別紙２

Results report

１． Title of Research and Development： Investigation of cortical memory circuits in normal and disease model mice using synaptic optogenetics.

２． Principal Investigator：Haruo Kasai (Lahoratory of Structural Physiology, Graduate School of Medicine, The University of Tokyo, Professor)

３． Counterpart Principal investigator：Kevin Fox (Department of Neuroscience, Cardiff University, Professor (UK)）

４． Results of Research and Development:

Kasai Lab has eventually succeeded in publishing the memory probe (AS-probe) work in Nature (Article, 525:333-338) by performing the following experiments to persuade the referees. The key issue is the mechanisms how recently enlarged spines are selectively labeled by the probe. We have found three major mechanisms underlying the staining. 1) DTE (dendritic transportation element) allows very efficient transport of mRNA into the dendrites, so that proteins synthesis is suppressed in the soma. If proteins are translated in soma, they are transported to dendrite non-selectively, and incorporated into every spines irrespective of whether they undergo enlargement or not. This is because the persistent presence of the proteins in the dendrites equilibrate protein level throughout the dendrites. If DTE is added to 3'UTR region, protein synthesis is mostly induced in the dendrites. 2) Stimulation of spines causes enlargement and local protein synthesis, and resulting new proteins are inserted effectively into enlarged spines. On the other hands, the rest of spines are not stained with the probe, because protein turnover in a spine was slow, and 3) the excess proteins left in the dendrites are effectively scavenged by ubiquitin proteasomal system, and the resting spines cannot be labeled afterward. When we shrunk the labeled spines by photoactivation of PaRac1, the score of the motor task which was used to label the spines was markedly reduced. To test the task specificity of spines, we sequentially performed two tasks, and found that only the second task where the AS labeling was remaining was impaired. When we looked at the spines with the second task, they were mostly different from those of the first task. This is collaborated by the finding that the same spines tended to be potentiated by the first task after photoactivation, unlike the distinct second task. Thus, specific learning tasks mobilize distinctive sets of spines.

In order to label the entire memory circuits, we also labeled and identify synaptic contact and neuritis pathways leading to AS labeled spins. By trying this, we have found several specific problems to be overcome, which should be addressed in the future investigations.

Fox laboratory has started the molecular works for the AS probes, but has met with initial trouble shooting with the core technique such as plasmid amplification, and AAV vector formations. Via e-mail, and the joint workshop held in Cambridge (UK), we have addressed the problems, and have found ways to solve them. The joint workshop was also a good chance to communicate Japanese and UK laboratories, particularly by participation of many young scientists from laboratories of two countries. I think our collaboration has already succeeded in stimulating communication between Japanese and British laboratories.

別紙２

Results report

１．Title of Research and Development : Role of TLRs in patients with severe complicated malaria due to *Plasmodium vivax* and the development of diagnostic method for predicting the severity

２．Principal Investigator : Fumie Kobayashi (Professor; Department of Infectious Diseases, Kyorin University School of Medicine)

３．Counterpart Principal investigator : Rakesh Sehgal (Professor & Head; Department of Medical Parasitology, Postgraduate Institute of Medical Education & Research (India))

４．Results of Research and Development:

Severe cases of malaria are mainly caused by human malaria parasite, *Plasmodium falciparum*, leading to death if not promptly treated. Although *P. vivax* has long been considered as a benign infection, there has been an increase in the reported cases of severe malaria due to *P. vivax* in recent few years. However, the pathogenesis in severe vivax malaria is not fully understood. To investigate pathogenesis in severe vivax malaria, the clinical data related to the vivax malaria were collected at Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India. The informed consent was taken from patients before taking the clinical history and blood samples. Clinical data were analyzed in line with WHO criteria for severe malaria. Analysis of data obtained from the 90 *P. vivax* malaria patients revealed that 48% of patients showed the same clinical manifestation as severe malaria. The clinical manifestations observed in severe vivax malaria are severe anemia, thrombocytopenia, renal failure, jaundice, respiratory distress, and hypoglycemia to multi-organ dysfunction. Especially, thrombocytopenia was found in 75% of severe vivax malaria patients, suggesting that thrombocytopenia may be associated with increasing severity in vivax malaria.

The severity in vivax malaria patients may be attributed to the various factors, such as parasitic, host and environmental factors. In this study, we focused on the host factors responsible for the causation of severe complicated vivax malaria. We first investigated the various toll like receptors (TLRs), such as TLR4 and TLR9, in 90 subjects with *P. vivax* malaria. As results, we found that the nucleotide change was observed in all the targeted toll like receptor genes with varying frequency. In the available sequences, mutations in the TLR4 and in the TLR 9 were observed in 19 of 80 subjects and in 8 of 35 subjects, respectively. These findings suggest that the mutations in toll like receptors might be associated with pathogenesis of severe vivax malaria.

To investigate pathogenesis in severe vivax malaria, we tried to establish attenuated rodent malaria parasites and examined key factors determining the severity of malaria. *Plasmodium* parasites are unable to synthesize purine rings de novo. Thus, they rely on the host for a supply of purine nucleosides and then synthesize purine nucleotides through a purine salvage pathway. We demonstrated that the growth and virulence of *P. berghei* (*Pb*) ANKA, a high virulent-rodent malaria parasite, were suppressed by purine restriction. In mice infected with purine restricted parasites, progression of thrombocytopenia was delayed compared with mice infected with intact parasite. Subsequent purine restriction resulted in a substantial reduction in ATP levels relative to intact parasites, suggesting that the ATP levels in parasites might be useful for predicting the disease severity during malaria. Moreover, the attenuated parasites were readily cleared by wild-type mice, but not by TLR2/4/9-deficient mice and γδT cell-deficient mice. These results suggest that polymorphism or mutation of immune factors may be associated with the severity of malaria due to *P. vivax*.

Further analyses of clinical cases with severe vivax malaria (Indian side) and mouse malaria models (Japanese side) would contribute to understanding the pathogenesis and developing a diagnostic method for predicting the severity due to *P. vivax*.

別紙２

Results report

１． Title of Research and Development： "Improvement of the accuracy for differential diagnosis against viral infection – DENV, CHIKV, and FluV- by spreading of high quality RDT in India

２． Principal Investigator：Takeshi Kurosu （National Institute of Infectious Diseases）

３． Counterpart Principal investigator：Sujatha Sunil （International Centre for Genetic Engineering and Biotechnology（Country））

４． Results of Research and Development:

We aim to develop the rapid diagnosis test kits for dengue, chikungunya and influenza viruses and enlighten importance of early diagnosis. Patients with these diseases showed similar symptom at early stage; however, they have to receive different treatments.

1. Development of a diagnosis kit for dengue virus infection, epidemiological study and virological study: To make a good rapid diagnosis kit, most important issue is to obtain good antibodies which react efficiently with pathogens. We immunized mice with dengue virus infected cells. After several trials, we changed the protocol for immunization and obtained hybridomas producing antibodies. We stared characterization of antibodies. For epidemiological study, virus characterization is important; however, there is no method to classify virus in virulence using cultured cells. To solve this problem, we have established a new approach. Dengue viruses exhibited various virulence in mice lacking type I and II interferon systems. This approach will be useful to understand virulence.
2. Development of a diagnosis kit for chikungunya virus (CHIKV): Modified test stript (stick type) kit was produced. We had meeting with Indian counterpart about selection and criteria for evaluation. Indian counterpart performed a part of sequence analysis of CHIKV. For virus characterization, we performed to develop mouse model for arthritis. In India, there are many cases of co-infection between dengue virus and CHIKV, therefore, we assumed that co-infection may exacerbate arthritis because co-infection possibly enhances levels of cytokines compared with single infection with CHIKV. By using mouse model system, we will test this hypothesis.
3. Evaluation of influenza kit: For evaluation of kit, it is important to compare with conventional method. For this, we invited Indian young researchers to Japan and had training for diagnosis. During this year, a new matter of concern arose. In India, there were many death cases by H1N1 infection. In other countries, it was not observed, which means this was India-specific observation. Indian government wants to investigate. So Dr. Gaind’s team at Safdarjung Hostipal has establish diagnosis to distinguish H1N1. For this reason, Indian young researchers learnt diagnosis of H1N1 during their stay in Japan. Additionally, Japanese side and Indian side had agreement to examine a new rapid diagnosis method based on nucleic acid detection for H1N1.

Regarding international exchange, we invited two young researchers to Japan from January to March and two investigators to join symposium and have meeting. By presentation each other, we had exchanged information on epidemiological situation of infection diseases and idea. At symposium, four Japanese researchers and four Indian researchers gave presentation about progress of projects.

別紙２

Results report

１．Title of Research and Development：Single molecule imaging of synaptic protein dynamics in neurodegeneration

２．Principal Investigator：Akihiro Kusumi

(Professor, Institute for Integrated Cell-Material Sciences, Kyoto University)

３．Counterpart Principal investigator：Giovanna Mallucci

(Department Chair and Professor, Department of Clinical Neurosciences, University of Cambridge (U.K.))

４．Results of Research and Development:

In many neurodegenerative diseases, impaired synaptic structural plasticity, which leads to synapse loss and ultimately neurodegeneration, have often been found. Among many studies detecting the relationship between impaired synaptic structural plasticity and neurodegenerative processes, the PI of the present project on the U.K. side, Prof. Giovanna Mallucci, discovered very clear evidence for the relationship. When the brain or neuron is cooled to 17˚C, many synapses are lost, but when they are rewarmed, synapses reform – a physiological form of structural plasticity. However, Prof. Mallucci found that such synaptic reformation upon rewarming does not occur in many neurodegenerative diseases. This shows that, by understanding the synapse reforming processes and mechanisms, we might be able to discover how synapses are impaired in neurodegenerative diseases. The objective of the present investigation is thus to understand the synaptic structural plasticity in the cooling-rewarming process. For this purpose, we image the entrance into and exiting from synapses of various synaptic molecules at the level of single molecules in the cooling-rewarming cycle.

During the previous fiscal year (FY14), Prof. Mallucci's group established that a heat-shock protein called RNA-Binding Motif protein 3 (RBM3) works as a switch for synaptic regeneration upon warming (Peretti et al. 2015 Nature). This opened a new possibility to regulate synaptic structural plasticity in both positive and negative ways. In FY15, we advanced the present project, incorporating this new finding.

In this fiscal year (FY15), the Mallucci lab established the conditions for synaptic regeneration in the cycle of cooling and rewarming using primary neuron culture obtained from the mouse hippocampus. While this experiment was ongoing, two young researchers from the Kusumi lab visited the Mallucci lab, and helped to establish the proper conditions, so that the same system works in the Kusumi lab. It has now become possible to perform single-molecule imaging of synaptic structural plasticity using the primary neuron culture in the Kusumi lab.

Meanwhile, the Kusumi group advanced the method for specifically labeling AMPA receptor with fluorescent probe molecules (GluA1 and GluA2; submitted). They also developed methods to simultaneously observe the clusters of Homer1b and single GluA1 and GluA2 molecules.

Previously, we only paid attention to proteins as synaptic molecules. However, in-out of synaptic molecules often depends on their diffusion within the plasma membrane, and therefore, we decided to observe the movements of lipids, entering and exiting from synapses. For this purpose, we developed fluorescent ganglioside analogs (submitted). In particular, the direct interaction of a ganglioside GM1 and AMPA receptor has been proposed. Therefore, we will examine the behaviours of fluorescent GM1 in the cycle of cooling and rewarming.

別紙２

Results report

１．Title of Research and Development： Identification of biomarkers for infection-induced reactive arthritis based on the inflammation amplifier

２．Principal Investigator：Masaaki Murakami（Professor, Molecular Neuroimmunology, Institute for Genetic Medicine, Hokkaido University）

３．Counterpart Principal investigator：Ramnath Misra（Dean, Professor & Head, Clinical Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow（India））

４．Results of Research and Development:

Reactive arthritis (ReA) is a sterile inflammation of joints, triggered by infection at a distant site i.e. gastro-intestinal tract with *Salmonella*, *Yersenia*, *Shigella*, or *Campylobacter*. It is a major health problem causing acute and chronic arthritis in adolescent and young adults. Since *Salmonella typhimurium* is one of the commonest causes of bacterial diarrhea in India, therefore incidence rate of occurring ReA is expected to be high. Indeed, in sporadic ReA, the Misra laboratory has reported that *Salmonella typhimurium* accounts for one-third of cases in our community, in contrast to *Yersinia* and *Chlamydia* in Western countries. However, there are challenges in this disease, as there are no diagnostic tests as well as diagnostic biomarker for prognistification. One factor limiting the development of specific biomarker and therapy of ReA is the absence of a good animal model for the study of the molecular mechanisms involved.

The Murakami laboratory has discovered a chronic inflammation inducing machinery “inflammation amplifier” in local tissues. The inflammation amplifier induces excessive productions of inflammatory chemokines and growth factors in non-immune cells including endothelial cells, fibroblasts, glia cells, and epithelial cells, which is activated by the simultaneous activation of NFκB and STATs in response to cytokines such as interleukin (IL)-17A and IL-6. Dr Misra has found that presence of these cytokines in large quantities in synovial fluids of ReA patients. Therefore, the Japan-India collaboration by these laboratories will lead to establish a ReA animal model to study ReA pathogenesis and develop a biomarker and therapeutic strategy to ReA.

Dr Murakami already established a ReA mouse model using F759 knock-in mice infected with *Salmonella* in Osaka University. After moving his laboratory to Hokkaido University, the Murakami group improved the ReA model by injecting serum components derived from *Salmonella*-infected mice at the ankle joints of mice during the fiscal year of 2015. This phenomenon can explain joint inflammation by infection at the distant site (i.e. gastro-intestinal tract). Therefore, the pathogenesis of the serum component-induced arthritis will be extensively studied next year. In addition, the evidence of the amplifier activation in ReA patient was further supported by the observation that the amplifier-target soluble factors were elevated in sera of the patients in India.

In this fiscal year, a young researcher from the Indian side joined in the Murakami laboratory and learned experimental techniques to induce the ReA mouse model and analyze it. He will continue to stay in Japan next year to study the mechanism of disease induction by the serum components. In February 2015, Dr Misra visited the Murakami laboratory to discuss the Japan-India collaborative project for the fiscal year of 2016.

別紙２

Results report

１．Title of Research and Development：The development of the functional organization in visual cortex

２．Principal Investigator：

Kenichi Ohki　（Professor, Department of Molecular Physiology, Kyushu University）

３．Counterpart Principal investigator：

Matthias Kaschube（Professor, Department of Computational Neuroscience and Computational Vision, Frankfurt Institute for Advanced Studies and Goethe University（Germany））

４．Results of Research and Development:

The functional organization of the visual cortex describes the layout of tuning properties in large numbers of individual neurons. How it develops from synaptic connectivity is a central question of neuroscience. Recent studies in mice suggest that significant changes in the functional organization occur after eye opening. Moreover, recent technological advancements provide the unique opportunity to monitor these changes simultaneously in large numbers of cells. In this proposal we will perform two-photon calcium imaging of large populations of neurons in the developing mouse visual cortex to study how the functional organization changes during normal development. As a complementary effort, we will develop a computational circuit model, to understand which properties of the cortical network and of its feed-forward inputs account for the observed changes. To directly measure how visual response properties in individual neurons reorganize during development, we will make chronic two-photon recordings in mice around the time of eye opening. We study the network model over time, aiming to identify candidate mechanisms of cortical reorganization from a quantitative comparison between model and experiment. The proposed research will significantly broaden our understanding of the factors guiding the formation and maturation of the functional organization of the visual cortex.

This year, the Japan team developed a wide-field calcium imaging method using macro-zoom microscope, and found that patterns of spontaneous activity in the mouse cerebral cortex change dramatically during normal development. Furthermore, they developed a method for chronic two-photon calcium imaging and chronically observed changes of response selectivity of neurons in mouse visual cortex. The German team received the data of spontaneous activity in the mouse cerebral cortex during development and started the analysis of the data.

別紙２

Results report

１．Title of Research and Development： Bioinformatics platform for predicting autologous cell therapy efficacy in patients with heart failure

２．Principal Investigator：Yoshiki Sawa（Professor, Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine）

３．Counterpart Principal investigator：Esko Kankuri（Docent, Pharmacology, Institute of Biomedicine, University of Helsinki（Finland））

４．Results of Research and Development:

Our achievement of this year is to establish disease-specific induced pluripotent stem (iPS) cell from the patient of congenital cardiovascular disease. We decided to research Dilated Cardiomyopathy (DCM) and differentiation into cardiomyocyte was induced by adding cytokines such as Activin A and Bone morphogenetic protein 4. (Reference: Nature Biotechnology, 25 (9) (Sep 2007), pp. 1015–1024 and Journal of Molecular and Cellular Cardiology 50 (2011) 327–332). After differentiation, the purity of cardiomyocyte was 10 – 20 %. We are considering that some improvements will be needed in the differentiation process. On the other hand, we already developed the new method to purify iPS-derived cardiomyocyte from the cardiomyocyte-induced iPS cell population in the first year of this project. Because one of the extra cellular matrix has affinity to cardiomycyte, cardiomyocyte can be easily separated from other cells without invasions in a short time. This technique is already applied domestic patent as we reported before (2014-188180). This year, we added some new data that shows our technique is superior in the point of high yield and low cytotoxicity than the conventional cell purification methods. Then we applied this method to international application (PCT/JP2015/076072). The researchers in Helsinki contributed actively to carrying out this research such as giving information about protocol to the researchers in Osaka. Antti Siltanen, a researcher of University of Helsinki is now writing a paper about this research. Furthermore, Keitaro Domae, a young researcher of Osaka University went to Helsinki from 23rd February to 18th March. He learned recent development of treatment for heart failure in Helsinki. Especially he discussed surgical stratagem in treating for heart failure with Ari Harjula, Professor of Cardiothoracic Surgery, Helsinki University Central Hospital and his colleague. He also shares the recent development of basic research of cardiac regenerative therapy in both University of Helsinki and Osaka University. . On 10th March, scientific meeting hold at University of Helsinki. The participants from Finland were Professor Harjula, Dr. Kankuri, Dr. Kankainen, and Dr. Siltanen. The participants from Japan were Dr. Miyagawa, Dr. Fukushima and Dr. Domae. We discussed actively about current status of cardiac regenerative therapy in Osaka University and University of Helsinki, and the application of bioinformatics into clinical setting of cardiac regenerative therapy. We also confirm the facilitation of exchange and cooperation between Helsinki and Osaka University. Our young researchers joined this meeting and discuss and interact with researchers of Helsinki University. We also confirmed the research plan and cooperation in the next year.

別紙２

Results report

１．Title of Research and Development： Privacy-preserving genomic data analysis for personalized medicine (PRIVAGEN)

２．Principal Investigator：Kana Shimizu （Senior Research Scientist, National Institute of Advanced Industrial Science and Technology）

３．Counterpart Principal investigator：Antti Honkela（Academy Research Fellow（Finland））

４．Results of Research and Development:

In this fiscal year, the Japanese team has focused on developing cryptographic protocols for searching genome information.

1) Privacy-preserving allele information search system

We implemented a novel system in which the user can search allele information without showing his/her query to the server while the server only returns the search result. To achieve practical performance, we developed an improved protocol whose communication overhead is $O(\sqrt{N})$ and replaced the previous protocol whose overhead is $O(N)$ by the new one. In addition to implementing searching module, we also implemented authentication module, secure communication module and GUI both for user and server applications to construct an entire searching system. We have installed the implemented system in the human genome databank to test an efficiency of the system.

2) Privacy-preserving genome sequence search

We designed and implemented a novel protocol which enables to search a similar genome sequence from a database. The method is based on a new approach that combines efficient string data structures such as the (positional) Burrows-Wheeler transform with a cryptographic technique called an oblivious transfer. The proposed method is order of magnitude faster than existing algorithms for finding substring match. In an experiment using 2184 aligned haploid genomes from the 1000 Genomes Project, our algorithm was able to perform typical queries within $\fallingdotseq$4.6 s and $\fallingdotseq$10.8 s for client and server side, respectively, on laptop computers. We also published the source code of the proposed algorithm at gitHub. The proposed method can be used in a wide range of applications.

