Rapid quantification of extracellular neurotransmitters in mouse brain by PESI/MS/MS and longitudinal data analysis using the R and Stan-based Bayesian state-space model

Title

Renewal of the established microdialysis technique – make it possible with ambient ionization mass spectrometry and Bayesian statistical modelling -

Key Points

- PESI/MS/MS achieved rapid quantitation of L-Glu and GABA in microdialysates with sufficient reproducibility.
- Continuous monitoring of L-Glu and GABA in mouse striatum was achieved for 30 min with 1 min temporal resolution.
- Bayesian state-space model was highly effective in evaluating the time-series data obtained from a mouse by microdialysis.

Summary

We developed a methodology for rapid quantification of L-glutamic acid (L-Glu) and gamma-aminobutyric acid (GABA) in the mouse striatum by probe electrospray ionization mass spectrometry (PESI/MS/MS), which is an ambient ionization mass spectrometry, and longitudinal data analysis using the R and Stan-based Bayesian state-space model. We constructed a quantitative method for L-Glu and GABA, where the analysis time was just 0.5 min per a sample. Validation results also demonstrated high reproducibility and quantitativity of the method. To evaluate the feasibility of the method, microdialyses were performed on free-moving mice that were stimulated by high-K+-induced depolarization under different sampling conditions: 1) every 5 min for 150 min and 2) every 1 min for 30 min.

We applied the R and Stan-based Bayesian state-space model to each mouse's time-series data considering autocorrelation, and the model successfully detected abnormal changes in the L-Glu and GABA

levels in each mouse. In particular, a 1-min temporal resolution was achieved using this method, thereby successfully monitoring microenvironmental changes in the extracellular L-Glu and GABA of the mouse striatum.

In conclusion, this methodology using PESI/MS/MS and Bayesian state-space model allowed easy monitoring of neurotransmitters at high temporal resolutions and appropriate data interpretation considering autocorrelation of time-series data, which will reveal hidden pathological mechanisms of brain diseases, such as Parkinson's disease and Huntington's disease in the future.

Research Background

Neurotransmitters play an important role in neural communications in the brain. Moreover, L-Glu and GABA are mainly involved in the excitatory and inhibitory neural communications, respectively. Microdialysis is an established technique for collecting neurotransmitters in an extracellular region, and it can be applied to living animals. In microdialysis, a semipermeable membrane is mounted in a microdialysis probe, and the probe is implanted in the brain, enabling monitoring of the extracellular levels of neurotransmitters in a conscious free-moving animal. To quantitate the neurotransmitters in the microdialysates, which generally contain high concentration salts, the following analytical techniques were reported: liquid chromatography and liquid chromatography mass spectrometry.

