

# An illustrated guide to landmarks in histological rat and mouse brain images

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The brain is the most complex structure known, with large numbers of interconnected regions, subregions and zones. The structural and functional architecture of brain regions differ across regions, and is often the focus of experimental studies in rat and mouse models for development, aging and disease. The anatomical localization of neuroscientific data is thus crucial for interpretation of neuroscience data. For histological images, efficient interpretation and analysis based on neuroanatomical information can be achieved by spatial registration of images to reference atlases (see, e.g. Puchades et al., 2019, PLOS one; Yates et al., 2019, Front. Neuroinf.). However, accurate spatial registration critically depends on neuroanatomical knowledge, which is necessary to identify anatomical locations and determine the spatial location and orientation of histological images. In this context, prominent and specific brain regions are highly useful and can be thought of as “landmark” structures that readily can be recognized in different images of brain tissue.

With this guide, we provide an overview of landmark structures in rat and mouse brains. We have annotated a selection of coronal, sagittal, and horizontal images and provide descriptions of the illustrated landmark structures. These landmarks provide a useful basis for getting an overview of murine brain anatomy in general and for spatial registration of brain images to reference atlases in particular. The images used in this guide are taken from publicly available datasets downloaded from the EBRAINS infrastructure and the [Allen Institute web portal](#), the references for which are listed below (click the headline to jump to the landmark descriptions):

- **Coronal rat brain images**
  - o Leergaard, T. B., Lillehaug, S., Dale, A., & Bjaalie, J. G. (2018). Atlas of normal rat brain cyto- and myeloarchitecture [Data set]. Human Brain Project Neuroinformatics Platform. <https://doi.org/10.25493/C63A-FEY> | [View original dataset](#)
- **Sagittal mouse brain images**
  - o Boccara, C., Kjonigsen, L., Hammer, I., Bjaalie, J. G., Leergaard, T. B., & Witter, M. P. (2019). Sagittal and horizontal section images showing neuronal nuclei, calbindin and parvalbumin staining in the rat hippocampal region [Data set]. Human Brain Project Neuroinformatics Platform. <https://doi.org/10.25493/JQ8F-TNF> | [View original dataset](#)
  - o The Allen mouse brain in situ hybridization database (<https://mouse.brain-map.org/>)
- **Horizontal mouse brain images**
  - o Yetman, M., Lillehaug, S., Bjaalie, J. G., Leergaard, T. B., & Jankowsky, J. (2018). Neurospine tetracycline-transactivator expression: horizontal sections (case 2877) [Data set]. Human Brain Project Neuroinformatics Platform. <https://doi.org/10.25493/AYBB-BXV> | [View original dataset](#)
  - o Boccara, C., Kjonigsen, L., Hammer, I., Bjaalie, J. G., Leergaard, T. B., & Witter, M. P. (2019). Sagittal and horizontal section images showing neuronal nuclei, calbindin and parvalbumin staining in the rat hippocampal region [Data set]. Human Brain Project Neuroinformatics Platform. <https://doi.org/10.25493/JQ8F-TNF> | [View original dataset](#)
  - o Papp EA, Leergaard TB, Calabrese E, Johnson GA, Bjaalie JG (2014) Waxholm Space atlas of the Sprague Dawley rat brain. NeuroImage 97, 374-386. DOI: 10.1016/j.neuroimage.2014.04.001

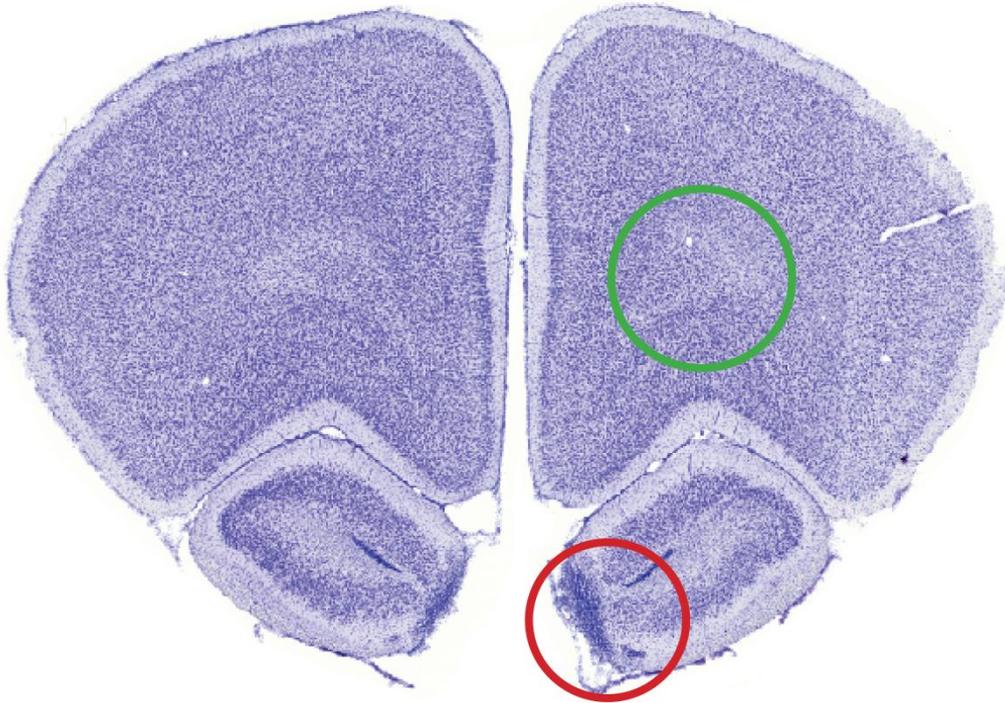
## Landmarks in the coronal plane (anterior to posterior)

| <u>Landmark</u>                              | <u>Description</u>  |
|--|---|
| <b>Granular layers of the olfactory bulb</b> | The most anterior section where the granular layer of the olfactory bulb is last seen (Figure 1, red)   |
| <b>Anterior corpus callosum</b>              | The most anterior section where the corpus callosum is visible (Figure 1, green)  |
| <b>Anterior nucleus accumbens</b>            | The most anterior section where the nucleus accumbens is visible (Figure 2, red)  |
| <b>Anterior striatum</b>                     | The most anterior section where the striatum is visible (Figure 2, green)   |
| <b>Genu corpus callosum</b>                  | The most anterior section in which the corpus callosum is continuous across the midline (Figure 3)  |
| <b>Merging of anterior commissure</b>        | The most anterior section in which the anterior commissure is continuous across the midline (Figure 4)  |
| <b>Anterior hippocampus</b>                  | The most anterior section where the hippocampus can be distinguished (Figure 5). The rest of the anterior part of the hippocampus has a characteristic profile and is well suited to determine anteroposterior location |
| <b>Amygdalar nuclei</b>                      | A triangle shaped nuclei compilation that can be seen through many sections medially in the brain going anterior to posterior (Figure 6)  |
| <b>Optic chiasm/Optic tract</b>              | The development of the optic chiasm into optic tract, and the change in shape of optic tract when moving posteriorly (Figure 7, red)  |
| <b>Habenular nuclei/habenular commissure</b> | The most posterior section where habenular nuclei develops into habenular commissure (Figure 8, red)  |
| <b>Posterior commissure</b>                  | Most anterior section where posterior commissure appears dorsally to 3rd ventricle (Figure 9)   |
| <b>Splenium corpus callosum</b>              | The most anterior section where the corpus callosum is no longer continuous across the midline (Figure 10) Note that in transgenic mice, an asymmetry with the template is often observed                               |
| <b>Ventral dentate gyrus</b>                 | The most anterior section where ventral dentate gyrus is visible (Figure 11)  |

