DIVISION OF BRAIN BIOLOGY

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Our research is focused on understanding the formation and evolution of the brain and on the mechanisms underlying memory.Our approach is threefold. 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 are studying the mechanisms underlying learning behaviors by examining gene expression. Thirdly, we are studying a new transcriptional factor in which both sense and antisense RNA are expressed in the brain.

I. Genes expressed in specific areas of the neocortex

The neocortex is most remarkably evolved in the primate and plays the major role in higher brain functions. It is divided into distinct functional and anatomical areas and it has been a matter of debate 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.

1) Examining 1088 genes by microarray analysis, most genes showed only less 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 that are expressed among the different areas of the human neocortex are very similar. However, the question remained whether 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 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.

In this year [2005], we also reported on the features of *occ1* expression in mammalian neocortices. Although *occ1* is abundantly expressed in the primary visual cortex, we found that there is another mode of expression of *occ1*: sparsely expressed throughout the entire neocortex. Therefore, there are two different modes of *occ1* expression (Figure1). One mode is the expression in excitatory cells in the primary sonsory areas, particularly in visual cortex, which receive thalamocortical projections. The other mode is the expression in inhibitory GABAergic cells that are spread throughout all layers of entire neocortex. We found that among inhibitory neurons only Parvalbumin (PV) positive neurons expressed *occ1*.

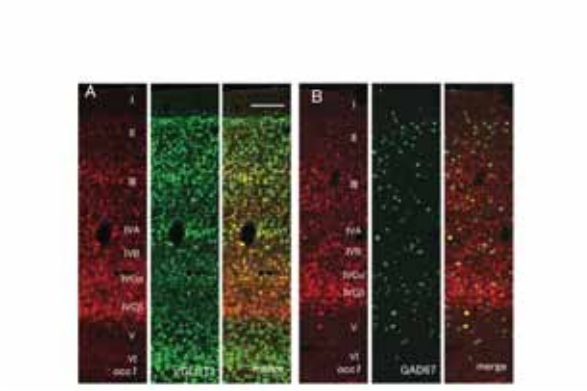

Figure 1. Expression pattern of *occ1*. *occ1* RNA is markedly expressed in the visual cortex and expressed in both excitatory (A:vGlut positive) and inhibitory (B:GAD) neurons (Takahata *et al*. Cerebral Cortex, 2005 Sep 8; [Epub ahead of print]).

As previously reported, *occ1* expression in the primary visual cortex is activity-dependent. In monkeys whose eyes are monocularly deprived by TTX injection, the *occ1* expression is markedly decreased in the monocularly deprived column. We then examined which mode of *occ1* expression is activity-dependent. *occ1* expression in excitatory neurons is decreased in the monocularly deprived column but not in inhibitory interneurons. Since primate brains have distinct features from other mammalian brains, we thought the expression profiles in primates might be different from those of other mammals. The most striking difference was observed in the expression in excitatory neurons in primaly sensory areas, particularly in the visual cortex. No clear border was observed in V1 and V2 in mammals (mice, rabbits, ferrets) other than those of macaques and marmosets. These results suggest the *occ1* expression in excitatory neurons was aquired in a specific step of evolution from an ancestor to the primate.

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 depending on the types of the tasks. 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 to analyze 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).

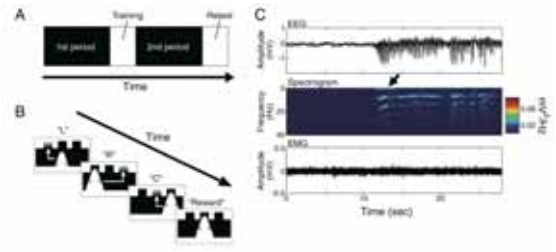

Figure 2. Properties of 7-12 Hz oscillations.(A) Schedule for experiments under task conditions of EEG and EMG recordings. (B) Illustration of one-trial schedule in sequential nose-poking task. In this task, when rats were responded to a predetermined sequence of the direction of lights (e.g., L-R-C), they were rewarded by a food pellet. (C) Top panel: an EEG signal from a frontal region during 7-12 Hz oscillations. Middle panel: an interpolated spectrogram of the EEG signal. These oscillations consist of basic frequences (7-12 Hz) with their harmonics. An arrow indicates slow-wave activities (<4Hz) observed in the initial stage. Bottom: a stimultaneous EMG signal. (These data are shown in Sakata *et al*. Neuroscience *134*, 1099-1111, 2005)

Various oscillations are observed depending on brain-states. Spike wave complexes (Sws), 7-12 Hz cortical oscillations with harmonics in awake but immobile rats, have been widely regarded as a model of paroxysmal activities in absence epilepsy. However, several studies have suggested that SWs in the primary somatosensory cortex are analogous to human mu rhythms. Because SWs have been frequently observed depending on vigilance levels, SWs in rats might represent normal brain-states related to the sleep-waking cycle. To elucidate behavioral contexts to induce SWs and temporal relations between SWs and neuronal ensemble activities, we recorded local field potentials (LFP) and multi-unit activities (MUAs) in the medial prefrontal cortex and electroencephalogram (EEG) in the bilateral regions of rats. Long-term recordings of EEG revealed that SWs were prominently generated in frontal and parietal regions and that SWs frequently followed non-REM sleeps. Occurrence probabilities of SWs significantly increased after the rats performed cognitive tasks. Our results suggest that SWs are one of the brain-state-specific oscillations rather than pathological activities. We also observed that MUAs were organized into phase-locked patterns in cycles of these oscillations. MUAs recorded from electrodes apart to each other were synchronized during SWs (Sakata *et al.* 2005a).

