**Responses to the Editor’s comments**

**Thank you for your very helpful and considered comments. They are much appreciated. We have completely changed manuscript according to below indications. Please find revised manuscript, which we trust will prove satisfactory.**

1. Some protocol steps need more explanation; see notes in the attached revised manuscript (there are several outside of the protocol section as well).

**Ans:** We have completely revised protocol steps according to your attached comment in revised manuscript. We showed the revised parts by red colored character.

2. Please combine all panels of Figures 1 and 2 into a single file. Note that we don’t have a size limit, except for the file size.

**Ans:** We have combined all panels of Figure 1 and 2 into single file.

3. You mention that inulin is ‘a marker of intravascular volume space’; why then remove any blood vessels at all (Discussion, step 4.4)?

**Ans:** As you indicated, the expression of removing blood vessels on the surface of the brain was not appropriate. We carefully wiped off blood adhering to the surface of the brain with a cotton swab moistened with saline solution to eliminate at least the influence of 14C-inulin in the blood exist on the surface of the brain. Since capillary vessels have a network structure in the brain, 14C-inulin in the blood remaining in the intravascular volume space may be overestimated as their own brain distribution. In the intravenous administration, the intravascular volume in the brain can be corrected by measuring the amount of 14C-inulin in the brain. Because inulin is a water-soluble macromolecule, it does not penetrate the BBB under the normal condition. In the case of nasal administration, it is difficult to distinguish between inulin amount in the brain and intravascular inulin amount in the brain. Because there are two pathways in the nose. One is a pathway distributed directly in the brain from the nose. The other is the pathway transferred from the nose to the blood.

And we have revised 4.4. to “Wipe off blood adhering to the surface of the brain with a cotton swab moistened with saline solution to eliminate at least the influence of 14C-inulin in the blood exist on the surface of the brain.”

**Thank you very much for your consideration of our revised manuscript. We look forward to hearing from you at your earliest convenience.**

**Sincerely**