upon visual stimulation, demonstrating the inputs-evoked dynamic regulation of gene expression profile in marmoset V1 (Published in Nakagami *et al*., Front. Neural Circuits. 7:43, 2013).

III. Genes selectively expressed in the visual cortex of the OLD World monkey

Among the genes selectively expressed in the primary visual cortex, this year we have reported a new class of gene, SEMA 7A, whose expression is different from previously reported V1-selective gene expressions such as those of OCC1/FSTL1, HTR1B and HTR2A in that it is already expressed in the mid-embryonic stage (embryonic day 83 in macaque monkey) at the time when thalamocortical projections start. In addition, in contrast to the V1 selective gene we previously reported, SEMA 7A shows only week activity-dependent gene expression when examined by monocular inhibition by TTX injection into one eye (Komatsu *et al*., Published as a book Chapter, *In* Cortical Development, Eds. Kageyama R and Yamamori T Springer, Tokyo, 263-276). These features of SEMA 7A suggest a different role in primate cortical development from those suggested in mice, such as roles in axon branching and/or presynaptic punctate formation in the thalamocortical projections. We therefore are currently working to explore possible functions that explain the result in monkeys.

Publication List

〔Original papers〕

- Hata, K., Mizukami, H., Sadakane, O., Watakabe, A., Ohtsuka, M., Takaji, M., Kinoshita, M., Isa, T., Ozawa. K., and Yamamori, T. (2013). DNA methylation and methyl-binding proteins control differential gene expression in distinct cortical areas of macaque monkey. J. Neurosci. *33*, 19704-19714.
- Moritoh, S., Komatsu, Y., Yamamori, T., and Koizumi, A. (2013). Diversity of retinal ganglion cells identified by transient GFP transfection in organotypic tissue culture of adult marmoset monkey retina. PLoS ONE *8*, e54667.
- Nakagami, Y., Watakabe, A., and Yamamori, T. (2013). Monocular inhibition reveals temporal and spatial changes in gene expression in the primary visual cortex of marmoset. Front. Neural Circuits. *7*, 43.

〔Review article〕

- Komatsu, K., Toita, S., Ohtsuka, M., Takahata, T., Tochitani, S., and Yamamori, T. (2013). Genes selectively expressed in the visual cortex of the Old World monkey. *In* Cortical Development (Eds. Kageyama, R., and Yamamori, T.) Springer, Tokyo, 263-276.