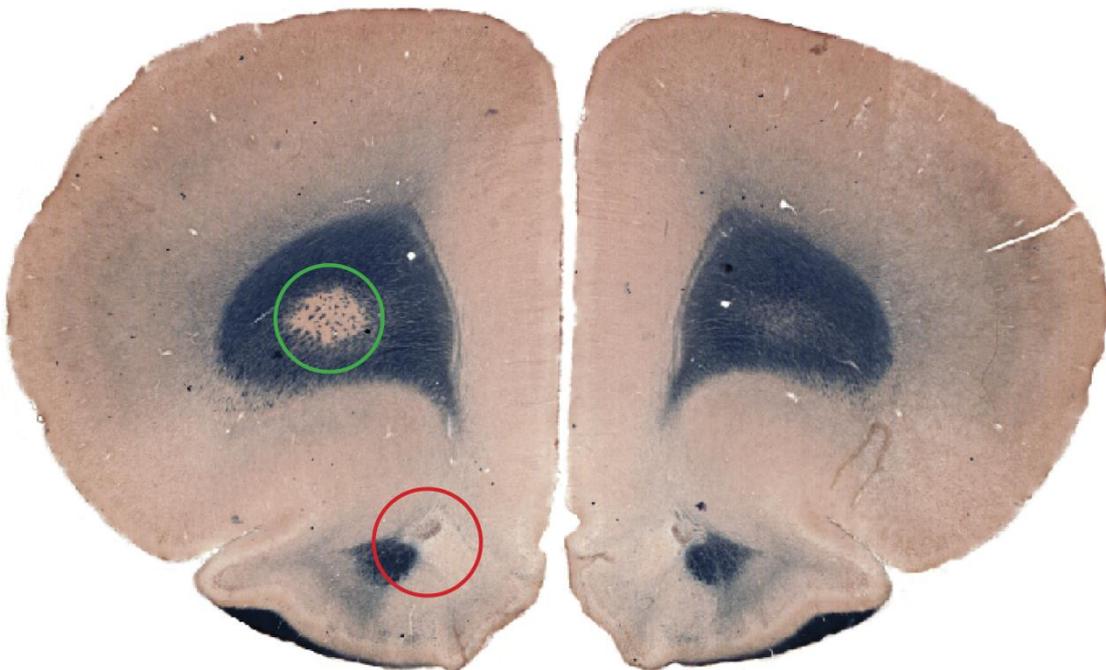
|  |   |
|--|---|
| <b>Posterior mammillary nucleus</b>                    | The most anterior section where the nucleus is still visible (Figure 12)  |
| <b>Posterior end of thalamus</b>                       | The most anterior section in which the thalamus is no longer visible (Figure 13)  |
| <b>Posterior superior colliculus</b>                   | The most posterior section showing the posterior superior colliculus (Figure 14)  |
| <b>Posterior dentate gyrus</b>                         | The most posterior section showing the dentate gyrus (Figure 15)  |
| <b>Posterior subiculum</b>                             | The most posterior section showing the posterior subiculum (Figure 16)  |
| <b>Inferior colliculus</b>                             | The most anterior section in which the superior colliculus is no longer visible and the inferior colliculus occupies the dorsal-most part of the brain stem (Figure 17)   |
| <b>Anterior pontine nuclei</b>                         | The most anterior section in which the grey matter of the pontine nuclei can be discerned among the distinct transverse fibres of the pons (Figure 18)  |
| <b>Separation of inferior colliculus and brainstem</b> | The first section posteriorly where the inferior colliculus has separated from the brainstem and cerebellum in visible medially (Figure 19)   |
| <b>Posterior inferior colliculus</b>                   | The most posterior section where the tissue of inferior colliculus is still visible (Figure 19)   |
| <b>Facial nerve</b>                                    | The section where facial nerve is seen across the whole brainstem (Figure 20)   |
| <b>Cerebellar nuclei</b>                               | The most anterior and posterior sections after the separation of brainstem and cerebellum in which the nuclei are visible (Figure 21)   |
| <b>Separation of cerebellum and brainstem</b>          | The first section posteriorly where cerebellum and brainstem are no longer connected (Figure 22)  |
| <b>Posterior cerebellum</b>                            | The last section where tissue of cerebellum is still visible (Figure 23). Note that this should only be used carefully in the case of physical sections, as small parts of the posterior cerebellum might have been lost during mounting. |
| <b>Inferior olive, anterior and posterior end</b>      | The most anterior and the most posterior sections in which the inferior olive is visible (Figure 24 and 26, respectively)   |
| <b>Merging of tissue around central canal</b>          | The section where the tissue by the 4th ventricle closes and the central canal is created (Figure 25)   |

**Illustration of landmarks**

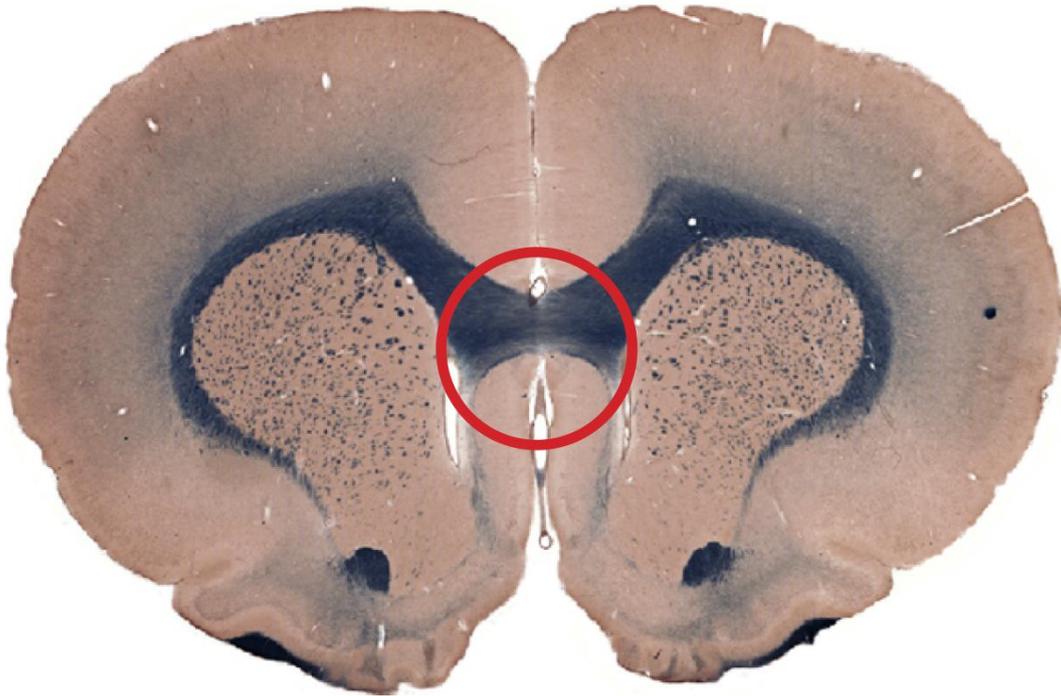
**Figure 1: Cell layers of olfactory bulb (red) / Anterior corpus callosum (green)**



