



Fukushima University

Cluster of Agricultural Sciences, Faculty of Food and Agricultural Sciences

Response sheet Reviewer #1

1) Reviewer #1: Was the TMPy-derivatation reasonably enough? Please show if there are any evidence of heating the mixture for 60°C, 10min.

Between each sample, how much if there were any difference between the efficiency of TMPy-derivatation?

A. Thank you for your comments. To determine optimized reaction condition of TMPy, different TMPy reaction conditions with the targets were considered. One is imaging experiment has achieved without incubation. Without incubation, target molecules did not detect and image clearly, indicating that TMPy needed to be under moist state in order to react with target molecules.

The other one is imaging experiment has achieved with methanol/ water (30/70 v/v) condition. Under this condition, target molecule has migrated from original position due to moist-state. Thus, our suggested condition (methanol/water (70/30 v/v) solution at 60 °C. for 10 min.) is most suitable to react TMPy derivatization reagent on section. To avoid misunderstanding for broad reader, we added this result in revised manuscript (red character).

2) The elimination half-time of L-DOPA is around 0.75 to 1.5 hours. How will the pharmacodynamics change with the administration of NSD-1015, before euthanasia?

A. Thank you for your advice. In this time, L-DOPA was not affected by an elimination half-time. Because forty minutes following injection of NSD-1015, L-DOPA or D<sub>3</sub>- L-DOPA (100 mg/kg) was individually i.p. injected. After 120 min, all mice were euthanized by cervical dislocation. Thus, an exogenous L-DOPA or D<sub>3</sub>-L-DOPA marginally metabolized (eliminated half-time). Therefore, exogenous L-DOPA delivered and accumulated without metabolism at brain stem (BS). In the previous manuscript (Line 127-129), our sentences was misleading for experimental procedure. Thus, we revised in this section.



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Response sheet for Reviewer #2

Reviewer #2: - Authors introduced a unique way to image the brain's neurotransmitters by mass spectroscopy. The localization of the concentrations of the neurotransmitters were achieved by nucleophilic reaction of pyrylium structures. The topics were scientifically designed and results have shown meaningful brain images. However, there are issues which should be resolved before consideration of publication.

1. IMS sounds very powerful to surpass the capabilities based on immuno or immunochemical binding methods, which lacks specificity. Then, how about other bioprobes such as recent one, aptamer?

A. Thank you for kind suggestion. There are some reports for novel imaging method using aptamer. Most of the reports involve fluorescent reagent or nanoparticle aptamers modified with nucleic acids. Certainly, these results were selective and sensitive. Somehow, we described IMS advantages compared with aptamer imaging, IMS could image small molecules such as catechol amines, and gives us exhaustive data for omics analysis. Thus, we may find biomarker molecules from IMS data. In revised manuscript, we added new references for aptamer imaging as other bioimaging method.

2. TMPy labeling on brain section should accompany unwanted monoamine compounds in brain. Authors should describe how the background reaction from the TMPy could be removed or minimized.

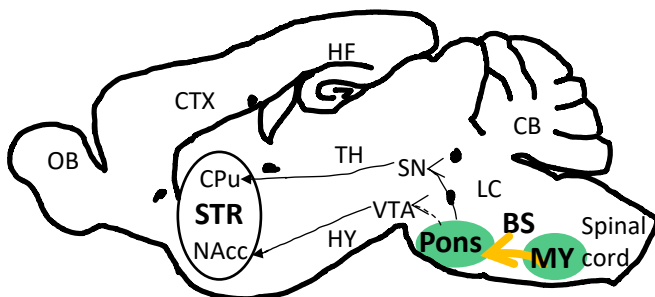
A. Thank you for your comment. IMS selects one  $m/z \pm 0.05$  signal and image target molecules. Thus, other unwanted monoamine which is difference  $m/z$  compared with that of target did not image as background. In this paper we imaged known monoamines which knew localization area. (ie. Dopamine localized caudate putamen (CPu) in STR.)

To avoid ion suppression by unwanted monoamines, we confirmed ion

intensity at several section points as pre-measurement. In pre-measurement, we set laser power ( $<60 \text{ J/m}^2$ ) to avoid over saturated ion count.

3. There are no synergistic aspects other than endogenous or exogenous neurotransmitters between two methods of TMPy labeling and deuterium-substituted neurotransmitters?

A. Thank you for your question. TMPy labeling enhanced detection limit (Support information 1). In present manuscript, we described the transportation route of L-DOPA (Line 308-322) and dopamine by endogenous or exogenous L-DOPA. In addition, we re-discussed the effect deuterium L-DOPA. Striatum (STR) was divided into two regions, caudate putamen (CPu) and nucleus accumbens (NAcc). Generally, the CPu and NAcc receives DA input primarily from the SN and ventral tegmental area (VTA), respectively. The DA which received from SN and VTA differentially works to action or reward forecast, respectively. From IMS data, DA localized at whole STR indicating that treated-L-DOPA transported from both VTA and SN (Fig. 3d). In contrast,  $\text{D}_3$ -DA was mainly imaged at CPu, indicating  $\text{D}_3$ -L-DOPA preferentially transported to SN. Subsequently,  $\text{D}_3$ -DA which was  $\text{D}_3$ -L-DOPA metabolite transported to CPu from SN (Fig. 3i). Thus, we hypothesized  $\text{D}_3$ -L-DOPA may correlates with an action although we should examine this phenomenon mechanism using other animal model (ie. Parkinson's disease model). We added discussion in revised manuscript (Line 338-347) and illustration of projection of catechol amine in brain in Figure 3.



Referred Figure; Projection of catechol amine from Figure 3



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4. The process of TMPy labeling need a relatively high temperature of 60°C. Is there any other available temperature around mouse's vital tone?

A. Thank you for kind suggestion. We have tried to react TMPy in other condition. The imaging experiment has achieved without incubation. Target molecules did not detect and image clearly, indicating that TMPy could not react with target molecules under room temperature. The merit of reaction at 60 °C is a vapor condition. To enhance the reaction efficiency of TMPy on sections, the TMPy sprayed sections were placed into a dedicated container and allowed to react at 60 °C for 10 min. The container contains two channels in the central partition to wick moisture from the wet filter paper region to the sample section region. The filter paper is soaked with 1 mL of methanol/water (70/30 v/v) or methanol/water (30/70 v/v) and is placed next to the section inside the container. The container is then completely sealed to maintain humidity levels. Resulting, target monoamines detected and imaged in methanol/water (70/30 v/v) condition. In the case of methanol/water (30/70 v/v) that is high humidity, target molecules could react to TMPy, although obscure image was obtained due to that a migration of target molecules on section occurred. Thus, we adopted the condition in methanol/water (70/30 v/v) at 60 °C for 10 min. we revised manuscript in material and method (Line 140-146) and Line294-303, and added the information of dedicated container in supporting information 2.

5. Introduction needs broader range of descriptions about brain imaging or brain mapping, for example, including fMRI, ETC.

A. Thank you for your kind suggestion. As other imaging method, we cited fMRI in revised manuscript. Due to this citation, we clearly described the differentiation of character of IMS.