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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 study 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 the 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 the 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.

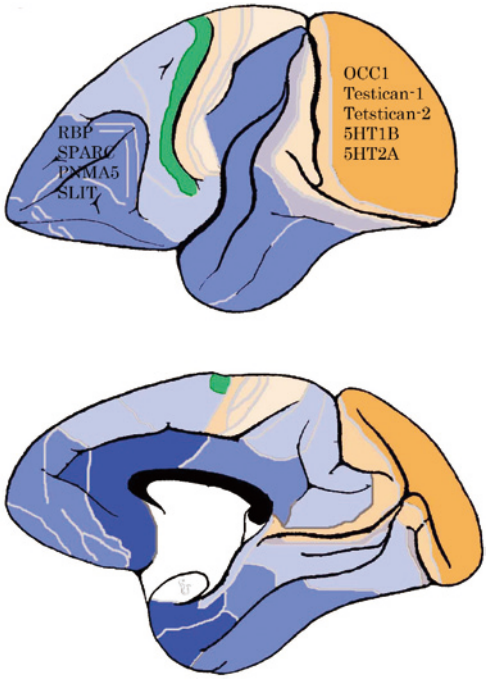

Figure 1. The expression of visual area specific genes (orange color) and association area specific genes (blue) and gdf7 (green) are schematically illustrated. Top and bottom views are medial and lateral surfaces, respectively. (cited from Yamamori & Rockland, Neurosci. Res., 55, 11-27, 2006)

This year, I reviewed our studies over the last ten years and more (Yamamori, 2011). The main discussions and conclusions are the functional significance of the two groups of the genes as shown in Figure 1. The first group of genes are those highly expressed in the primary sensory areas, particularly in the primary visual area (V1). In collaboration with Prof. Hiromichi Sato, Osaka University, we have previously shown that 5HT1B works by enhancing signal to noise (S/N) ratio and 5HT2A works as a gain controller (Figure 2). Recently, it has been reported that, fstl1 (follistatin-like 1), the mouse homologue of OCC1, is highly expressed in mouse dorsal root ganglion (DRG). The function in DRG has been revealed by the other group, showing that Fstl1 is directly bound to Na, K. ATPase, which suppresses sensory evoked presynaptic transmission by enhancing K+ influx and suppressing voltage dependent Ca++ influx (Li et al., Neuron 69, 974-987, 2011). This mechanism likely also works in primate V1 where OCC1 (macaque homologue of fstl1) is abundantly expressed. The

Note: Those members appearing in the above list twice under different titles are members whose title changed during 2011. The former title is indicated by an asterisk (*).

major difference between macaque V1 and mouse tissues that highly express OCC1 and fstl1, respectively, is that expression of OCC1 in V1 is activity-dependent whereas fstl1 expression in mouse tissues is not activity-dependent (Takahata et al., J. Chem Neuroanat., 35, 146-157, 2008).

These data of 5HT1B, 5HT2A and OCC1 expressions and functions strongly suggest that these genes control or modulate inputs from the retina in activity-dependent manners at the expression and functional levels (Figure 2). Such expression patterns specific to primates have come about during the course of primate evolution (Takahata et al., 2011).

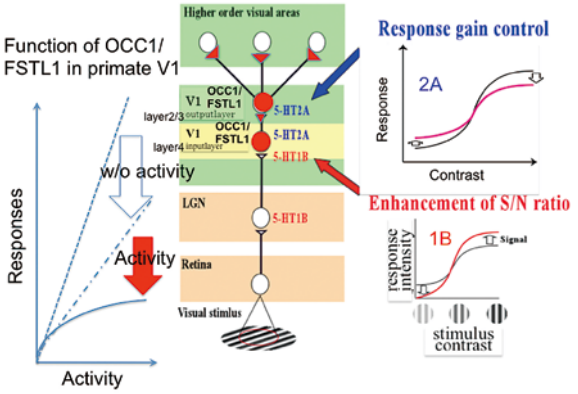

Figure 2. Roles of 5HT1B, 5HT2A and OCC1 in primate V1. Right: Possible roles of OCC1 in primate V1. As shown in rodent DRG, OCC1 is likely bound to Na, K, ATPase (NKA) and suppresses visual stimuli evoked synaptic transmission. The suppression is further enhanced by activity-dependent gene expression. Left: Responses to a stimulus with a low contrast are suppressed by the activation of 5HT1B and are enhanced by that of 5HT2A. At a high stimulus contrast, the effects are reversed. S/N ratio analysis demonstrated that the activation of 5HT1B contributes to the increase in S/N ratio. We predict that 5HT2A works as a gain controller to compensate for the gain or loss caused by 5HT1B receptors at a certain stimulus contrast. The left picture is drawn by Drs. Hiromichi Sato and Satoshi Shimegi (Osaka University) and is cited from Yamamori, T., Progress in Neurobiology 94, 201-222, 2011.

