

Supplementary Materials: Thalamus Manual Segmentation Protocol

Gustavo Retuci Pinheiro^{1*}, Lorenza Brusini², Diedre Carmo¹, Renata Prôa^{1,3}, Thays Abreu¹, Simone Appenzeller⁴, Gloria Menegaz², and Leticia Rittner¹

1. Introduction

This document is supplementary material to the article "Thalamus Segmentation Using Deep Learning with Diffusion MRI Data: An Open Benchmark". It describes a thalamus segmentation protocol. The protocol considers both T1w and diffusion images, thus, taking advantage of diffusion properties of the brain tissue to distinguish the structures. In particular, the diffusion is considered through the FA (Fractional Anisotropy), however, other diffusion indices could be used and even combined.

For the protocol to work properly, the images from different MRI sequences must be aligned. If the acquisition sequences do not deliver the images in the same space, the alignment could be done by registering the images. Some popular tools for this task are the DIPY [1] and FSL-FLIRT [2]. In this tutorial, we used the ITK-SNAP [3] as a tool to draw the structures, but any other tool that has the option of opening multiple images simultaneously and drawing over them could be used for this purpose. For standardization, the left and right thalamus were assigned as label 10 and 49, respectively, following the look up table employed for FreeSurfer [4] and FSL.

Besides using diffusion combined with T1 to enhance the thalamus borders, another key element of this protocol is the use of multiple views to help the specialist to decide the level of contrast belonging to the thalamus.

2. Procedure

- **Step 1:** Load the images T1w (Fig. S1) and FA (Fig. S2) overlaying them with 50% transparency (Fig. S3). We can clearly see, for example, that where the thalamus neighbors the internal capsule the contrast in the T1w image is very dim, but it is clear in the FA image. When the two images are combined, the contrast is enhanced for the whole thalamus border.
- **Step 2:** Using the axial view, draw the borders of the thalamus in the slices where the borders are the easiest to identify (Figs. S4 and S5). The following landmarks were taken into consideration following the publication by [5]:
 - Medial border: the upper part of the lateral wall of the third ventricle and by the middle commissure that connects both thalami;
 - Lateral border: genu and the posterior limb of the internal capsule;
 - Anterior border: anterior commissure;
 - Posterior border: atrium of the lateral ventricle or the tail of the hippocampus, or both structures;
 - Superior border: lateral ventricles in anterior slices and the fornix in posterior slices.
 - Inferior border: *zona incerta* and the internal capsule.

This procedure should be done for at least one slice at the top of the thalamus, one in the mid-axial slice, and one near the bottom of the structure. For each segmented slice, the projection of the partial segmentation should appear on the other views, helping on the definition on what contrast levels should be considered as the borders. Conventionally, use the label 49 for the left thalamus.

- **Step 3:** With some slice defined on the axial view, the threshold of the thalamus borders on the sagittal view is considerably easier to spot, thus, the borders can now be drawn. With at least one slice drawn on the sagittal view, it is possible to proceed to the coronal view (Fig. S6).



Figure S1. T1w image.

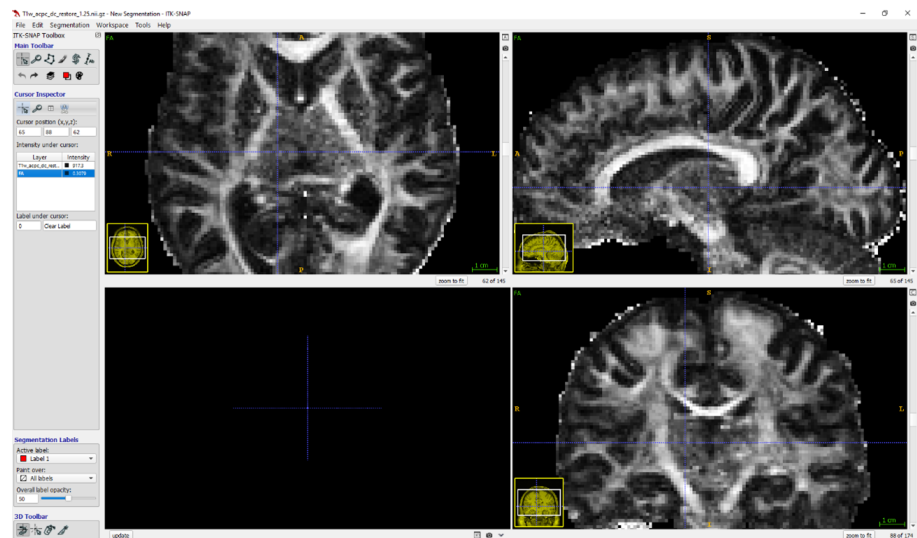


Figure S2. FA image.

