DIVISION OF BRAIN BIOLOGY

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In order to understand the formation and evolution of the brain and the mechanisms underlying memory, we are focusing primarily on two issues. Firstly, we are studying the genes that are expressed in specific areas of the primate neocortex. We have obtained genes that show marked differences within primate neocortical areas. Secondly, we study the mechanisms underlying learning behaviors by examining gene expression.

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

The neocortex is most remarkably evolved in the anatomical areas and it has been a matter of debate to what extent areas of the neocortex are genetically and environmentally determined. It is also puzzling why, during the evolution of mammals, the neocortex was markedly expanded while the number of the genes in the mammal was little changed. In order to elucidate these questions, we studied gene expression within different areas of the neocortex. In the last several years, we reported the following findings,which are schematically illustrated in Fig. 1 and Fig. 2.

1) Examining 1088 genes by microarray analysis, most genes showed less than two fold difference in their expressions among the three neocortical (frontal, motor and visual) areas. Only one gene showed more than three fold difference and another one was between two and three fold difference within the three areas (Watakabe *et al*. Mol. Brain Res. *88*, 74-82, 2001). These results suggest that the genes expressed among the different areas of the human neocortex are very similar. However, the question remained whether or not there are any genes that show marked neocortical area difference.

2) In order to answer this question, we have employed differential display methods and found three genes that indicated area specific expressions.

i) One gene, designated *occ1*, is specifically expressed in the occipital cortex, particularly in V1 area, in the primate brain. We also demonstrated that *occ1* expression was markedly increased postnataly in V1.

ii) 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).

iii) *Rbp* (retinol-binding protein) is preferentially expressed in association and higher areas in the neocortex (Komatsu *et al*. 2005). *Rbp* also shows characteristic features. a) Its expression is high in sensory association and higher association areas and limbic areas, but low in the primary sensory areas. Expression is complementary to that of *occ1* and to parvalbumin immunoreactivity (PV-IR) in primary sensory areas. b) In early sensory pathways, the expression is limited to superficial layers

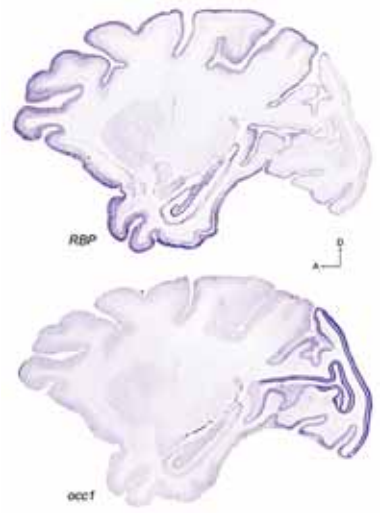

Figure1. Expression pattern of *occ1 and Rbp*. *occ1* RNA is markedly expressed in the visual cortex and expressed in primary sensory areas, particularly in visual cortex whereas primate and plays the major role in higher brain functions [?]. It is divided into distinct functional and Rbp is preferentially expressed in association areas (This Figure is cited from Komatsu et al., Cereb. Cortex, 15, 96-108, 2005)

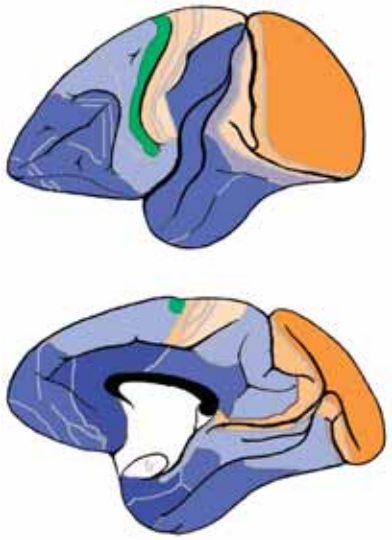

Figure 2. The expression of occ1 (orange color)m Rbp (blue) and gdf7 (green) are schematicaly illustrated in Brodmannís area figure in the guenon monkey. Top and bottom views are medial and laetral surfaces, respectively. (The figure is cited from Yamamori & Rockland, Neurosci. Res., 55, 11-27, 2006).

only (in particular, layer 2). With progression into higher sensory areas, the expression is expanded into layers 3 and then 5. c) In higher-order association areas, *Rbp* is expressed throughout all layers except layer 4. d) This characteristic distribution of *Rbp* is mainly formed during postnatal development. *Rbp* probably regulates the concentration of retinoic acid (RA) by the delivery of retinol, which is converted into RA in cells. Although the role of RA in the mature brain is not yet known, the characteristic expression of *Rbp* within association areas may provide a clue to the molecular basis of the formation and function of the association areas