our group has reported that PESI/MS/MS (Fig. 1) could be applied to the direct analysis of compounds in various biological specimens（K. Zaitsu* et al., Analytical Chemistry 2016. Y. Hayashi, K. Zaitsu* et al., Analytica Chimica Acta 2017. K. Zaitsu* et al. Analytical Chemistry 2018. K. Zaitsu* et al. Analytical Chemistry 2020. K. Hisatsune, K. Zaitsu* et al. ACS Omega 2020.). PESI/MS/MS enables us to execute the direct analysis of targeted compounds without the time-consuming pretreatment. Moreover, it reduces the volume of the sample in spite of high concertation salts in microdialysates, improving the temporal resolution of the microdialysis technique.
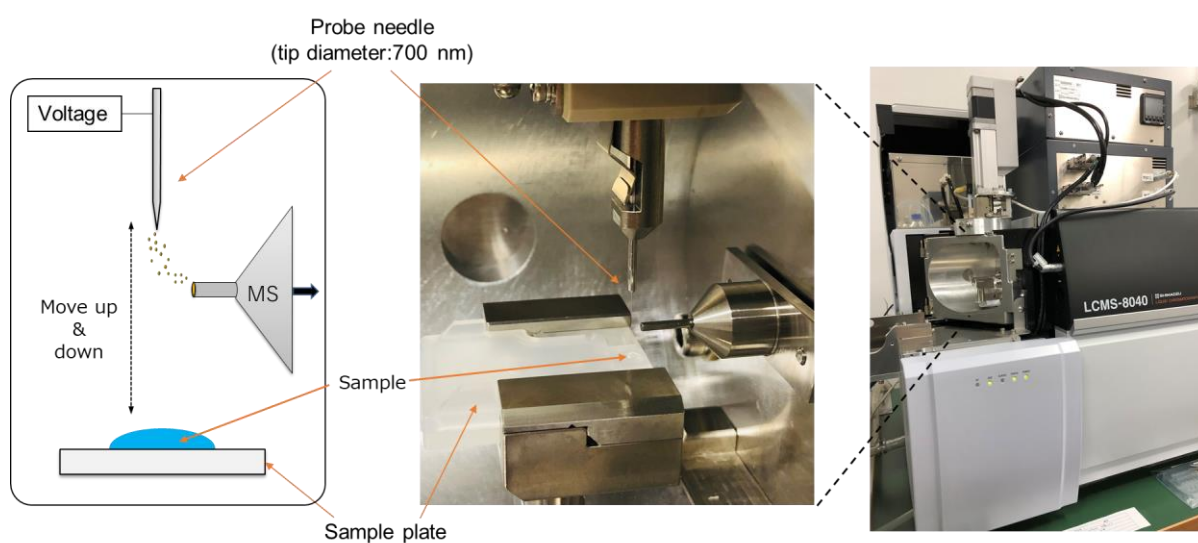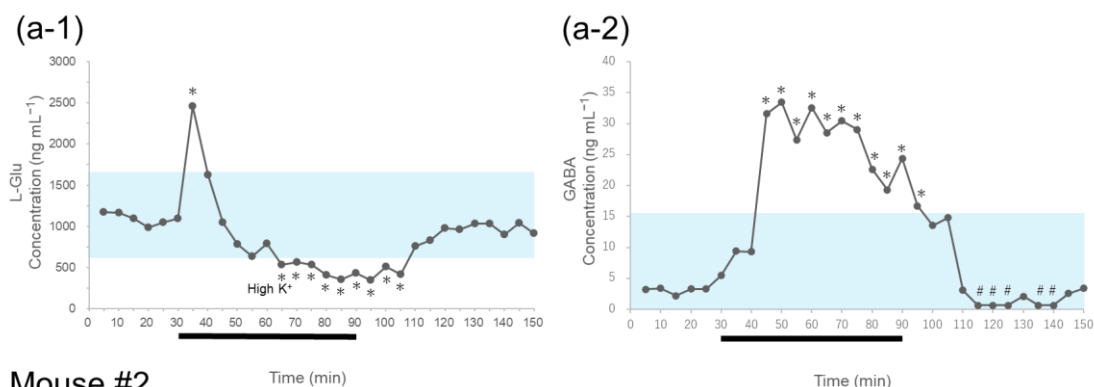

Fig. 1 Schematics of PESI/MS/MS.

Evidently, time-series data of neurotransmitters and metabolites are obtained from the microdialysis technique, and thus, appropriate longitudinal data analysis is mandatory for interpreting the data. In previous studies, statistical analyzes such as analysis of variance parametric test were performed for each time point data, which were obtained by averaging different individuals' values at each time point. However, this approach is somewhat inappropriate for evaluating time-series data, which show autocorrelation for each individual. Alternatively, it is acceptable to use a Bayesian state-space model for such time-series data, though no report has applied the Bayesian state-space model to time-series data obtained by microdialysis.

Research Results

We developed quantitative method for L-Glu and GABA using PESI/MS/MS, where the analysis time was just 0.5 min per a sample. We constructed the calibration curves for L-Glu and GABA to evaluate the quantitativity of the method. Validation results demonstrated high reproducibility and quantitativity of the method (accuracy and precision: 0.4%–7.5% and 1.7%–5.4% for L-Glu and 0.1%–4.8% and 2.1%–5.7% for GABA). To evaluate the feasibility of this method, extracellular L-Glu and GABA were collected from free-moving mice by in vivo microdialysis. We monitored the local releases of L-Glu and GABA from the presynaptic terminal by high-K+-induced depolarization under different sampling conditions: 1) every 5 min for 150 min and 2) every 1 min for 30 min. The results are shown in Fig. 2 and 3. Also, we adopted the Bayesian state-space model and determined the 95% credible interval (CI). Based on the 95% CI, we detected abnormal changes in each mouse, which were caused by high-K+-induced depolarization.
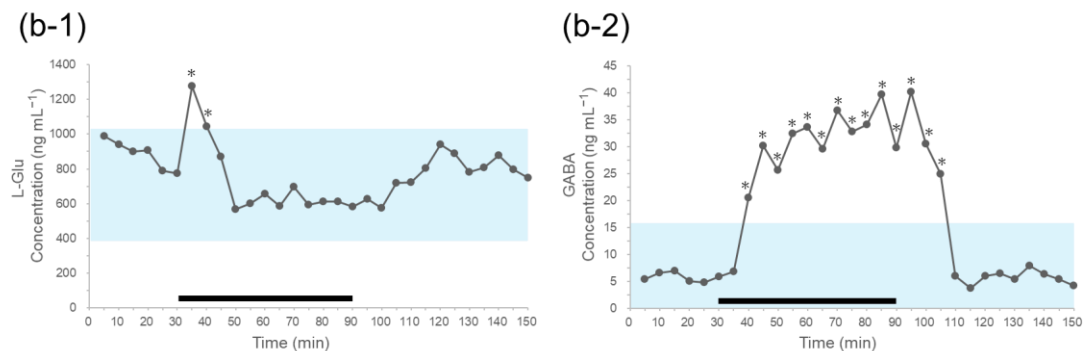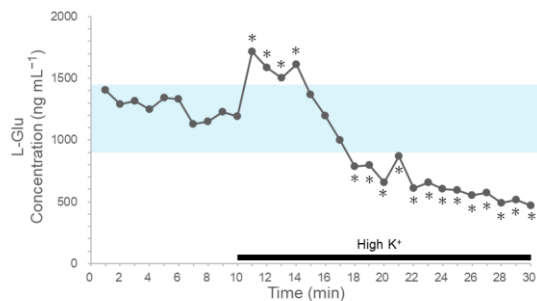

