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研究課題名（和文）Shedding Light on the Role of Glia in Autism Spectrum Disorders(ASD) via Multifunctional Fibers

研究課題名（英文）Shedding Light on the Role of Glia in Autism Spectrum Disorders(ASD) via Multifunctional Fibers

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研究成果の概要（和文）：脳の中には、神経細胞やグリア細胞があり、それらによって複雑な回路が形成されて様々な信号が細胞間で伝達されている。これまで、脳機能のメカニズムについては神経細胞を中心に研究されてきたが、最近の研究ではグリア細胞が脳の正常活動に重要な様々な役割を担っていることが報告されている。しかし、グリア細胞がやり取りするのは繊細な化学信号だけであり、それらの活動を精密に記録できる新たな技術 が必要である。本研究は熱延伸技術で作製された多機能ファイバと、イメージセンサを組み合わせることで、生体埋め込み型の 新しいpH可視化プローブを開発し、脳内の複数点においてpH変化を同時に高感度で測定することを可能にした。

研究成果の学術的意義や社会的意義
新規開発したオールインワンプローブ型のイオン可視化ツールは、多機能ファイバとバイオセンサを組み合わせることにより、多機能ファイバ本来の機能にイオンイメージング機能を付与し、ファイバや半導体センサ単体だ けでは実現できない飛躍的な機能を集積した発明になります。この pH 可視化プローブを利用し、世界で初め て、脳深部において、細胞や組織を蛍光標識することなく、pH 変化をリアルタイムで可視化することに成功し ました。 本研究で開発したイオン可視化ツールは、斬新な脳機能研究を発展させることができ、これまでに解明されてな い病気の原因や治療法の研究において重要な貢献になると考えています。

研究成果の概要（英文）：The cells -neurons and glial cells- inside of our brain communicate via chemicals as their languages, however we are far away from understanding it, to unravel the complexity of the brain in health and disease. Here we developed a new type of brain implants based on flexible and multimodal fibers for reading brain chemicals. In addition to the conventional electrical recording and optical delivery capabilities, we stretched the functional boundary of fiber-based neural probes with the multiplexed sensing of intrinsic brain chemical signals, such as pH. Such probe is based on a multimodal fiber coupled with biochemical imaging sensors - light-addressable potentiometric sensor (LAPS).
Such effort in innovating fiber-based chemical sensing may open new possibilities of a new class of in vivo multiplexed chemical sensing and imaging technologies, allowing for investigating intrinsic chemical signals in vivo with behaviors in unprecedented details.

研究分野：医工学

キーワード：chemical sensing　pH microscope　multifunctional fibers　ASD

１．研究開始当初の背景

In-brain chemistry actively regulate brain functions from cellular to circuit, system and behavior level in health and disease. The imbalanced chemical releases are correlated with various brain disorders, among which, neurodevelopmental disorders, such as autism spectrum disorders, are highly associated with the chronically imbalanced chemical release from neuron or glial cells in regulating synaptic formation and function. But the detailed mechanisms are poorly understood due to the lack of available technological tools to study intrinsic chemical release in vivo correlating with circuit dynamics and behavior. In this research, We aimed to further incorporate the biochemical sensing into multifunctional fibers for in vivo neurochemistry together with electrical and optical functions. Impacts will be towards understanding brains in both health and disease, particularly ASDs, to ultimately develop new therapeutic targets.
Combining such multifunctional fiber platform with transgenic mice models with light-sensitive opsins expressed specifically in astrocyte, comprehensive investigation of astrocytic roles in ASDs becomes possible through optical manipulation of astrocyte, chemical sensing, and electrical recording of neuronal activities from cellular, circuit to behavior associated with autism, seizures and other brain disorders.

２．研究の目的

This research aims to innovate multifunctional fibers, which integrate novel biochemical sensing components together with electrical and optical functions into a single flexible polymer fiber based neural probe. Such probes allow us to evaluate the chemical dynamics in brain mediating electrical response of neurons at local and circuit level underlying various brain disorders such as anxious phenotype of the ASDs or seizure models.