3) Long-term preservation of the personal genome

The genome is inheritable to offspring and thus the protection period of genomic data could be very long. Despite that, most of the conventional studies use security parameters of the cryptosystem which were originally designed to handle other types of personal information such as a bank account and a phone number whose ideal protection period is much shorter than that of genome information. In our study, we address the problem of long term genomic data protection and suggest a novel approach that combines an information-theoretically secure method and a computationally secure method. We targeted on an allele frequency search problem which is modeled by 1 out of N oblivious transfer (1-N OT), and designed the novel 1-N OT protocol that achieves a long protection period while keeping utility.

In addition to above research results, we organized a workshop PRIVAGEN 2016 together with Finnish researchers. The workshop aimed to facilitate discussion among researchers in diverse fields including bioinformatics, genome ethics, machine learning, data-mining and cryptography. The PRIVAGEN was held as an official satellite workshop of GIW/InCoB 2016 which was the international conference of Bioinformatics, and there were 80 participants from more than 10 countries. We also organized a session for discussing the effect of new legislation of personal information in Japan on genome information analyses at IIBMP 2015 which was the largest domestic conference of Bioinformatics in Japan.

In this fiscal year, we published four referred journal papers and one paper for a domestic conference, and won three awards (IIBMP 2015 Excellent Research Award, IIBMP2015 Best presentation award, AIST president award (research)).

別紙２

Results report

1. Title of Research and Development : Amazon fruits nano-supplements development: nutrigenomic and nutrigenetic effects on aging and health

2. Principal Investigator : Toshiro Aigaki (Professor, Department of Biological Sciences, Tokyo Metropolitan University)

3. Counterpart Principal investigator : Ivana Cruz (Professor, Health Science Center, Federal University of Santa Maria, (Brazil))

4. Results of Research and Development:

The goal of this project is to understand the functionality of the Amazonian Guaraná fruit (*Paullinia cupana*) at the molecular, cellular and organismal levels, and to develop nano-supplements that promotes health and longevity in humans. The Japanese team uses the fruit fly Drosophila as an experimental model system, whereas the Brazilian team works on humans and cultured cells. We exchange researchers and information between the two teams to promote the collaborative project efficiently. We made the following achievements this year.

I. Identification of genes affected by Guaraná fruit (GF) intake

We performed microarray experiments to investigate the effects of GF intake on gene expression in *Drosophila*. Flies were transferred from normal media to those containing GF powder at the concentration of 10mg/ml and kept for 24 hours. Control flies have been kept in normal media. Then mRNA was extracted and analyzed for gene expression with Agilent microarrays. We identified 195 and 203 genes whose expression levels were up- or down-regulated, respectively. Among the identified genes, four genes were selected and subjected to quantitative RT-PCR. The results were consistent with those of microarray.

II. Metabolomic changes induced by GF intake

Flies were treated with none (control), GF, caffeine, or catechin for 24hrs as described above, and extracted with 75% acetonitrile, and the soluble fractions were subjected to metabolomic analyse using an LC/MS system. Metabolomic profiles of GF treated flies were clearly different from those of control, indicating that GF has an impact on the metabolism of the animals. GF contains a high level of caffeine, and the metabolomics profiles were relatively close to that of GF-treated flies, suggesting some of the effects of GF may be caused by caffeine.

III. Tolerance to heavy metal (methyl mercury).

Flies were raised on medium containing methyl mercury with or without GF. No fly has developed from the medium with methyl mercury, whereas approximately 30 % flies hatched from the food containing both methyl mercury and GF. The data demonstrate that GF confers the tolerance to methyl mercury.

IV. Evaluation of nano-supplement containing GF

Nano particles containing GF (nano-supplement) was examined for their biological activity using *Drosophila*. Flies were kept on media with carrier only (control) or with GF nano-supplement, and measured their lifespan. There was no significant difference in adult life span between the two groups. Since the amount of GF in the nano-supplement was low, we need to develop nano-supplement containing a higher concentration of GF.

別紙２

Results report

１． Title of Research and Development： Cortically-triggered robotic hand orthosis for home-based therapy and assistance in activities of daily living

２． Principal Investigator：Jumpei Arata (Associate Professor in Department of Mechanical Engineering, Faculty of Engineering, Kyushu University)

３． Counterpart Principal investigator：Roger Gassert (Associate Professor in Department of Health Sciences and Technology, ETH Zurich (Switzerland)

４． Results of Research and Development:

This project aims to develop and evaluate a brain-triggered robotic hand orthosis to provide assistance and therapy in activities of daily living (ADL) for neurological patients and aging persons with severe hand impairment. For the first time, a wearable brain-robot interface (BRI) and an innovative, lightweight robotic hand orthosis will be combined to enable this novel mode of therapy and assistance in the clinic and at home.

In the current stage of the project, we have developed a preliminary BRI combined with the robotic hand orthosis and a commercialized NIRS device. The signal from NIRS device is currently processed by a simple method (Linear Discriminant Analysis) and will be further investigated in the next year. A method for adapting the robot on individual patients is under investigation, showing positive feasibility on a preliminary stage prototype that consists of a commercialized 3D scanner and a CAD algorithm that directly output the 3D model data to be printed by a 3D printer.

別紙２

Results report

１． Title of Research and Development：

Preemptive cancer treatments based on detection and elimination of precancerous cells using cell competition and supercompetition markers

２．Principal Investigator：**Yasuyuki Fujita** （Professor at Institute for Genetic Medicine, Hokkaido University）

３．Counterpart Principal investigator：**Eduardo Moreno** （Professor at Department of Institute of Cell Biology, Bern University）

４． Results of Research and Development:

The aim of this research project is to establish a novel and innovative cancer preventive medicine by studying cell competition between normal and transformed epithelial cells in a comprehensive manner through close collaborations between Japanese and Swiss groups. In this year, we have obtained the following outcomes.

1) By using various approaches, we have explored molecular mechanisms whereby cell competition between normal and transformed epithelial cells are regulated. Especially, by using a variety of biochemical cell fractionation methods and quantitative mass-spectrometry (SILAC), we have successfully identified multiple (potential) cell competition regulators.
In particular, we have found that mitochondrial activity is substantially decreased in RasV12-transformed cells when they are surrounded by normal epithelial cells. Increased expression of PDKs is responsible for the down-regulation of mitochondrial activity. Addition of DCA (Dichloroacetate), an inhibitor of PDKs, significantly suppresses the apical extrusion of RasV12-transformed cells, suggesting that PDK-mediated mitochondrial down-regulation plays a positive role in the elimination of transformed cells. Furthermore, expression of LDH is enhanced in RasV12-transformed cells surrounded by normal cells, and suppression of LDH activity leads to formation of basal protrusions. These data suggest that the Warburg effect-like phenotype can occur at the initial stage of carcinogenesis, which plays a tumor-suppressive role by promoting elimination of transformed cells from epithelial tissues. By further developing this new research field, we would create a novel type of cancer treatment: eradication of transformed cells by enhancing a defensive force of neighbouring normal epithelial cells.

2) In June, 2015, one PhD student in Fujita lab stayed at Dr. Moreno's lab for one month and acquired the technique of *Drosophila* genetics. In addition, Prof. Fujita (PI in the Japanese side) also visited Dr. Moreno's lab, and intensive discussion was held between the two groups as to the mutual research progress and upcoming collaborations. Thus, since the start of this collaborative project, close interactions and mutual exchange of young scientists have been promoted, which profoundly help them equip international sense.

別紙２

Results report

１． Title of Research and Development： Impedance regulation during energy transfer motor tasks: from human experiments to computational modeling and robotics

２． Principal Investigator : Dr. Ganesh Gowrishankar (Researcher, Center for Information and Neural Networks (CiNet); National Institute of Information and Communications Technology)

３． Counterpart Principal investigator : Dr. Patrick van der Smagt (Professor, Biomimetic Robotics and Machine learning, Technical university of Munich (Germany))

４． Results of Research and Development:

We planned and achieved the aims of the project over a series of steps-

1) We developed a behavioral experiment using the NICT TVINS manipulandum to examine the behavior of humans during impact tasks.
2) We developed a new analytical method to measure human arm impedance (both stiffness and damping) during impacts.
3) We examined the human behaviors in these tasks and found that humans can utilize haptic feedback during impacts to change their impedance and optimize the energy transfer during impacts. **This result was presented in the NCM conference.**
4) We analyzed the human behaviors computationally, and determined how the haptic feedback during impacts is used to optimize subsequent impacts. **This result will be submitted as a full journal paper.**
5) Using these results we developed a robot algorithm that enables robots to perform impacts like humans. This robot algorithm was implemented in Germany this year. **The paper will be submitted in the next months.**
6) In addition to the initial project goals, we also examined an additional issue of tool embodiment during fast movements. Embodiment issues are closely linked to control, and in this experiment, again with the NICT TVINS manipulandum we isolated a new embodiment process. **The results were published in Nature Communications in 2014.**
7) Finally we also examined learning in virtual impact (penalty kick) task to isolate for the first time, interactions between choice learning and motor learning by humans. **This result was presented in the NCM conference and has been submitted as a full journal paper.**
8) On the German side, the collaborators worked on algorithms to estimate human arm impedance from recordings of electromyography (EMG). **These were presented in the NCM conference**
9) The German side developed a new fast manipulandum specialized for human impedance measurements.

別紙２

Results report

１． Title of Research and Development: Bio-functionalization of Ti-based materials for osseointegrated implants

２. Principal Investigator: Takao Hanawa (Professor, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University)

３． Counterpart Principal investigator: Luís Augusto Sousa Marques da Rocha (Universidade Estadual Paulista, Faculdade de Ciências de Bauru Department and job title at the affiliated institution (Brazil))

４． Results of Research and Development:

The main aim of this project is to develop a new generation of bio-multifunctional Ti-based surfaces for osseointegrated implants. In particular the goal is to play with different surface modification techniques in order to obtain surfaces capable of being biologically selective (promoting, simultaneously, an adequate adhesion of osteoblastic cells while minimizing the possibilities of colonization by unwanted microorganisms), together with high corrosion and tribocorrosion resistance. Special focus will be given towards the understanding of the underlying mechanisms governing each phenomenon. This project aims at interrelating three complementary research activities, namely: (1) electrochemical surface treatments and sputtering techniques to obtain surfaces with different micro and nano-topographies, structures and chemistry (doping with bioactive and antimicrobial species), (2) in particular, micro-nano-porous and nano-tubular surfaces will be produced. Further, immobilization of organic PEG, RGD and/or collagen molecules will be carried out by electrodeposition techniques, (3) investigation of the corrosion and tribocorrosion mechanisms of the surfaces through the integration of electrochemical corrosion techniques in tribological tests, and (4) investigation of the mechanisms governing the adhesion of osteoblastic cells and microorganisms with the different surfaces. Surface parameters governing the competition between osteoblastic cells and microorganisms to adhere to the surface will be studied in detail.

The multi-disciplinary team integrating this proposal bridges Materials Engineering, Biomedical Science and Biology. It is expected that this team will be able of progressing in the scientific understanding of the fundamental mechanisms governing osseointegration, microbial colonization and tribocorrosion behavior. Hereto an integrated approach will be followed addressing the interactions between these mechanisms. By doing so, guidelines will become available for the future production of improved implant multifunctional surfaces. Also, the involvement of young MSc and PhD students that will benefit from the networking and complementary expertise of the Brazilian and Japanese teams will bring an added value for their carrier progression. Finally, collaborative high quality publications will be expected throughout the project.

The purpose of the project was to create a next generation multi-functional titanium surface with osseointegration. In particular, surface modification techniques strongly adhering osteoblast and simultaneously preventing bacterial adhesion has been focused. In this budget year, an acquirement of antibacterial property due to the addition of oxide layer containing Ag, an animal evaluation of micro-arc oxidation (MAO), characterization of structure of a Ti-Zr-Mo alloy, investigation of the possibility of MAO treatment of the alloy, immobilization of a new functional molecule to Ti surface were attempted.

In Ag-containing technique and evaluation of antibacterial property, the relation between the Ag concentration in the oxide layer and antibacterial property was elucidated. In addition, both bone formation and antibacterial properties that are opposite properties were appeared. MAO treatment was effective according to the results of animal test. Furthermore, polarization treatment of MAO oxide layer accelerated bone formation on the oxide. On the other hand, β-type Ti-15Zr-7.5Mo alloy was melted and formed, followed by the evaluation of mechanical property. Also, utility of MAO treatment against Ti-15Zr-7.5Mo alloy was investigated. The Ti-15Zr-7.5Mo alloy consisted of β phase and α" phase and ω phase. Vickers hardness of the alloy was larger than that of Ti-6Al-4V alloy and Young's modulus of the alloy was as the same as that of Ti-6Al-4V alloy. MAO treatment was also effective to Ti-15Zr-7.5Mo alloy because porous oxide layer was formed on the Ti-15Zr-7.5Mo alloy. Therefore, the Ti-15Zr-7.5Mo alloy has good mechanical property as a medical material. It is possible to obtain both good mechanical property and hard tissue compatibility in the Ti-15Zr-7.5Mo alloy because porous oxide layer was easily formed on the alloy by MAO treatment. In addition, immobilization of MPC polymer on Ti was performed and the effect of the immobilization was appeared.

別紙２

Results report

１．Title of Research and Development : Pathogenic mechanism underlying neurodevelopmental disorder in schizophrenia

２．Principal Investigator：Kozo Kaibuchi（Department of Cell Pharmacology, Nagoya University, Professor）

３．Counterpart Principal investigator : Orly Reiner （Department of Molecular Genetics, Weizmann Institute of Science, Professor （Israel）

４．Results of Research and Development:

Schizophrenia is a chronic brain disease, which imposes one of the greatest burdens on patients, their relatives and public health care. However, the molecular mechanisms underlying the pathophysiology of this disease are poorly defined and the diagnosis is still done by clinical interviews without the assistance of objective biological tests. Nevertheless, neurodevelopmental abnormalities have been considered as part of the pathophysiology of schizophrenia. The project combines an interdisciplinary approach of scientists from Japan and Israel, where the Japan-research group (JPN group) has identified rare missense single nucleotide variants (namely *NDE1*-S214F) in *NDE1* in patients as well as rare exonic duplications in *NDE1* and the small GTPase regulators such as ARHGAP26 and RAPGEF1. The JPN group generated the mutated constructs, expressed them in primary hippocampal neurons and analyzed differential protein complexes from different subcellular fractions by Mass spectrometry. The overexpression and knockdown studies revealed that RAPGEF1 was involved in the dendritogenesis of immature neurons. The expression of constitutive active Rap1 altered the dendritic complexicity. These findings suggest that Rap1 signaling is implicated in the pathology of schizophrenia via neurodevelopemnt. The JPN group found that expression of *NDE1*-S214F allele inhibited axonal outgrowth of hippocampal neurons (Kimura et al, Schizophrenia Bull, 41(3), 2015). To clarify the molecular pathology caused by *NDE1*-S214F allele, the JPN group tried to investigate NDE1-interactome using a NDE1-affinity column chromatography and identified more than 100 of NDE1-interactors. Furthermore, the pulldown samples from the immobilized with wildtype NDE1 or NDE1-S214F protein were subjected to the quantitative mass spectrometry. We found that more than 10 molecules were affected in the binding to NDE1 by the NDE-1S214F mutation.

In this joint project, the Israel-research group (ISR group) employed in the *in utero* electroporation and the genome-editing techniques for studying neuronal proliferation, migration and connectivity. To evaluate the pathophysiological meaning of *NDE1*-S214F expression in the developing brain, ISR group introduced the NDE1-S214F construct by in utero electroporation. The neurons expressing NDE1-S214F were impaired in cortical migration compared to the neurons expressing wildtype NDE1. This result suggests that the *NDE1*-S214F allele is involved in the cortical development. Recently, ISR group generated a knock-in mouse carrying *NDE1*-S214F allele by a genome-editing technique. When ISR group performed the immunoblotings of NDE1 using the brain lysates of wildtype or the mutant mice, there is little difference in the expression levels of NDE1 from both mice. In this collaborative project, we indicated that Rap1 signaling and the NDE1-interactome are implicated in the pathophysiology of schizophrenia.

別紙２

Results report

１．Title of Research and Development: Teleassistance for Seniors with Dementia – A Novel Concept for Safety

２．Principal Investigator: Hirokazu Kato (Professor at Graduate School of Information Science, Nara Institute of Science and Technology)

３．Counterpart Principal investigator: Petri Pulli (Professor at Department of Information Processing Science, Faculty of Information Technology and Electrical Engineering, University of Oulu (Finland))

４．Results of Research and Development:

We designed a tele-assistive system for the elderly with mild dementia so that they can walk outdoor safely by support of remote caregivers through the Internet. In order to realize the system, we focused on three elements as research topics; 1) development of a navigation system for elderly walking, 2) development of a health management system based on biomedical signals, and 3) development of a remote assistive system. These developments have been done through user studies.

1) Development of a navigation system for elderly walking

- Navigation display system with LASER light onto real surfaces

  We have implemented visual information display systems with LASER light onto surfaces by using walking aids or pendant typed wearable devices (Fig. A1, A2). We confirmed that it is not practical under the strong sunlight and it has a possibility to be dangerous due to the elderly behavior with LASER. As a result, we moved on the different method that is the development of smart MEGANE (“Megane” is the Japanese name of glasses).

- Smart Megane with LED lights for navigating the elderly

  We put LEDs on edges of glasses and designed the lighting patterns (Fig. A3). In addition, we re-implement the glasses to reduce the weight (Fig. A4). Through user studies with young and elderly participants, we confirmed that it has possibility to be a navigation system for walking out although there is a necessity of redesigning some lighting patterns. Our system could show that it is one of the methods to support the elderly to walk out safely.

2) Development of a health management system based on biomedical signals

- Sensor vest: wearable biomedical signal sensor

  It is easy to recording electrocardiograms with sensor vest, while conventional electrocardiographs require to attach several electrodes onto the subject bodies. We investigated the qualities of sensing signals from sensor vests, and validated the quality was enough good in standing and lying position (Fig. B). Also, we confirmed that the quality of signal will be declined in walking.