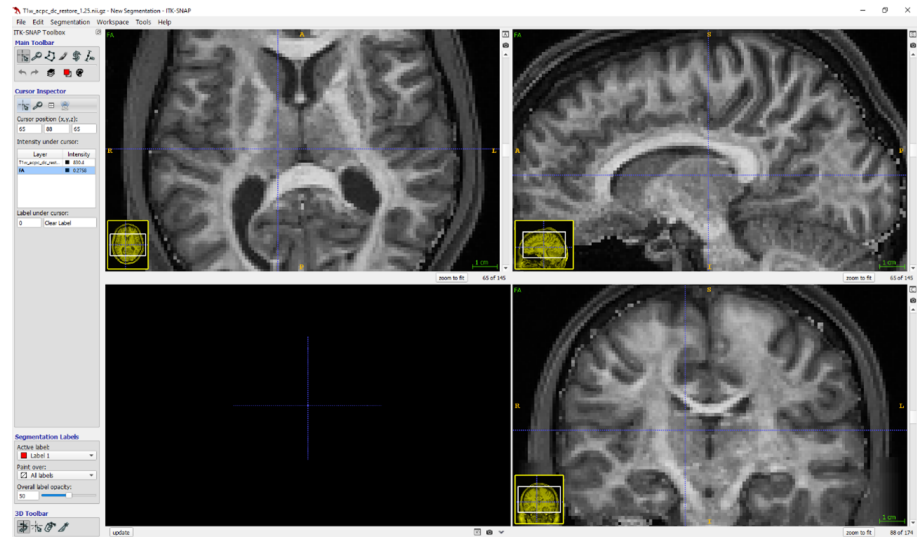


Figure S3. T1w and FA images combined.

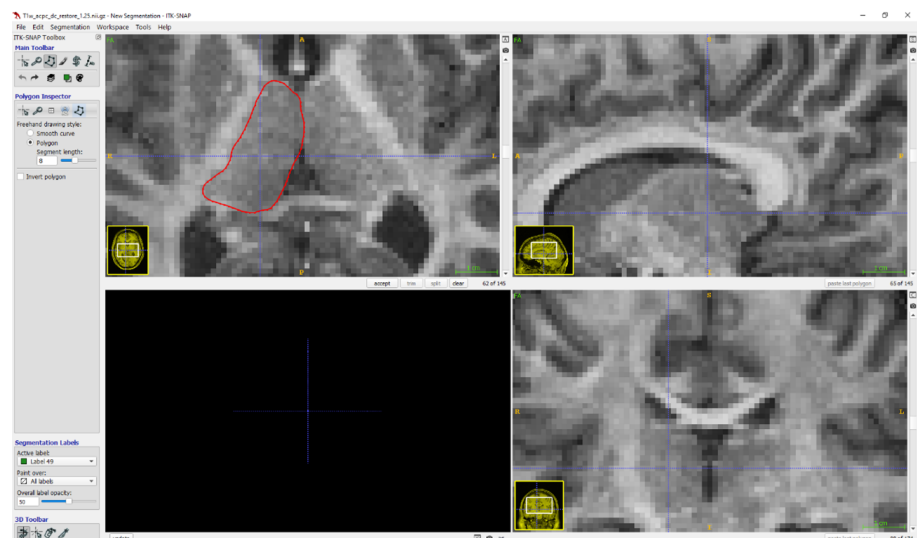


Figure S4. Outline of right thalamus on axial view.