**Figure 2: Anterior striatum (dorsal striatum in green, nucleus accumbens in red)**



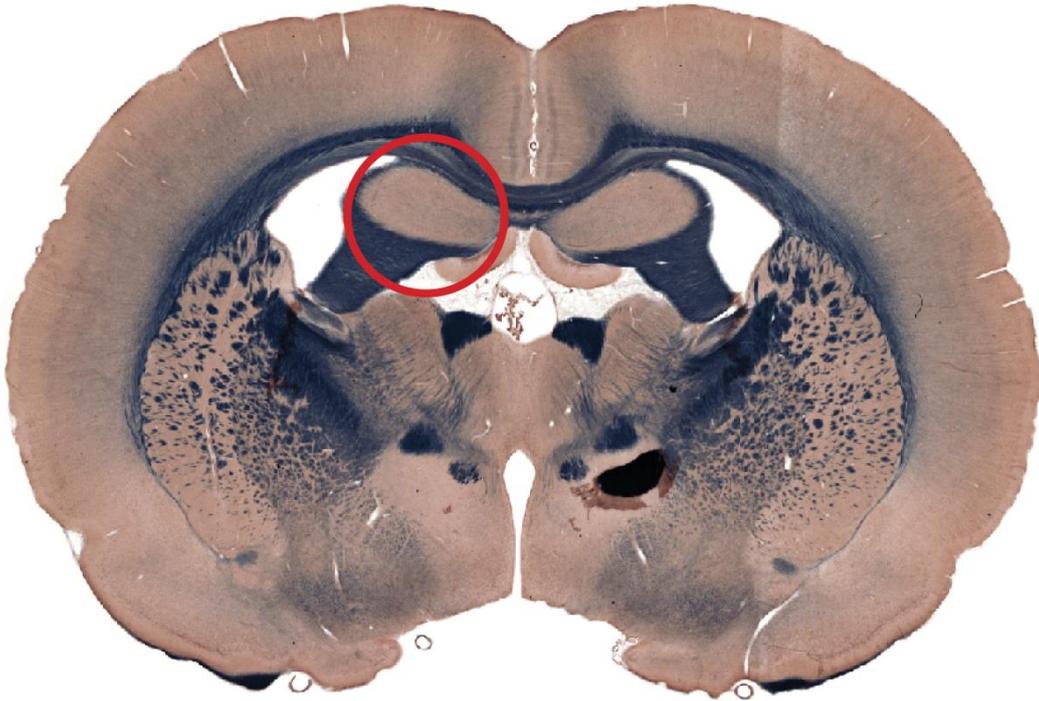
**Figure 3: Genu of corpus callosum**



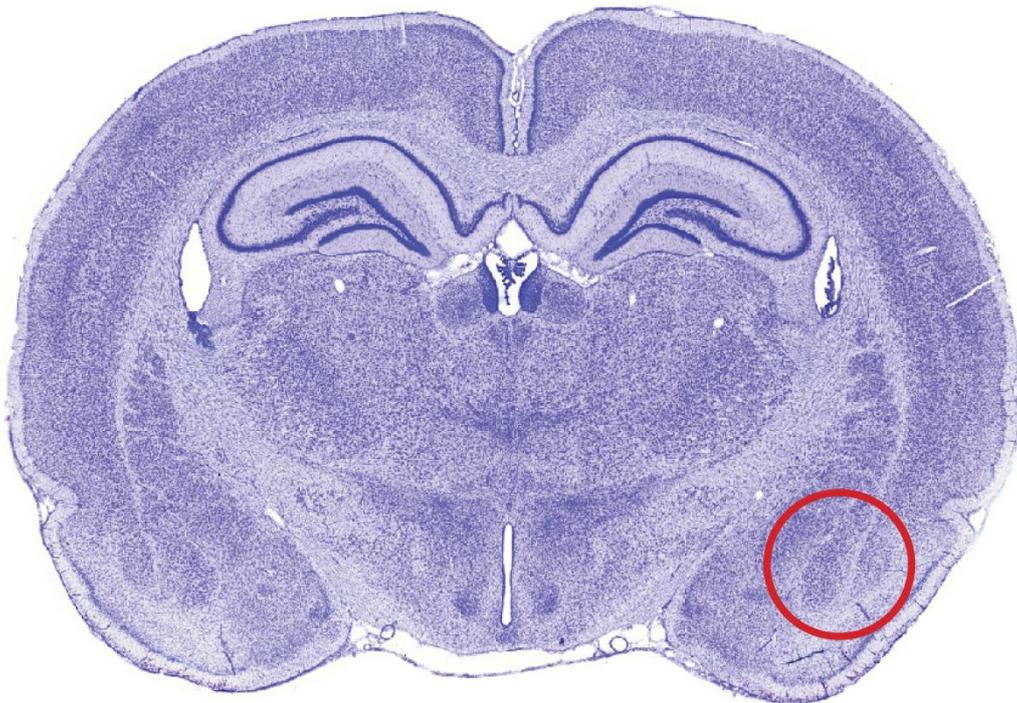
**Figure 4: Merging of anterior commissure**



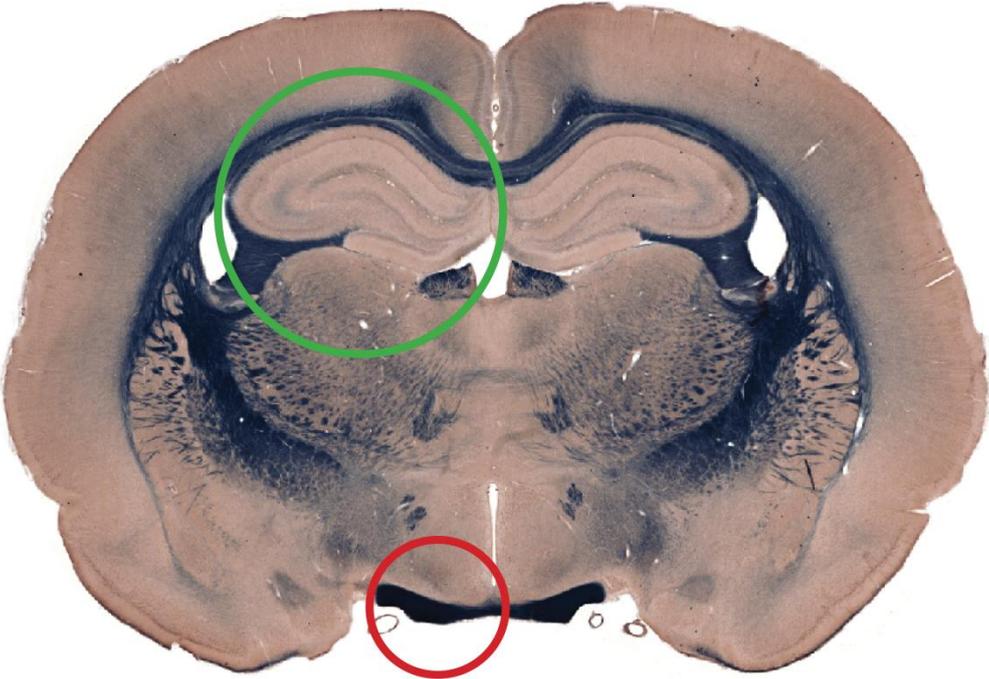
**Figure 5: Anterior hippocampus**



**Figure 6: Amygdala**



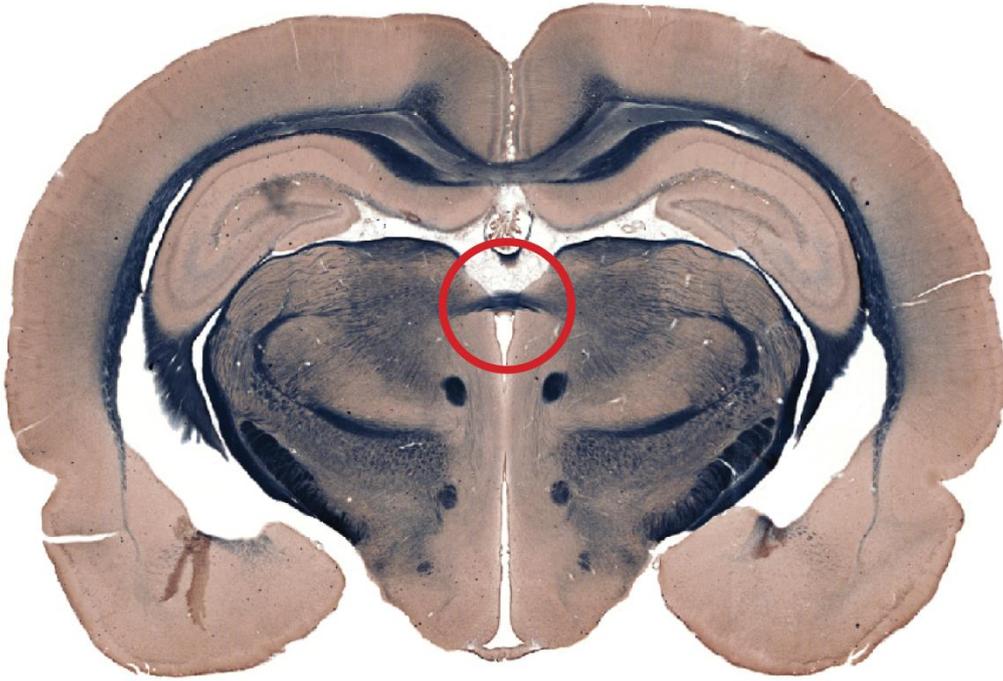
**Figure 7: Optic tract in red, mid-level of the anterior hippocampus in green**