３．研究の方法

In this research, we were able to develop a new class of in vivo chemical sensing devices, in particular, an all-in-one pH probe for spatially-resolved and label-free pH sensing in vivo, based on a field-effect pH sensor (**Fig. 1**), i.e., a light-addressable potentiometric sensor (LAPS)[1], coupled to a flexible multimodal fiber[2]. A readout photocurrent from the LAPS, elicited from a modulated light source, registers the localized surface potential change, proportional to the pH change. Upon simultaneous illuminations at multi-spot by a plurality of light sources with different modulation frequencies, pH changes at multiple designated spots are obtained via demultiplexing this photocurrent. To enable its in vivo applications, we combined the LAPS with a multimodal fiber fabricated by the convergence thermal drawing. Such fiber seamlessly integrates a multicore optical waveguide in the center for the light delivery, surrounded by electrodes for leading out photocurrent and serving as a pseudo-reference electrode, respectively. Such hybrid all-in-one pH probes can measure pH changes at 14 pixels simultaneously with a spatial resolution of 250 μm and a temporal resolution of 30 Hz. The pH sensitivity was characterized as 57.5±2.2 mV/pH homogeneously across all measurable pixels. Such

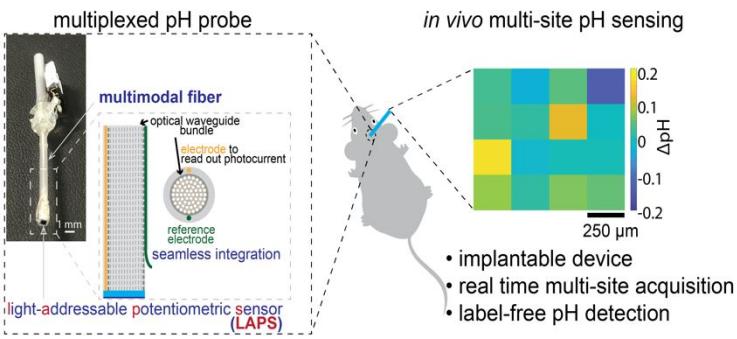

Figure 1 Miniature multiplexed pH probe and its in vivo applications

probes have been implanted into the hippocampal formation of rats and their capabilities to capture pH changes at multiple pixels were evaluated at both physiological and pathological conditions. Technologies developed here represents a new class of in vivo chemical sensing technologies enabling the spatially-resolved investigation of intrinsic chemical signals in deep brain structures with high spatial and temporal resolutions.

3.1. Fiber fabrication

To fabricate multimodal fibers, we built our customized fiber fabrication tower, which additionally incorporated with the convergence drawing system. In this work, we first prepared a preform, which hosted an imaging core, working and reference electrodes. Each of image pixels was based on commercially available polymer optical fiber (ESKA CK-20, Mitsubishi Chemical Corp.). To prepare the preform, hundreds of sections of this polymer optical fiber with a length of 25 cm were cut and prepared. They were bundled tightly and confined within a poly(methyl methacrylate) (PMMA) tube which had an inner diameter of 12 mm and an outer diameter of 16 mm. In order to incorporate electrodes into the final fiber, a layer of polycarbonate (PC) was rolled around the bundle of PC/PMMA optical fibers and consolidated at 180 °C in vacuum. We then machined four slots on the outer PC surface of the preform, two of them had cross-sectional dimensions of 1.5 mm × 1.5 mm with the other two of 2 mm × 1.5 mm. Then another layer of PC film was rolled on top to achieve a preform size of 20 mm. Then we placed the preform into the furnace of the customized fiber tower and heated at 230 °C. During the drawing process, a copper wire (Oyadei.com, diameter: 0.05 mm) and silver wire (Nilaco Corporation, diameter: 0.1 mm) were fed into the preform and consequently combined with the fiber together (**Fig. 2**), resulting in a fiber with hundreds of pixels, one Cu electrode as a working electrode, one Ag electrode to be used as a pseudo reference electrode, and two additional channels for general purposes (**Fig. 2b**).

