News Release

Title

In vivo real-time monitoring system using probe electrospray ionization/tandem mass spectrometry (PESI/MS/MS) for metabolites in mouse brain

Key Points

- World's first success of in vivo real-time monitoring of metabolites in a living mouse brain.
- Due to the development of a newly movable stage, which enables to fix a living mouse and control the sampling point in the brain, we succeeded in the real-time monitoring of metabolites in a living mouse brain for 3 hours.
- To validate the system's practicality, we monitored metabolites in the brain of an energy-metabolism disruption model mouse in real time, resulting in capturing synchronized alteration of energy-metabolism related metabolites. The newly developed "in vivo real-time monitoring system" is highly expected to be applied to analysis of brain disease such as Alzheimer's disease. In addition, the system is able to directly estimate "quality of homeostasis".

Summary

The research group of Assoc. Prof. Kei Zaitsu and Senior Assist. Prof. Yumi Hayashi (In Vivo Real-Time Omics Laboratory, Nagoya University), and Shimadzu Corporation have succeeded in developing a novel analytical system for in vivo real-time monitoring of metabolites in a living mouse brain using probe electrospray ionization/tandem mass spectrometry (PESI/MS/MS).

Metabolome analysis (metabolomics) has recently been applied to life sciences. In 2016, our group developed a new analytical method to detect intact metabolites in mouse liver by PESI/MS/MS without sample preparation. Our team has also succeeded in intact analysis of metabolites in mouse brain using the same methodology.

In the present study, we have applied this technique to a living mouse brain, resulting in real-time monitoring of metabolites in a living mouse brain (Fig. 1). PESI uses an extremely thin solid needle (tip diameter: 700 nm) for simultaneous sampling and ionization, though there is a need to control the sampling point and to fix a mouse in place for in vivo real-time monitoring of metabolites in a living mouse brain. Finally, our team developed a new movable stage and achieved in vivo real-time monitoring of metabolites in a living mouse brain for 3 hours.

In addition, we succeeded in capturing the synchronized alteration of energy-metabolism related metabolites in an energy-disruption model mouse, proving practicality of our system.

Our established "in vivo real-time monitoring system" will highly contribute not only to analytical improvement in the real-time metabolomics field, but also to pathophysiological analysis for brain diseases such as Alzheimer's disease or cognitive impairment.

Our study has been published in *Analytical Chemistry* online on March 19, 2018.

Summary 2

Our research group succeeded in the development of an in vivo real-time monitoring system for metabolites in a living mouse brain. In this system, we used probe electrospray ionization/tandem mass spectrometry (PESI/MS/MS) and a movable stage, which is able to fix a mouse and control the sampling point. The present system achieved real-time monitoring of metabolites in a living mouse brain for 3 hours, and the system will highly contribute not only to analytical improvement in the real-time metabolomics field, but also to pathophysiological analysis for brain diseases such as Alzheimer's disease or cognitive impairment.

Research Background

Expanding the capability of analytical methods, mass spectrometric applications combined with ambient ionization techniques have been developed over the last decade. Probe electrospray ionization (PESI) is one of the ambient ionization techniques, first invented by Prof. K. Hiraoka in 2007. Uniqueness of this ionization technique lies in the use of a thin needle (700 nm tip diameter) as the probe to work for both sampling and ionization units. The research group of Assoc. Prof. Kei Zaitsu and Senior Assist. Prof. Yumi Hayashi (In Vivo Real-Time Omics Laboratory, Nagoya University), and Shimadzu Corporation was the first to develop the combinational use of PESI and tandem mass spectrometry (MS/MS) and to report PESI/MS/MS in achieving higher specific identification of hepatic metabolites (K. Zaitsu, Y. Hayashi et al., *Anal. Chem.* 2016, 88(7), 3556-3561.). In addition, the team succeeded in the intact analysis of metabolites in a dissected mouse brain sample without sample preparation by PESI/MS/MS (Y. Hayashi, K. Zaitsu et al. *Anal. Chim, Acta*, 2017, 983, 160-165.). These studies strongly suggested that PESI/MS/MS can achieve real-time monitoring of metabolites in the brain of a living mouse, if we construct a dedicated system combing the appropriate manipulation unit with PESI/MS/MS.