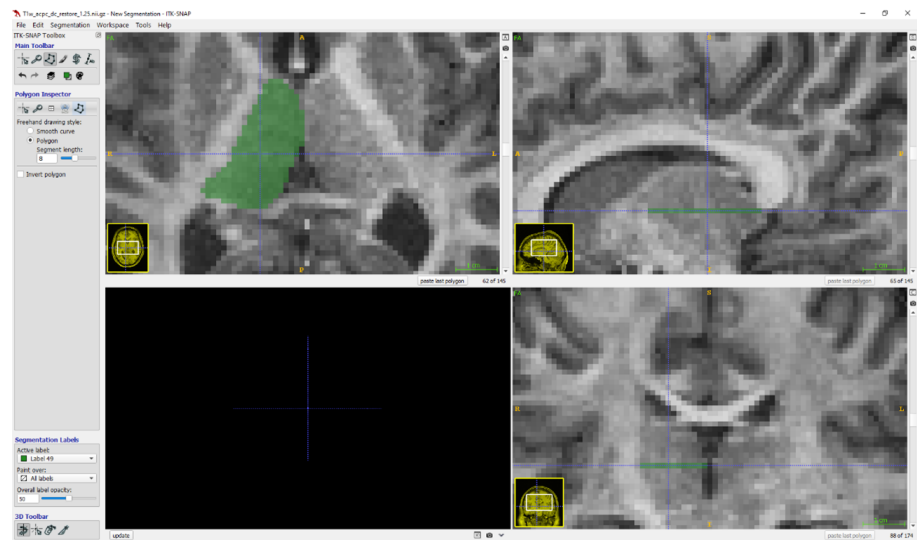


Figure S5. Filled thalamus slice on axial view and projection on coronal and sagittal views.

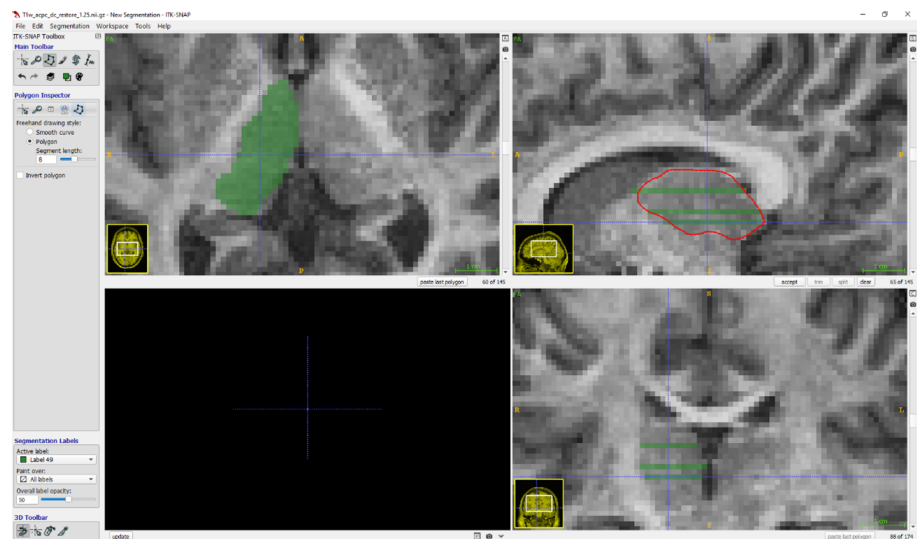


Figure S6. Filled thalamus slice on axial view, projection of thalamus filled slices on coronal and sagittal views, and thalamus outline on sagittal view.

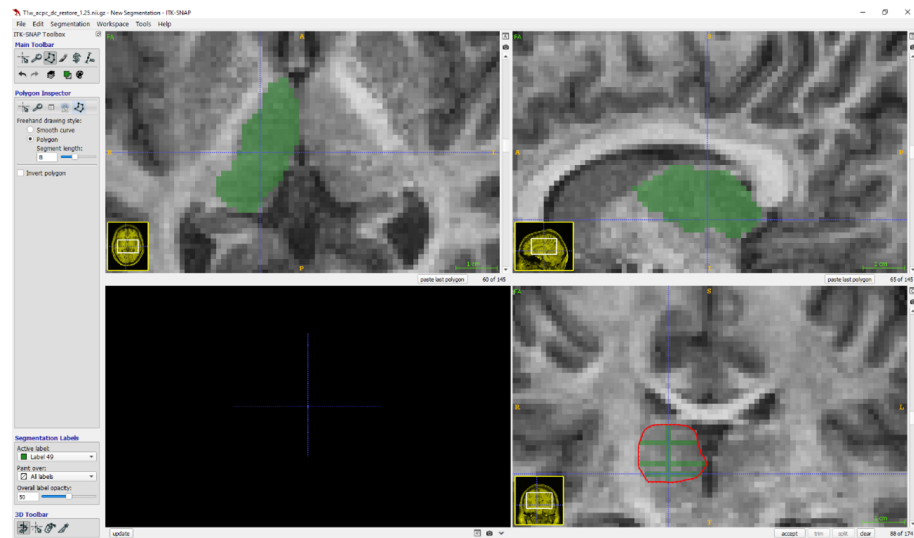


Figure S7. Filled thalamus slice on axial and sagittal views, projection of thalamus filled slice on coronal view, and thalamus outline on coronal view.

