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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 mammalian evolution, the neocortex was markedly expanded while the total number of genes in the mammal was little changed. In order to answer 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 Figure 1.

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. The question remained, however, whether or not there are any genes that show marked neocortical area difference.

2) In order to answer this question, we 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 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

iv) 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).

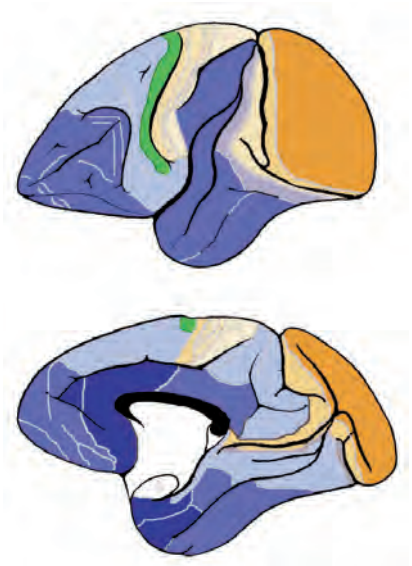

Figure 1. The expression of *occ1* (orange color) m *Rbp* (blue) and *gdf7* (green) are schematicaly illustrated in Brodmann's area figure in the guenon monkey. Top and bottom views are medial and lateral surfaces, respectively. (The figure is cited from Yamamori & Rockland, Neurosci. Res., *55*, 11-27, 2006).

v) *occ1* is strongly expressed in the mouse LGN. We then monocularly deprived activity 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 (Figure 2; Takahata *et al*., J. Chem Neuroanat., in press).

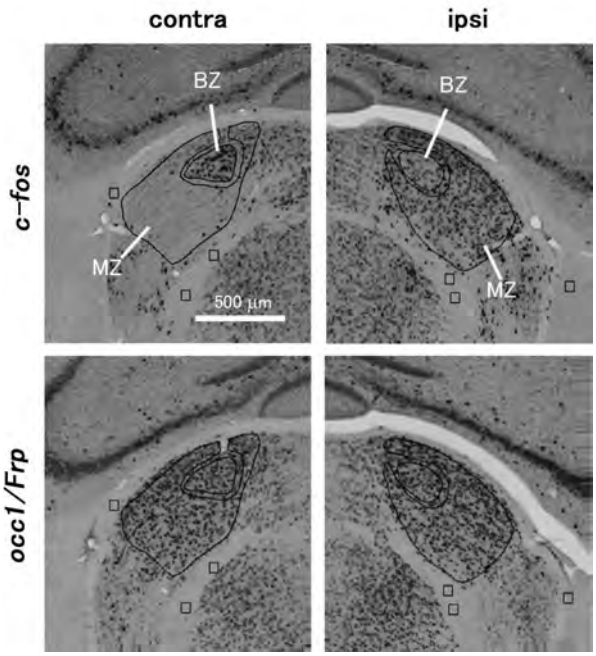

Figure 2. The expression of *c-fos* (top) and *occ1/Frp* (bottom) in mouse LGN are shown. In the monocular zone (MZ) of the contra LGN to the deprived eye, *c-fos* mRNA expression was dramatically decreased, whereas the *occ1/Frp* mRNA expression is not affected. (The original figure is shown in Takahata *et al*., J. Chem Neuroanat., 2007 Sep 16; [Epub ahead of print]).

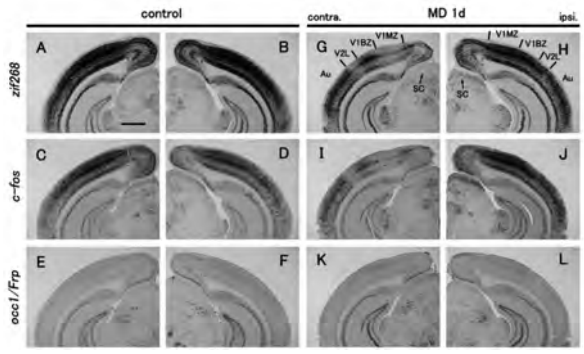

Figure 3. The expression of *zif268* (top), *c-fos* (middle) and *occ1/Frp* (bottom) in mouse visual cortex are shown. In the contra visual cortex (V1MZ: V1 monocular zone) and V2L (lateral secondary visual cortex), the expression of *zif268* and *c-fos* to the deprived eye (MD, 1 day after monocular deprivation), *c-fos* mRNA expression was dramatically decreased, whereas the *occ1/Frp* mRNA expression is not affected. (The original figure is shown in Takahata *et al*., J. Chem Neuroanat., 2007 Sep 16; [Epub ahead of print]).

vi) In the visual cortex of the mouse, it is known that monocular deprivation reduces the expression of immediate early genes such as *c-fos* and *zif268*. However, the expression of *occ1/Frp* in the LGN and visual cortex was not affected by monocular deprivation experiments (Figure 3; Takahata *et al*., J. Chem. Neuroanat., in press).