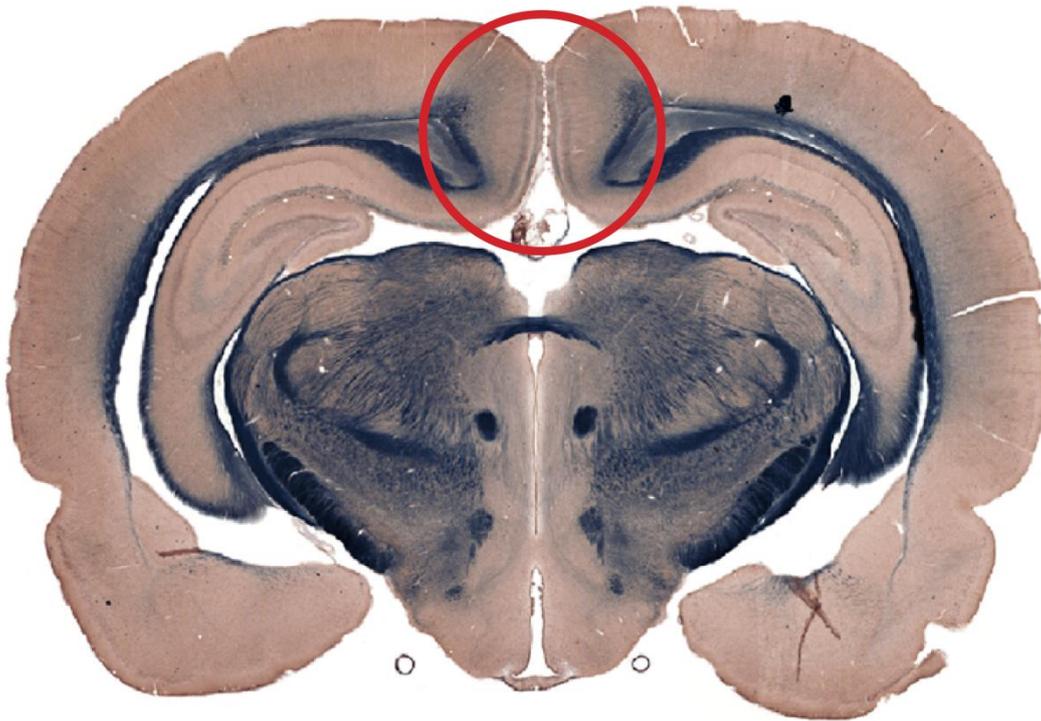
**Figure 8: Habenular commissure**



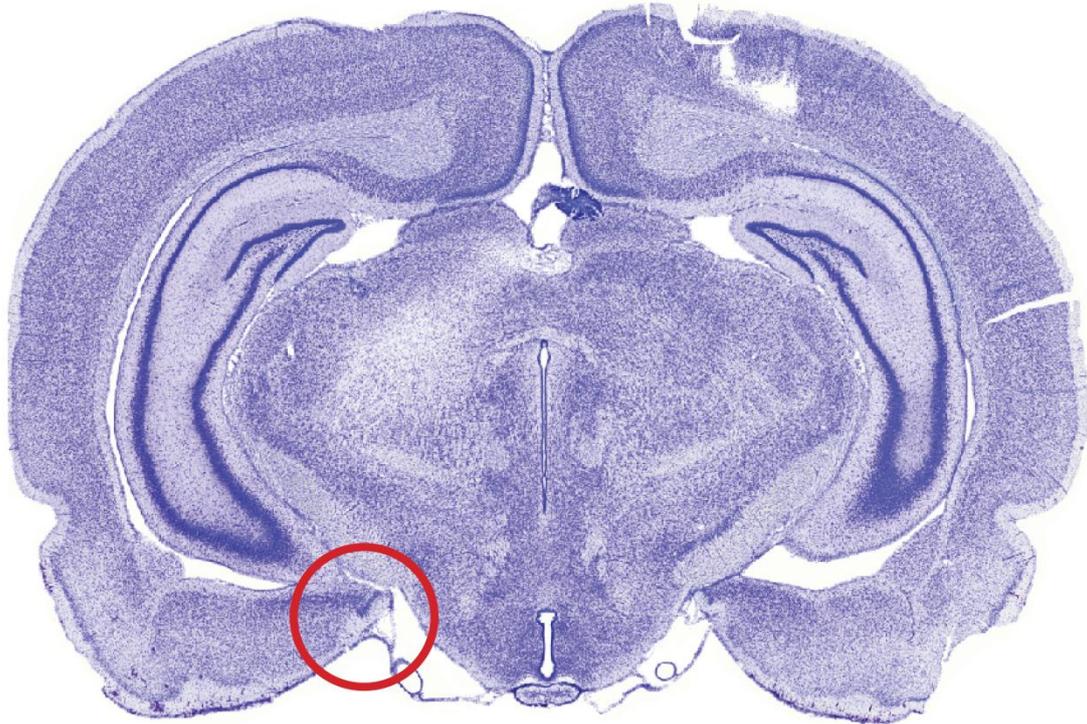
**Figure 9: Posterior commissure**



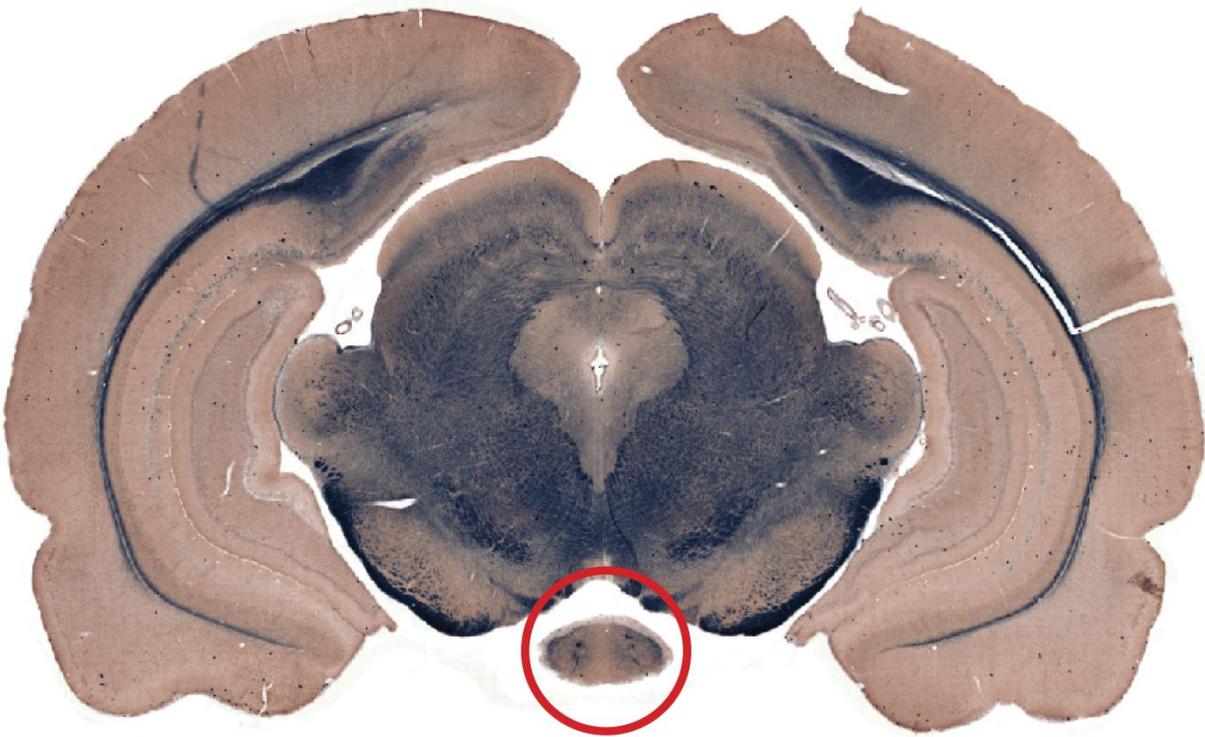
**Figure 10: Splenium of the corpus callosum**