Research Results

As shown in Fig. 2, we developed a new manipulation apparatus, which is able to fix a mouse and control the sampling point in the brain, and constructed an in vivo real-time monitoring system. The manipulation system (Fig. 3) was constructed with an x-y-z axial free-movable stage (manual control for x- and y-axes, electric control for z-axis, each spatial resolution: 3 μm), which can be manually moved to the front of the ion source, and a control device. According to our previous study, a special sample cup, which is fixed directly onto the mouse's skull, was used for supplying ethanol to the needle tip for enhancing ionization efficiency. To hold the cup in place, a fixing arm is set on the stage. Also, to prevent hypothermia of the anesthetized mouse, a rubber heater is set on the stage surface. In vivo real-time monitoring was executed for a living mouse brain using the system, succeeding in 8 metabolites such as glucose and citric acid for 3 hours (Fig. 4). In this case, an energy-metabolism suppressant was administered to the mouse 3 min after the analysis started. As a result, glycolysis was enhanced, while TCA-cycle intermediates were also fluctuated. These phenomena were not observed in a control mouse, demonstrating practicality of the system. In addition, neither remarkable traumatic injury nor edema were observed on the brain surface, especially around the probe-insertion position, examined 3 hours after in vivo real-time monitoring.

Research Summary and Future Perspective

In the present study, we have developed an in vivo real-time monitoring system for metabolites in a living mouse brain. The present system will highly contribute not only to analytical improvement in the real-time metabolomics field, but also to pathophysiological analysis for brain disease such as Alzheimer's disease or cognitive impairment. In addition, the system will be applied to estimate the quality of homeostasis.

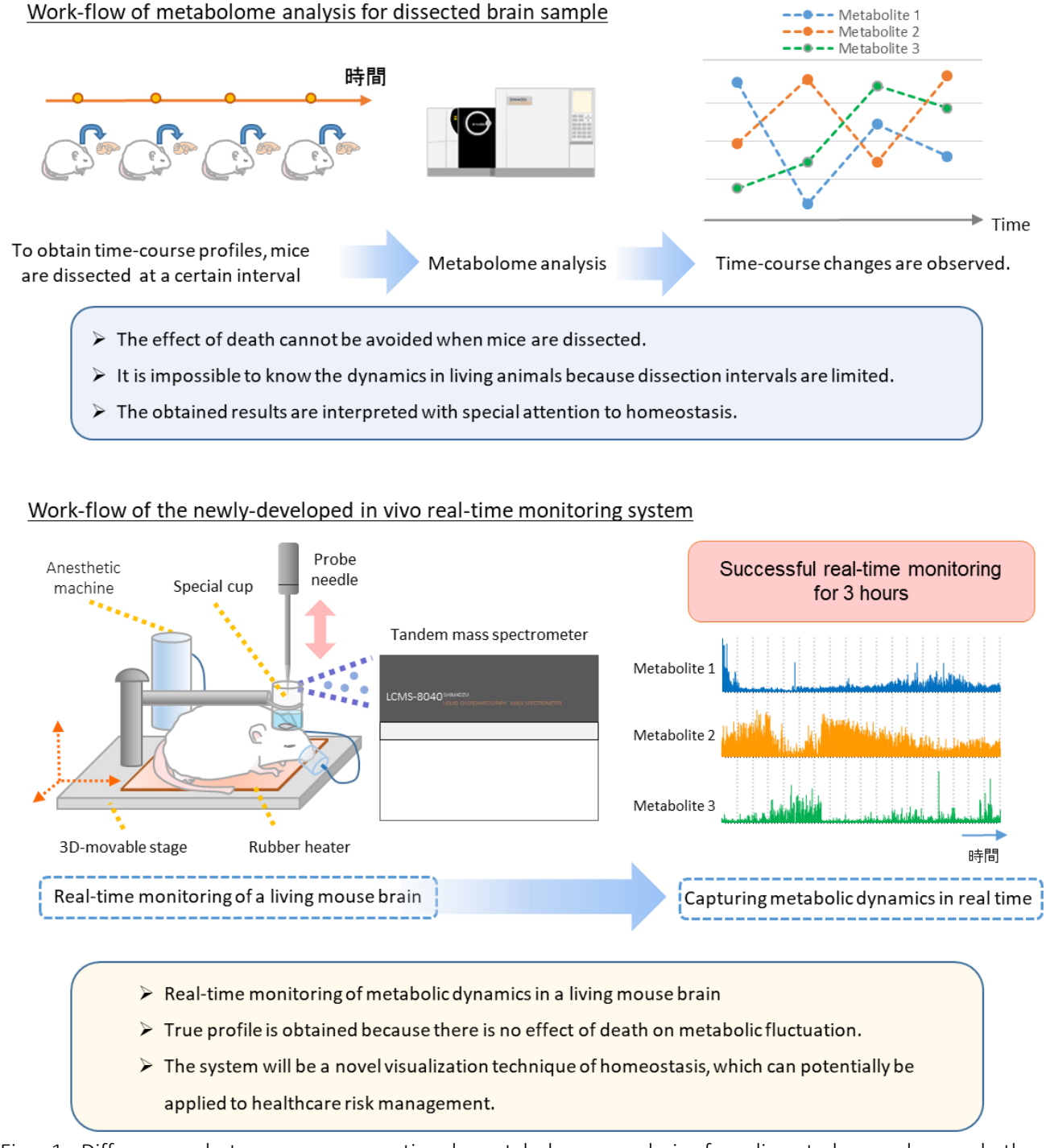

Fig. 1 Differences between a conventional metabolome analysis for dissected samples and the newly-developed monitoring method.