Fig. 2 Time-course changes in extracellular (a-1 and b-1) L-Glu and (a-2 and b-2) GABA levels in the microdialysate every 5 min. At 30 min, high-K+ aCSF was maintained for 60 min (black bar). 95% CI of the first
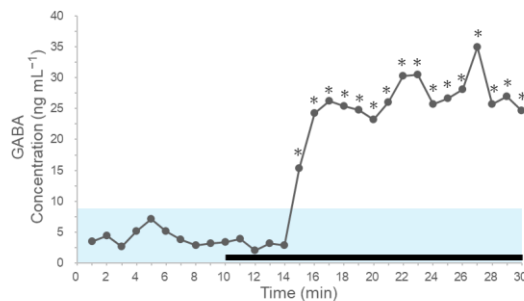
ten-point data is shown in blue. Asterisk sign (*) indicates values that are outside the 95% CI. Hash sign (#) in (a-2) indicates values that are under LLOQ but over LLOD.
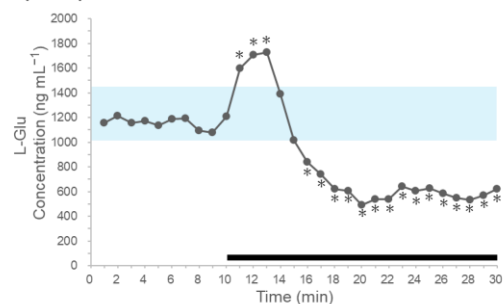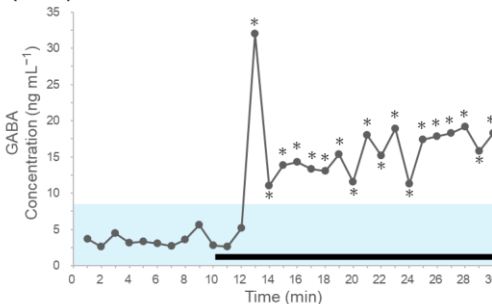

Fig. 3 Time-course changes in (a-1 and b-1) L-Glu and (a-2 and b-2) GABA levels in the microdialysate every 1 min. At 10 min, high-K+ aCSF was maintained for 20 min (black bar). The 95% CI of the first ten-point data is shown in blue. Asterisk sign (*) indicates values that are outside the 95% CI.

The L-Glu level of mouse no. 1 was below 95% CI for ca. 40 min (65-105 min in Fig. 2a-1), while the L-Glu level of mouse no. 2 was within 95% CI (Fig. 2b-1). However, L-Glu levels of mice nos. 3, and 4 were below 95% CI after the levels decreased (Fig. 3a-1 and b-1). These results suggested that the measurement with a temporal resolution of 1 min was required to capture the subtle changes in the L-Glu levels by high-K+-induced depolarization. Also, the Bayesian state-space model was highly effective in evaluating the time-series data obtained by microdialysis.

Research Summary and Future Perspective

This methodology using PESI/MS/MS and the Bayesian state-space model allowed the easy monitoring of neurotransmitters at high temporal resolutions and appropriate data interpretation considering autocorrelation of time-series data, which will reveal the hidden pathological mechanisms of brain diseases like PD and HD in the future. In addition, we have developed a new device for on-line connection of microdialysis and PESI/MS/MS (patent pending), which will enable us to perform in vivo real-time monitoring of extracellular biogenic compounds in mouse brain.

Publication

Rapid quantification of extracellular neurotransmitters in mouse brain by PESI/MS/MS and longitudinal data analysis using the R and Stan-based Bayesian state-space model.

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