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 tothe other direction (Kitsukawa *et al*. SFN Meeting, 2002). The pegs can be changed with [?] various patterns as desired. The task required of the mouse thus can be regarded as representing a procedural learning. We examined various areas of 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 been progressed. However, their roles in behavioral tasks have remained obscure. We are currently examining the altered behavior under the pharmacological treatments that affect the metabolism of the interneurons in the striatum.

III. A transcriptional factor (zfh-5) both of which sense and antisense RNA are expressed in the brain

Antisense RNAs have recently been reported to be expressed much more than previously thought. However, the roles of antisense RNAs have been widely unknown, particularly in the brain. We found that the antisense strand of zfh-5, the gene for a novel transcription factor that we identified, was also transcribed in the developing mouse brain, in a manner complementary to the expression of zfh-5 mRNA (Figure3; Komine *et al*. 2005). Using gene-targeting approach, we showed that the expression of zfh-5 mRNA was negatively regulated by this antisense RNA. Our observations suggest that the suppression mechanism by the zfh-5 antisense RNA differs from those by previously known antisense RNAs.

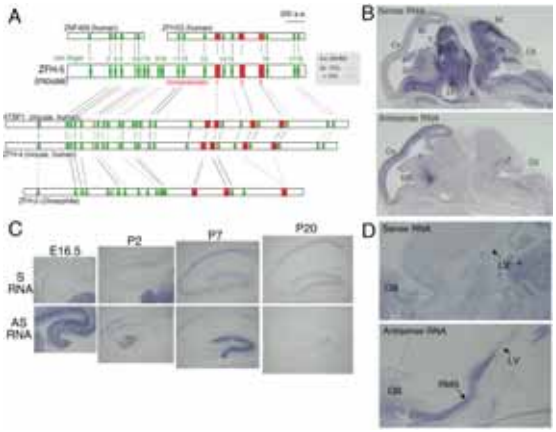

Figure 3. *zfh-5* expression in *ΔASC* mutant. (**A**) A pair of wild-type (+/+) and homozygous (*ΔASC/ΔASC: deleted in antisence control region*) littermates was used. In the *ΔASC/ΔASC* embryo (E16.5), the antisense RNA was almost depleted and the amount of the sense RNA increased in various regions of the brain compared with the same age of +/+ embryo. Arrows: pontine nuclei. (**B**) In the *ΔASC/ΔASC* mutant mice (P8), the ectopic expression of the sense RNA was prominent. (**C**) Two sets of RNAs (each lane contains 1 μg of polyA$^+$ RNA) from wild-type (+/+), hetero- (+/*Δ*) and homozygous (*Δ*/*Δ*) mice were electrophoresed and each set was hybridized with a strand-specific probe. As the amount of the antisense RNA decreased (+/+>+/*Δ*>*Δ*/*Δ*), that of the sense RNA including the full-length mRNA (indicated by arrowheads) increased (D) The expression patterns of sense and antisense RNA in RMSs (Rostal migratony stream) (The figure is shown in Komine *et al*. Mol. Cell Neurosci. 2005 Oct 27; [Epub ahead of print])

Publication List:

Original papers

Komatsu, Y., Watakabe, A., Hashikawa, T., Tochitani, S., and Yamamori, T. (2005). Retinol-binding protein gene is highly expressed in higher-order association areas of the primate neocortex. Cereb. Cortex *15*, 96-108.

Komine, Y., Nakamura, K., Katsuki, M., and Yamamori, T. (2005). Novel transcription factor zfh-5 is negatively regulated by its own antisense RNA in mouse brain. Mol. Cell Neurosci. (online publication)

Sakata, S., Komatasu, Y., and Yamamori, T. (2005b). Local design principles of mammalian cortical networks. Neurosci. Res. *51*, 309-315.

Sakata, S., Yamamori, T., and Sakurai, Y. (2005a). 7-12 Hz cortical oscillations: Behavioral context and dynamics of prefrontal neuronal ensembles. Neuroscience *134*, 1099-1111.

Takahata, T., Komatsu, Y., Watakabe, A., Hashikawa, T., Tochitani, S., and Yamamori, T. (2005). Activity-dependent expression of *occ1* in excitatory neurons is a characteristic feature of the primate visual cortex. Cereb. Cortex (online publication)

Review article

Watakabe, A., Komatsu, W., Nawa, H., and Yamamori, T. (2005). Gene expression profiling of primate neocortex: molecular neuroanatomy of cortical areas. Genes Brain Behav. in press.