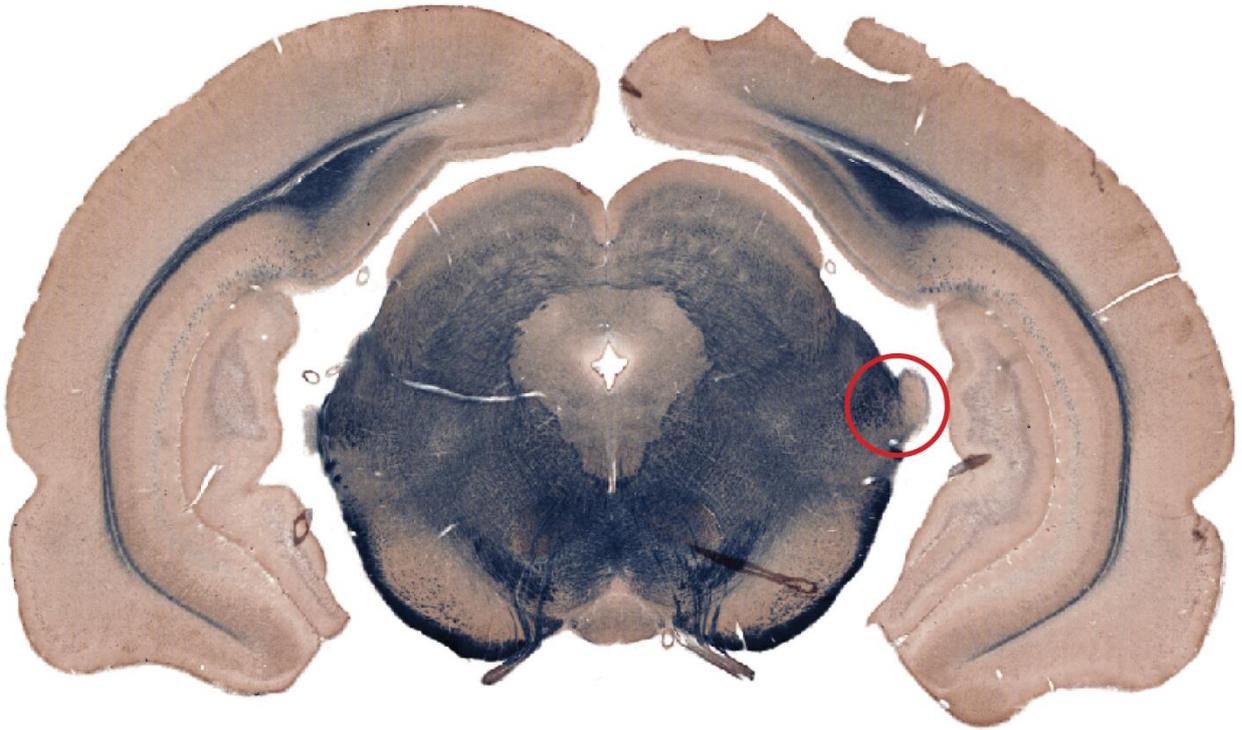
**Figure 11: Ventral dentate gyrus**



**Figure 12: Posterior end of mammillary nucleus**



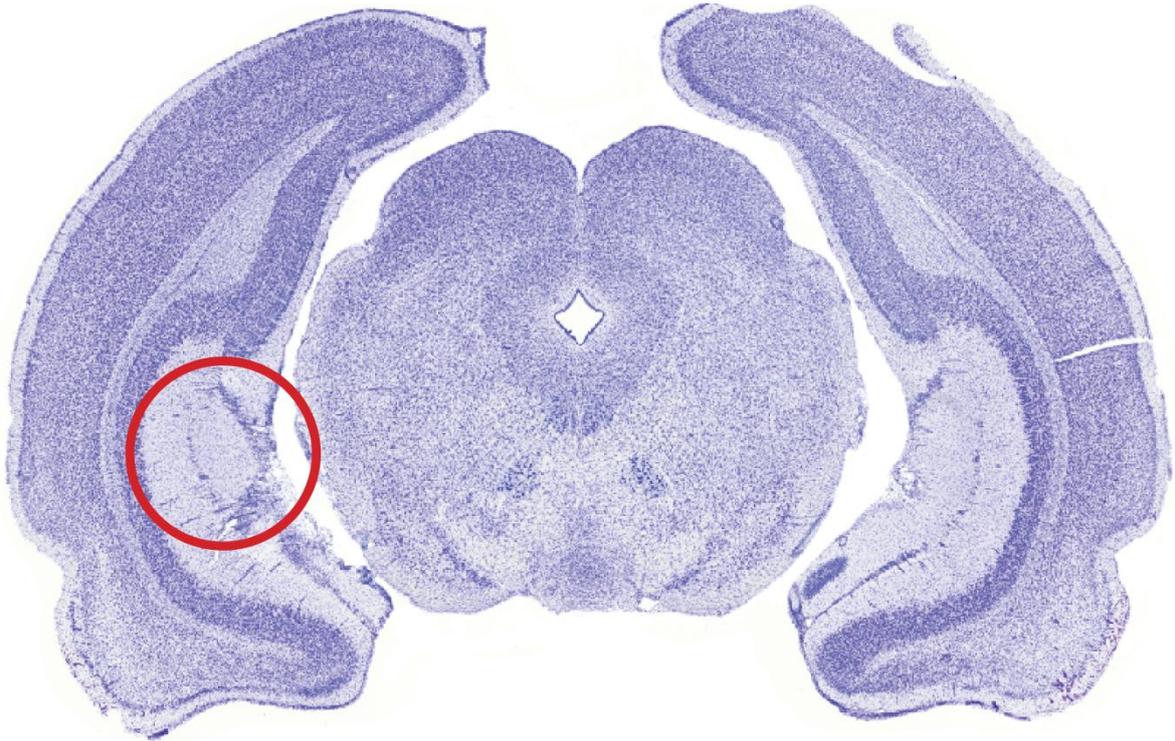
**Figure 13: Posterior end of thalamus**



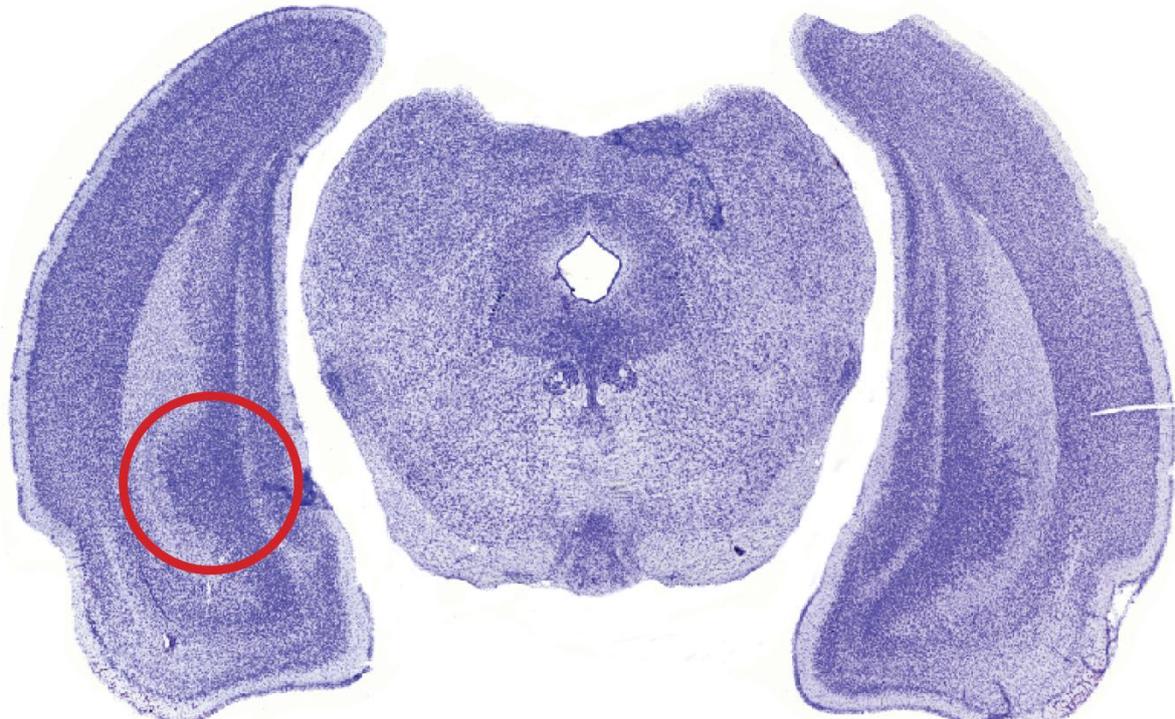
**Figure 14: Posterior end of superior colliculus**



