DIVISION OF BRAIN BIOLOGY †

Professor
YAMAMORI, Tetsuo

Associate Professor
WATAKABE, Akiya

Assistant Professor: *KOMINE, Yuriko*
SADAKANE, Osamu

This year we have reported the following results: (1) Long-term two-photon calcium imaging, (2) *in vivo* dendritic spines in marmoset neocortex, and (3) expression patterns of immediate early genes in the cerebellum.

I. Long-Term Two-Photon Calcium Imaging of Neuronal Populations with Subcellular Resolution in Adult Non-human Primates.

Two-photon imaging with genetically encoded calcium indicators (GECIs) enables long-term observation of neuronal activity in vivo. However, there are very few studies of GECIs in primates. Here, in collaboration with Professor Matsuzaki's laboratory, we report a method for long-term imaging of a GECI, GCaMP6f, expressed from adeno-associated virus vectors in cortical neurons of the adult common marmoset (Callithrix jacchus), a small New World primate. We used a tetracycline-inducible expression system to robustly amplify neuronal GCaMP6f expression and up- and downregulate it for more than 100 days. We succeeded in monitoring spontaneous activity not only from hundreds of neurons three-dimensionally distributed in layers 2 and 3 but also from single dendrites and axons in layer 1. Furthermore, we detected selective activities from somata, dendrites, and axons in the somatosensory cortex responding to specific tactile stimuli. Our results provide a way to investigate the organization and plasticity of cortical microcircuits at subcellular resolution in non-human primates (Published in Cell Rep. 13, 1989-1999, 2015).

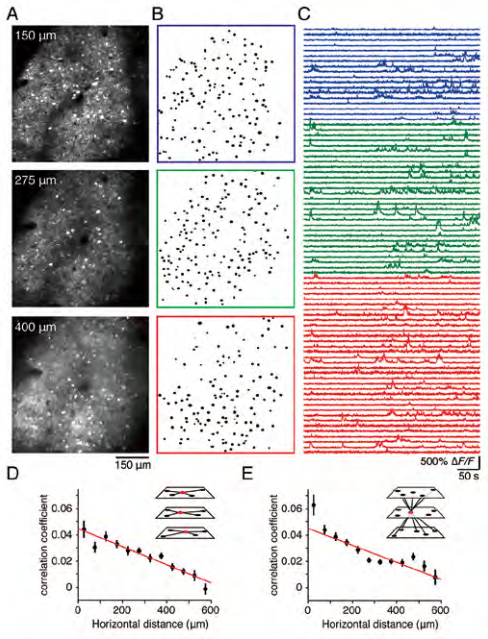

Figure 1. Three-Dimensional Imaging of Neuronal Population Activity in L2 and L3

(A) Representative two-photon images of GCaMP6f at a depth of 150 µm (top), 275 µm (middle), and 400 µm (bottom) from the cortical surface. These fields have the same horizontal location.

(B) ROI for activity analysis at each depth.

(C) Δ*F*/*F* traces of all active neurons over 400 s. Blue, green, and red indicate the traces of neurons at a depth of 150 µm, 275 µm, and 400 µm, respectively.

(D) Relation between pairwise correlation coefficient and horizontal cellular distance for 1,120 pairs of active neurons located at the same depth (from the same color traces shown in C). Red line indicates the linear regression line. Pairs were grouped every 50 µm. The error bars denote SEM.

(E) Relation between pairwise correlation coefficient and horizontal cellular distance for 1,508 pairs of active neurons located at different depths (from blue and green traces and from green and red traces; shown in C). (Cited from Sadakane et al., Published in Cell Rep. 13, 1989-1999, 2015)

II. In Vivo Two-Photon Imaging of Dendritic Spines in Marmoset Neocortex

Two-photon microscopy in combination with a technique involving the artificial expression of fluorescent protein has enabled the direct observation of dendritic spines in living brains. However, the application of this method to primate brains has been hindered by the lack of appropriate labeling techniques for visualizing dendritic spines. Here, we developed an adeno-associated virus vector-based fluorescent protein expression system for visualizing dendritic spines in vivo in the marmoset neocortex. For the clear visualization of each spine, the expression of reporter fluorescent protein should be both sparse and strong. To fulfill these requirements, we amplified fluorescent signals using the tetracycline transactivator (tTA)-tetracycline-responsive element system and by titrating down the amount of Thy1S promoter-driven tTA for sparse expression. By this method, we were able to visualize dendritic spines in the marmoset cortex by two-photon microscopy in vivo and analyze the turnover of spines in the prefrontal cortex. Our results demonstrated that short spines in the marmoset cortex tend to change more frequently than long spines. The comparison of in vivo samples with fixed samples showed that we did not detect all existing spines by our method. Although we found glial cell proliferation, the damage of tissues caused by window construction was relatively small, judging from the comparison of spine length between samples with or without window construction. Our new labeling technique for two-photon imaging to visualize in vivo dendritic spines of the marmoset neocortex can be applicable to examining circuit reorganization and synaptic plasticity in primates (Published in eNeuro. 2(4) ENEURO.0019-15, 2015).