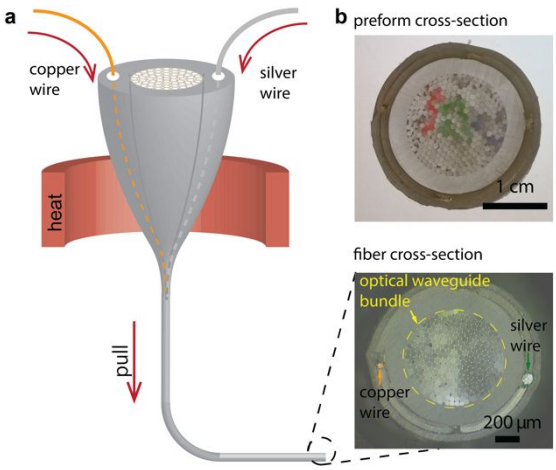

Figure 2 Fiber fabrication process with the convergence of microwires.

3.2. Miniature pH probe assembly

First, the Ag wire within the multimodal fiber was exposed at 1–2 mm away from the tip of the multimodal fiber. It was done via removing the polymer cladding with a surgical scalpel under a microscope. Then the LAPS chip was connected to the tip with the Cu wire of the fiber by silver paint (SPI supplies, Inc.). The exposed edges of the LAPS chip were then covered with medical epoxy (McMaster-Carr). At the backend of the fiber, the Cu and Ag electrodes were exposed from the fiber side by removing the polymer cladding and connected to microwires using silver paint. Another 3D-printed optical ferrule with a length of 6.5 mm, an outer diameter of 2.5 mm and an inner diameter of 1 mm was connected to the fiber with the epoxy. Then the flat surface of ferrule end was prepared by polishing via a fiber polishing kit or fresh cut via razor blades, which enables the optimal optical coupling. The last step of the device assembly (**Fig. 3a**) involved coating the exposed silver wire with the Ag/AgCl paste (ALS Co., Ltd), which was cured at room temperature overnight to ensure a good adhesion and proper functions as a pseudo reference electrode.

4．研究成果

4.1. Multiplex pH sensing performance in vitro

We evaluated the multiplexed pH-sensing feature of the all-in-one pH probe prior to its in vivo deployment. In the standard pH buffer solutions from pH 4 to 7 where there were almost no interference from Cl^- concentrations, we observed the normalized I–V curve shifts in response to pH at all 14 working pixels with an example of them shown in Fig. 3b.

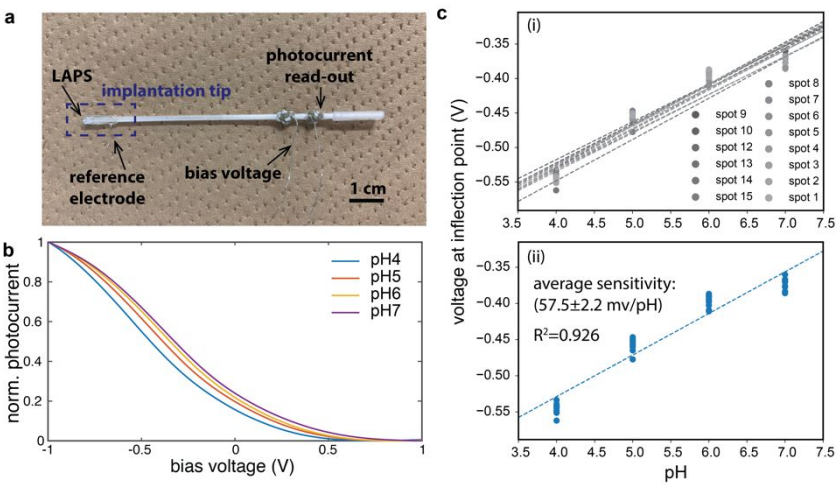

Figure 3. Miniature pH probe and characterization of pH sensitivity in vitro.

The main reason that standard pH buffer solutions up to pH of 7 were chosen is because the physiological pH in brain ranges from 7.1 to 7.4 and pathological conditions lead to more acidified brain. It is more of interests to calibrate the acidic spectrum of pH detection.