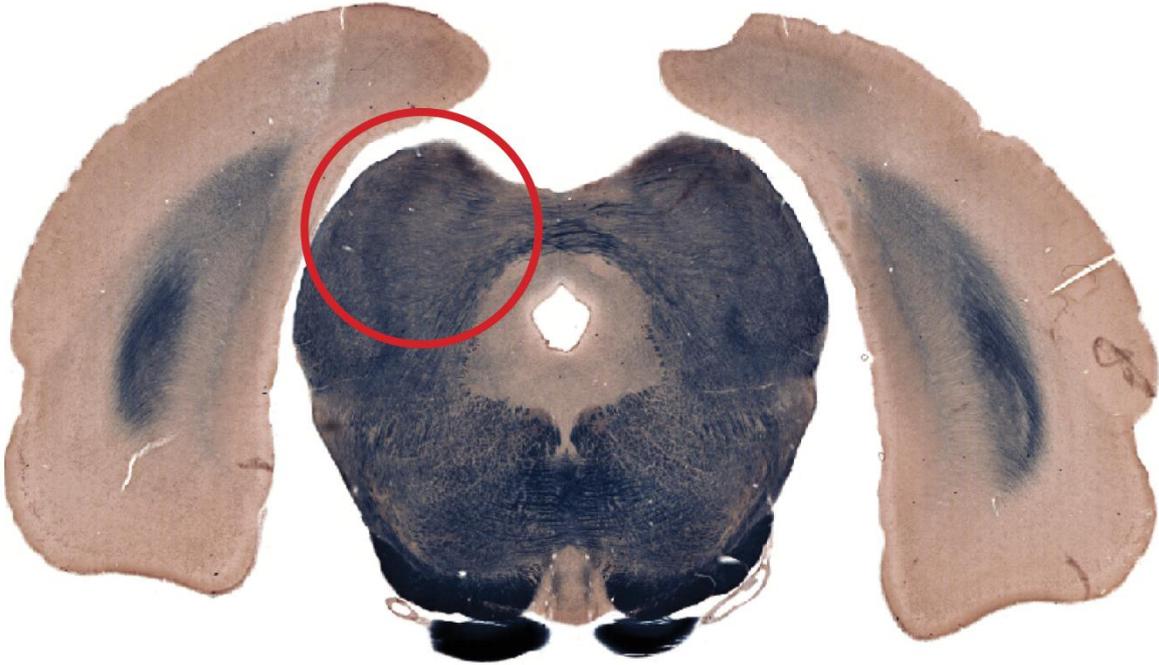
**Figure 15: Posterior end of dentate gyrus**



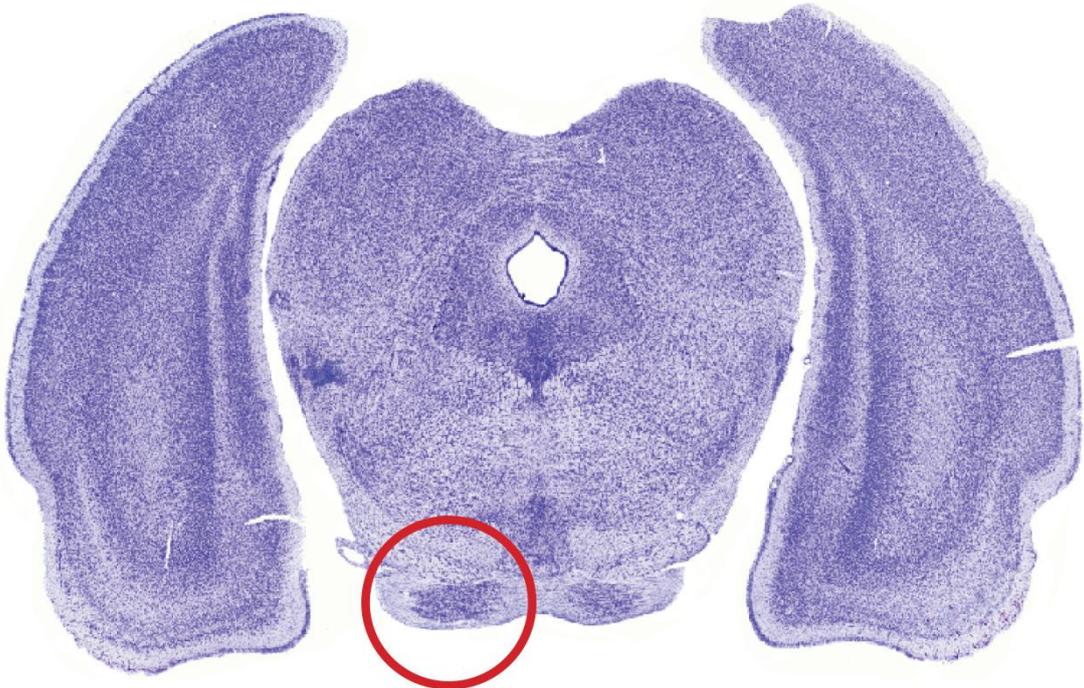
**Figure 16: Posterior end of subiculum**