3) Development of a remote assistive system

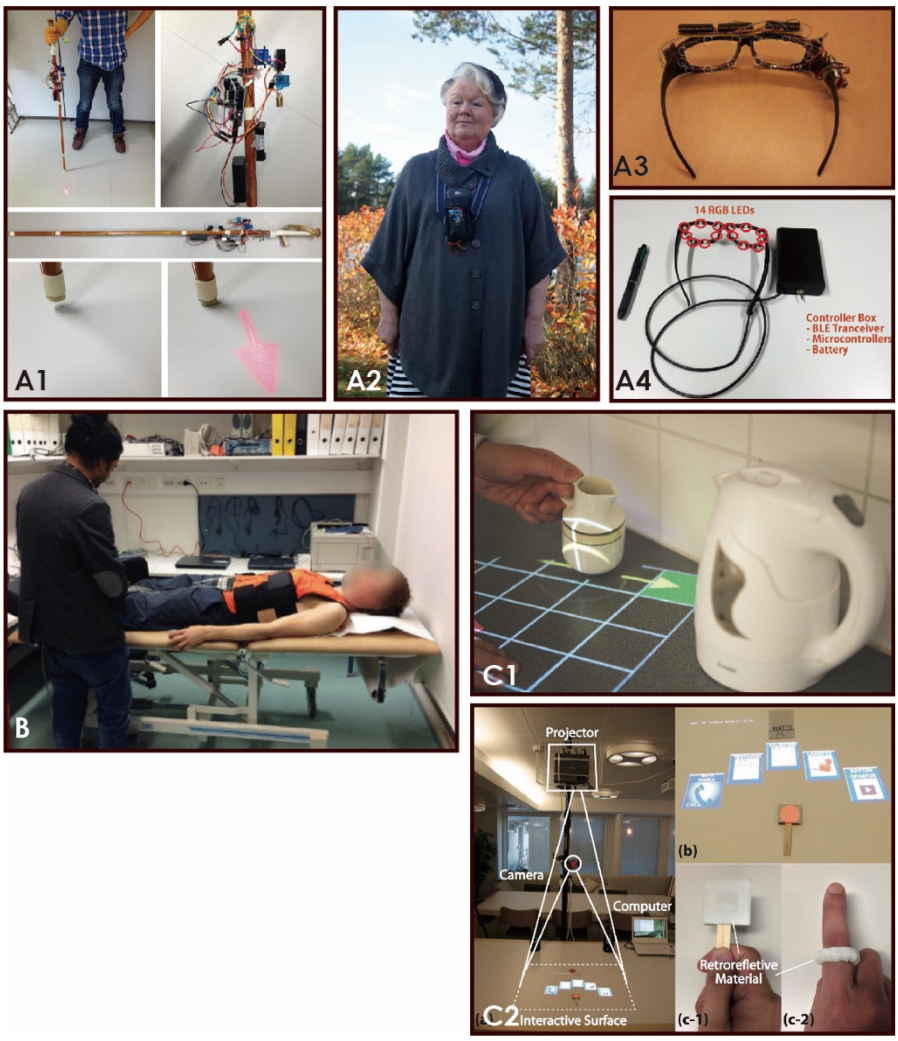
- Projection visual information displays

  We can show visual information onto active spaces of the elderly by using a projector. In this view point, we have developed projection systems for kitchen activity as one of the elderly working location. We have implemented a projection system that displays assistive information from the remote caregivers, and a projection system that provides input interfaces for the elderly to use (Fig. C1, C2).

- Promoting communication with the remote assistance

  We focused on increasing communication which might have possibility to reduce the symptoms of dementia. As our target situation, there is a main task and we were trying to make the elderly speak out by giving

additional information. Then, we tried to find a suitable trigger as additional information, words, through user studies. We implemented an experimental system with several types of words, then conducted user studies with young and elderly participants. As a result, we confirmed that the participants tend to speak out more when showing question form than memorial information.



Our system. A1: Cane-type navigation system, A2: Pendant-type navigation system, A3: Smart Megane (first prototype), A4: Smart Megane (light model), B: Sensor vest, C1: Projection remote assistive system, C2: Projection visual input interface.

In addition to the research and development such as above, research exchange was located important activity in this program. Therefore, we have exchanged some students and researchers every year positively. Also, we collaborate with each researcher and publish/make presentations at some international conferences. We believe we achieved the purpose for research exchange enough. In addition, we could expand our network to connect to other researchers through international conferences.

別紙２

Results report

１． Title of Research and Development：Research and Development of Dependable Wireless Medical Network Using Highly Reliable Body Area Network

２． Principal Investigator：Ryuji Kohno(Professor, Division of Physics, Electrical and Computer Engineering, Graduate School of Engineering, Yokohama National University）

３． Counterpart Principal investigator：Jari Iinatti（Professor, Faculty of Information Technology and Electrical Engineering, University of Oulu (Finland)）

４． Results of Research and Development:

This joint project between Yokohama National University and University of Oulu has promoted various academic and industrial collaboration in interdisciplinary field between medicine and information communication technology (ICT), so-called “Medical ICT.” Major theoretical results are (1) joint optimization of wireless medical network technologies in physical, MAC and network layers such as hybrid ARQ corresponding various QoS requirement of medical data, dependable MAC protocol and (2) dependable wireless remote feedback loop control for medical vital (glucose) sensors and actuators(insulin pump). Major industrial contribution are (3) promotion of amendment of IEEE and ETSI international standards for wireless medical Body Area Netwlork(BAN), and (4) implementation of prototypes of dependable wireless BAN. This project held 2015 and 2016 international symposia on medical ICT in Kamakura, Japan and Boston, USA.

別紙２

Results report

１．Title of Research and Development： THE ROLE OF HIV IN PRE-ECLAMPSIA

２．Principal Investigator：Tadashi Konoshita（University of Fukui Faculty of Medical Sciences, Clinical Professor）

３． Counterpart Principal investigator：Jagidesa Moodley（University of KwaZulu-Natal, Professor（Republic of South Africa））

４．Results of Research and Development:

This collaborative research aimed at evaluation of the effects of HIV infection and genetic variants in system such as the renin–angiotensin system (a very important endocrine system that regulates blood pressure) on pre-eclampsia, a specific model for hypertension which often causes serious symptoms such as convulsion and loss of consciousness and is prevalent in South Africa. Specifically, the Japanese researchers were in charge of provision of techniques and knowledge for assays of genetic variants, and the South African researchers were in charge of provision of subjects suffering from pre-eclampsia and clinical evaluation. By working together, this project contributed to development of a new interventional treatment for pre-eclampsia.

At the beginning 2 years, DNA samples were recruited in the Republic of South Africa. Considering the statistical significance level and power, about 600 blood samples were collected and DNAs were extracted. A South Africa side researcher was invited to Japan side laboratory, and he analyzed 7 renin-angiotensin system genetic variants (renin, angiotensin, ACE, AT1, AT2, CYP11B2 and ENPEP). Without any main trouble, he persuade his task. At the crude analysis step, we obtained some statistical significance results between 2 of the genes and pre-eclampsia. It is for the first time that the 7 genes of the RAS were examined at the same sample series.

On the other hand, we tried to establish a measurement system for one of new component of the RAS. Especially, by monoclonal antibody, a high sensitivity system was aimed. We made hybridomas and screened the activity for the protein. We selected several clones and tried several combinations for sandwich ELISA assay system. Finally we selected one combination of the antibodies. We tested the reproductivity of the assay and optimized the condition. Now we have almost established the new assay system for a new component of the RAS.

We are preparing to present the obtained results at some international congresses and to publish them in some international journals.

別紙２

Results report

１． Title of Research and Development： RNA-based novel approaches for discovery of ALS biomarker

２． Principal Investigator : Shin Kwak（Division of Clinical Biotechnology, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, visiting scientist）

３． Counterpart Principal investigator : Erez Levanon (Faculty of Life Sciences , Bar-llan University, principal investigator (Israel))

４． Results of Research and Development:

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease affecting middle-aged individuals in their motor functions and lack of effective therapy leads them to eventual death by respiratory muscle weakness within a few years of onset. More than 90% of ALS occurs in a sporadic fashion, and there are no reliable biomarkers for the disease. Recently it has been demonstrated that deficient adenosine to inosine (A-I) conversion (RNA editing) of pre-mRNA resulting from down-regulation of RNA editing enzyme called adenosine deaminase acting on RNA 2 (ADAR2) occurs in the motor neurons in the majority of patients with ALS in a disease-specific manner. Notably, this molecular abnormality is a cause of both death of motor neurons and TDP-43 pathology, the most reliable neuropathological hallmark of ALS, in the mouse model （ADAR2$^{flox/flox}$/VChAT-Cre, or AR2 mice）mimicking these ALS-specific molecular abnormalities. These lines of evidence indicate that ADAR2 down-regulation is involved in the pathogenesis of sporadic ALS.

Because demonstration of ADAR2 down-regulation in peripherally accessible organs would be a pathomechanism-associated ALS biomarker, we aim to search for a biomarker that reflects down-regulation of ADAR2 in the patients' body fluids. RNAs and proteins are secreted as such or packed in exosomes from individual cells into the body fluids, including blood and cerebro-spinal fluid (CSF) and RNAs in exosomes and circlar RNAs (circRNA) in body fluids are known to be very stable. A-I conversion occurs actively at numerous positions in the mammalian central nervous system and ADAR2 is the major executive enzyme. Down-regulation of ADAR2 in the ALS motor neurons results in the failure of RNA editing at numerous ADAR2-mediated positions in RNAs, including both coding and non-coding regions. If we could detect some of these RNAs with unedited at ADAR2 positions in the body fluids, they likely become biomarkers for sporadic ALS because of the selectivity of ADAR2 down-regulation in sporadic ALS.

Conditional ADAR2 knockout mice（AR2 mice）mimic the molecular abnormalities seen in the motor neurons of ALS patients and exhibit ALS phenotype. ADAR2-lacking motor neurons in the AR2 mice express RNAs devoid of RNA editing at the ADAR2-mediated positions. We therefore started by isolating single motor neurons from AR2 mice and wild-type mice with a laser microdisecter to extract motor neuron-specific RNAs. We dissected more than 2,000 motor neurons from an individual mouse (n=3 for each group). We extracted RNAs from the single motor neuron tissues using the SMARTer Stranded RNA-Seq Kit（Clontech Laboratories, Inc）to prepare samples for RNA-seq.

After removing all variants present in catalogues of DNA differences in SNP databases, those caused by sequence mapping artifacts such as read alignment across splice junctions, gene duplications, and homopolymer stretches and rare SNPs, research collaborators in Israel scrutinized the RNA-seq data on A-I positions. We then searched

for ADAR2 positions among these A-I positions and selected candidate RNAs by tentative criteria that the proportion of edited RNA was 1) more than 10% of the transcript in the wild-type mice and 2) significantly lower in the AR2 mice than in the wild-type mice.

We then searched for the human homolog of these candidate mouse RNAs and tested whether human homolog of RNAs have ADAR2 positions in the human-derived cultured cells by siRNA knockdown and overexpression of ADAR2. We are now examining the presence of these candidate RNAs in human body fluids.

別紙２

Results report

１．Title of Research and Development： Neural circuit mechanisms of reinforcement learning

２．Principal Investigator：Kenji Morita （Graduate School of Education, The University of Tokyo, Lecturer）

３．Counterpart Principal investigator：Abigail Morrison （Jülich Research Centre, Professor（Germany））

４．Results of Research and Development:

(1) Models of corticostriatal CCS and CPn cells

Estimations of the parameters of the computational models of crossed-corticostriatal (CCS) and corticopontine (CPn) cells (on the NEURON simulation environment) based on the detailed anatomical and physiological data (obtained by Dr. Morishima and Dr. Kawaguchi) have been done by Dr. Kondo, and parameter sets that could reproduce the experimental results have been obtained. Then, using the models with the obtained parameter sets (two for each cell type), simulations of the cases where the cells receive synaptic inputs that oscillate as a population with various frequencies have been done by Dr. Kondo, and conditions for the generation of "doublets" (two action potentials with short intervals) have been clarified.

(2) Model of cortico-basal ganglia circuit

Simulations of the cases where the direct or indirect pathway of the basal ganglia is blocked in the cortico-basal ganglia circuit have been elaborated, and it has been shown that the hypothesis that these two pathways represent reward predictions with time difference (Morita et al., 2012, Trends Neurosci) could account for the experimental results that the blockade of the direct or indirect pathway respectively impaired learning based on positive values (or prediction errors) and the initial phase of reversal learning, which is considered to be based on negative prediction errors. The manners the blockade of each basal ganglia pathway relates to addiction have also been examined through simulations, and it has been shown that these manners can significantly depend on the degree of exploration (over exploitation) upon choice.

(3) Model of the temporal changes of dopamine concentration

Model of the cortico-basal ganglia circuit implementing reinforcement learning with the assumption that dopamine represents reward prediction error has been elaborated by incorporating the assumption that plastic changes of the strengths of cortico-striatal synapses decay in time (by Kato and Morita). It has been shown that various temporal patterns of dopamine signals can be reproduced by the model. Also, based on simulations and analyses of the model, we have proposed a circuit/synapse-level mechanism of the ever-suggested link between dopamine and motivation (paper under review).

(4) Explorations of integrative mechanisms

Reviews of the studies that have been conducted by the Japanese and German groups, including the abovementioned studies, and also related studies done by other researchers on the neural circuit mechanisms of reinforcement learning, have been done by Morita and the member of the German group (Dr. Morrison and Dr. Jitsev) with a special focus on value-based action selection (decision making), and have been published as a joint review article. In the article, we have proposed hypotheses on how reinforcement learning algorithms (Q-learning and SARSA) can be implemented in the neural circuits, as well as on potential computational roles of the complex patterns of cortical and striatal neural activity suggested in recent experimental and theoretical studies.

別紙２

Results report

１． Title of Research and Development：Protective and subversive mechanisms of macrophage genes in Mycobacterium tuberculosis infection

２． Principal Investigator：Harukazu Suzuki (Center for Life Science Technologies, Group Director)

３．Counterpart Principal investigator : Frank Brombacher（University of Cape Town, Professor （South Africa））

４． Results of Research and Development:

This research aims at identification and analyses of macrophage genes that work protective or subversive in Mycobacterium tuberculosis (Mtb) infection. For this purpose, we compared gene expression dynamics of macrophage in Mtb infection with that of cytokine-induced macrophage activations. The main experimental task of both parties were selection of candidate genes using already obtained high quality transcriptome data (Japan), Creation of the constructs for the perturbation experiments (Japan), gene expression analysis of the perturbation samples (Japan), Mtb infection experiments using macrophages and experimental mice (South Africa) and preparation of samples for the gene expression analysis (South Africa). Because we have already had a great progress in our analysis, we focused on writing papers as much as possible in the last fiscal year, with minimum additional wet experiments.

In the stimulation by pathogen infection including Mtb, the activated macrophage cells, as well as classically activated one, express various host protective factor genes involved in inflammation, such as *Tnf*, etc. We successfully identified that Batf2 is a novel transcription factor (TF) involved in gene expression of those factors. In addition, we found that Batf2 associates with Irf1, another TF induced by IFNg, and induces inflammatory genes, which was published in *J. Immunology*. Further, including the above results, we have published a review article in *Oncotarget*. Furthermore, in vivo functional analysis of Batf2 is going on using the Batf2 KO mice. We have already obtained interesting results for the Mtb infection response in the KO mice and aim at publication in earlier timing.

We also analyzed transcriptional regulation dynamics of classical and alternative activations in macrophage cells. Surprisingly, the analysis revealed that almost same set of transcription factor binding motifs are involved in both activations, although those two activations shows quite different features. The results suggest that same TFs are involved in the different activations. Further, using the comprehensive promoter-level expression profiles, we successfully identified novel TF genes, peripheral genes and lncRNAs that are considered to play important role in those activations, which was published in *Nucleic Acids Research*. In addition, we explored effect of the alternative activation to Mtb infection by using the IL4 receptor KO mice that does not occur alternative activation. We found that alternative activation in macrophage cells has almost no effect in Mtb infection, which was published in *PLosONE*.

In addition to the above achievement, we are analyzing large scale time course expression data in Mtb-infected macrophages, which was obtained by the deepCAGE method. Because we have almost completed the analysis, we aim at the paper publication in earlier timing. Further, we found that Batf, a family TF of Batf2, is also transiently induced in pathogen (Mtb)-infected macrophages. We are now analyzing the function of Batf in Mtb infection. Finally, although we had a plan to have a workshop with the collaborator in the last half of the fiscal year, it was not held. It is because we decided in our discussion that we took paper publication work as our first priority.

別紙２

Results report

１．Title of Research and Development：Development of A Comprehensive System for Assessment of Sarcopenia, Osteoporosis and Joint Dysfunction

２．Principal Investigator：Masaki Takao（Associate Professor, Department of Orthopaedic Medical Engineering, Osaka University Graduate School of Medicine, Japan）

３．Counterpart Principal investigator： Guoyan Zheng（PrivatDozent, Institute for Surgical Technology and Biomechanics ,University of Bern（Switzerland））

４．Results of Research and Development:

This project aims to develop a comprehensive system to assess musculoskeletal conditions of high-risk elderly patients of mobility loss, fall and hip fracture by considering osteoporosis, sarcopenia and joint dysfunction simultaneously. Patients with hip arthritis or with femoral neck fracture have been recruited by the Japanese team. 2D X-ray images and 3D QCT of patients' hips have been acquired. The Japanese team managed to develop automatic system to extract 3D musculoskeletal models from the 3D data. The Swiss team has been developing a system for biomechanical hip modeling and for reconstructing 3D musculoskeletal models from 2D X-ray images.

In this fiscal year, Japanese team managed to develop statistical model of insertion of muscle fibers on the pelvic and femoral bones. The team consisted of a medical team from Osaka University and an engineering team from Nara Institute of Science and Technology. Last fiscal year, we had performed a preliminary cadaver study in Singapore to get the three dimensional data of muscle insertion using our original computer navigation system. We improved the computer navigation system and revised the experiment protocol. In January, 2016, we performed another cadaver study in Johns Hopkins University using 8 cadavers. We developed statistical atlas of muscle insertion area on the pelvic and femoral bones. We could not perform prospective clinical study to predict falls from 3D musculoskeletal model due to delay in developing automatic segmentation system of musculoskeletal models from 3D CT data. On the other hand, we performed a retrospective study to develop a large-scale database of 1142 patients with hip disorders including radiographs, 3D-CT data and operation data acquired using navigation system. The NAIST engineering team developed automatic matching system between 2D radiographs and 3D CT data of pelvic and femoral bones. Using the software, Osaka University medical team analyzed pelvic flexion in supine and standing positions in 474 patients.

Swiss engineering team of Bern University has succeeded in reconstruction of 3D pelvic model from 2D X-ray images using dataset from our database. By applying automatic planning system of total hip arthroplasty to their system, they succeeded in automatic cup implant planning based on 2D/3D radiographic pelvis reconstruction (Schumann S, 2015). In addition, they reported that they succeeded in reconstruction of muscle model of rectus femoris from 2D-Xray images.

On August 29th in 2015, we held mini symposium entitled "International Symposium on Musculoskeletal Simulation – from Big Data to Robot-assisted Rehabilitation" in Osaka University Nakanoshima center. We reported our achievements each other and discussed the future direction of combined research. This symposium was open and the number of participants was 32.

Reference: Schumann S, Sato Y, Nakanishi Y, Yokota F, Takao M, Sugano N, Zheng G. Cup Implant Planning Based on 2-D/3-D Radiographic Pelvis Reconstruction-First Clinical Results. IEEE Trans Biomed Eng. 2015 Nov;62(11):2665-73.

別紙２

Results report

１． Title of Research and Development : Genetic and pathogenic diversity of *Pantoea ananatis* strains

２． Principal Investigator : Yuichi Takikawa （Professor, Graduate School of Science and Technology, Shizuoka University）

３． Counterpart Principal investigator : Teresa A. Coutinho （Professor, Microbiology and Plant Pathology, Natural and Agricultural Sciences, University of Pretoria（South Africa））

４． Results of Research and Development:

This research aimed to clarify the genetic and pathogenic diversity of *Pantoea ananatis* and to develop a method identifying the pathogenic strain infecting important crops such as rice, corn and onions, in collaborative work between Japanese and South African researchers.

We had revealed the presence of a new pathogenicity determining genetic locus in *P. ananatis*, which was named as PASVIL (*P. ananatis* specific virulence locus). RT-PCR techniques were applied to determine the operons in PASVIL. More than 3 operons having 16 ORFs could be identified and the putative promoter regions were suggested. Introduction of the entire PASVIL region to a non-pathogenic *P. ananatis* strain lead to acquisition of pathogenicity on tobacco and onions. Using this PASVIL introduction system together with in vitro transposon insertion technique revealed that not the all ORFs in PASVIL were required for pathogenicity but some were dispensable. Construction of a plasmid vector for site-directed mutagenesis enabled us to disrupt any genes and to assay their contributions to pathogenicity. Some PCR primer sets could detect PASVIL and its homologue in *P. ananatis* and *P. agglomerans* strains. These primers were considered as very useful tools for rapid detection of pathogenic strains in *Pantoea* without time-consuming bioassay methods. A PASVIL homologue found in *P. agglomerans* has ca. 80% sequence homology with PASVIL of *P. ananatis*, suggesting that considerable time has passed since the horizontal transfer of PASVIL took place between theses two species.