In the results (**Fig. 3b**), the normalized I–V curve reflects the state of the space charge region in semiconductor from inversion, depletion and accumulation, in response to the bias voltage supplied from the integrated pseudo reference electrode. In addition, these I–V curves shift towards an increased bias voltage with higher pH values. We performed the normalization of I–V response to eliminate the influence from variance in light intensities from the light delivery module.

From these I–V curves obtained at 14 working pixels, we further calculated pH sensitivity across all pixels based on the linear regression fitting of their inflection voltage versus pH. All pixels displayed a similar linearity (**Fig. 3c**–i), which means the sensor has a homogenous sensing performance across its working pixels. Then an average pH sensitivity of 57.5±2.2 mV/pH was calculated based on the linear regression of the average inflection voltage to pH. (**Fig. 3c**). Such value, close to Nernstian limit, is consistent with standalone LAPS devices with their measurements performed with a conventional glass-based aqueous reference electrode.

4.2. Multiplexed pH sensing in vivo in physio-/pathological brain states

To evaluate capabilities of recording multiplexed physiological pH changes in the brain, sensory stimuli — toe pinches were applied to either left or right lower limb of subject rats (**Fig. 4a**) under anesthesia. Representative imaging frames in Fig. 4c shows the pH changes during one recording session. Toe pinches triggered observable and active pH changes in the localized area in the septal hippocampus comparing to the recording prior to and after the toe pinch, though the subject rat had no pedal reflex. Such pH changes can be clearly captured in recording traces of the entire session from all pixels (**Fig. 4d**(i)). The period when the toe pinch was applied was indicated along with traces. pH changes induced by the toe pinch, though subtle, were statistically significant and they quickly returned back to the baseline (**Fig. 4d**(ii)). In addition, both acidification and alkalization were observed among different pixels, suggesting these subtle fluctuations in pH changes during the toe pinch were physiological events in the brain, ruling out possibilities of electrical artifacts.

We also demonstrated abilities of our probe in the multiplexed pH sensing in pathological

conditions, where the optical activation of ChR2 in the septal–temporal axis of hippocampal formation was applied in Thy1.2-ChR2V rats[3]. A robust optogentically induced seizure model was adopted here in order to show that our pH probe can potentially facilitate the detailed investigation of pH changes in relationship with optically induced seizures with high spatial and temporal resolutions. As illustrated in **Fig. 4b**, an optical fiber was implanted in the temporal part of the hippocampal formation of Thy1.2-ChR2V rats. Upon optical activation of ChR2 in the temporal hippocampus, recurrent seizures would be generated and propagating in between of temporal and septal hippocampus. Via the miniature pH probe implanted in the septal hippocampus, the localized pH changes were acquired at multiple pixels with sampling frequency of 30 Hz. It allows to evaluate local pH changes in response to the ChR2 activation occurring at its longitudinally connected hippocampal circuits. Repetitive light pulses were delivered to the temporal hippocampus to induce recurrent seizure genesis, from our pH probe, we recorded the corresponding pH changes at the septal hippocampus in **Fig. 4e**. The optogenetic activation indicated the approximate onset of induced seizures. After around 120 s, there were both strong acidification observed at certain pixels, with the pixel 10 as an example. The observed pH changes were in accordance with literatures, where it has been reported as a characteristic that seizures induce in-brain pH acidification and this acidosis further terminates epileptic activity. In the meanwhile, alkalization occurs at neighboring pixels, notably, pixel 7, 8 and 15, showing potential effect of the local brain homeostasis to regulate ionic balance and further mediate brain functions.