†: This laboratory was closed on 31 March, 2015.

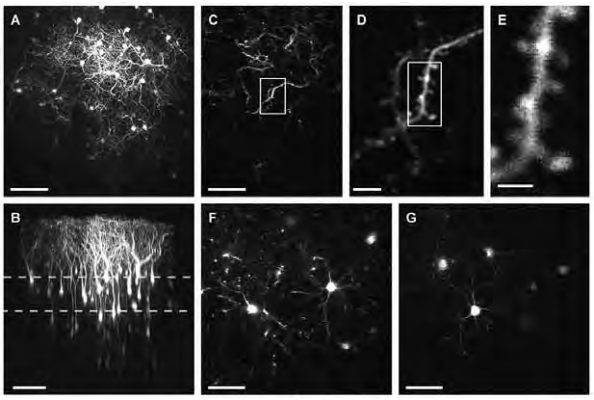

Figure 2. Dendritic spines imaged by *in vivo* two-photon microscopy. ***A***, Maximum intensity projection of the images acquired by *in vivo* two-photon imaging of marmoset cortex. ***B***, Side view of three-dimensional reconstruction of the images of the same site shown in ***A***. The depths of the areas shown in ***F*** and ***G*** are indicated by dashed lines. ***C***, Image plane near pial surface. ***D***, Magnified image of the boxed area in ***C***. ***E***, Magnified image of boxed area in ***D*** showing dendritic spines. ***F***, Image plane at a depth of 220 μm showing soma and basal dendrites. ***G***, Image plane at a depth of 330 μm. Scale bars: ***A***, ***B***, 100 μm; ***C***, 50 μm; ***D***, 5 μm; ***E***, 2 μm; ***F***, ***G***, 50 μm (Published in eNeuro. 2(4) ENEURO.0019-15, 2015)

III. Expression pattern of immediate early genes in the cerebellum of D1R KO, D2R KO, and wild type mice under vestibular-controlled activity

We previously reported the different motor abilities of D1R knockout (KO), D2R KO and wild-type (WT) mice. To understand the interaction between the cerebellum and the striatal direct and indirect pathways, we examined the expression patterns of immediate early genes (IEG) in the cerebellum of these three genotypes of mice. In the WT naive mice, there was little IEG expression. However, we observed a robust expression of c-fos mRNA in the vermis and hemisphere after running rota-rod tasks. In the vermis, c-fos was expressed throughout the lobules except lobule 7, and also in crus 1 of the ansiform lobule (Crus1), copula of the pyramis (Cop) and most significantly in the flocculus in the hemisphere. jun-B was much less expressed but more preferentially expressed in Purkinje cells. In addition, we observed significant levels of c-fos and jun-B expressions after handling mice, and after the stationary rota-rod task in naive mice. Surprisingly, we observed significant expression of c-fos and jun-B even 30 min after single weighing. Nonetheless, certain additional c-fos and jun-B expressions were observed in three genotypes of the mice that experienced several sessions of motor tasks 24 h after stationary rota-rod task and on days 1 and 5 after rota-rod tasks, but no significant differences in expressions after the running rota-rod tasks were observed among the three genotypes. In addition, there may be some differences 24 h after the stationary rota-rod task between the naive mice and the mice that experienced several sessions of motor tasks. (Published in Front Cell Dev Biol. 3:38, 2015).

Publication List

〔Original papers〕

- Nakamura, T., Sato, A., Kitsukawa, T., Sasaoka, T., and Yamamori, T. (2015). Expression pattern of immediate early genes in the cerebellum of D1R KO, D2R KO, and wild type mice under vestibular-controlled activity. Front. Cell Dev. Biol. *3*, 38.
- Sadakane, O., Masamizu, Y., Watakabe, A., Terada, S., Ohtsuka, M., Takaji, M., Mizukami, H., Ozawa, K., Kawasaki, H., Matsuzaki, M., and Yamamori, T. (2015). Long-term two-photon calcium imaging of neuronal populations with subcellular resolution in adult non-human primates. Cell Rep. *13*, 1989-1999.
- Sadakane, O., Watakabe, A., Ohtsuka, M., Takaji, M., Sasaki, T., Kasai, M., Isa, T., Kato, G., Nabekura, J., Mizukami, H., Ozawa, K., Kawasaki, H., and Yamamori, T. (2015). In vivo two-photon imaging of dendritic spines in marmoset neocortex. eNeuro doi: 10.1523/ENEURO.0019-15.2015