The Type VI protein secretion system gene sets had been also identified in the genome of P. ananatis strains. The significance of the Type VI secretion systems in plant pathogenesis was assayed by disrupting the genes using site-directed mutagenesis. Our study revealed that the disruption of Type VI secretion system of *P. ananatis* strain isolated from rice in Japan did not abolish its pathogenicity, but significantly reduced its ability to compete with other bacteria when they were co-cultivated. In contrast, the Type VI secretion system of P. ananatis strain isolated from onion in South Africa was shown to be indispensable for pathogenicity. These results indicated that the significance of the Type VI secretion system depends on bacterial strains used and that the system might not serve as an index of plant pathogenicity.

In tobacco plants, expression of some resistance-related genes of plant was assayed after injection of the pathogenic strain of *P. ananatis*. RT-PCR analysis demonstrated that the kind of genes induced was similar to that induced in hypersensitive reaction (HR), but the induction was delayed by ca. 12 hours compared to HR. At the same time, PASVIL of *P. ananatis* was shown to be strongly induced when the bacterium was introduced into tobacco tissues.

Phylogenetic study on *Pantoea* spp. revealed their core genome and specification of each species. Even in *P. ananatis*, there is significant diversity among the strains and the pathogenic strains were shown to be scattered in diverse groups.

In conclusion, we revealed the pathogenicity determinants and diversity of *P. ananatis*, and developed a method to discriminate its pathogenic population.

別紙２

Results report

１． Title of Research and Development： Mechanism of circadian clock based on clock genes

２． Principal Investigator：Name　Toru Takumi（Senior Team Leader, RIKEN Brain Science Institute）

３． Counterpart Principal investigator：Name　Grigory Bordyugov（Research Scientist, Institute for Theoretical Biology, Charite（Germanry））

４． Results of Research and Development:

The physiological and behavioral rhythms of all life on earth are bound to the earth's rotational cycle of ~24 h. This fundamental rhythm is also affected by the planet's slanted rotational axis, which causes seasonal variations in the length of the day. How life has adapted to anticipate this yearly rhythm is still debated. The mammalian suprachiasmatic nucleus (SCN) forms not only the master circadian clock, but also a seasonal clock. This neural network of ~10,000 circadian oscillators encodes season-dependent daylength changes through a largely unknown mechanism. We show that region-intrinsic changes in the SCN fine-tune the degree of network synchrony, and reorganize the phase relationship among circadian oscillators to represent daylength. We measure oscillations of the clock gene *Bmal1*, at single-cell and regional levels, in cultured SCN explanted from animals raised under short or long days. Coupling estimation using the Kuramoto framework reveals that the network has couplings that can be both phase-attractive (synchronizing) and repulsive (desynchronizing). The phase gap between the dorsal and ventral regions increases, and the overall period of the SCN shortens with longer daylength. We find that one of the underlying physiological mechanisms is the modulation of the intracellular chloride concentration, which can adjust the strength and polarity of the ionotropic γ-aminobutyric acid receptor ($GABA_A$) mediated synaptic input. We show that increasing daylength changes the pattern of chloride transporter expression yielding more excitatory GABA synaptic input, and that blocking $GABA_A$ signaling or the chloride transporter disrupts the unique phase and period organization induced by the daylength. We test the consequences of this tunable GABA coupling in the context of excitation-inhibition balance through detailed realistic modeling. These results indicate that the network encoding of seasonal time is controlled by modulation of intracellular chloride, which determines the phase relationship among and period difference between the dorsal and ventral SCN.

別紙２

Results report

１．Title of Research and Development：Evaluation of different extracts and isolated compounds from Brazilian Propolis in obesity and diabetes via in vitro and in vivo assay.

２．Principal Investigator：Je Tae Woo　（Department Biological Chemistry, Chubu University; Professor）

３．Counterpart Principal investigator：Jairo Kenupp Bastos（Pharmaceutical Sciences, University of São Paulo; Professor（Brasil））

４．Results of Research and Development:

Propolis is widely used as a functional food and its various physiological activities are also being accumulated for scientific evidence. We have reported that one of the cinnamic acid derivatives, contained in the characteristic of Brazilian green propolis, improves insulin resistance in the cellular level and in the animal level. However, there are still questions about the detailed mechanisms of its action. In addition, it is desired to improve bioavailability of propolis products for the practical use.

The main objective of our research was to develop a new functional food that prevents and/or improves obesity and diabetes. Lipid nanoparticles of Brazilian green propolis formulations were made for improving in vivo absorption and then the stability and sustained release were evaluated.

Secondly, as our second objective, in order to obtain scientific evidence of the Brazilian green propolis and develop it as a high value-added product, we screened new functions of the ingredients of Brazilian green propolis in cell culture systems. As a result, we found a novel effect of cinnamic acid derivative compounds from Brazilian green propolis on adipocyte and pancreatic β cells, which might reveal a part of the mechanism of antidiabetic action.

This Japan-Brazil joint project promoted propolis research for scientific evidence and contributed in training for international young researchers by collaborating with foreign scientists.

別紙２

Results report

１．Title of Research and Development : Late Life Depression: Molecular Basis and Neural Networks

２． Principal Investigator：Shigeto Yamawaki（Professor and Chairman, Department of Psychiatry and Neurosciences, Hiroshima University）

３．Counterpart Principal Investigator：Bernard Lerer（Professor of Psychiatry, Director Biological Psychiatry Laboratory, Hadassah- Hebrew University Medical Center（Israel））

４．Results of Research and Development

The principal objective of this project is to gain an understanding of the neurobiological basis of late life depression (LLD), a serious and highly prevalent age dependent psychiatric disorder. Individuals with LLD experience greater functional disability and cognitive decline than similarly aged people without depression and greater morbidity and mortality from medical illness. When compared with early-onset MDD, LLD is associated with a higher prevalence of dementia. Because of increasing lifespan in Japan and Israel the social and economic cost of LLD is extremely high and rising. We hypothesized that an interaction between exposure to chronic stress, brain white matter damage and age related changes underlies the development of LLD. In the current project we are testing our hypotheses by a systematic series of experiments in mouse models and by translational studies in humans. The Japanese group is assessing brain structure and function in individuals with major depression and healthy controls using MRI in conjunction with online-behavioral tasks and measures of chronic stress. We are also evaluating biomarker profiles in human blood samples from these subjects. Thus far we have examined resting-state functional magnetic resonance imaging (fMRI) activity in hippocampus and serum concentrations of BDNF in 12 patients with MDD and 14 healthy controls (HCs). To model effects of age, the participants were classified as elderly (age $\geq$50) including 6 MDD and 7 HCs and younger age group including 6 MDD and 7 HCs. Preliminary analysis revealed that patients with depression exhibited significant decreases of low frequency fluctuation in the hippocampus bilaterally. Among the patients with MDD, the serum concentration of BDNF was positively correlated with low frequency fluctuation in the right hippocampus suggesting a protective effect of BDNF on the hippocampal dysfunction. Further data collection and analyses are necessary to examine the age related changes. The Israeli group is employing mouse models that explore the interaction between age and exposure to chronic stress. To model effects of age they are studying young vs. old male and female mice. In the experiment completed thus far young and old female mice were exposed to chronic stress for 5 weeks and then evaluated with extensive behavioral testing of cognitive, depressive and anxious phenotypes. Clear evidence has emerged for a differential effect of stress on young and old mice, particularly with regard to tests that reflect hippocampal function. They found that stress adversely affects cognitive function in old mice while the performance of young mice is actually enhanced. There was a similar differential effect of stress on anxiety, which showed the young but not the old mice to be resilient to stress. They have obtained brain tissue samples from these mice and these are being examined by Japanese group for biomarker correlates of the behavioral effects in order to determine the mechanisms underlying the differences between young and old mice in their response to stress. At this stage of the project the correspondence of hippocampal-related findings in the animal and in human studies is intriguing as is the potential role of BDNF which will be examined together with other biomarkers in both the animal and human components of the research.

別紙２

Results report

１． Title of Research and Development： The influence of feature salience over microsaccades in normal and blindsight humans and monkeys: an experimental and theoretical investigation

２. Principal Investigator：Masatoshi Yoshida（Assistant Professor , National Institute for Physiological Sciences）

３. Counterpart Principal investigator：Ziad M. Hafed（Junior Research Group Leader, Eberhard-Karls-Universität Tübingen, Werner Reichardt-Centrum für integrative Neurowissenschaften (CIN)（Germany））

４． Results of Research and Development:

Microsaccades are tiny saccadic eye movements that take place during periods of gaze fixation. Even though the detailed mechanisms for microsaccade generation, as well as their functional role in vision, are not fully understood, recent research has revealed that these eye movements can act as an important proxy for larger saccades in understanding several aspects of perception and cognition. One such aspect concerns the relationship between selective visual attention and eye movements, in which it was shown that microsaccades, in contrast to their classic description as random and spontaneous, are related to attention. The neural mechanisms for such a relationship are not fully understood, and its characteristics in pathological states of vision are completely unexplored. The purpose of the proposed research is to understand how feature salience that is commonly included in models of selective visual attention can influence microsaccades.

1. Animal experiments

Monkeys were trained to make a fixation to a spot on a display. By using retinal stabilization, we examined how a peripheral cue affects microsaccades. We also tested with a free-viewing experiment in which normal monkeys and monkeys with lesion in V1 or the superior temporal gyrus viewed movie clips. We found that the number of saccades toward the affected hemifield was reduced in the V1-lesioned monkeys.

2. Human experiments

Subjects with normal vision participated in a Posner task in which a pre-cue is presented and a saccadic target appeared immediately after that. When the time difference between the cue and the saccadic target was varied, the saccadic reaction time was modified (inhibition-of-return (IOR) effect). We analyzed microsaccades during the trials and found evidence that the rhythmic process under microsaccade generation can explain the IOR effect.

In a free viewing task, one subject with a damage in V1 and six control subjects participated in the experiment. We measured eye movements during the free-viewing task and a fixation task with the same movie clip and found that the direction of microsaccades during the fixation task is anti-correlated with the direction of saccades during the free-viewing task.

3. Neural network model

We constructed computational models of the superior colliculus based on a neural-field model and a spiking-neuron network model. We found that the models worked as a saliency detector and are able to replicate the effect of saliency on microsaccades (published in Frontier is Systems Neuroscience 2016). Based on these finding, we constructed a model about neural network of saliency computation and proposed the model in a reviw paper submitted to the theme issue "Auditory and Visual Scene Analysis" of Philosophical Transactions B.

別紙２

Results report

１．Title of Research and Development：

Genomics, bioinformatics and systems medicine to facilitate therapy of ovarian cancer

２．Principal Investigator： Johji Inazawa, Professor, Tokyo Medical and Dental University

３．Counterpart Principal investigator：Olli P. Kallioniemi, Professor, Institute for Molecular Medicine Finland, University of Helsinki

４．Results of Research and Development:

Ovarian cancer has a poor prognosis and a high mortality rate. Finland has a relatively high morbidity of ovarian cancer. On the other hand, Japan and other Asian countries have a high morbidity of platinum-resistant ovarian cancer including clear cell carcinoma. Identification of new therapeutic targets is critical for the implementation of personalized and effective ovarian cancer treatment.

The aim of our study is the establishment of the ovarian cancer therapy by exploring new drugs and including known molecule targeted drugs, that is, drug repositioning (DR). The results of our study of 2016 are as follows.

1. International collaboration of biobank in gynecological cancer

Finland has established biobank facilities which link medical records under the biobank law. In 2015, the Japanese group visited Auria biobank (Turku), and Helsinki biobank (Helsinki) to participate the conference on international joint research.

2. The understanding of ovarian cancer cell system with bioinformatics and identification of candidate targets for ovarian cancer therapeutics.

Japanese and Finnish groups have been detecting intracellular molecular changes occurring through the pathway in ovarian carcinogenesis, which may lead to drug repositioning (DR).

3. Genome analysis on ovarian cancer

Genome analysis was performed in ovarian cancer tissues collected in Tokyo Medical and Dental University Bioresource Research Center and Keio Women’s Health Biobank (KWB) to explore genomic alterations specific for ovarian cancer.

4. Primary culture for gynecological cancer and drug sensitivity and resistant testing

Primary culture with tissues and ascites from ovarian cancer patient was performed to detect *ex vivo* drug sensitivity of the cells.

5. Analysis for the ovarian cancer pathogenesis through impaired autophagy

P62 protein expression, which is regarded to the hub of autophagy function, was analyzed in ovarian cancer.

6. Ovarian cancer metabolome analysis

Possible involvement of metabolic alterations in refractory ovarian cancer pathogenesis was discussed.

7. Japanese - Finnish symposiums

Members from both countries participated in the meeting of Japan-Finland Cooperative Scientific Research as part of the FY 2015 Strategic International Research Cooperative Program (SICP) at Sapporo in Japan. A Finnish leader, Professor Olli Kallioniemi showed Finnish Biobamk system and its utility in precision medicine in Finland as a presentator at the 34th International Cancer Symposium held at Sapporo (25-27 June 2015).

Results report

1. Title of Research and Development: Testing computational models of learning from social, real, and fictive feedback in human and nonhuman primates.

2. Principal Investigator: Masaki Isoda (Associate Professor; Department of Physiology, Kansai Medical University School of Medicine)

3. Counterpart Principal investigator: Markus Ullsperger (Professor; Department of Neuropsychology, Faculty of Natural Sciences, Otto-von-Guericke Universität Magdeburg (Germany))

4. Results of Research and Development:

The goal of this consortium is to develop computational models of learning and decision making and to test them in two biological systems, humans and macaques. In fiscal year 2015, the Japanese team studied activity of single dopamine neurons in the midbrain of monkeys performing a socially-oriented, Pavlovian conditioning paradigm, which allowed us to study the neural mechanisms underlying learning from real and social feedback. In the paradigm, two monkeys facing each other were presented with a conditioned stimulus (CS) that differently signaled the probability of each monkey's upcoming reward. The reward feedback was given first to the nonrecorded animal (designated as *other*) and, 1 s later, to the recorded animal (designated as *self*). The monkeys were alternately conditioned in two contextually different trial blocks: i.e., one block in which the other's-reward probability was variable depending on the CS and the self-reward probability was constant, and another block in which the self-reward probability was now variable and the other's-reward probability was constant. Consistent with previous studies, the animals placed higher value on the CS predicting a higher probability of *one's own* reward. Interestingly, the animals placed lower value on the CS predicting a higher probability of *other's* reward. Thus, the prospect of others' reward profoundly affected the value of one's own reward. Single-unit recording was then made from presumed dopamine neurons (n = 229) in the substantia nigra pars compacta and the ventral tegmental area. About half of the neurons (n = 115) showed a significant positive correlation between the phasic CS response and the self-reward probability. Among them, the activity of 80 neurons was not influenced by the other's-reward probability, while that of 35 neurons additionally showed a significant negative correlation with the other's-reward probability as if representing the subjective value. There were few neurons that selectively encoded the other's-reward probability. Crucially, however, dopamine neurons as a population became more discriminative for the other's-reward probabilities as they discriminated more clearly between the self-reward probabilities. These findings suggest that dopamine neurons incorporate self-reward information and other's-reward information in a single value scale. This is in sharp contrast to firing properties of medial prefrontal neurons, which distinctly encoded the self-reward information or other's-reward information. Our data demonstrate that midbrain dopamine neurons and medial prefrontal neurons participate in different aspects of learning from real and social feedback.

Meanwhile the German team developed a social instrumental learning task enabling to compare learning from experienced outcomes of own actions and observational learning from outcomes observed for actions taken by another player. With appropriate simplification this task can be easily transferred to a setting allowing to study observational learning in macaques. Participants were required to learn the stimulus-outcome contingencies for an adapted three-armed bandit task. In each block, their aim was to learn over the course of 30 trials, which of three stimuli would most often lead to a positive outcome. The participants would play together, with the acting and observing player switching roles regularly. A first task version using blockwise role switches successfully demonstrated the ability of participants to learn from own action outcomes as well as from observation. However, the block structure caused difficulties in modelling the behaviour with reinforcement learning models. Therefore, role switching every 1-3 trials was introduced to engage the participant during the observation stage more fully than the previous task structure. It was also expected to ease modelling and be more suitable for a follow-up experiment using fMRI than the former design. Sixty-four channel EEG was recorded from both participants as they performed the task. An adapted Q-learning algorithm was fit to participants' choices in this task. Comparable model estimated learning rates were obtained for trials in which the same player acted consecutively, relative to when players switched from an observing to an acting role. This suggested that participants used similar computational algorithms to weight the outcomes they received from making and observing choices on each trial. Model-based single-trial EEG analysis using multiple robust regression revealed that reward prediction errors were represented in the feedback-related negativity (FRN) around 280 ms after the feedback. Furthermore, P3a and P3b amplitudes correlated with the experienced or observed outcomes. The role (actor/observer) had a sustained positive-going effect at centroparietal electrodes from 200-600 ms such that experienced outcomes elicited a generally more positive event-related potential. Moreover, the factors role and prediction error interacted significantly at the latencies of the FRN and P3b, such that both potentials were larger when participants experienced outcomes of their own actions. These findings suggest that while the general learning mechanisms are similar for own and observed action outcomes, own experiences have a stronger impact on the participants. Based on these findings the computational model will be modified to allow differential learning rates for own and observed action outcomes. In summary, the newly developed task has proven well-suited to study observational learning and can now be transferred to animal research and functional magnetic resonance imaging in humans. Moreover, it will be modified to investigate the influence of social context, for example competitive vs. cooperative settings.

別紙２

Results report

１． Title of Research and Development： Autonomous Learning of Active Depth Perception: from Neural Models to Humanoid Robots

２． Principal Investigator：Sungmoon Jeong (School of Information Science, Assistant Professor, Japan Advanced Institute of Science and Technology

３． Counterpart Principal investigator：Jochen Triesch (Department of Neuroscience, Professor, Frankfurt Institute for Advanced Studies, Germany)

４． Results of Research and Development:

During the first few months of their postnatal development, humans and other animals autonomously learn how to use such depth perception cues like disparity, motion parallax and optical flow. Thereafter, they continue to adapt and self-calibrate their vision to compensate for growth of the eye, head, and body, but the underlying neural mechanisms are still largely unknown. Providing robots with similar abilities to autonomously learn and self-calibrate sensori-motor loops for active perception would make them more autonomous and robust. Therefore, in this research period, we proposed a system for the autonomous self-calibration of active depth perception based on motion parallax using a single moving camera. Our system is based on active efficient coding (AEC) framework for the autonomous self-calibration of active perception which autonomously learns to represent image motion and perform compensatory eye rotations to keep the object fixated during lateral movement − thereby learning to actively estimate the object’s distance.

First, to validate our research, we implemented an active binocular vision hardware framework by using two dimensional linear actuators to realize human’s body movements and the two camera with pan-tilts unit for mimicking the eye’s rotation. Second, the new cost function was designed by considering the active efficient coding theory with intrinsic motivation concept to simultaneously train eye rotation (action) and sensory representation (perception) of the autonomous robot under the motion parallax phenomena. The intrinsically motivated visual system can generate a suitable eye movement to increase the redundancy between successive images for understanding of the motion parallax phenomena. Third, the generated eye movements was transferred to the input of the depth estimator as a goal-directed visual learning system to autonomously estimate the depth between the observer and an arbitrary object. An autonomous depth estimator was developed by two layer feed-forward artificial neural network to map between the eye movements and the object’s distance.

Finally, we had validated our proposed model by using computer simulation and real robot experiments. The motion parallax based self-calibrated visual framework achieved good results with less than 0.1 estimation error of eye rotation degree in the computer simulation and 0.05 estimation error in the real robot experiments. And also, it can estimate accurate depth information from eye movements with less than 7% depth estimation error in the computer simulation and less than 3% depth estimation error in the real robot experiments. Moreover, the proposed framework can successfully estimate depth and generate eye movements to keep the object at the center of gaze by autonomous self-calibration when we apply a perturbation to the system.