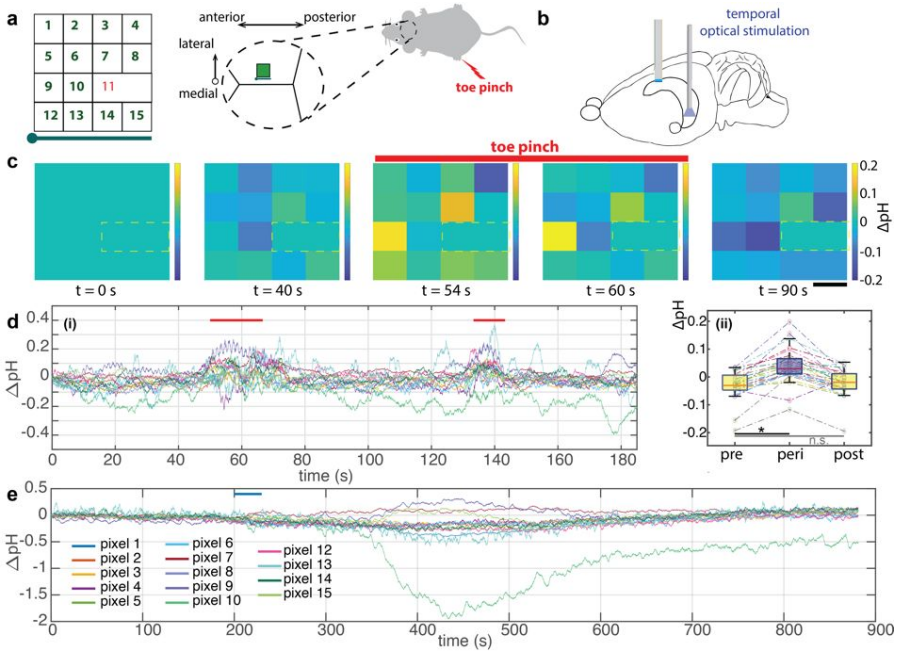

Figure 4 Real-time *in vivo* multiplexed pH acquisition.

References:

1. Hafeman, D. G., Parce, J. W., & McConnell, H. M. (1988). Light-addressable potentiometric sensor for biochemical systems. *Science*, *240*(4856), 1182-1185.
2. Park, S., Guo, Y., Jia, X. et al. One-step optogenetics with multifunctional flexible polymer fibers. Nat Neurosci 20, 612–619 (2017).
3. Osawa, S. I., Iwasaki, M., Hosaka, R., Matsuzaka, Y., Tomita, H., Ishizuka, T., ... & Mushiake, H. (2013). Optogenetically induced seizure and the longitudinal hippocampal network dynamics. *PloS one*, *8*(4), e60928.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　1件／うちオープンアクセス　3件）

１．著者名 Yuanyuan Guo*, Carl Frederik Werner, Shoma Handa, Mengyun Wang, Tomokazu Ohshiro, Hajime Mushiake, Tatsuo Yoshinobu	４．巻 174
２．論文標題 Miniature multiplexed label-free pH probe in vivo	５．発行年 2021年
３．雑誌名 Biosensors and Bioelectronics	６．最初と最後の頁 112870
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bios.2020.112870	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Shan Jiang, Dipan C Patel, Jongwoon Kim, Shuo Yang, William A Mills, Yujing Zhang, Kaiwen Wang, Ziang Feng, Sujith Vijayan, Wenjun Cai, Anbo Wang, Yuanyuan Guo, Ian F Kimbrough, Harald Sontheimer, Xiaoting Jia	４．巻 11
２．論文標題 Spatially expandable fiber-based probes as a multifunctional deep brain interface	５．発行年 2020年
３．雑誌名 Nature Communications	６．最初と最後の頁 1-14
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-020-19946-9	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 T. Yoshinobu, D. Sato, Y. Guo, C. F. Werner and K. Miyamoto	４．巻 19
２．論文標題 Modeling of the Return Current in a Light-Addressable Potentiometric Sensor	５．発行年 2019年
３．雑誌名 Sensors	６．最初と最後の頁 4566,4578
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/s19204566	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Y. Guo *, C.F. Werner, A. Canales, L. Yu, X. Jia, P. Anikeeva* and T. Yoshinobu	４．巻 15
２．論文標題 Polymer-fiber-coupled field-effect sensors for label-free deep brain recordings	５．発行年 2020年
３．雑誌名 PLOS ONE	６．最初と最後の頁 －
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pone.0228076	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計12件（うち招待講演　2件／うち国際学会　8件）