The second group of genes is selectively expressed in the association areas of the primate neocortex. Among the association area selective-expression genes, RBP, PNMA5, SPARC, and SLT2 are expressed in layers 2, 3, 5 and 6 in association areas and most abundantly prefrontal cortex and higher sensory association areas. SLIT1, on the other hand, is expressed in layers 2, 4 and 5 and most abundantly in layer 4 in the prefrontal cortex and to a lesser extent in the sensory association areas (Sasaki et al., Cerab. Cortex. 20, 2496-2510, 2010). Although the functions of these association area-selective genes are unknown, SLIT1 works to enhance dendritic branching in developing cortical neurons in rodents and thus presumably has a similar function in the primate neocortex.

II. Multisensory Information Facilitates Reaction Speed by Enlarging Activity Difference between Superior Colliculus Hemispheres in Rats

Animals can orient responses faster to multisensory stimuli than to unisensory stimuli. We have been working on studies of how animals can respond to multisensory stimulation in collaboration with Professor Yoshio Sakurai (Kyoto University) using an experimental system that evaluates audio-visual discrimination tasks in rats (Sakata et al., Exp Brain Res. 159, 409-417, 2004). Using this system, we identified the brain areas of V2L that are specifically involved in multisensory (visual and auditory) stimuli (Hirokawa et al., Neuroscience, 1402-1417, 2008). These studies were further extended to the superior colliculus (SC).

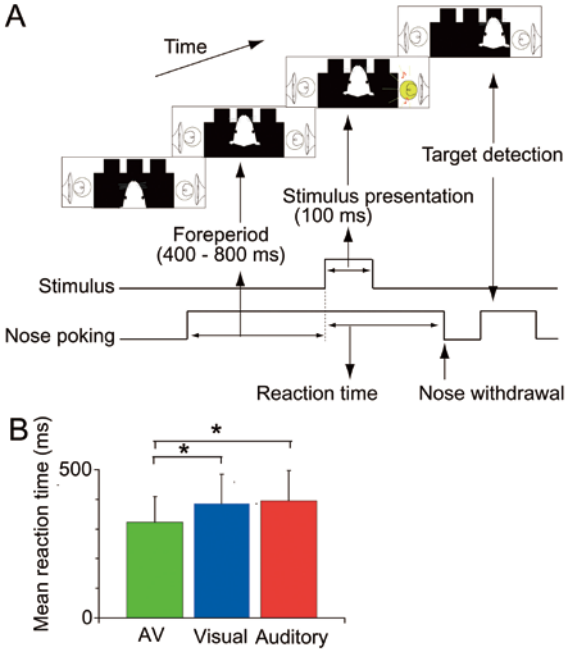

Figure 3. Two-alternative spatial discrimination tasks based on auditory and/or visual cues.
A) Timing of task events. Nose poking into the central hole initiated a trial. After a variable foreperiod, a cue stimulus was delivered from the left or right, randomly chosen from visual, auditory and audiovisual stimuli. Rats responded to the stimulus by withdrawing from the central hole and selected the direction of the cue stimulus by poking their heads into the hole ipsilateral to the stimulus. (B) Mean reaction time for each type of stimulus across sessions and rats (*p<0.001 in ANOVA and post hoc Tukey test). Error bars, standard deviation. This figure is cited from Hirokawa et al., Plos One, 2011.

The superior colliculus (SC), which receives multiple inputs from different sensory modalities, is thought to be involved in the initiation of orienting responses. However, the mechanism by which multisensory information facilitates orienting responses had not yet been understood. We demonstrate that multisensory information modulates competition among SC neurons to elicit faster responses. We conducted multiunit recordings from the SC of rats performing a two-alternative spatial discrimination task using auditory and/or visual stimuli. We found that a large population of SC neurons showed direction-selective activity before the onset of movement in response to the stimuli irrespective of stimulation modality. Trial-by-trial correlation analysis showed that the premovement activity of many SC neurons increased with faster reaction speed for the contraversive movement, whereas the premovement activity