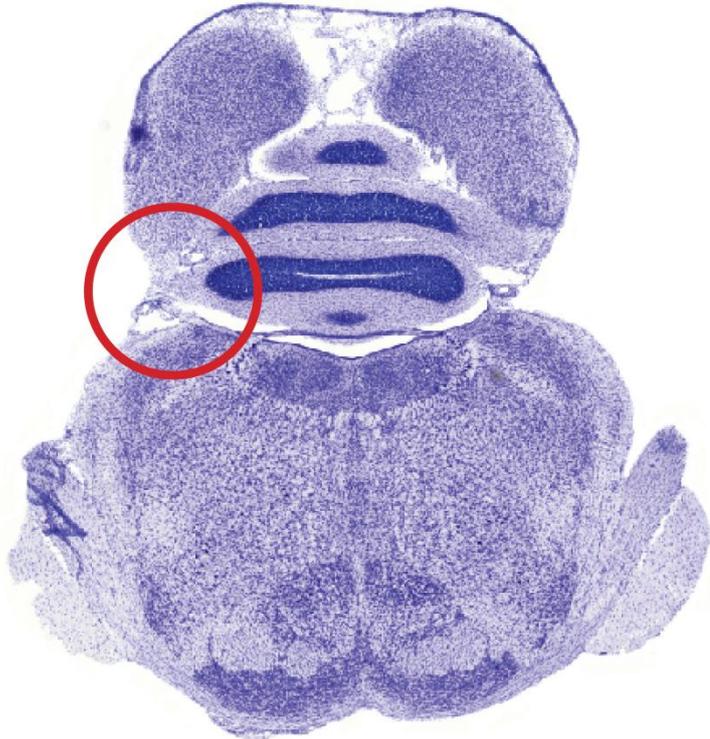
**Figure 17: Inferior colliculus**



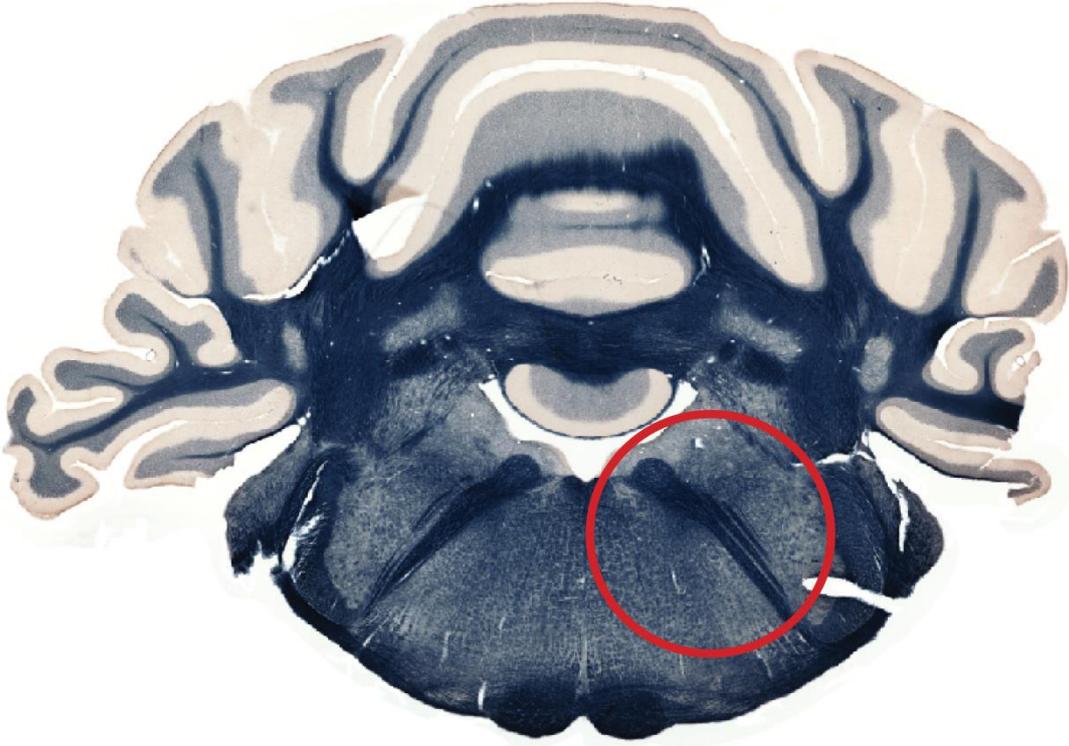
**Figure 18: Anterior pons**



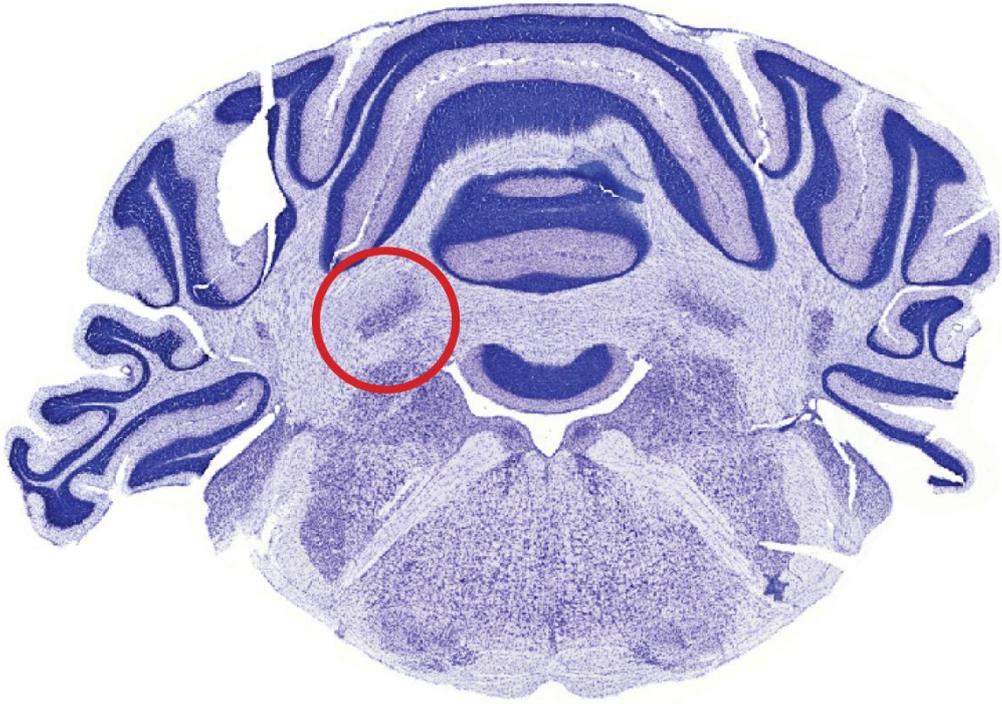
**Figure 19: Separation of inferior colliculus and brainstem / Posterior inferior colliculus**



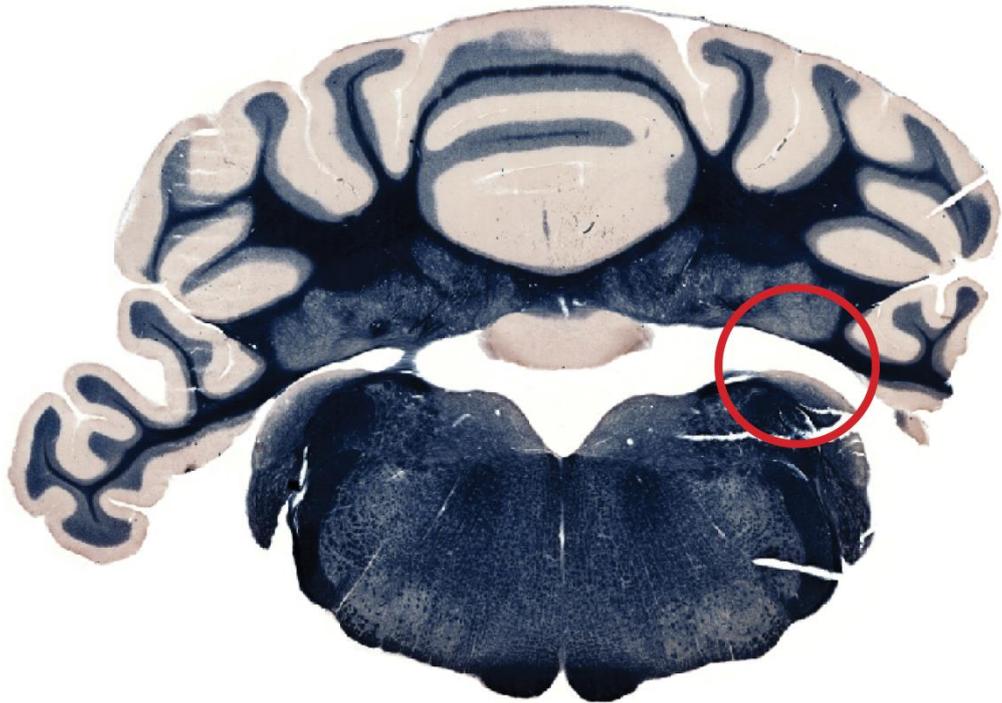
**Figure 20: Facial nerve**



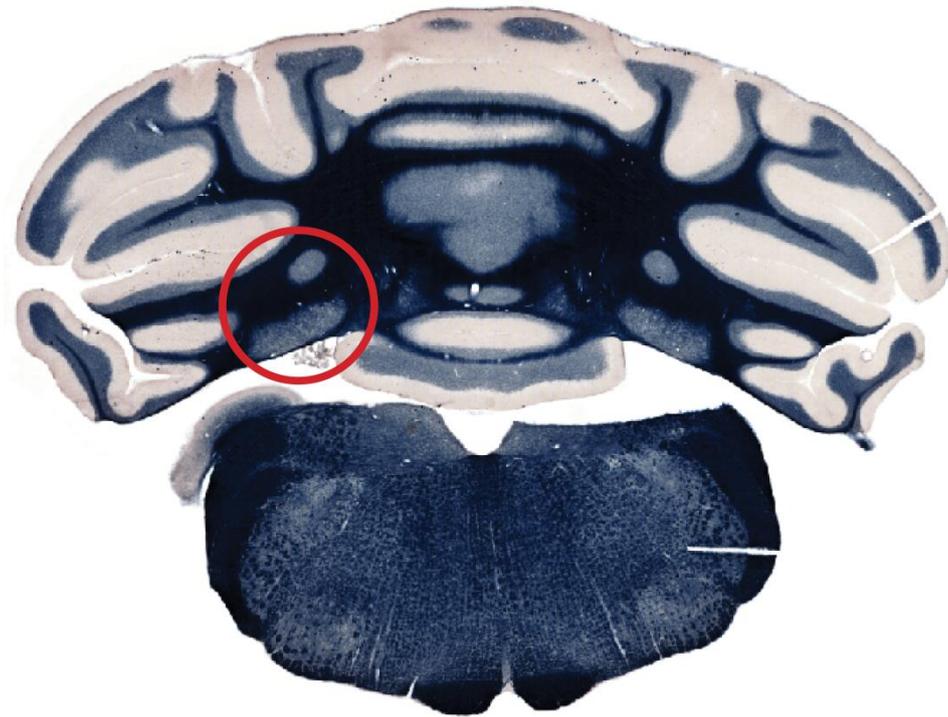
**Figure 21: Anterior part of cerebellar nuclei**