別紙２

Results report

１． Title of Research and Development：

Decoding of in vivo two-photon imaging data in mouse motor cortex.

２． Principal Investigator：

Yukiyasu Kamitani, Professor, Graduate School of Informatics, Kyoto University, Japan.

３． Counterpart Principal investigator：

Takashi Sato, Junior group leader, Center for Integrative Neuroscience, University of Tübingen, Germany.

４． Results of Research and Development:

We performed multi-variate pattern analysis to two-photon microscopy data that were obtained by Dr. Sato's group in Germany. In our preliminary analysis before 2015, we observed that mice performed a movement task more quickly when the motor cortex exhibited sparse and stable neural activity. In 2015, we obtained data from multiple mice for quantitative analysis with additional experiments where excitatory and inhibitory cells were recorded separately. In the first analysis, we confirmed the reproducibility of the results on additional six mice. In the second analysis, the similar tendency was observed for both excitatory and inhibitory cells in terms of the stability and sparseness of motor cortical activity.

To conduct the above analyses in a tight collaboration with Dr. Sato's group in Germany, a researcher in Dr. Kamitani's group visited Dr. Sato's laboratory in University of Tübingen in February. As a result, we performed the analysis work efficiently, and enabled the members in Dr. Sato's group to do preliminary analyses in their own side by providing a set of computer programs for the analysis made in Dr. Kamitani's group.

別紙２

Results report

１．Title of Research and Development : Role of TLRs in patients with severe complicated malaria due to *Plasmodium vivax* and the development of diagnostic method for predicting the severity

２．Principal Investigator : Fumie Kobayashi (Professor; Department of Infectious Diseases, Kyorin University School of Medicine)

３．Counterpart Principal investigator : Rakesh Sehgal (Professor & Head; Department of Medical Parasitology, Postgraduate Institute of Medical Education & Research (India))

４．Results of Research and Development:

Severe cases of malaria are mainly caused by human malaria parasite, *Plasmodium falciparum*, leading to death if not promptly treated. Although *P. vivax* has long been considered as a benign infection, there has been an increase in the reported cases of severe malaria due to *P. vivax* in recent few years. However, the pathogenesis in severe vivax malaria is not fully understood. To investigate pathogenesis in severe vivax malaria, the clinical data related to the vivax malaria were collected at Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India. The informed consent was taken from patients before taking the clinical history and blood samples. Clinical data were analyzed in line with WHO criteria for severe malaria. Analysis of data obtained from the 90 *P. vivax* malaria patients revealed that 48% of patients showed the same clinical manifestation as severe malaria. The clinical manifestations observed in severe vivax malaria are severe anemia, thrombocytopenia, renal failure, jaundice, respiratory distress, and hypoglycemia to multi-organ dysfunction. Especially, thrombocytopenia was found in 75% of severe vivax malaria patients, suggesting that thrombocytopenia may be associated with increasing severity in vivax malaria.

The severity in vivax malaria patients may be attributed to the various factors, such as parasitic, host and environmental factors. In this study, we focused on the host factors responsible for the causation of severe complicated vivax malaria. We first investigated the various toll like receptors (TLRs), such as TLR4 and TLR9, in 90 subjects with *P. vivax* malaria. As results, we found that the nucleotide change was observed in all the targeted toll like receptor genes with varying frequency. In the available sequences, mutations in the TLR4 and in the TLR 9 were observed in 19 of 80 subjects and in 8 of 35 subjects, respectively. These findings suggest that the mutations in toll like receptors might be associated with pathogenesis of severe vivax malaria.

To investigate pathogenesis in severe vivax malaria, we tried to establish attenuated rodent malaria parasites and examined key factors determining the severity of malaria. *Plasmodium* parasites are unable to synthesize purine rings de novo. Thus, they rely on the host for a supply of purine nucleosides and then synthesize purine nucleotides through a purine salvage pathway. We demonstrated that the growth and virulence of *P. berghei* (*Pb*) ANKA, a high virulent-rodent malaria parasite, were suppressed by purine restriction. In mice infected with purine restricted parasites, progression of thrombocytopenia was delayed compared with mice infected with intact parasite. Subsequent purine restriction resulted in a substantial reduction in ATP levels relative to intact parasites, suggesting that the ATP levels in parasites might be useful for predicting the disease severity during malaria. Moreover, the attenuated parasites were readily cleared by wild-type mice, but not by TLR2/4/9-deficient mice and γδT cell-deficient mice. These results suggest that polymorphism or mutation of immune factors may be associated with the severity of malaria due to *P. vivax*.

Further analyses of clinical cases with severe vivax malaria (Indian side) and mouse malaria models (Japanese side) would contribute to understanding the pathogenesis and developing a diagnostic method for predicting the severity due to *P. vivax*.

別紙２

Results report

１． Title of Research and Development： "Improvement of the accuracy for differential diagnosis against viral infection – DENV, CHIKV, and FluV- by spreading of high quality RDT in India

２． Principal Investigator：Takeshi Kurosu （National Institute of Infectious Diseases）

３． Counterpart Principal investigator：Sujatha Sunil （International Centre for Genetic Engineering and Biotechnology（Country））

４． Results of Research and Development:

We aim to develop the rapid diagnosis test kits for dengue, chikungunya and influenza viruses and enlighten importance of early diagnosis. Patients with these diseases showed similar symptom at early stage; however, they have to receive different treatments.

1. Development of a diagnosis kit for dengue virus infection, epidemiological study and virological study: To make a good rapid diagnosis kit, most important issue is to obtain good antibodies which react efficiently with pathogens. We immunized mice with dengue virus infected cells. After several trials, we changed the protocol for immunization and obtained hybridomas producing antibodies. We stared characterization of antibodies. For epidemiological study, virus characterization is important; however, there is no method to classify virus in virulence using cultured cells. To solve this problem, we have established a new approach. Dengue viruses exhibited various virulence in mice lacking type I and II interferon systems. This approach will be useful to understand virulence.
2. Development of a diagnosis kit for chikungunya virus (CHIKV): Modified test stript (stick type) kit was produced. We had meeting with Indian counterpart about selection and criteria for evaluation. Indian counterpart performed a part of sequence analysis of CHIKV. For virus characterization, we performed to develop mouse model for arthritis. In India, there are many cases of co-infection between dengue virus and CHIKV, therefore, we assumed that co-infection may exacerbate arthritis because co-infection possibly enhances levels of cytokines compared with single infection with CHIKV. By using mouse model system, we will test this hypothesis.
3. Evaluation of influenza kit: For evaluation of kit, it is important to compare with conventional method. For this, we invited Indian young researchers to Japan and had training for diagnosis. During this year, a new matter of concern arose. In India, there were many death cases by H1N1 infection. In other countries, it was not observed, which means this was India-specific observation. Indian government wants to investigate. So Dr. Gaind’s team at Safdarjung Hostipal has establish diagnosis to distinguish H1N1. For this reason, Indian young researchers learnt diagnosis of H1N1 during their stay in Japan. Additionally, Japanese side and Indian side had agreement to examine a new rapid diagnosis method based on nucleic acid detection for H1N1.

Regarding international exchange, we invited two young researchers to Japan from January to March and two investigators to join symposium and have meeting. By presentation each other, we had exchanged information on epidemiological situation of infection diseases and idea. At symposium, four Japanese researchers and four Indian researchers gave presentation about progress of projects.

別紙２

Results report

１．Title of Research and Development：Single molecule imaging of synaptic protein dynamics in neurodegeneration

２．Principal Investigator：Akihiro Kusumi

(Professor, Institute for Integrated Cell-Material Sciences, Kyoto University)

３．Counterpart Principal investigator：Giovanna Mallucci

(Department Chair and Professor, Department of Clinical Neurosciences, University of Cambridge (U.K.))

４．Results of Research and Development:

In many neurodegenerative diseases, impaired synaptic structural plasticity, which leads to synapse loss and ultimately neurodegeneration, have often been found. Among many studies detecting the relationship between impaired synaptic structural plasticity and neurodegenerative processes, the PI of the present project on the U.K. side, Prof. Giovanna Mallucci, discovered very clear evidence for the relationship. When the brain or neuron is cooled to 17˚C, many synapses are lost, but when they are rewarmed, synapses reform – a physiological form of structural plasticity. However, Prof. Mallucci found that such synaptic reformation upon rewarming does not occur in many neurodegenerative diseases. This shows that, by understanding the synapse reforming processes and mechanisms, we might be able to discover how synapses are impaired in neurodegenerative diseases. The objective of the present investigation is thus to understand the synaptic structural plasticity in the cooling-rewarming process. For this purpose, we image the entrance into and exiting from synapses of various synaptic molecules at the level of single molecules in the cooling-rewarming cycle.

During the previous fiscal year (FY14), Prof. Mallucci's group established that a heat-shock protein called RNA-Binding Motif protein 3 (RBM3) works as a switch for synaptic regeneration upon warming (Peretti et al. 2015 Nature). This opened a new possibility to regulate synaptic structural plasticity in both positive and negative ways. In FY15, we advanced the present project, incorporating this new finding.

In this fiscal year (FY15), the Mallucci lab established the conditions for synaptic regeneration in the cycle of cooling and rewarming using primary neuron culture obtained from the mouse hippocampus. While this experiment was ongoing, two young researchers from the Kusumi lab visited the Mallucci lab, and helped to establish the proper conditions, so that the same system works in the Kusumi lab. It has now become possible to perform single-molecule imaging of synaptic structural plasticity using the primary neuron culture in the Kusumi lab.

Meanwhile, the Kusumi group advanced the method for specifically labeling AMPA receptor with fluorescent probe molecules (GluA1 and GluA2; submitted). They also developed methods to simultaneously observe the clusters of Homer1b and single GluA1 and GluA2 molecules.

Previously, we only paid attention to proteins as synaptic molecules. However, in-out of synaptic molecules often depends on their diffusion within the plasma membrane, and therefore, we decided to observe the movements of lipids, entering and exiting from synapses. For this purpose, we developed fluorescent ganglioside analogs (submitted). In particular, the direct interaction of a ganglioside GM1 and AMPA receptor has been proposed. Therefore, we will examine the behaviours of fluorescent GM1 in the cycle of cooling and rewarming.

別紙２

Results report

１．Title of Research and Development： Identification of biomarkers for infection-induced reactive arthritis based on the inflammation amplifier

２．Principal Investigator：Masaaki Murakami（Professor, Molecular Neuroimmunology, Institute for Genetic Medicine, Hokkaido University）

３．Counterpart Principal investigator：Ramnath Misra（Dean, Professor & Head, Clinical Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow（India））

４．Results of Research and Development:

Reactive arthritis (ReA) is a sterile inflammation of joints, triggered by infection at a distant site i.e. gastro-intestinal tract with *Salmonella*, *Yersenia*, *Shigella*, or *Campylobacter*. It is a major health problem causing acute and chronic arthritis in adolescent and young adults. Since *Salmonella typhimurium* is one of the commonest causes of bacterial diarrhea in India, therefore incidence rate of occurring ReA is expected to be high. Indeed, in sporadic ReA, the Misra laboratory has reported that *Salmonella typhimurium* accounts for one-third of cases in our community, in contrast to *Yersinia* and *Chlamydia* in Western countries. However, there are challenges in this disease, as there are no diagnostic tests as well as diagnostic biomarker for prognistification. One factor limiting the development of specific biomarker and therapy of ReA is the absence of a good animal model for the study of the molecular mechanisms involved.

The Murakami laboratory has discovered a chronic inflammation inducing machinery “inflammation amplifier” in local tissues. The inflammation amplifier induces excessive productions of inflammatory chemokines and growth factors in non-immune cells including endothelial cells, fibroblasts, glia cells, and epithelial cells, which is activated by the simultaneous activation of NFκB and STATs in response to cytokines such as interleukin (IL)-17A and IL-6. Dr Misra has found that presence of these cytokines in large quantities in synovial fluids of ReA patients. Therefore, the Japan-India collaboration by these laboratories will lead to establish a ReA animal model to study ReA pathogenesis and develop a biomarker and therapeutic strategy to ReA.

Dr Murakami already established a ReA mouse model using F759 knock-in mice infected with *Salmonella* in Osaka University. After moving his laboratory to Hokkaido University, the Murakami group improved the ReA model by injecting serum components derived from *Salmonella*-infected mice at the ankle joints of mice during the fiscal year of 2015. This phenomenon can explain joint inflammation by infection at the distant site (i.e. gastro-intestinal tract). Therefore, the pathogenesis of the serum component-induced arthritis will be extensively studied next year. In addition, the evidence of the amplifier activation in ReA patient was further supported by the observation that the amplifier-target soluble factors were elevated in sera of the patients in India.

In this fiscal year, a young researcher from the Indian side joined in the Murakami laboratory and learned experimental techniques to induce the ReA mouse model and analyze it. He will continue to stay in Japan next year to study the mechanism of disease induction by the serum components. In February 2015, Dr Misra visited the Murakami laboratory to discuss the Japan-India collaborative project for the fiscal year of 2016.

別紙２

Results report

１．Title of Research and Development：The development of the functional organization in visual cortex

２．Principal Investigator：

Kenichi Ohki　（Professor, Department of Molecular Physiology, Kyushu University）

３．Counterpart Principal investigator：

Matthias Kaschube（Professor, Department of Computational Neuroscience and Computational Vision, Frankfurt Institute for Advanced Studies and Goethe University（Germany））

４．Results of Research and Development:

The functional organization of the visual cortex describes the layout of tuning properties in large numbers of individual neurons. How it develops from synaptic connectivity is a central question of neuroscience. Recent studies in mice suggest that significant changes in the functional organization occur after eye opening. Moreover, recent technological advancements provide the unique opportunity to monitor these changes simultaneously in large numbers of cells. In this proposal we will perform two-photon calcium imaging of large populations of neurons in the developing mouse visual cortex to study how the functional organization changes during normal development. As a complementary effort, we will develop a computational circuit model, to understand which properties of the cortical network and of its feed-forward inputs account for the observed changes. To directly measure how visual response properties in individual neurons reorganize during development, we will make chronic two-photon recordings in mice around the time of eye opening. We study the network model over time, aiming to identify candidate mechanisms of cortical reorganization from a quantitative comparison between model and experiment. The proposed research will significantly broaden our understanding of the factors guiding the formation and maturation of the functional organization of the visual cortex.

This year, the Japan team developed a wide-field calcium imaging method using macro-zoom microscope, and found that patterns of spontaneous activity in the mouse cerebral cortex change dramatically during normal development. Furthermore, they developed a method for chronic two-photon calcium imaging and chronically observed changes of response selectivity of neurons in mouse visual cortex. The German team received the data of spontaneous activity in the mouse cerebral cortex during development and started the analysis of the data.

別紙２

Results report

１．Title of Research and Development： Bioinformatics platform for predicting autologous cell therapy efficacy in patients with heart failure

２．Principal Investigator：Yoshiki Sawa（Professor, Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine）

３．Counterpart Principal investigator：Esko Kankuri（Docent, Pharmacology, Institute of Biomedicine, University of Helsinki（Finland））

４．Results of Research and Development:

Our achievement of this year is to establish disease-specific induced pluripotent stem (iPS) cell from the patient of congenital cardiovascular disease. We decided to research Dilated Cardiomyopathy (DCM) and differentiation into cardiomyocyte was induced by adding cytokines such as Activin A and Bone morphogenetic protein 4. (Reference: Nature Biotechnology, 25 (9) (Sep 2007), pp. 1015–1024 and Journal of Molecular and Cellular Cardiology 50 (2011) 327–332). After differentiation, the purity of cardiomyocyte was 10 – 20 %. We are considering that some improvements will be needed in the differentiation process. On the other hand, we already developed the new method to purify iPS-derived cardiomyocyte from the cardiomyocyte-induced iPS cell population in the first year of this project. Because one of the extra cellular matrix has affinity to cardiomycyte, cardiomyocyte can be easily separated from other cells without invasions in a short time. This technique is already applied domestic patent as we reported before (2014-188180). This year, we added some new data that shows our technique is superior in the point of high yield and low cytotoxicity than the conventional cell purification methods. Then we applied this method to international application (PCT/JP2015/076072). The researchers in Helsinki contributed actively to carrying out this research such as giving information about protocol to the researchers in Osaka. Antti Siltanen, a researcher of University of Helsinki is now writing a paper about this research. Furthermore, Keitaro Domae, a young researcher of Osaka University went to Helsinki from 23rd February to 18th March. He learned recent development of treatment for heart failure in Helsinki. Especially he discussed surgical stratagem in treating for heart failure with Ari Harjula, Professor of Cardiothoracic Surgery, Helsinki University Central Hospital and his colleague. He also shares the recent development of basic research of cardiac regenerative therapy in both University of Helsinki and Osaka University. . On 10th March, scientific meeting hold at University of Helsinki. The participants from Finland were Professor Harjula, Dr. Kankuri, Dr. Kankainen, and Dr. Siltanen. The participants from Japan were Dr. Miyagawa, Dr. Fukushima and Dr. Domae. We discussed actively about current status of cardiac regenerative therapy in Osaka University and University of Helsinki, and the application of bioinformatics into clinical setting of cardiac regenerative therapy. We also confirm the facilitation of exchange and cooperation between Helsinki and Osaka University. Our young researchers joined this meeting and discuss and interact with researchers of Helsinki University. We also confirmed the research plan and cooperation in the next year.

別紙２

Results report

１．Title of Research and Development： Privacy-preserving genomic data analysis for personalized medicine (PRIVAGEN)

２．Principal Investigator：Kana Shimizu （Senior Research Scientist, National Institute of Advanced Industrial Science and Technology）

３．Counterpart Principal investigator：Antti Honkela（Academy Research Fellow（Finland））

４．Results of Research and Development:

In this fiscal year, the Japanese team has focused on developing cryptographic protocols for searching genome information.

1) Privacy-preserving allele information search system

We implemented a novel system in which the user can search allele information without showing his/her query to the server while the server only returns the search result. To achieve practical performance, we developed an improved protocol whose communication overhead is $O(\sqrt{N})$ and replaced the previous protocol whose overhead is $O(N)$ by the new one. In addition to implementing searching module, we also implemented authentication module, secure communication module and GUI both for user and server applications to construct an entire searching system. We have installed the implemented system in the human genome databank to test an efficiency of the system.

2) Privacy-preserving genome sequence search

We designed and implemented a novel protocol which enables to search a similar genome sequence from a database. The method is based on a new approach that combines efficient string data structures such as the (positional) Burrows-Wheeler transform with a cryptographic technique called an oblivious transfer. The proposed method is order of magnitude faster than existing algorithms for finding substring match. In an experiment using 2184 aligned haploid genomes from the 1000 Genomes Project, our algorithm was able to perform typical queries within $\fallingdotseq$4.6 s and $\fallingdotseq$10.8 s for client and server side, respectively, on laptop computers. We also published the source code of the proposed algorithm at gitHub. The proposed method can be used in a wide range of applications.

3) Long-term preservation of the personal genome

The genome is inheritable to offspring and thus the protection period of genomic data could be very long. Despite that, most of the conventional studies use security parameters of the cryptosystem which were originally designed to handle other types of personal information such as a bank account and a phone number whose ideal protection period is much shorter than that of genome information. In our study, we address the problem of long term genomic data protection and suggest a novel approach that combines an information-theoretically secure method and a computationally secure method. We targeted on an allele frequency search problem which is modeled by 1 out of N oblivious transfer (1-N OT), and designed the novel 1-N OT protocol that achieves a long protection period while keeping utility.

In addition to above research results, we organized a workshop PRIVAGEN 2016 together with Finnish researchers. The workshop aimed to facilitate discussion among researchers in diverse fields including bioinformatics, genome ethics, machine learning, data-mining and cryptography. The PRIVAGEN was held as an official satellite workshop of GIW/InCoB 2016 which was the international conference of Bioinformatics, and there were 80 participants from more than 10 countries. We also organized a session for discussing the effect of new legislation of personal information in Japan on genome information analyses at IIBMP 2015 which was the largest domestic conference of Bioinformatics in Japan.