- **Step 4:** After defining slices on axial and sagittal views, it is possible to define the thalamus borders on the coronal view, specially the top and bottom borders that have the lowest contrasts (Fig. S7).
 - **Step 5:** At this point, a rough segmentation of the thalamus is achieved, but containing gaps among the segmented slices. To fill the gaps, the specialist should change the slices on each view and draw the outlines based on the projections of the slices drawn on other views. A 3D visualization of the current state of the segmentation is useful to spot gaps and to evaluate the shape of the segmentation (Figs. S8 and S9).
 - **Step 6:** Finally, it is recommended to check all slices, one by one and for all views, looking for voxels that were mistakenly marked, deleting them (Fig. S10). Again, the 3D visualization can help to spot those points as they usually appear as spikes.
 - **Step 7:** The same procedure described above should be repeated for the right thalamus (Figs. S11, S12, and S13). As a standard, use the label 10 assigned to the right thalamus.
1. Garyfallidis, E.; Brett, M.; Amirbekian, B.; Rokem, A.; Van Der Walt, S.; Descoteaux, M.; Nimmo-Smith, I. Dipy, a library for the analysis of diffusion MRI data. *Front. in Neuroinform.* **2014**, *8*, 8. <https://doi.org/10.3389/fninf.2014.00008>.
 2. Patenaude, B.; Smith, S.M.; Kennedy, D.N.; Jenkinson, M. A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage* **2011**, *56*, 907–922.
 3. Yushkevich, P.A.; Piven, J.; Cody Hazlett, H.; Gimpel Smith, R.; Ho, S.; Gee, J.C.; Gerig, G. User-Guided 3D Active Contour Segmentation of Anatomical Structures: Significantly Improved Efficiency and Reliability. *Neuroimage* **2006**, *31*, 1116–1128.
 4. Bruce, F. Freesurfer. *NeuroImage* **2012**, *62*, 774–781.
 5. Burggraaff, J.; Liu, Y.; Prieto, J.C.; Simoes, J.; de Sitter, A.; Ruggieri, S.; Brouwer, I.; Lissenberg-Witte, B.I.; Rocca, M.A.; Valsasina, P.; et al. Manual and automated tissue segmentation confirm the impact of thalamus atrophy on cognition in multiple sclerosis: A multicenter study. *NeuroImage: Clinical* **2021**, *29*, 102549.

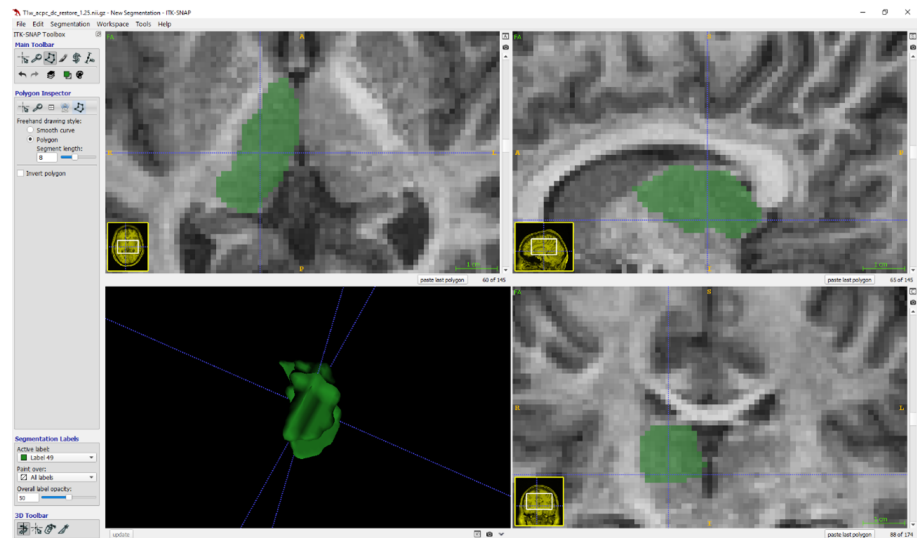


Figure S8. Filled thalamus slices on axial, coronal, and sagittal views, and 3D render of thalamus partial segmentation.