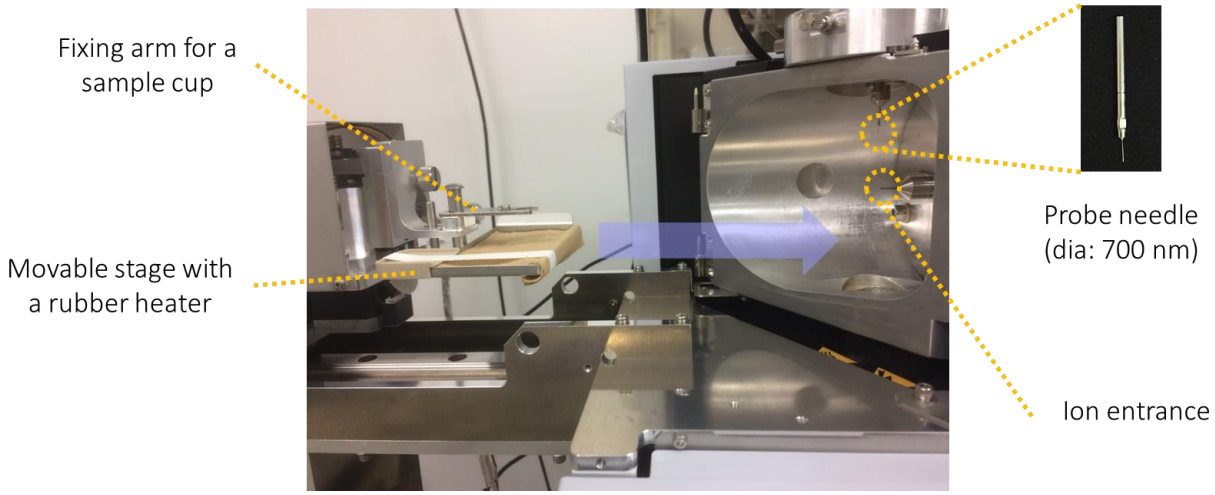

Fig. 2 Photograph of a newly-developed in vivo real-time monitoring system.

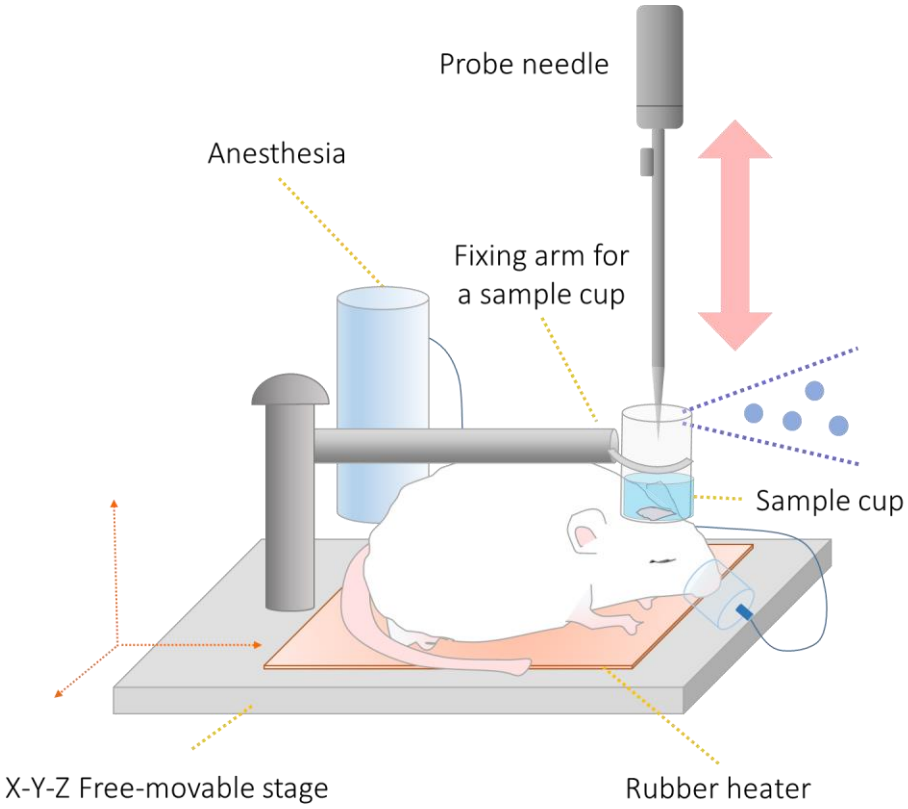

Fig. 3 Schematic image of a free-movable stage.