This year we reported other features of *occ1* expression in mammalian brains. Firstly, we compared the *occ1* expression in subcortical areas and found certain nuclei strongly expressed *occ1*. Interestingly, in most of the nuclei that *occ1* is strongly expressed in monkeys, the authologue of *occ1* is similarly expressed in mice, which suggests that the occ1 expression in subcortical nuclei is generally well conserved during mammalian evolution (except for a few nuclei such as LGN). Secondly, we examined activity dependent expression of occ1 in mice. occ1 is strongly expressed in the mouse LGN. We then monocularly deprived by enucleation or TTX injection and examined *occ1* expression in LGN. Contrary to the monkey primary visual cortex, *occ1* expression was not affected by monocular deprivation. This is a clear contrast with other well known activity dependent gene expressions such as c-Fos expression. This result thus suggests that activity dependency of *occ1* has been acquired during evolution of the primate brain (Takahata et al., Society for Neuroscience in North America, 2006).

As pointed out by Brodmann 100 years ago, all mammalian brains consist of six layers, which structure is a fundamental frame work of the mammalian neocortex. It thus may give us an important information for understanding evolution of the brain in mammals by comparing the layer specific gene expression pattern. We examined the expression patterns of four layer-specific genes in monkey and mouse cortices by fluorescence double in situ hybridization (Fig. 3). Based on their coexpression profiles, we were able to distinguish several subpopulations of deep layer neurons. One group was characterized by the expression of ER81 and the lack of Nurr1 mRNAs and mainly localized to layer 5. In monkeys, this neuronal group was further subdivided by 5-HT2C receptor mRNA expression. The 5-HT2C+/ER81+ neurons were located in layer 5B in most cortical areas but they intruded layer 6 in the primary visual area (V1). Another group of neurons, in monkey layer 6, was characterized by Nurr1 mRNA expression and was further subdivided as Nurr1+/CTGF- and Nurr1+/CTGF+ neurons in layers 6A and 6B, respectively. The Nurr1+/CTGF+ neurons coexpressed ER81 mRNA in monkeys but not in mice. On the basis of tracer injections in three monkeys, we found that the Nurr1+ neurons in layer 6A send some corticocortical, but not corticopulvinar, projections. Although the Nurr1+/CTGF- neurons were restricted to lateral regions in the mouse cortex, they were present throughout the

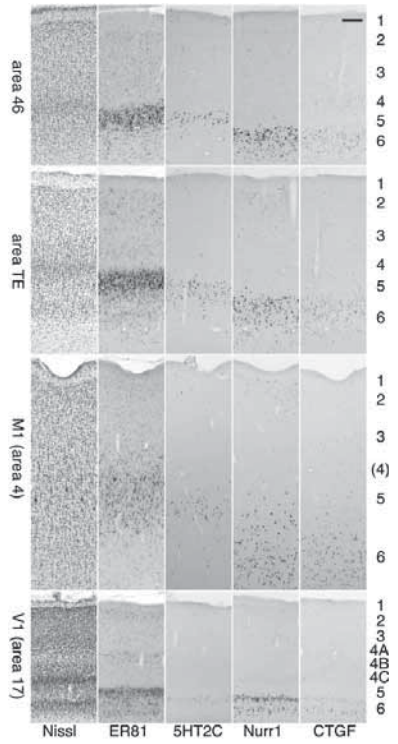

Figure 3. In situ hybridization patterns of layer-specific genes in various areas of monkey cortex. Adjacent sections of the monkey brain were processed for non-radioactive ISH with the probes indicated at the bottom of the panel. Here, we present data for area 46 on the bank of the principle sulcus, area TE on the inferior temporal gyrus, the primary motor cortex (M1 or Brodmann area 4) on the anterior bank of the central sulcus, and the primary visual cortex (V1 or Brodmann area 17) on the operculum of the occipital cortex. The numbers on the right indicate the layers determined by the adjacent Nissl staining. Scale bar on top right corner, 500 μm. (The figure is cited from Watakabe et al., Cereb Cortex. 2006 Oct 25; [Epub ahead of print])

monkey cortex. Thus, we revealed an architectonic heterogeneity across areas and species for the neuronal layer specific gene expression analysis (Watakae et al., Cereb Cortex. 2006 Oct 25; [Epub ahead of print Cereb]).

We also found another layer specific expression in mouse and monkey neocortex; that is, we showed that Semaphorin 3E (Sema3E), a class 3 member of the semaphorin family, exhibits highly layer specific expression in the mature neocortices of monkeys and mice. In macaque monkeys, Sema3E mRNA was restricted to layer VI across the entire neocortex. In all the areas examined, Sema3E-positive neurons were a subpopulation of VGluT1-positive excitatory neurons, but their percentages varied between 34% and 63% in the motor cortex and area TE, respectively. In the mouse cortex, Sema3E mRNA was also enriched in deeper layers, but, unlike the monkeys, it was expressed also in layer Vb. In addition, a subset of GABAergic interneurons in layers I-VI expressed Sema3E mRNA in mice but not in monkeys. In an in vitro binding assay, Plexin D1 bound to Sema3E but not to other members of class 3 semaphorins. Double ISH for Plexin D1 and Sema3E genes showed that these two genes exhibit

complementary patterns in mice and monkeys, although there are some species differences in lamina expression patterns. These results suggest that Sema3E and Plexin D1 may play a conserved role in regulating the interaction between upper and deep layer neurons in mice and monkeys (Watakabe et al., J. Comp. Neurol., 499, 258-273, 2006).