In this fiscal year, we published four referred journal papers and one paper for a domestic conference, and won three awards (IIBMP 2015 Excellent Research Award, IIBMP2015 Best presentation award, AIST president award (research)).

別紙２

Results report

1. Title of Research and Development : Amazon fruits nano-supplements development: nutrigenomic and nutrigenetic effects on aging and health

2. Principal Investigator : Toshiro Aigaki (Professor, Department of Biological Sciences, Tokyo Metropolitan University)

3. Counterpart Principal investigator : Ivana Cruz (Professor, Health Science Center, Federal University of Santa Maria, (Brazil))

4. Results of Research and Development:

The goal of this project is to understand the functionality of the Amazonian Guaraná fruit (*Paullinia cupana*) at the molecular, cellular and organismal levels, and to develop nano-supplements that promotes health and longevity in humans. The Japanese team uses the fruit fly Drosophila as an experimental model system, whereas the Brazilian team works on humans and cultured cells. We exchange researchers and information between the two teams to promote the collaborative project efficiently. We made the following achievements this year.

I. Identification of genes affected by Guaraná fruit (GF) intake

We performed microarray experiments to investigate the effects of GF intake on gene expression in *Drosophila*. Flies were transferred from normal media to those containing GF powder at the concentration of 10mg/ml and kept for 24 hours. Control flies have been kept in normal media. Then mRNA was extracted and analyzed for gene expression with Agilent microarrays. We identified 195 and 203 genes whose expression levels were up- or down-regulated, respectively. Among the identified genes, four genes were selected and subjected to quantitative RT-PCR. The results were consistent with those of microarray.

II. Metabolomic changes induced by GF intake

Flies were treated with none (control), GF, caffeine, or catechin for 24hrs as described above, and extracted with 75% acetonitrile, and the soluble fractions were subjected to metabolomic analyse using an LC/MS system. Metabolomic profiles of GF treated flies were clearly different from those of control, indicating that GF has an impact on the metabolism of the animals. GF contains a high level of caffeine, and the metabolomics profiles were relatively close to that of GF-treated flies, suggesting some of the effects of GF may be caused by caffeine.

III. Tolerance to heavy metal (methyl mercury).

Flies were raised on medium containing methyl mercury with or without GF. No fly has developed from the medium with methyl mercury, whereas approximately 30 % flies hatched from the food containing both methyl mercury and GF. The data demonstrate that GF confers the tolerance to methyl mercury.

IV. Evaluation of nano-supplement containing GF

Nano particles containing GF (nano-supplement) was examined for their biological activity using *Drosophila*. Flies were kept on media with carrier only (control) or with GF nano-supplement, and measured their lifespan. There was no significant difference in adult life span between the two groups. Since the amount of GF in the nano-supplement was low, we need to develop nano-supplement containing a higher concentration of GF.

別紙２

Results report

１． Title of Research and Development： Cortically-triggered robotic hand orthosis for home-based therapy and assistance in activities of daily living

２． Principal Investigator：Jumpei Arata (Associate Professor in Department of Mechanical Engineering, Faculty of Engineering, Kyushu University)

３． Counterpart Principal investigator：Roger Gassert (Associate Professor in Department of Health Sciences and Technology, ETH Zurich (Switzerland)

４． Results of Research and Development:

This project aims to develop and evaluate a brain-triggered robotic hand orthosis to provide assistance and therapy in activities of daily living (ADL) for neurological patients and aging persons with severe hand impairment. For the first time, a wearable brain-robot interface (BRI) and an innovative, lightweight robotic hand orthosis will be combined to enable this novel mode of therapy and assistance in the clinic and at home.

In the current stage of the project, we have developed a preliminary BRI combined with the robotic hand orthosis and a commercialized NIRS device. The signal from NIRS device is currently processed by a simple method (Linear Discriminant Analysis) and will be further investigated in the next year. A method for adapting the robot on individual patients is under investigation, showing positive feasibility on a preliminary stage prototype that consists of a commercialized 3D scanner and a CAD algorithm that directly output the 3D model data to be printed by a 3D printer.

別紙２

Results report

１． Title of Research and Development：

Preemptive cancer treatments based on detection and elimination of precancerous cells using cell competition and supercompetition markers

２．Principal Investigator：**Yasuyuki Fujita** （Professor at Institute for Genetic Medicine, Hokkaido University）

３．Counterpart Principal investigator：**Eduardo Moreno** （Professor at Department of Institute of Cell Biology, Bern University）

４． Results of Research and Development:

The aim of this research project is to establish a novel and innovative cancer preventive medicine by studying cell competition between normal and transformed epithelial cells in a comprehensive manner through close collaborations between Japanese and Swiss groups. In this year, we have obtained the following outcomes.

1) By using various approaches, we have explored molecular mechanisms whereby cell competition between normal and transformed epithelial cells are regulated. Especially, by using a variety of biochemical cell fractionation methods and quantitative mass-spectrometry (SILAC), we have successfully identified multiple (potential) cell competition regulators.
In particular, we have found that mitochondrial activity is substantially decreased in RasV12-transformed cells when they are surrounded by normal epithelial cells. Increased expression of PDKs is responsible for the down-regulation of mitochondrial activity. Addition of DCA (Dichloroacetate), an inhibitor of PDKs, significantly suppresses the apical extrusion of RasV12-transformed cells, suggesting that PDK-mediated mitochondrial down-regulation plays a positive role in the elimination of transformed cells. Furthermore, expression of LDH is enhanced in RasV12-transformed cells surrounded by normal cells, and suppression of LDH activity leads to formation of basal protrusions. These data suggest that the Warburg effect-like phenotype can occur at the initial stage of carcinogenesis, which plays a tumor-suppressive role by promoting elimination of transformed cells from epithelial tissues. By further developing this new research field, we would create a novel type of cancer treatment: eradication of transformed cells by enhancing a defensive force of neighbouring normal epithelial cells.

2) In June, 2015, one PhD student in Fujita lab stayed at Dr. Moreno's lab for one month and acquired the technique of *Drosophila* genetics. In addition, Prof. Fujita (PI in the Japanese side) also visited Dr. Moreno's lab, and intensive discussion was held between the two groups as to the mutual research progress and upcoming collaborations. Thus, since the start of this collaborative project, close interactions and mutual exchange of young scientists have been promoted, which profoundly help them equip international sense.

別紙２

Results report

１． Title of Research and Development： Impedance regulation during energy transfer motor tasks: from human experiments to computational modeling and robotics

２． Principal Investigator : Dr. Ganesh Gowrishankar (Researcher, Center for Information and Neural Networks (CiNet); National Institute of Information and Communications Technology)

３． Counterpart Principal investigator : Dr. Patrick van der Smagt (Professor, Biomimetic Robotics and Machine learning, Technical university of Munich (Germany))

４． Results of Research and Development:

We planned and achieved the aims of the project over a series of steps-

1) We developed a behavioral experiment using the NICT TVINS manipulandum to examine the behavior of humans during impact tasks.
2) We developed a new analytical method to measure human arm impedance (both stiffness and damping) during impacts.
3) We examined the human behaviors in these tasks and found that humans can utilize haptic feedback during impacts to change their impedance and optimize the energy transfer during impacts. **This result was presented in the NCM conference.**
4) We analyzed the human behaviors computationally, and determined how the haptic feedback during impacts is used to optimize subsequent impacts. **This result will be submitted as a full journal paper.**
5) Using these results we developed a robot algorithm that enables robots to perform impacts like humans. This robot algorithm was implemented in Germany this year. **The paper will be submitted in the next months.**
6) In addition to the initial project goals, we also examined an additional issue of tool embodiment during fast movements. Embodiment issues are closely linked to control, and in this experiment, again with the NICT TVINS manipulandum we isolated a new embodiment process. **The results were published in Nature Communications in 2014.**
7) Finally we also examined learning in virtual impact (penalty kick) task to isolate for the first time, interactions between choice learning and motor learning by humans. **This result was presented in the NCM conference and has been submitted as a full journal paper.**
8) On the German side, the collaborators worked on algorithms to estimate human arm impedance from recordings of electromyography (EMG). **These were presented in the NCM conference**
9) The German side developed a new fast manipulandum specialized for human impedance measurements.

別紙２

Results report

１． Title of Research and Development: Bio-functionalization of Ti-based materials for osseointegrated implants

２. Principal Investigator: Takao Hanawa (Professor, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University)

３． Counterpart Principal investigator: Luís Augusto Sousa Marques da Rocha (Universidade Estadual Paulista, Faculdade de Ciências de Bauru Department and job title at the affiliated institution (Brazil))

４． Results of Research and Development:

The main aim of this project is to develop a new generation of bio-multifunctional Ti-based surfaces for osseointegrated implants. In particular the goal is to play with different surface modification techniques in order to obtain surfaces capable of being biologically selective (promoting, simultaneously, an adequate adhesion of osteoblastic cells while minimizing the possibilities of colonization by unwanted microorganisms), together with high corrosion and tribocorrosion resistance. Special focus will be given towards the understanding of the underlying mechanisms governing each phenomenon. This project aims at interrelating three complementary research activities, namely: (1) electrochemical surface treatments and sputtering techniques to obtain surfaces with different micro and nano-topographies, structures and chemistry (doping with bioactive and antimicrobial species), (2) in particular, micro-nano-porous and nano-tubular surfaces will be produced. Further, immobilization of organic PEG, RGD and/or collagen molecules will be carried out by electrodeposition techniques, (3) investigation of the corrosion and tribocorrosion mechanisms of the surfaces through the integration of electrochemical corrosion techniques in tribological tests, and (4) investigation of the mechanisms governing the adhesion of osteoblastic cells and microorganisms with the different surfaces. Surface parameters governing the competition between osteoblastic cells and microorganisms to adhere to the surface will be studied in detail.

The multi-disciplinary team integrating this proposal bridges Materials Engineering, Biomedical Science and Biology. It is expected that this team will be able of progressing in the scientific understanding of the fundamental mechanisms governing osseointegration, microbial colonization and tribocorrosion behavior. Hereto an integrated approach will be followed addressing the interactions between these mechanisms. By doing so, guidelines will become available for the future production of improved implant multifunctional surfaces. Also, the involvement of young MSc and PhD students that will benefit from the networking and complementary expertise of the Brazilian and Japanese teams will bring an added value for their carrier progression. Finally, collaborative high quality publications will be expected throughout the project.

The purpose of the project was to create a next generation multi-functional titanium surface with osseointegration. In particular, surface modification techniques strongly adhering osteoblast and simultaneously preventing bacterial adhesion has been focused. In this budget year, an acquirement of antibacterial property due to the addition of oxide layer containing Ag, an animal evaluation of micro-arc oxidation (MAO), characterization of structure of a Ti-Zr-Mo alloy, investigation of the possibility of MAO treatment of the alloy, immobilization of a new functional molecule to Ti surface were attempted.

In Ag-containing technique and evaluation of antibacterial property, the relation between the Ag concentration in the oxide layer and antibacterial property was elucidated. In addition, both bone formation and antibacterial properties that are opposite properties were appeared. MAO treatment was effective according to the results of animal test. Furthermore, polarization treatment of MAO oxide layer accelerated bone formation on the oxide. On the other hand, β-type Ti-15Zr-7.5Mo alloy was melted and formed, followed by the evaluation of mechanical property. Also, utility of MAO treatment against Ti-15Zr-7.5Mo alloy was investigated. The Ti-15Zr-7.5Mo alloy consisted of β phase and α" phase and ω phase. Vickers hardness of the alloy was larger than that of Ti-6Al-4V alloy and Young's modulus of the alloy was as the same as that of Ti-6Al-4V alloy. MAO treatment was also effective to Ti-15Zr-7.5Mo alloy because porous oxide layer was formed on the Ti-15Zr-7.5Mo alloy. Therefore, the Ti-15Zr-7.5Mo alloy has good mechanical property as a medical material. It is possible to obtain both good mechanical property and hard tissue compatibility in the Ti-15Zr-7.5Mo alloy because porous oxide layer was easily formed on the alloy by MAO treatment. In addition, immobilization of MPC polymer on Ti was performed and the effect of the immobilization was appeared.

別紙２

Results report

１．Title of Research and Development : Pathogenic mechanism underlying neurodevelopmental disorder in schizophrenia

２．Principal Investigator：Kozo Kaibuchi（Department of Cell Pharmacology, Nagoya University, Professor）

３．Counterpart Principal investigator : Orly Reiner （Department of Molecular Genetics, Weizmann Institute of Science, Professor （Israel）

４．Results of Research and Development:

Schizophrenia is a chronic brain disease, which imposes one of the greatest burdens on patients, their relatives and public health care. However, the molecular mechanisms underlying the pathophysiology of this disease are poorly defined and the diagnosis is still done by clinical interviews without the assistance of objective biological tests. Nevertheless, neurodevelopmental abnormalities have been considered as part of the pathophysiology of schizophrenia. The project combines an interdisciplinary approach of scientists from Japan and Israel, where the Japan-research group (JPN group) has identified rare missense single nucleotide variants (namely *NDE1*-S214F) in *NDE1* in patients as well as rare exonic duplications in *NDE1* and the small GTPase regulators such as ARHGAP26 and RAPGEF1. The JPN group generated the mutated constructs, expressed them in primary hippocampal neurons and analyzed differential protein complexes from different subcellular fractions by Mass spectrometry. The overexpression and knockdown studies revealed that RAPGEF1 was involved in the dendritogenesis of immature neurons. The expression of constitutive active Rap1 altered the dendritic complexicity. These findings suggest that Rap1 signaling is implicated in the pathology of schizophrenia via neurodevelopemnt. The JPN group found that expression of *NDE1*-S214F allele inhibited axonal outgrowth of hippocampal neurons (Kimura et al, Schizophrenia Bull, 41(3), 2015). To clarify the molecular pathology caused by *NDE1*-S214F allele, the JPN group tried to investigate NDE1-interactome using a NDE1-affinity column chromatography and identified more than 100 of NDE1-interactors. Furthermore, the pulldown samples from the immobilized with wildtype NDE1 or NDE1-S214F protein were subjected to the quantitative mass spectrometry. We found that more than 10 molecules were affected in the binding to NDE1 by the NDE-1S214F mutation.

In this joint project, the Israel-research group (ISR group) employed in the *in utero* electroporation and the genome-editing techniques for studying neuronal proliferation, migration and connectivity. To evaluate the pathophysiological meaning of *NDE1*-S214F expression in the developing brain, ISR group introduced the NDE1-S214F construct by in utero electroporation. The neurons expressing NDE1-S214F were impaired in cortical migration compared to the neurons expressing wildtype NDE1. This result suggests that the *NDE1*-S214F allele is involved in the cortical development. Recently, ISR group generated a knock-in mouse carrying *NDE1*-S214F allele by a genome-editing technique. When ISR group performed the immunoblotings of NDE1 using the brain lysates of wildtype or the mutant mice, there is little difference in the expression levels of NDE1 from both mice. In this collaborative project, we indicated that Rap1 signaling and the NDE1-interactome are implicated in the pathophysiology of schizophrenia.

別紙２

Results report

１．Title of Research and Development: Teleassistance for Seniors with Dementia – A Novel Concept for Safety

２．Principal Investigator: Hirokazu Kato (Professor at Graduate School of Information Science, Nara Institute of Science and Technology)

３．Counterpart Principal investigator: Petri Pulli (Professor at Department of Information Processing Science, Faculty of Information Technology and Electrical Engineering, University of Oulu (Finland))

４．Results of Research and Development:

We designed a tele-assistive system for the elderly with mild dementia so that they can walk outdoor safely by support of remote caregivers through the Internet. In order to realize the system, we focused on three elements as research topics; 1) development of a navigation system for elderly walking, 2) development of a health management system based on biomedical signals, and 3) development of a remote assistive system. These developments have been done through user studies.

1) Development of a navigation system for elderly walking

- Navigation display system with LASER light onto real surfaces

  We have implemented visual information display systems with LASER light onto surfaces by using walking aids or pendant typed wearable devices (Fig. A1, A2). We confirmed that it is not practical under the strong sunlight and it has a possibility to be dangerous due to the elderly behavior with LASER. As a result, we moved on the different method that is the development of smart MEGANE (“Megane” is the Japanese name of glasses).

- Smart Megane with LED lights for navigating the elderly

  We put LEDs on edges of glasses and designed the lighting patterns (Fig. A3). In addition, we re-implement the glasses to reduce the weight (Fig. A4). Through user studies with young and elderly participants, we confirmed that it has possibility to be a navigation system for walking out although there is a necessity of redesigning some lighting patterns. Our system could show that it is one of the methods to support the elderly to walk out safely.

2) Development of a health management system based on biomedical signals

- Sensor vest: wearable biomedical signal sensor

  It is easy to recording electrocardiograms with sensor vest, while conventional electrocardiographs require to attach several electrodes onto the subject bodies. We investigated the qualities of sensing signals from sensor vests, and validated the quality was enough good in standing and lying position (Fig. B). Also, we confirmed that the quality of signal will be declined in walking.

3) Development of a remote assistive system

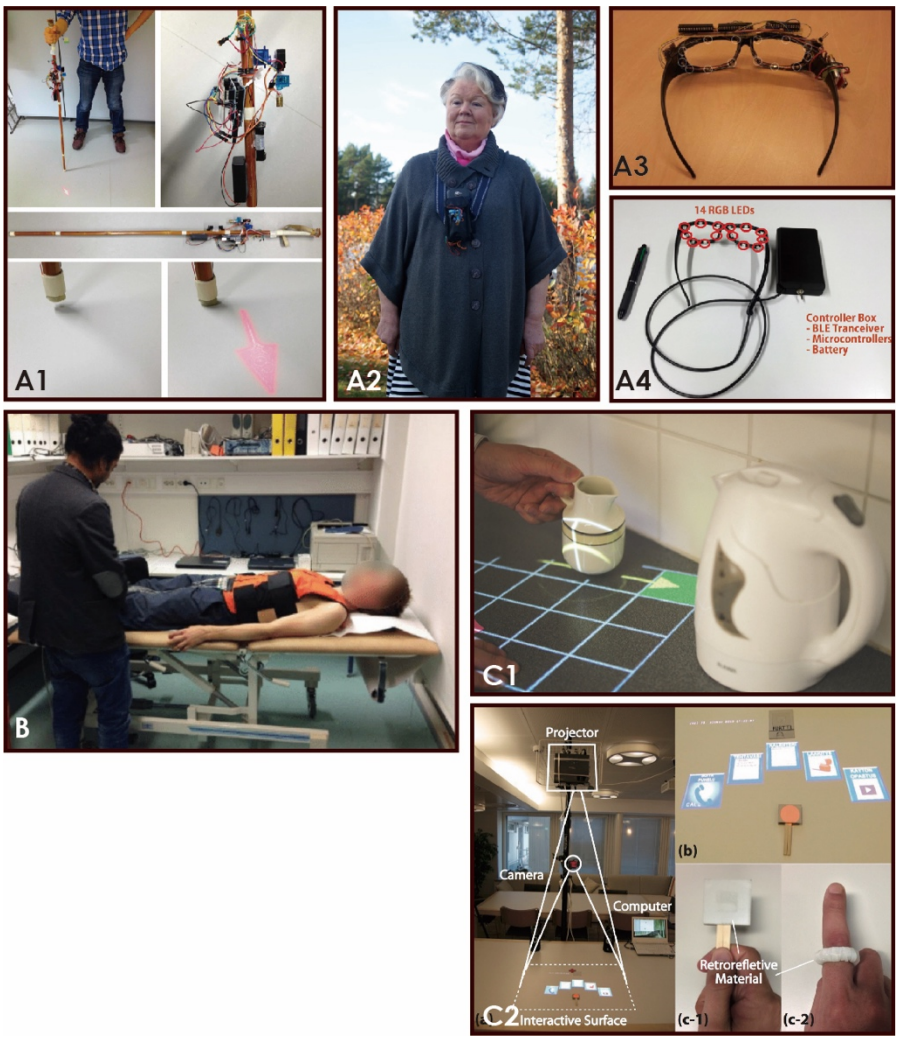
- Projection visual information displays

  We can show visual information onto active spaces of the elderly by using a projector. In this view point, we have developed projection systems for kitchen activity as one of the elderly working location. We have implemented a projection system that displays assistive information from the remote caregivers, and a projection system that provides input interfaces for the elderly to use (Fig. C1, C2).

