1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

To the best of our abilities, we have proofread carefully.

2. The highlighted protocol steps are over the 2.75 page limit (including headings and spacing). Please reduce the amount of highlighted protocol steps.

We revised the highlighting of the protocol to meet the requirement.

3. For steps that are done using software, a step-wise description of software usage must be included in the step. Please mention what button is clicked on in the software, or which menu items need to be selected to perform the step.

We included more details on the specific clicks and menu items of the imaging software:

5.1. Measure optical densities (ODs) of each calibration standard from the [3H]microscale with an image analysis software.

5.1.1. Select an area of equal size for each point of the [3H]microscale using a tool for ‘region creation’ from the menu item ‘region determination’. Assign a number to each selected area by clicking on ‘number’ under the menu item ‘label’.

5.1.2. Export OD values for each point of the calibration standard by clicking on the menu items ‘file’, ‘export’ and ‘2D region report’. Transfer OD values to a spreadsheet and normalize by the size of the selected area. Perform linear regression to obtain a standard curve for further densitometric analysis.

Note: Make sure that the selected areas are labelled in order to identify matching OD values and samples.

5.2. Perform quantification of autoradiograms using the proprietary imaging software by selecting the region of interest (ROI) using a ‘region creation’ tool in every section and measuring its ODs. Select the same region in every section by creating a template for the region of interest, which is copied and manually adjusted to minor variations in brain anatomy for each autoradiogram. Identify the anatomy of the ROI by comparison of autoradiograms with a brain atlas (e.g.18). When multiple treatments are compared, perform the analysis blinded and randomized in order to avoid biased selection of ROIs.

5.3. Export OD values and sizes of selected areas into a spreadsheet by clicking on the menu items ‘file’, ‘export’ and ‘2D region report’.

4. Calculation steps cannot be filmed unless a graphical user interface is involved and detailed software usage is provided.

We removed the highlighting from the steps involving calculations.

5. Please remove all headers from Discussion. Headers removed

6. Step 2.3: What’s the temperature and time for incubation?

Preincubation temperature and time depends on the nature of the radioligand and the target. For the GHB analogue HOCPCA, pre-incubation is performed for 30 min at room temperature:

2.3.1. Cover plastic trays with lid in order to avoid evaporation and pre-incubate at relevant temperature (for GHB protocol pre-incubate for 30 min at RT) under constant gentle (20 rpm) shaking on a plate shaker.

7. 2.5: What’s the temperature and time for incubation?

Again, incubation temperature and time depends on the nature of the radioligand and the target. For the GHB analogue HOCPCA, incubation is performed for 1 h at room temperature:

2.5. To avoid dehydration, immediately incubate sections with relevant concentration of radioligand in assay buffer under desired conditions (for GHB protocol, 1 nM [3H]HOCPCA for 1 h at RT) by covering sections completely with the radioligand solution (700 µL for 3-4 rodent coronal sections).

8. 2.8: Please write this step in imperative tense.

2.8. Transfer slides to a fixator containing paraformaldehyde (PFA) powder for overnight fixation with PFA vapours at RT in order to protect the integrity of the ligand-target complex.