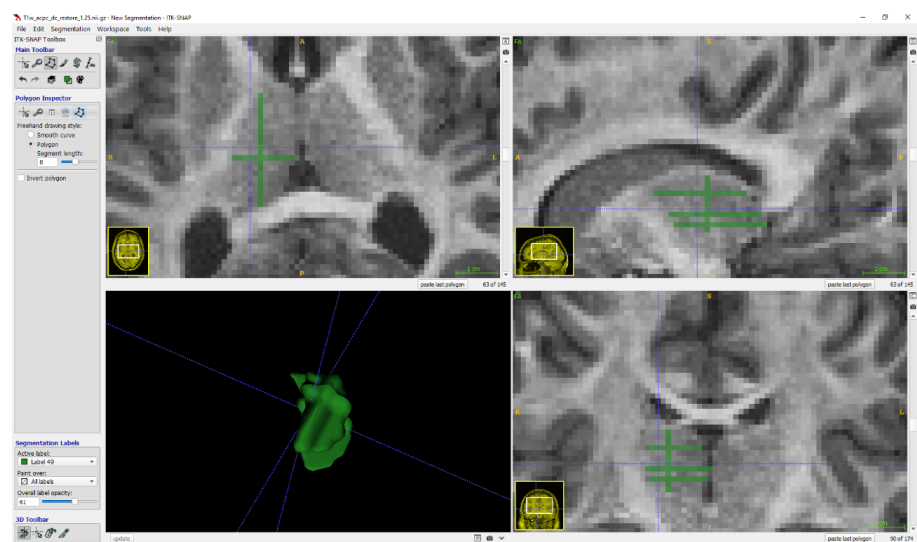


Figure S9. Projection of thalamus filled slices on coronal, axial, and sagittal views, and a 3D visualization of the current state of the thalamus segmentation.

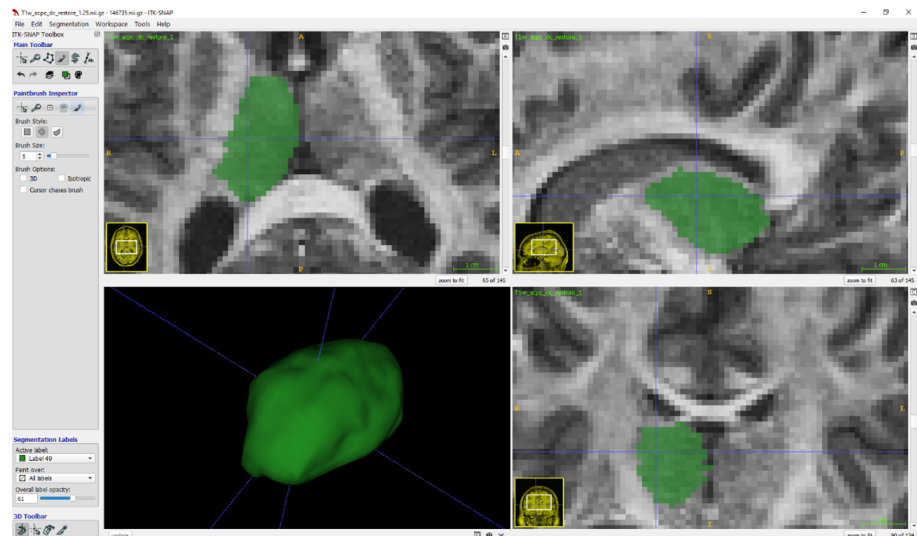


Figure S10. Segmentation of the left thalamus on 3 views (axial, sagittal, and coronal) and a 3D representation of the segmentation.