- Promoting communication with the remote assistance

  We focused on increasing communication which might have possibility to reduce the symptoms of dementia. As our target situation, there is a main task and we were trying to make the elderly speak out by giving

additional information. Then, we tried to find a suitable trigger as additional information, words, through user studies. We implemented an experimental system with several types of words, then conducted user studies with young and elderly participants. As a result, we confirmed that the participants tend to speak out more when showing question form than memorial information.



Our system. A1: Cane-type navigation system, A2: Pendant-type navigation system, A3: Smart Megane (first prototype), A4: Smart Megane (light model), B: Sensor vest, C1: Projection remote assistive system, C2: Projection visual input interface.

In addition to the research and development such as above, research exchange was located important activity in this program. Therefore, we have exchanged some students and researchers every year positively. Also, we collaborate with each researcher and publish/make presentations at some international conferences. We believe we achieved the purpose for research exchange enough. In addition, we could expand our network to connect to other researchers through international conferences.

別紙２

Results report

１． Title of Research and Development：Research and Development of Dependable Wireless Medical Network Using Highly Reliable Body Area Network

２． Principal Investigator：Ryuji Kohno(Professor, Division of Physics, Electrical and Computer Engineering, Graduate School of Engineering, Yokohama National University）

３． Counterpart Principal investigator：Jari Iinatti（Professor, Faculty of Information Technology and Electrical Engineering, University of Oulu (Finland)）

４． Results of Research and Development:

This joint project between Yokohama National University and University of Oulu has promoted various academic and industrial collaboration in interdisciplinary field between medicine and information communication technology (ICT), so-called “Medical ICT.” Major theoretical results are (1) joint optimization of wireless medical network technologies in physical, MAC and network layers such as hybrid ARQ corresponding various QoS requirement of medical data, dependable MAC protocol and (2) dependable wireless remote feedback loop control for medical vital (glucose) sensors and actuators(insulin pump). Major industrial contribution are (3) promotion of amendment of IEEE and ETSI international standards for wireless medical Body Area Netwlork(BAN), and (4) implementation of prototypes of dependable wireless BAN. This project held 2015 and 2016 international symposia on medical ICT in Kamakura, Japan and Boston, USA.

別紙２

Results report

１．Title of Research and Development： THE ROLE OF HIV IN PRE-ECLAMPSIA

２．Principal Investigator：Tadashi Konoshita（University of Fukui Faculty of Medical Sciences, Clinical Professor）

３． Counterpart Principal investigator：Jagidesa Moodley（University of KwaZulu-Natal, Professor（Republic of South Africa））

４．Results of Research and Development:

This collaborative research aimed at evaluation of the effects of HIV infection and genetic variants in system such as the renin–angiotensin system (a very important endocrine system that regulates blood pressure) on pre-eclampsia, a specific model for hypertension which often causes serious symptoms such as convulsion and loss of consciousness and is prevalent in South Africa. Specifically, the Japanese researchers were in charge of provision of techniques and knowledge for assays of genetic variants, and the South African researchers were in charge of provision of subjects suffering from pre-eclampsia and clinical evaluation. By working together, this project contributed to development of a new interventional treatment for pre-eclampsia.

At the beginning 2 years, DNA samples were recruited in the Republic of South Africa. Considering the statistical significance level and power, about 600 blood samples were collected and DNAs were extracted. A South Africa side researcher was invited to Japan side laboratory, and he analyzed 7 renin-angiotensin system genetic variants (renin, angiotensin, ACE, AT1, AT2, CYP11B2 and ENPEP). Without any main trouble, he persuade his task. At the crude analysis step, we obtained some statistical significance results between 2 of the genes and pre-eclampsia. It is for the first time that the 7 genes of the RAS were examined at the same sample series.

On the other hand, we tried to establish a measurement system for one of new component of the RAS. Especially, by monoclonal antibody, a high sensitivity system was aimed. We made hybridomas and screened the activity for the protein. We selected several clones and tried several combinations for sandwich ELISA assay system. Finally we selected one combination of the antibodies. We tested the reproductivity of the assay and optimized the condition. Now we have almost established the new assay system for a new component of the RAS.

We are preparing to present the obtained results at some international congresses and to publish them in some international journals.

別紙２

Results report

１． Title of Research and Development： RNA-based novel approaches for discovery of ALS biomarker

２． Principal Investigator : Shin Kwak（Division of Clinical Biotechnology, Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo, visiting scientist）

３． Counterpart Principal investigator : Erez Levanon (Faculty of Life Sciences , Bar-llan University, principal investigator (Israel))

４． Results of Research and Development:

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease affecting middle-aged individuals in their motor functions and lack of effective therapy leads them to eventual death by respiratory muscle weakness within a few years of onset. More than 90% of ALS occurs in a sporadic fashion, and there are no reliable biomarkers for the disease. Recently it has been demonstrated that deficient adenosine to inosine (A-I) conversion (RNA editing) of pre-mRNA resulting from down-regulation of RNA editing enzyme called adenosine deaminase acting on RNA 2 (ADAR2) occurs in the motor neurons in the majority of patients with ALS in a disease-specific manner. Notably, this molecular abnormality is a cause of both death of motor neurons and TDP-43 pathology, the most reliable neuropathological hallmark of ALS, in the mouse model （ADAR2$^{flox/flox}$/VChAT-Cre, or AR2 mice）mimicking these ALS-specific molecular abnormalities. These lines of evidence indicate that ADAR2 down-regulation is involved in the pathogenesis of sporadic ALS.

Because demonstration of ADAR2 down-regulation in peripherally accessible organs would be a pathomechanism-associated ALS biomarker, we aim to search for a biomarker that reflects down-regulation of ADAR2 in the patients' body fluids. RNAs and proteins are secreted as such or packed in exosomes from individual cells into the body fluids, including blood and cerebro-spinal fluid (CSF) and RNAs in exosomes and circlar RNAs (circRNA) in body fluids are known to be very stable. A-I conversion occurs actively at numerous positions in the mammalian central nervous system and ADAR2 is the major executive enzyme. Down-regulation of ADAR2 in the ALS motor neurons results in the failure of RNA editing at numerous ADAR2-mediated positions in RNAs, including both coding and non-coding regions. If we could detect some of these RNAs with unedited at ADAR2 positions in the body fluids, they likely become biomarkers for sporadic ALS because of the selectivity of ADAR2 down-regulation in sporadic ALS.

Conditional ADAR2 knockout mice（AR2 mice）mimic the molecular abnormalities seen in the motor neurons of ALS patients and exhibit ALS phenotype. ADAR2-lacking motor neurons in the AR2 mice express RNAs devoid of RNA editing at the ADAR2-mediated positions. We therefore started by isolating single motor neurons from AR2 mice and wild-type mice with a laser microdisecter to extract motor neuron-specific RNAs. We dissected more than 2,000 motor neurons from an individual mouse (n=3 for each group). We extracted RNAs from the single motor neuron tissues using the SMARTer Stranded RNA-Seq Kit（Clontech Laboratories, Inc）to prepare samples for RNA-seq.

After removing all variants present in catalogues of DNA differences in SNP databases, those caused by sequence mapping artifacts such as read alignment across splice junctions, gene duplications, and homopolymer stretches and rare SNPs, research collaborators in Israel scrutinized the RNA-seq data on A-I positions. We then searched

for ADAR2 positions among these A-I positions and selected candidate RNAs by tentative criteria that the proportion of edited RNA was 1) more than 10% of the transcript in the wild-type mice and 2) significantly lower in the AR2 mice than in the wild-type mice.

We then searched for the human homolog of these candidate mouse RNAs and tested whether human homolog of RNAs have ADAR2 positions in the human-derived cultured cells by siRNA knockdown and overexpression of ADAR2. We are now examining the presence of these candidate RNAs in human body fluids.

別紙２

Results report

１．Title of Research and Development： Neural circuit mechanisms of reinforcement learning

２．Principal Investigator：Kenji Morita （Graduate School of Education, The University of Tokyo, Lecturer）

３．Counterpart Principal investigator：Abigail Morrison （Jülich Research Centre, Professor（Germany））

４．Results of Research and Development:

(1) Models of corticostriatal CCS and CPn cells

Estimations of the parameters of the computational models of crossed-corticostriatal (CCS) and corticopontine (CPn) cells (on the NEURON simulation environment) based on the detailed anatomical and physiological data (obtained by Dr. Morishima and Dr. Kawaguchi) have been done by Dr. Kondo, and parameter sets that could reproduce the experimental results have been obtained. Then, using the models with the obtained parameter sets (two for each cell type), simulations of the cases where the cells receive synaptic inputs that oscillate as a population with various frequencies have been done by Dr. Kondo, and conditions for the generation of "doublets" (two action potentials with short intervals) have been clarified.

(2) Model of cortico-basal ganglia circuit

Simulations of the cases where the direct or indirect pathway of the basal ganglia is blocked in the cortico-basal ganglia circuit have been elaborated, and it has been shown that the hypothesis that these two pathways represent reward predictions with time difference (Morita et al., 2012, Trends Neurosci) could account for the experimental results that the blockade of the direct or indirect pathway respectively impaired learning based on positive values (or prediction errors) and the initial phase of reversal learning, which is considered to be based on negative prediction errors. The manners the blockade of each basal ganglia pathway relates to addiction have also been examined through simulations, and it has been shown that these manners can significantly depend on the degree of exploration (over exploitation) upon choice.

(3) Model of the temporal changes of dopamine concentration

Model of the cortico-basal ganglia circuit implementing reinforcement learning with the assumption that dopamine represents reward prediction error has been elaborated by incorporating the assumption that plastic changes of the strengths of cortico-striatal synapses decay in time (by Kato and Morita). It has been shown that various temporal patterns of dopamine signals can be reproduced by the model. Also, based on simulations and analyses of the model, we have proposed a circuit/synapse-level mechanism of the ever-suggested link between dopamine and motivation (paper under review).

(4) Explorations of integrative mechanisms

Reviews of the studies that have been conducted by the Japanese and German groups, including the abovementioned studies, and also related studies done by other researchers on the neural circuit mechanisms of reinforcement learning, have been done by Morita and the member of the German group (Dr. Morrison and Dr. Jitsev) with a special focus on value-based action selection (decision making), and have been published as a joint review article. In the article, we have proposed hypotheses on how reinforcement learning algorithms (Q-learning and SARSA) can be implemented in the neural circuits, as well as on potential computational roles of the complex patterns of cortical and striatal neural activity suggested in recent experimental and theoretical studies.

別紙２

Results report

１． Title of Research and Development：Protective and subversive mechanisms of macrophage genes in Mycobacterium tuberculosis infection

２． Principal Investigator：Harukazu Suzuki (Center for Life Science Technologies, Group Director)

３．Counterpart Principal investigator : Frank Brombacher（University of Cape Town, Professor （South Africa））

４． Results of Research and Development:

This research aims at identification and analyses of macrophage genes that work protective or subversive in Mycobacterium tuberculosis (Mtb) infection. For this purpose, we compared gene expression dynamics of macrophage in Mtb infection with that of cytokine-induced macrophage activations. The main experimental task of both parties were selection of candidate genes using already obtained high quality transcriptome data (Japan), Creation of the constructs for the perturbation experiments (Japan), gene expression analysis of the perturbation samples (Japan), Mtb infection experiments using macrophages and experimental mice (South Africa) and preparation of samples for the gene expression analysis (South Africa). Because we have already had a great progress in our analysis, we focused on writing papers as much as possible in the last fiscal year, with minimum additional wet experiments.

In the stimulation by pathogen infection including Mtb, the activated macrophage cells, as well as classically activated one, express various host protective factor genes involved in inflammation, such as *Tnf*, etc. We successfully identified that Batf2 is a novel transcription factor (TF) involved in gene expression of those factors. In addition, we found that Batf2 associates with Irf1, another TF induced by IFNg, and induces inflammatory genes, which was published in *J. Immunology*. Further, including the above results, we have published a review article in *Oncotarget*. Furthermore, in vivo functional analysis of Batf2 is going on using the Batf2 KO mice. We have already obtained interesting results for the Mtb infection response in the KO mice and aim at publication in earlier timing.

We also analyzed transcriptional regulation dynamics of classical and alternative activations in macrophage cells. Surprisingly, the analysis revealed that almost same set of transcription factor binding motifs are involved in both activations, although those two activations shows quite different features. The results suggest that same TFs are involved in the different activations. Further, using the comprehensive promoter-level expression profiles, we successfully identified novel TF genes, peripheral genes and lncRNAs that are considered to play important role in those activations, which was published in *Nucleic Acids Research*. In addition, we explored effect of the alternative activation to Mtb infection by using the IL4 receptor KO mice that does not occur alternative activation. We found that alternative activation in macrophage cells has almost no effect in Mtb infection, which was published in *PLosONE*.

In addition to the above achievement, we are analyzing large scale time course expression data in Mtb-infected macrophages, which was obtained by the deepCAGE method. Because we have almost completed the analysis, we aim at the paper publication in earlier timing. Further, we found that Batf, a family TF of Batf2, is also transiently induced in pathogen (Mtb)-infected macrophages. We are now analyzing the function of Batf in Mtb infection. Finally, although we had a plan to have a workshop with the collaborator in the last half of the fiscal year, it was not held. It is because we decided in our discussion that we took paper publication work as our first priority.

別紙２

Results report

１．Title of Research and Development：Development of A Comprehensive System for Assessment of Sarcopenia, Osteoporosis and Joint Dysfunction

２．Principal Investigator：Masaki Takao（Associate Professor, Department of Orthopaedic Medical Engineering, Osaka University Graduate School of Medicine, Japan）

３．Counterpart Principal investigator： Guoyan Zheng（PrivatDozent, Institute for Surgical Technology and Biomechanics ,University of Bern（Switzerland））

４．Results of Research and Development:

This project aims to develop a comprehensive system to assess musculoskeletal conditions of high-risk elderly patients of mobility loss, fall and hip fracture by considering osteoporosis, sarcopenia and joint dysfunction simultaneously. Patients with hip arthritis or with femoral neck fracture have been recruited by the Japanese team. 2D X-ray images and 3D QCT of patients' hips have been acquired. The Japanese team managed to develop automatic system to extract 3D musculoskeletal models from the 3D data. The Swiss team has been developing a system for biomechanical hip modeling and for reconstructing 3D musculoskeletal models from 2D X-ray images.

In this fiscal year, Japanese team managed to develop statistical model of insertion of muscle fibers on the pelvic and femoral bones. The team consisted of a medical team from Osaka University and an engineering team from Nara Institute of Science and Technology. Last fiscal year, we had performed a preliminary cadaver study in Singapore to get the three dimensional data of muscle insertion using our original computer navigation system. We improved the computer navigation system and revised the experiment protocol. In January, 2016, we performed another cadaver study in Johns Hopkins University using 8 cadavers. We developed statistical atlas of muscle insertion area on the pelvic and femoral bones. We could not perform prospective clinical study to predict falls from 3D musculoskeletal model due to delay in developing automatic segmentation system of musculoskeletal models from 3D CT data. On the other hand, we performed a retrospective study to develop a large-scale database of 1142 patients with hip disorders including radiographs, 3D-CT data and operation data acquired using navigation system. The NAIST engineering team developed automatic matching system between 2D radiographs and 3D CT data of pelvic and femoral bones. Using the software, Osaka University medical team analyzed pelvic flexion in supine and standing positions in 474 patients.

Swiss engineering team of Bern University has succeeded in reconstruction of 3D pelvic model from 2D X-ray images using dataset from our database. By applying automatic planning system of total hip arthroplasty to their system, they succeeded in automatic cup implant planning based on 2D/3D radiographic pelvis reconstruction (Schumann S, 2015). In addition, they reported that they succeeded in reconstruction of muscle model of rectus femoris from 2D-Xray images.

On August 29th in 2015, we held mini symposium entitled "International Symposium on Musculoskeletal Simulation – from Big Data to Robot-assisted Rehabilitation" in Osaka University Nakanoshima center. We reported our achievements each other and discussed the future direction of combined research. This symposium was open and the number of participants was 32.

Reference: Schumann S, Sato Y, Nakanishi Y, Yokota F, Takao M, Sugano N, Zheng G. Cup Implant Planning Based on 2-D/3-D Radiographic Pelvis Reconstruction-First Clinical Results. IEEE Trans Biomed Eng. 2015 Nov;62(11):2665-73.

別紙２

Results report

１． Title of Research and Development : Genetic and pathogenic diversity of *Pantoea ananatis* strains

２． Principal Investigator : Yuichi Takikawa （Professor, Graduate School of Science and Technology, Shizuoka University）

３． Counterpart Principal investigator : Teresa A. Coutinho （Professor, Microbiology and Plant Pathology, Natural and Agricultural Sciences, University of Pretoria（South Africa））

４． Results of Research and Development:

This research aimed to clarify the genetic and pathogenic diversity of *Pantoea ananatis* and to develop a method identifying the pathogenic strain infecting important crops such as rice, corn and onions, in collaborative work between Japanese and South African researchers.

We had revealed the presence of a new pathogenicity determining genetic locus in *P. ananatis*, which was named as PASVIL (*P. ananatis* specific virulence locus). RT-PCR techniques were applied to determine the operons in PASVIL. More than 3 operons having 16 ORFs could be identified and the putative promoter regions were suggested. Introduction of the entire PASVIL region to a non-pathogenic *P. ananatis* strain lead to acquisition of pathogenicity on tobacco and onions. Using this PASVIL introduction system together with in vitro transposon insertion technique revealed that not the all ORFs in PASVIL were required for pathogenicity but some were dispensable. Construction of a plasmid vector for site-directed mutagenesis enabled us to disrupt any genes and to assay their contributions to pathogenicity. Some PCR primer sets could detect PASVIL and its homologue in *P. ananatis* and *P. agglomerans* strains. These primers were considered as very useful tools for rapid detection of pathogenic strains in *Pantoea* without time-consuming bioassay methods. A PASVIL homologue found in *P. agglomerans* has ca. 80% sequence homology with PASVIL of *P. ananatis*, suggesting that considerable time has passed since the horizontal transfer of PASVIL took place between theses two species.

The Type VI protein secretion system gene sets had been also identified in the genome of P. ananatis strains. The significance of the Type VI secretion systems in plant pathogenesis was assayed by disrupting the genes using site-directed mutagenesis. Our study revealed that the disruption of Type VI secretion system of *P. ananatis* strain isolated from rice in Japan did not abolish its pathogenicity, but significantly reduced its ability to compete with other bacteria when they were co-cultivated. In contrast, the Type VI secretion system of P. ananatis strain isolated from onion in South Africa was shown to be indispensable for pathogenicity. These results indicated that the significance of the Type VI secretion system depends on bacterial strains used and that the system might not serve as an index of plant pathogenicity.

In tobacco plants, expression of some resistance-related genes of plant was assayed after injection of the pathogenic strain of *P. ananatis*. RT-PCR analysis demonstrated that the kind of genes induced was similar to that induced in hypersensitive reaction (HR), but the induction was delayed by ca. 12 hours compared to HR. At the same time, PASVIL of *P. ananatis* was shown to be strongly induced when the bacterium was introduced into tobacco tissues.

Phylogenetic study on *Pantoea* spp. revealed their core genome and specification of each species. Even in *P. ananatis*, there is significant diversity among the strains and the pathogenic strains were shown to be scattered in diverse groups.

In conclusion, we revealed the pathogenicity determinants and diversity of *P. ananatis*, and developed a method to discriminate its pathogenic population.