II. Gene expression under a declarative and a non-declarative memory

In order to study informational processing underlying the declarative and non-declarative memory at molecular and cellular levels in the brain, we established two behavioral systems.

1) We have been collaborating with professor Yoshio Sakurai (Kyoto University) who developed an audio-visual discrimination task (AVD-task) system. In this task, a rat was asked to choose either an audio cue (a high tone or low tone) or a visual cue (a light from the right or the left) to obtain a food pellet. We found that the visual and audio tasks enhanced the specific expression of c-Fos in the visual and audio cortices, respectively. Among the early visual and auditory pathways examined, c-Fos was specifically induced in the cortices but not in the earlier pathways, suggesting the neural modulation of the neocortex depends on the type of task. Interestingly, the task-dependent Fos expression was only observed in excitatory neurons in the relevant sensory cortices.

Although this AVD task system is quite powerful for analyzing the problem described above and useful for studying underlying molecular and cellular mechanisms because of the advantages of using rodents, one concern was that the auditory stimuli and visual stimuli were in different positions. Thus, we could not exclude the possibility that the difference between the auditory task and the visual task may not completely depend on the modality (visual Vs auditory) difference.

We wanted to solve this problem by placing auditory and visual stimuli in the same position. We also use nose-poking to measure the reaction time in which a rat responds to stimuli. By using this behavioral system, we were able to confirm amodal recognition of space which means that a rat can respond to a different modality (visual or auditory) if the stimuli are in the same position as previously reported in other systems. We also confirmed multisensory enhancement is indeed observed in rats. These results suggest that this new modified AVD system can be used to explore the molecular and cellular mechanisms underlying multisensory processing in rats (Sakata *et al*. Exp. Brain Res.,*159*, 409-417, 2004).

We studied multisensory processing further using a c-Fos mapping technique. We first developed a method to standerdize the cortex to quantitatively evaluate c-Fos expression by an automatic image analyzing system. Using this system, we found the secondary visual cortex (V2L) in rats is specifically activated under audiovisual multisensory stimulation. Injecting muscimol into V1, V2, V2L and superior colliculus (SC), we found that V2L is specifically involved in the stimulation of multisensory reaction (Hirokawa et al., 2006 Society for Neuroscience in North America).

2) The other task we developed is a wheel running system in which a water-deprived mouse is made to run to obtain water because the wheel with the pegs is turning in the other direction (Kitsukawa *et al*. SFN Meeting, 2002). The task required of the mouse can thus be regarded as representing a procedural learning. We examined various areas of the mouse brain following changes to the peg pattern. Among the areas examined, we found marked c-Fos expression in the striatum, cerebral cortex. The striatum, which is composed of projection neurons and several distinguished types of interneurons, is known to play an important role in reward-based learning. The characterization of these subtypes of interneurons has progressed. However, their roles in behavioral tasks have remained obscure. We are currently examining the altered behavior [that appears/develops?] under the pharmacological treatments that affect the metabolism of the interneurons in the striatum.

Publication List:

Original papers

Watakabe, A., Ohsawa, S., Hashikawa, T., and Yamamori, T. (2006). Binding and complementary expression patterns of semaphorin 3E and plexin D1 in the mature neocortices of mice and monkeys. J. Comp. Neurol. *499*, 258-273.

Takahata, T., Komatsu, Y., Watakabe, A., Hashikawa, T., Tochitani, S., and Yamamori, T. (2006). Activity-dependent expression of occ1 in excitatory neurons is a characteristic feature of the primate visual cortex. Cereb. Cortex *16*, 929-940.

Komine, Y., Nakamura, K., Katsuki, M., Yamamori, T., (2006). Novel transcription factor zfh-5 is negatively regulated by its own antisense RNA in mouse brain. Mol. Cell. Neurosci. *31*, 273-283.

Review articles

Yamamori, T., Rockland, K.S. (2006). Neocortical areas, layers, connections, and gene expression. Neurosci. Res. *55*,11-27.

Watakabe, A., Komatsu, Y., Nawa, H., Yamamori, T. (2006). Gene expression profiling of primate neocortex: molecular neuroanatomy of cortical areas. Genes Brain Behav. 5 Suppl *1*, 38-43.