1．発表者名
郭媛元

2．発表標題
極微細 pH 内視鏡の開発

3．学会等名
第3回COI学術交流会

4．発表年
2020年

1．発表者名
郭媛元

2．発表標題
Neuroelectronics

3．学会等名
FRIS Annual Meeting 2021／学際科学フロンティア研究所令和2年度成果報告会（国際学会）

4．発表年
2021年

1．発表者名
Yuanyuan Guo

2．発表標題
Biochemical Sensors Coupled with Multifunctional Fibers for Label-free Imaging in the Brain and Beyond

3．学会等名
13th Asian Conference on Chemical Sensors (ACCS 2019)（国際学会）

4．発表年
2019年

1．発表者名
Yuanyuan Guo

2．発表標題
Multimodal fibers and sensors for biological applications

3．学会等名
Institution Seminar, Institute of Nano- and Biotechnologies (INB), Aachen University of Applied Sciences, Julich, Germany
（招待講演）

4．発表年
2019年

１．発表者名
Yuanyuan Guo

２．発表標題
Multimodal approaches via fibers and sensors to the brain

３．学会等名
The Kick-off Symposium of Advanced Graduate Program for Future Medicine and Health Care（国際学会）

４．発表年
2019年

１．発表者名
Yuanyuan Guo

２．発表標題
Multimodal approaches via fibers and sensors to the brain

３．学会等名
通研共同プロジェクト研究会

４．発表年
2019年

１．発表者名
Yuanyuan Guo and Ko Matsui

２．発表標題
Study of astrocytes in modulating anxiety-related behaviour via multifunctional fibers

３．学会等名
Joint symposium of 10th Optogenetics Research Conference and Second International Symposium on Brain Information Dynamics 2018（国際学会）

４．発表年
2018年

１．発表者名
Yuanyuan Guo and Ko Matsui

２．発表標題
Study of astrocytes in modulating anxiety-related behavior via multifunctional fibers

３．学会等名
the 6th International Conference on Glial Biology in Medicine（招待講演）（国際学会）

４．発表年
2018年

１．発表者名
Yuanyuan Guo

２．発表標題
Multimodal approaches via fibers and sensors to the brain

３．学会等名
The Kick-off Symposium of Advanced Graduate Program for Future Medicine and Health Care（国際学会）

４．発表年
2019年

１．発表者名
Yuanyuan Guo

２．発表標題
Breaking the Wall of Brain-Machine Interface

３．学会等名
Falling Walls Lab Sendai（国際学会）

４．発表年
2018年

１．発表者名
Yuanyuan Guo

２．発表標題
Breaking the Wall of Brain-Machine Interface

３．学会等名
Falling Walls lab Berlin（国際学会）

４．発表年
2018年

１．発表者名
Yuanyuan Guo

２．発表標題
Multimodal approaches via fibers and sensors to the brain

３．学会等名
通研共同プロジェクト研究会

４．発表年
2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

東北大、多機能ファイバと半導体センサを複合した生体への適用が可能なpH可視化プローブを開発
https://www.nikkei.com/article/DGXLRSP602133_R21C20A2000000/
東北大，生体へ適用可能なpH可視化プローブを開発
https://optronics-media.com/news/20201221/71176/
生体への適用が可能なpH可視化プローブの開発 脳深部におけるpHのリアルタイム観察に成功
https://www.tohoku.ac.jp/japanese/2020/12/press20201221-01-ph.html
Eavesdropping on the pH levels inside the brain
https://medicalxpress.com/news/2020-12-eavesdropping-ph-brain.html
A New Device for Investigations of pH Dynamics
https://www.news-medical.net/news/20201224/New-device-for-investigating-intrinsic-extracellular-pH-dynamics-in-the-brain.aspx
郭 媛元助教 ACCSでYoung Scientist Awardを受賞
http://www.fris.tohoku.ac.jp/feature/topics/detail---id-670.html
郭 媛元助教 「Falling Walls Lab Sendai 2018」第1位獲得
https://www.fris.tohoku.ac.jp/feature/topics/detail---id-502.html
Falling Walls Lab Sendai Reports
https://www.tfc.tohoku.ac.jp/fwls2018/report.html

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関