These results suggest that activity dependency of *occ1* has been acquired during evolution of the primate brain (Takahata *et al*., Society for Neuroscience in North America, 2006, J. Chem Neuroanat., in press).

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 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 standardize 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).

Traditionally, multisensory integration was thought to occurr in higher neocortical areas by merging different modalities of primary sensory information. Our results suggest that the multisensory integration may in fact occur at a relatively "early sensory" area such as V2. Previous elecrophysiological studies also show that there exist multisensory areas in the secondary visual area and the boundary areas between two modal areas (Toldi *et al*., 1986; Barth *et al*., 1995; Wallace *et al*., 2004). This observation is consistent with our findings through the experiments using the newly developed behavioral system and c-Fos analyzing system. Therefore, we have demonstrated evidence for a role for the lateral secondary visual area (V2L) in multisensory informational processing under behavior conditions.

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. Their roles in behavioral tasks, however, have remained obscure. We are currently examining the altered behavior that appears under the pharmacological treatments that affect the metabolism of the interneurons in the striatum.

Ⅲ. Topological relationships between brain and social networks

Network theory has recently revealed that many networks (gene transcription, protein-protein interaction, The Internet, and sociological networks) share global and local properties (Albert and Barabasi, 2002; Barabasi, 2002; Barabasi and Albert, 1999; Milo *et al*., 2002, 2004; Watts and Strogatz, 1998). To understand the global and local design principles of mammalian cerebral cortical networks, we applied network-theoretical approaches to connectivity data from macaque and cat cortical networks. Firstly, we confirmed the "small world" property of these cortical networks. Secondly, we then compared them, based on the significance profile (SP) of thirteen possible network motifs in the real network compared to randomized networks. SPs of different mammalian cortical networks are highly conserved and robust. Our results thus suggest that there are constraints of neocortical development and evolution (Sakata and Yamamori, Neurosci. Res., 51, 309-315, 2005).

This year, we reported on the topological similarities between brain and social networks. The statistical relevance of specific tied structures differs between social "friendship" and "disliking" networks. This suggests a relation-type-specific topology of social networks. Overrepresented connected structures in brain networks are more similar to those in the friendship networks than to those in other networks (Figure 4). We found that, unlike what could be predicted by simply counting mutual connections, balanced and imbalanced reciprocal connections between nodes are significantly abundant and rare, respectively. We interpret these results as evidence of positive selection of balanced mutuality between nodes. These results also imply that there exist common underlying principles between the organization of the brain and social networks.

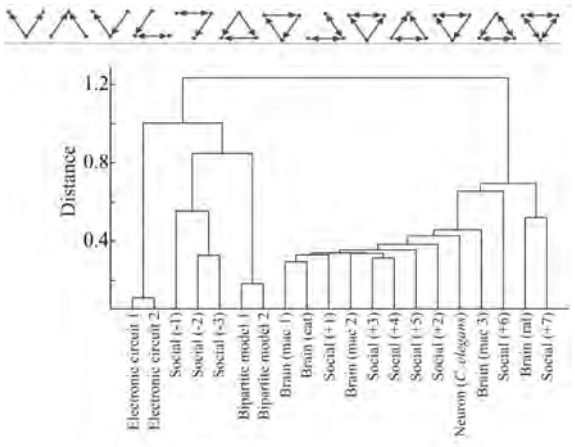

Figure 4. Hierarchical clustering of Triad Significance Profile (TSPs) (see thirteen possible motifs on the top panel) of complex networks. Note that brain and friendship networks fell into the same cluster. Abbreviations: mac. Macaque. The figures are cited from Sakata and Yamamori, Neural Networks., *20*, 12-21, 2007.

Publication List

〔Original papers〕

- Nakamura, K., Watakabe, A., Hioki, H., Fujiyama, F., Tanaka, Y., Yamamori, T., and Kaneko, T. (2007). Transiently increased colocalization of vesicular glutamate transporters 1 and 2 at single axon terminals during postnatal development of mouse neocortex: a quantitative analysis with correlation coefficient. Eur. J. Neurosci. *26*, 3054-3067.
- Sakata, S., and Yamamori, T. (2007). Topological relationships between brain and social networks. Neural Networks *20*, 12-21.
- Watakabe, A., Ichinohe, N., Ohsawa, S., Hashikawa, T., Komatsu, Y., Rockland, K.S., and Yamamori, T. (2007). Comparative analysis of layer-specific genes in mammalian neocortex. Cereb. Cortex *17*, 1918-1933.