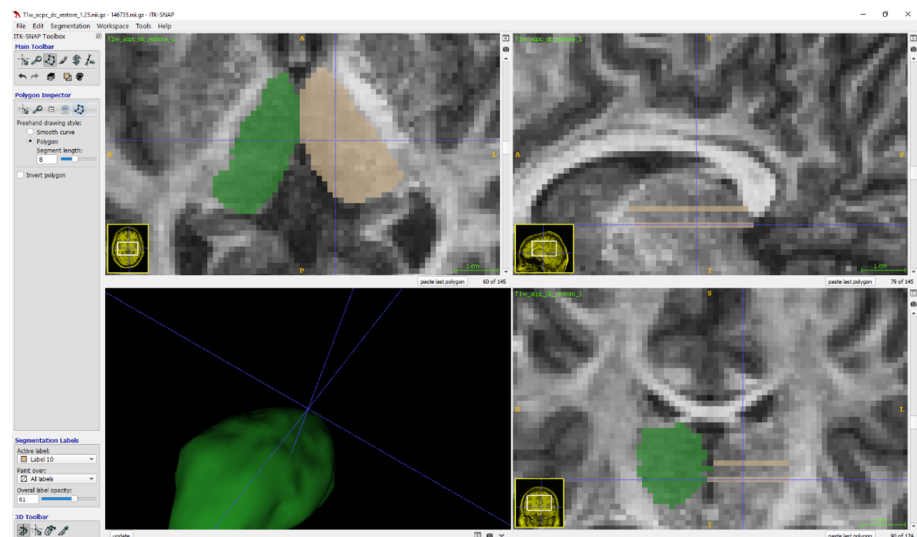


Figure S11. Segmentation of the left thalamus on 3 views, a 3D representation of the left thalamus, and two axial slices of the right thalamus.

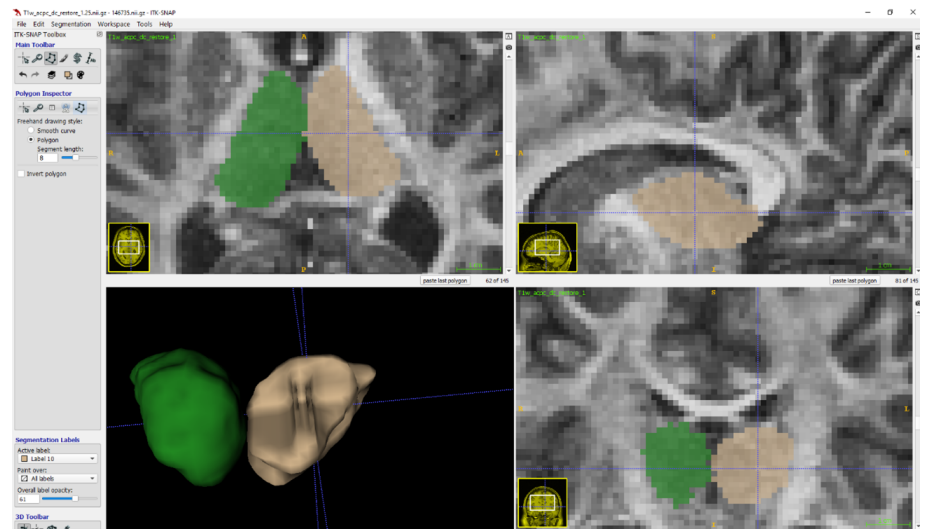


Figure S12. Segmentation of the left thalamus on 3 views, a 3D representation of the left thalamus, and partial segmentation of the right thalamus.

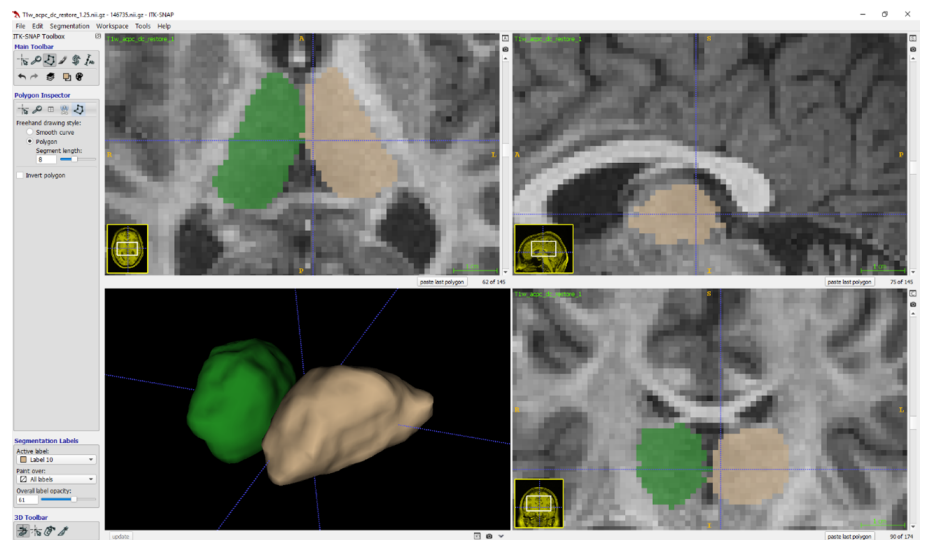


Figure S13. Segmentation of both Thalami on 3 views and a 3D representation of both segmentations.