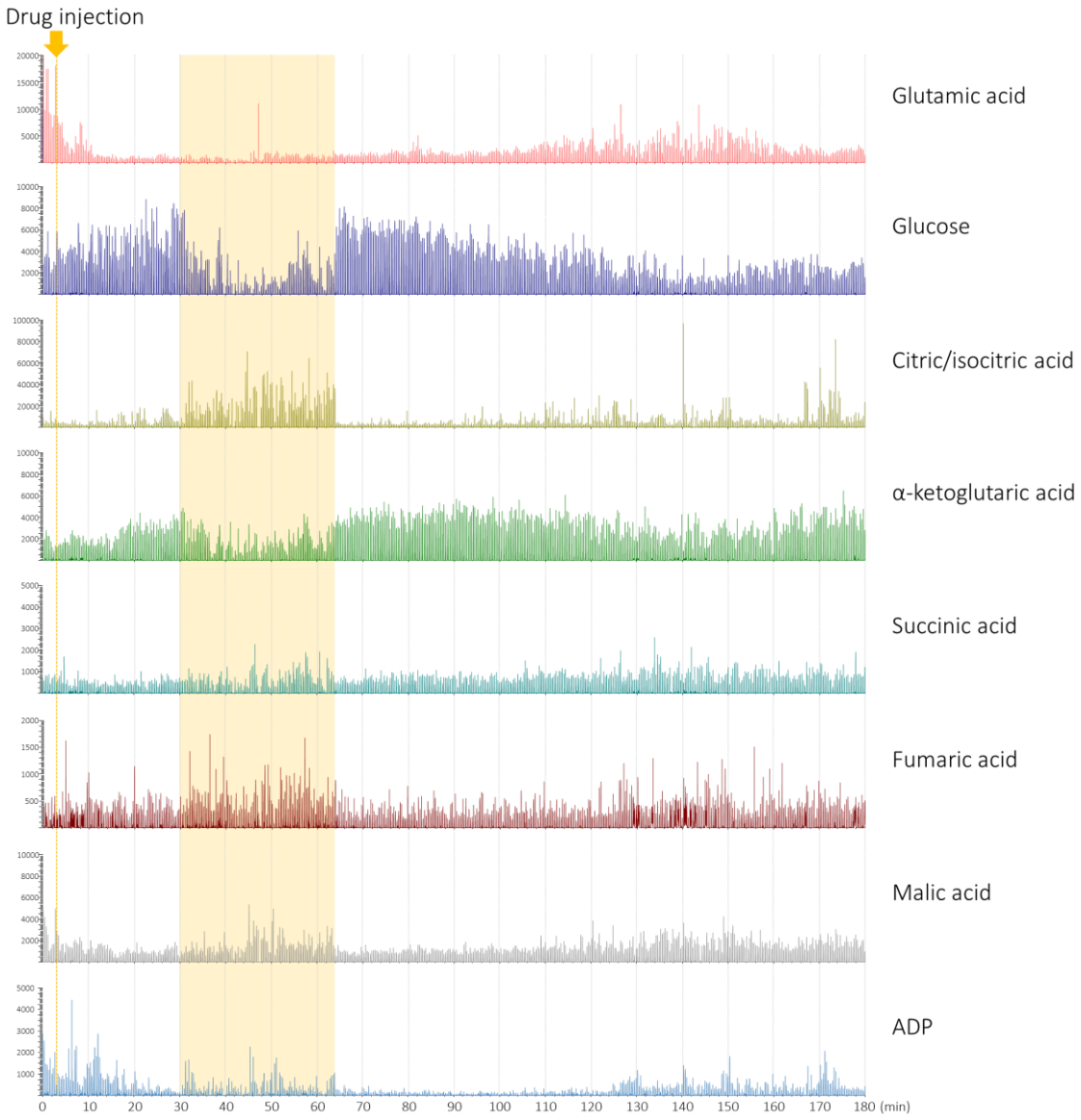

Fig. 4 In vivo real-time monitoring of metabolites in a living mouse brain for 3 hours.

Publication

"In vivo real-time monitoring system using probe electrospray ionization/tandem mass spectrometry (PESI/MS/MS) for metabolites in mouse brain"

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