別紙２

Results report

１． Title of Research and Development： Mechanism of circadian clock based on clock genes

２． Principal Investigator：Name　Toru Takumi（Senior Team Leader, RIKEN Brain Science Institute）

３． Counterpart Principal investigator：Name　Grigory Bordyugov（Research Scientist, Institute for Theoretical Biology, Charite（Germanry））

４． Results of Research and Development:

The physiological and behavioral rhythms of all life on earth are bound to the earth's rotational cycle of ~24 h. This fundamental rhythm is also affected by the planet's slanted rotational axis, which causes seasonal variations in the length of the day. How life has adapted to anticipate this yearly rhythm is still debated. The mammalian suprachiasmatic nucleus (SCN) forms not only the master circadian clock, but also a seasonal clock. This neural network of ~10,000 circadian oscillators encodes season-dependent daylength changes through a largely unknown mechanism. We show that region-intrinsic changes in the SCN fine-tune the degree of network synchrony, and reorganize the phase relationship among circadian oscillators to represent daylength. We measure oscillations of the clock gene *Bmal1*, at single-cell and regional levels, in cultured SCN explanted from animals raised under short or long days. Coupling estimation using the Kuramoto framework reveals that the network has couplings that can be both phase-attractive (synchronizing) and repulsive (desynchronizing). The phase gap between the dorsal and ventral regions increases, and the overall period of the SCN shortens with longer daylength. We find that one of the underlying physiological mechanisms is the modulation of the intracellular chloride concentration, which can adjust the strength and polarity of the ionotropic γ-aminobutyric acid receptor ($GABA_A$) mediated synaptic input. We show that increasing daylength changes the pattern of chloride transporter expression yielding more excitatory GABA synaptic input, and that blocking $GABA_A$ signaling or the chloride transporter disrupts the unique phase and period organization induced by the daylength. We test the consequences of this tunable GABA coupling in the context of excitation-inhibition balance through detailed realistic modeling. These results indicate that the network encoding of seasonal time is controlled by modulation of intracellular chloride, which determines the phase relationship among and period difference between the dorsal and ventral SCN.

別紙２

Results report

１．Title of Research and Development：Evaluation of different extracts and isolated compounds from Brazilian Propolis in obesity and diabetes via in vitro and in vivo assay.

２．Principal Investigator：Je Tae Woo　（Department Biological Chemistry, Chubu University; Professor）

３．Counterpart Principal investigator：Jairo Kenupp Bastos（Pharmaceutical Sciences, University of São Paulo; Professor（Brasil））

４．Results of Research and Development:

Propolis is widely used as a functional food and its various physiological activities are also being accumulated for scientific evidence. We have reported that one of the cinnamic acid derivatives, contained in the characteristic of Brazilian green propolis, improves insulin resistance in the cellular level and in the animal level. However, there are still questions about the detailed mechanisms of its action. In addition, it is desired to improve bioavailability of propolis products for the practical use.

The main objective of our research was to develop a new functional food that prevents and/or improves obesity and diabetes. Lipid nanoparticles of Brazilian green propolis formulations were made for improving in vivo absorption and then the stability and sustained release were evaluated.

Secondly, as our second objective, in order to obtain scientific evidence of the Brazilian green propolis and develop it as a high value-added product, we screened new functions of the ingredients of Brazilian green propolis in cell culture systems. As a result, we found a novel effect of cinnamic acid derivative compounds from Brazilian green propolis on adipocyte and pancreatic β cells, which might reveal a part of the mechanism of antidiabetic action.

This Japan-Brazil joint project promoted propolis research for scientific evidence and contributed in training for international young researchers by collaborating with foreign scientists.

別紙２

Results report

１．Title of Research and Development : Late Life Depression: Molecular Basis and Neural Networks

２． Principal Investigator：Shigeto Yamawaki（Professor and Chairman, Department of Psychiatry and Neurosciences, Hiroshima University）

３．Counterpart Principal Investigator：Bernard Lerer（Professor of Psychiatry, Director Biological Psychiatry Laboratory, Hadassah- Hebrew University Medical Center（Israel））

４．Results of Research and Development

The principal objective of this project is to gain an understanding of the neurobiological basis of late life depression (LLD), a serious and highly prevalent age dependent psychiatric disorder. Individuals with LLD experience greater functional disability and cognitive decline than similarly aged people without depression and greater morbidity and mortality from medical illness. When compared with early-onset MDD, LLD is associated with a higher prevalence of dementia. Because of increasing lifespan in Japan and Israel the social and economic cost of LLD is extremely high and rising. We hypothesized that an interaction between exposure to chronic stress, brain white matter damage and age related changes underlies the development of LLD. In the current project we are testing our hypotheses by a systematic series of experiments in mouse models and by translational studies in humans. The Japanese group is assessing brain structure and function in individuals with major depression and healthy controls using MRI in conjunction with online-behavioral tasks and measures of chronic stress. We are also evaluating biomarker profiles in human blood samples from these subjects. Thus far we have examined resting-state functional magnetic resonance imaging (fMRI) activity in hippocampus and serum concentrations of BDNF in 12 patients with MDD and 14 healthy controls (HCs). To model effects of age, the participants were classified as elderly (age $\geq$50) including 6 MDD and 7 HCs and younger age group including 6 MDD and 7 HCs. Preliminary analysis revealed that patients with depression exhibited significant decreases of low frequency fluctuation in the hippocampus bilaterally. Among the patients with MDD, the serum concentration of BDNF was positively correlated with low frequency fluctuation in the right hippocampus suggesting a protective effect of BDNF on the hippocampal dysfunction. Further data collection and analyses are necessary to examine the age related changes. The Israeli group is employing mouse models that explore the interaction between age and exposure to chronic stress. To model effects of age they are studying young vs. old male and female mice. In the experiment completed thus far young and old female mice were exposed to chronic stress for 5 weeks and then evaluated with extensive behavioral testing of cognitive, depressive and anxious phenotypes. Clear evidence has emerged for a differential effect of stress on young and old mice, particularly with regard to tests that reflect hippocampal function. They found that stress adversely affects cognitive function in old mice while the performance of young mice is actually enhanced. There was a similar differential effect of stress on anxiety, which showed the young but not the old mice to be resilient to stress. They have obtained brain tissue samples from these mice and these are being examined by Japanese group for biomarker correlates of the behavioral effects in order to determine the mechanisms underlying the differences between young and old mice in their response to stress. At this stage of the project the correspondence of hippocampal-related findings in the animal and in human studies is intriguing as is the potential role of BDNF which will be examined together with other biomarkers in both the animal and human components of the research.

別紙２

Results report

１． Title of Research and Development： The influence of feature salience over microsaccades in normal and blindsight humans and monkeys: an experimental and theoretical investigation

２. Principal Investigator：Masatoshi Yoshida（Assistant Professor , National Institute for Physiological Sciences）

３. Counterpart Principal investigator：Ziad M. Hafed（Junior Research Group Leader, Eberhard-Karls-Universität Tübingen, Werner Reichardt-Centrum für integrative Neurowissenschaften (CIN)（Germany））

４． Results of Research and Development:

Microsaccades are tiny saccadic eye movements that take place during periods of gaze fixation. Even though the detailed mechanisms for microsaccade generation, as well as their functional role in vision, are not fully understood, recent research has revealed that these eye movements can act as an important proxy for larger saccades in understanding several aspects of perception and cognition. One such aspect concerns the relationship between selective visual attention and eye movements, in which it was shown that microsaccades, in contrast to their classic description as random and spontaneous, are related to attention. The neural mechanisms for such a relationship are not fully understood, and its characteristics in pathological states of vision are completely unexplored. The purpose of the proposed research is to understand how feature salience that is commonly included in models of selective visual attention can influence microsaccades.

1. Animal experiments

Monkeys were trained to make a fixation to a spot on a display. By using retinal stabilization, we examined how a peripheral cue affects microsaccades. We also tested with a free-viewing experiment in which normal monkeys and monkeys with lesion in V1 or the superior temporal gyrus viewed movie clips. We found that the number of saccades toward the affected hemifield was reduced in the V1-lesioned monkeys.

2. Human experiments

Subjects with normal vision participated in a Posner task in which a pre-cue is presented and a saccadic target appeared immediately after that. When the time difference between the cue and the saccadic target was varied, the saccadic reaction time was modified (inhibition-of-return (IOR) effect). We analyzed microsaccades during the trials and found evidence that the rhythmic process under microsaccade generation can explain the IOR effect.

In a free viewing task, one subject with a damage in V1 and six control subjects participated in the experiment. We measured eye movements during the free-viewing task and a fixation task with the same movie clip and found that the direction of microsaccades during the fixation task is anti-correlated with the direction of saccades during the free-viewing task.

3. Neural network model

We constructed computational models of the superior colliculus based on a neural-field model and a spiking-neuron network model. We found that the models worked as a saliency detector and are able to replicate the effect of saliency on microsaccades (published in Frontier is Systems Neuroscience 2016). Based on these finding, we constructed a model about neural network of saliency computation and proposed the model in a reviw paper submitted to the theme issue "Auditory and Visual Scene Analysis" of Philosophical Transactions B.

別紙２

Results report

１．Title of Research and Development：

Genomics, bioinformatics and systems medicine to facilitate therapy of ovarian cancer

２．Principal Investigator： Johji Inazawa, Professor, Tokyo Medical and Dental University

３．Counterpart Principal investigator：Olli P. Kallioniemi, Professor, Institute for Molecular Medicine Finland, University of Helsinki

４．Results of Research and Development:

Ovarian cancer has a poor prognosis and a high mortality rate. Finland has a relatively high morbidity of ovarian cancer. On the other hand, Japan and other Asian countries have a high morbidity of platinum-resistant ovarian cancer including clear cell carcinoma. Identification of new therapeutic targets is critical for the implementation of personalized and effective ovarian cancer treatment.

The aim of our study is the establishment of the ovarian cancer therapy by exploring new drugs and including known molecule targeted drugs, that is, drug repositioning (DR). The results of our study of 2016 are as follows.

1. International collaboration of biobank in gynecological cancer

Finland has established biobank facilities which link medical records under the biobank law. In 2015, the Japanese group visited Auria biobank (Turku), and Helsinki biobank (Helsinki) to participate the conference on international joint research.

2. The understanding of ovarian cancer cell system with bioinformatics and identification of candidate targets for ovarian cancer therapeutics.

Japanese and Finnish groups have been detecting intracellular molecular changes occurring through the pathway in ovarian carcinogenesis, which may lead to drug repositioning (DR).

3. Genome analysis on ovarian cancer

Genome analysis was performed in ovarian cancer tissues collected in Tokyo Medical and Dental University Bioresource Research Center and Keio Women’s Health Biobank (KWB) to explore genomic alterations specific for ovarian cancer.

4. Primary culture for gynecological cancer and drug sensitivity and resistant testing

Primary culture with tissues and ascites from ovarian cancer patient was performed to detect *ex vivo* drug sensitivity of the cells.

5. Analysis for the ovarian cancer pathogenesis through impaired autophagy

P62 protein expression, which is regarded to the hub of autophagy function, was analyzed in ovarian cancer.

6. Ovarian cancer metabolome analysis

Possible involvement of metabolic alterations in refractory ovarian cancer pathogenesis was discussed.

7. Japanese - Finnish symposiums

Members from both countries participated in the meeting of Japan-Finland Cooperative Scientific Research as part of the FY 2015 Strategic International Research Cooperative Program (SICP) at Sapporo in Japan. A Finnish leader, Professor Olli Kallioniemi showed Finnish Biobamk system and its utility in precision medicine in Finland as a presentator at the 34th International Cancer Symposium held at Sapporo (25-27 June 2015).

Results report

1. Title of Research and Development: Testing computational models of learning from social, real, and fictive feedback in human and nonhuman primates.

2. Principal Investigator: Masaki Isoda (Associate Professor; Department of Physiology, Kansai Medical University School of Medicine)

3. Counterpart Principal investigator: Markus Ullsperger (Professor; Department of Neuropsychology, Faculty of Natural Sciences, Otto-von-Guericke Universität Magdeburg (Germany))

4. Results of Research and Development:

The goal of this consortium is to develop computational models of learning and decision making and to test them in two biological systems, humans and macaques. In fiscal year 2015, the Japanese team studied activity of single dopamine neurons in the midbrain of monkeys performing a socially-oriented, Pavlovian conditioning paradigm, which allowed us to study the neural mechanisms underlying learning from real and social feedback. In the paradigm, two monkeys facing each other were presented with a conditioned stimulus (CS) that differently signaled the probability of each monkey's upcoming reward. The reward feedback was given first to the nonrecorded animal (designated as *other*) and, 1 s later, to the recorded animal (designated as *self*). The monkeys were alternately conditioned in two contextually different trial blocks: i.e., one block in which the other's-reward probability was variable depending on the CS and the self-reward probability was constant, and another block in which the self-reward probability was now variable and the other's-reward probability was constant. Consistent with previous studies, the animals placed higher value on the CS predicting a higher probability of *one's own* reward. Interestingly, the animals placed lower value on the CS predicting a higher probability of *other's* reward. Thus, the prospect of others' reward profoundly affected the value of one's own reward. Single-unit recording was then made from presumed dopamine neurons (n = 229) in the substantia nigra pars compacta and the ventral tegmental area. About half of the neurons (n = 115) showed a significant positive correlation between the phasic CS response and the self-reward probability. Among them, the activity of 80 neurons was not influenced by the other's-reward probability, while that of 35 neurons additionally showed a significant negative correlation with the other's-reward probability as if representing the subjective value. There were few neurons that selectively encoded the other's-reward probability. Crucially, however, dopamine neurons as a population became more discriminative for the other's-reward probabilities as they discriminated more clearly between the self-reward probabilities. These findings suggest that dopamine neurons incorporate self-reward information and other's-reward information in a single value scale. This is in sharp contrast to firing properties of medial prefrontal neurons, which distinctly encoded the self-reward information or other's-reward information. Our data demonstrate that midbrain dopamine neurons and medial prefrontal neurons participate in different aspects of learning from real and social feedback.

Meanwhile the German team developed a social instrumental learning task enabling to compare learning from experienced outcomes of own actions and observational learning from outcomes observed for actions taken by another player. With appropriate simplification this task can be easily transferred to a setting allowing to study observational learning in macaques. Participants were required to learn the stimulus-outcome contingencies for an adapted three-armed bandit task. In each block, their aim was to learn over the course of 30 trials, which of three stimuli would most often lead to a positive outcome. The participants would play together, with the acting and observing player switching roles regularly. A first task version using blockwise role switches successfully demonstrated the ability of participants to learn from own action outcomes as well as from observation. However, the block structure caused difficulties in modelling the behaviour with reinforcement learning models. Therefore, role switching every 1-3 trials was introduced to engage the participant during the observation stage more fully than the previous task structure. It was also expected to ease modelling and be more suitable for a follow-up experiment using fMRI than the former design. Sixty-four channel EEG was recorded from both participants as they performed the task. An adapted Q-learning algorithm was fit to participants' choices in this task. Comparable model estimated learning rates were obtained for trials in which the same player acted consecutively, relative to when players switched from an observing to an acting role. This suggested that participants used similar computational algorithms to weight the outcomes they received from making and observing choices on each trial. Model-based single-trial EEG analysis using multiple robust regression revealed that reward prediction errors were represented in the feedback-related negativity (FRN) around 280 ms after the feedback. Furthermore, P3a and P3b amplitudes correlated with the experienced or observed outcomes. The role (actor/observer) had a sustained positive-going effect at centroparietal electrodes from 200-600 ms such that experienced outcomes elicited a generally more positive event-related potential. Moreover, the factors role and prediction error interacted significantly at the latencies of the FRN and P3b, such that both potentials were larger when participants experienced outcomes of their own actions. These findings suggest that while the general learning mechanisms are similar for own and observed action outcomes, own experiences have a stronger impact on the participants. Based on these findings the computational model will be modified to allow differential learning rates for own and observed action outcomes. In summary, the newly developed task has proven well-suited to study observational learning and can now be transferred to animal research and functional magnetic resonance imaging in humans. Moreover, it will be modified to investigate the influence of social context, for example competitive vs. cooperative settings.

別紙２

Results report

１． Title of Research and Development： Autonomous Learning of Active Depth Perception: from Neural Models to Humanoid Robots

２． Principal Investigator：Sungmoon Jeong (School of Information Science, Assistant Professor, Japan Advanced Institute of Science and Technology

３． Counterpart Principal investigator：Jochen Triesch (Department of Neuroscience, Professor, Frankfurt Institute for Advanced Studies, Germany)

４． Results of Research and Development:

During the first few months of their postnatal development, humans and other animals autonomously learn how to use such depth perception cues like disparity, motion parallax and optical flow. Thereafter, they continue to adapt and self-calibrate their vision to compensate for growth of the eye, head, and body, but the underlying neural mechanisms are still largely unknown. Providing robots with similar abilities to autonomously learn and self-calibrate sensori-motor loops for active perception would make them more autonomous and robust. Therefore, in this research period, we proposed a system for the autonomous self-calibration of active depth perception based on motion parallax using a single moving camera. Our system is based on active efficient coding (AEC) framework for the autonomous self-calibration of active perception which autonomously learns to represent image motion and perform compensatory eye rotations to keep the object fixated during lateral movement − thereby learning to actively estimate the object’s distance.

First, to validate our research, we implemented an active binocular vision hardware framework by using two dimensional linear actuators to realize human’s body movements and the two camera with pan-tilts unit for mimicking the eye’s rotation. Second, the new cost function was designed by considering the active efficient coding theory with intrinsic motivation concept to simultaneously train eye rotation (action) and sensory representation (perception) of the autonomous robot under the motion parallax phenomena. The intrinsically motivated visual system can generate a suitable eye movement to increase the redundancy between successive images for understanding of the motion parallax phenomena. Third, the generated eye movements was transferred to the input of the depth estimator as a goal-directed visual learning system to autonomously estimate the depth between the observer and an arbitrary object. An autonomous depth estimator was developed by two layer feed-forward artificial neural network to map between the eye movements and the object’s distance.

Finally, we had validated our proposed model by using computer simulation and real robot experiments. The motion parallax based self-calibrated visual framework achieved good results with less than 0.1 estimation error of eye rotation degree in the computer simulation and 0.05 estimation error in the real robot experiments. And also, it can estimate accurate depth information from eye movements with less than 7% depth estimation error in the computer simulation and less than 3% depth estimation error in the real robot experiments. Moreover, the proposed framework can successfully estimate depth and generate eye movements to keep the object at the center of gaze by autonomous self-calibration when we apply a perturbation to the system.

別紙２

Results report

１． Title of Research and Development：

Decoding of in vivo two-photon imaging data in mouse motor cortex.

２． Principal Investigator：

Yukiyasu Kamitani, Professor, Graduate School of Informatics, Kyoto University, Japan.

３． Counterpart Principal investigator：

Takashi Sato, Junior group leader, Center for Integrative Neuroscience, University of Tübingen, Germany.

４． Results of Research and Development:

We performed multi-variate pattern analysis to two-photon microscopy data that were obtained by Dr. Sato's group in Germany. In our preliminary analysis before 2015, we observed that mice performed a movement task more quickly when the motor cortex exhibited sparse and stable neural activity. In 2015, we obtained data from multiple mice for quantitative analysis with additional experiments where excitatory and inhibitory cells were recorded separately. In the first analysis, we confirmed the reproducibility of the results on additional six mice. In the second analysis, the similar tendency was observed for both excitatory and inhibitory cells in terms of the stability and sparseness of motor cortical activity.

To conduct the above analyses in a tight collaboration with Dr. Sato's group in Germany, a researcher in Dr. Kamitani's group visited Dr. Sato's laboratory in University of Tübingen in February. As a result, we performed the analysis work efficiently, and enabled the members in Dr. Sato's group to do preliminary analyses in their own side by providing a set of computer programs for the analysis made in Dr. Kamitani's group.