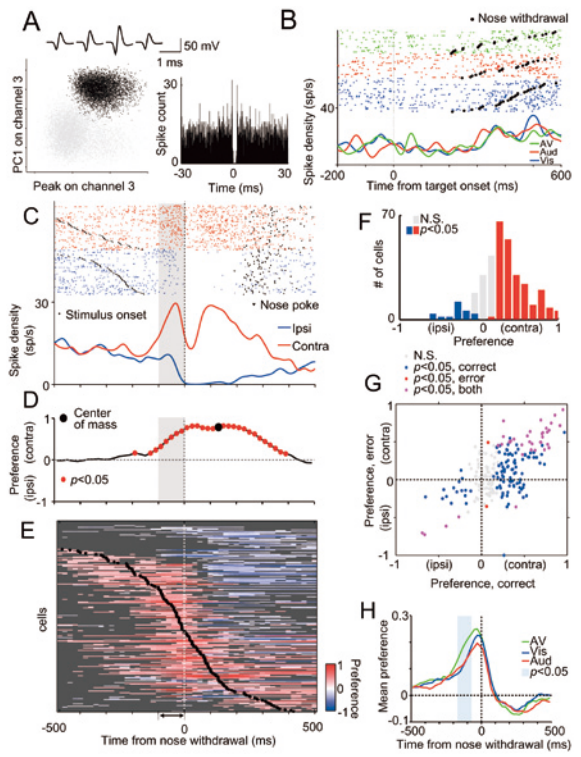

Figure 4. Direction preference preceding locomotion.
(A) Example of tetrode isolation for single unit. (Top) Average waveforms of spikes recorded on the four tetrode channels correspond to a cluster in black in the scatter plots below. The scatter plots indicate the peaks of waveforms from channel 3 plotted against principal component 1 (PC1) from channel 3 recorded from a tetrode. (Right) Corresponding autocorrelation functions with a window of ±30 ms. The bin size is 0.1 ms. (B) Rasters and spike density functions (SDFs) aligned to the onset of the target for isolated single unit (black cluster) during spatial discrimination task. The data were derived from only correct trials in responses to contralateral stimuli divided into visual, auditory and audiovisual stimulus conditions. Trials in the raster plot are sorted according to reaction time. (C) The data of the same neuron as above were aligned to movement onset and divided into ipsiversive and contraversive movement trials regardless of the modality of the stimulus. (D) Direction preference index of above unit was calculated using receiver operating characteristics (ROC) analysis for each time point ($p<0.05$, permutation test). (E) Direction preference curves for all cells (225 cells). Each row corresponds to one cell. Cells were sorted by the time of center of mass of significant positive preferences. Red and blue colors indicate preference indices with significant positive and negative values ($p<0.05$, permutation test), respectively, and gray points correspond to insignificant values. (F) Distribution of direction preference index in premovement period across population (225 cells). (G) Preference calculated for correct trials plotted against preference calculated for erroneous trials in premovement period. (H) Direction preferences calculated for each stimulus modality in each neuron and were averaged for each stimulus modality condition. The blue period shows a significant modulation of the preference under the multisensory condition (AV>unimodal, ANOVA with post hoc tukey test, $p<0.05$). This figure is cited from Hirokawa et al., 2011.

of another population of neurons decreased with faster reaction speed for the ipsiversive movement. When visual and auditory stimuli were presented simultaneously, the premovement activity of a population of neurons for the contraversive movement was enhanced, whereas the premovement activity of another population of neurons for the ipsiversive movement was depressed. Unilateral inactivation of SC using muscimol prolonged reaction times of contraversive movements, but it shortened those of ipsiversive movements. These findings suggest that the difference in activity between the SC hemispheres regulates the reaction speed of orienting responses, and multisensory information enlarges the activity difference resulting in faster responses (Hirokawa et al., 2011).

The SC has been considered as a brain region that is causally essential for integrating visual and auditory information, as demonstrated by a study of excitotoxic lesion of the SC in cats. On the other hand, our study showed that unilateral SC inactivation did not affect the facilitation of reaction speed to multisensory stimuli. It is therefore possible that different neural networks are responsive for accurate sensory detection and rapid response, respectively. In consistent with a line of this idea, we previously showed using the same technique as this study that the inactivation of the secondary visual cortex (V2L) suppresses the facilitation of reaction speed (Hirokawa et al., Neuroscience, 1402-1417, 2008).

Publication List

〔Original papers〕

- Hirokawa, J., Sadakane, O., Sakata, S., Bosch, M., Sakurai, Y., and Yamamori, T. (2011). Multisensory information facilitates reaction speed by enlarging activity difference between superior colliculus hemispheres in rats. PLoS ONE *6*, e25283.
- Kitsukawa, T., Nagata, M., Yanagihara, D., Tomioka, R., Utsumi, H., Kubota, Y., Yagi, T., Graybiel, A.M., and Yamamori, T. (2011). A novel instrumented multipeg running wheel system, Step-Wheel, for monitoring and controlling complex sequential stepping in mice. J Neurophysiol. *106*, 479-487.
- Rossini, L., Moroni. R.F., Tassi, L., Watakabe, A., Yamamori, T., Spreafico, R., and Garbelli, R. (2011). Altered layer-specific gene expression in cortical samples from patients with temporal lobe epilepsy. Epilepsia *52*, 1928-1937.

〔Original paper (E-publication ahead of print)〕

- Takahata, T., Shukla, R., Yamamori, T., and Kaas, J.H. Differential expression patterns of striate cortex-enriched genes among old world, new world, and prosimian primates. Cereb. Cortex. 2011 Nov. 7.

〔Review article〕

- Yamamori, T. (2011). Selective gene expression in regions of primate neocortex: implications for cortical specialization. Prog. Neurobiol. *94*, 201-222.