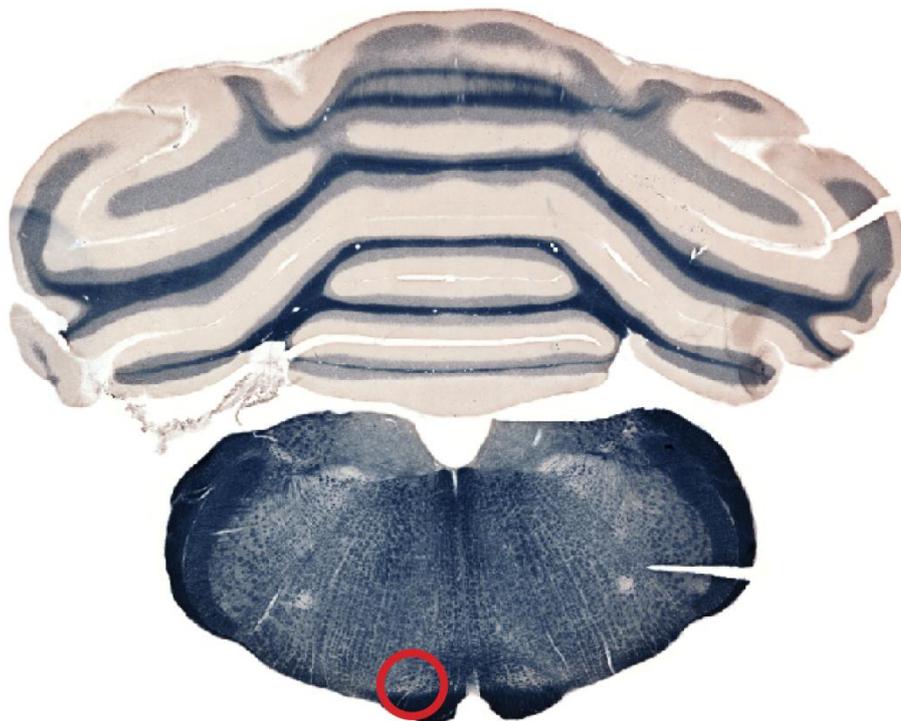
**Figure 22: Separation of cerebellum and brainstem**



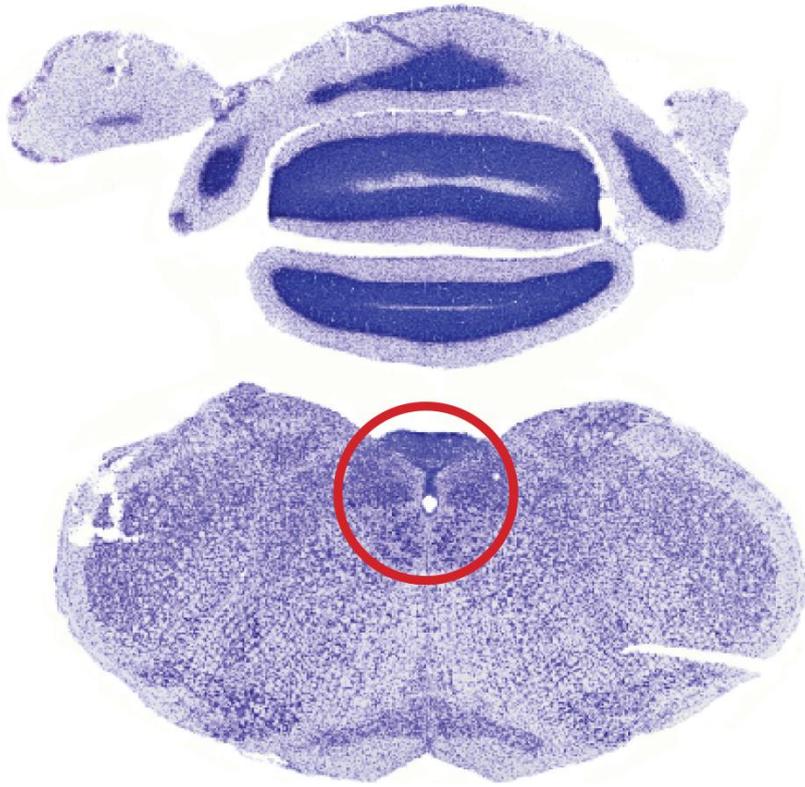
**Figure 23: Posterior part of the cerebellar nuclei**



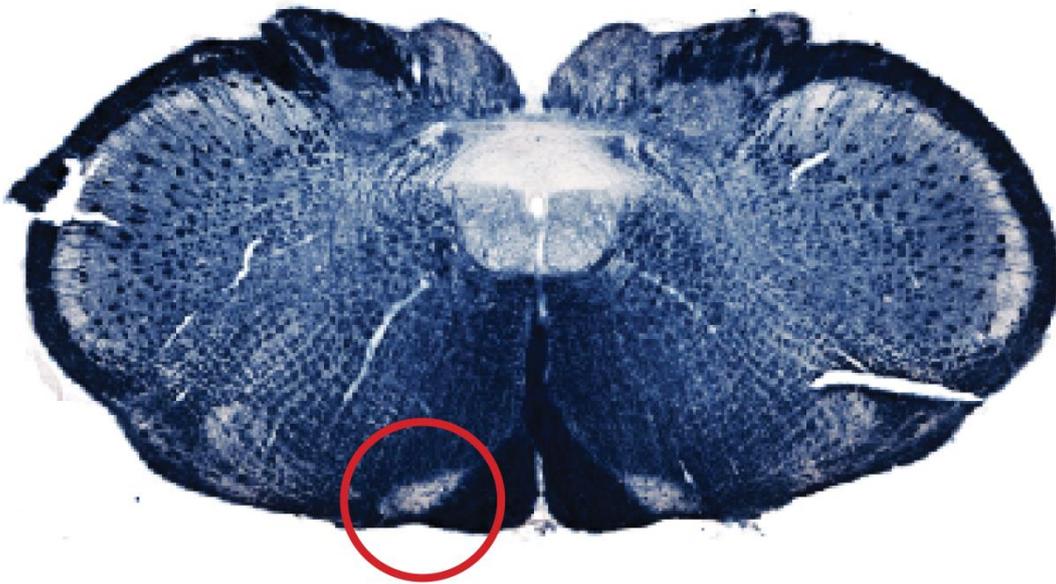
**Figure 24: Anterior inferior olive**



**Figure 25: Merging of the tissue around the central canal**



**Figure 26: Posterior inferior olive**



## Landmarks in the sagittal plane (lateral to medial)

| <u>Landmarks</u>   | <u>Description</u>  |
|--|---|
| <b>Lateral hippocampus</b>                                     | The first lateral section where the hippocampus is visible (Figure 1)   |
| <b>Lateral striatum</b>  | The first lateral section where the striatum is visible (Figure 2)  |
| <b>Lateral cerebellum</b>                                      | The first lateral section where cerebellar tissue becomes visible (Figure 3)  |
| <b>Internal capsule</b>  | The first lateral section where the internal capsule is visible (Figure 4)  |
| <b>Lateral globus pallidus</b>                                 | The first lateral section where the globus pallidus, external segment is visible (Figure 5)                               |
| <b>The x shaped CA3</b>  | The section where the cell layer of CA3 forms an x between the dorsal and ventral hippocampus (Figure 6, green)           |
| <b>Brainstem</b>   | The most lateral section where the brainstem is visible (Figure 6, red)   |
| <b>Cerebellar nuclei</b>                                       | The most lateral section where the cerebellar nuclei is visible (Figure 7)  |
| <b>Ventral geniculate nucleus</b>                              | The first section where the ventral geniculate nucleus (thalamus) is visible (Figure 8)                                   |
| <b>Separation of dorsal and ventral hippocampus</b>            | The most lateral section where the whole hippocampus is divided into a dorsal and a ventral part (Figure 9)               |
| <b>Separation of dorsal and ventral parahippocampal region</b> | The first section where the parahippocampal region separates into dorsal and ventral parts (Figure 10)                    |
| <b>Brachium of inferior colliculus</b>                         | The most lateral section where the brachium is visible ventrally to the para- and presubiculum (Figure 11, red)           |
| <b>Olfactory tubercle</b>                                      | The most lateral section where the olfactory tubercle is visible in the ventral part of the hemisphere (Figure 11, green) |
| <b>Olfactory bulb</b>  | The most lateral section where the olfactory bulb becomes visible (Figure 12)   |
| <b>Medial end of ventral hippocampus</b>                       | The most medial section where the ventral end of the hippocampus is still visible (Figure 13)                             |
| <b>Pontine nuclei</b>  | The most lateral section where the nuclei first is visible (Figure 14)  |

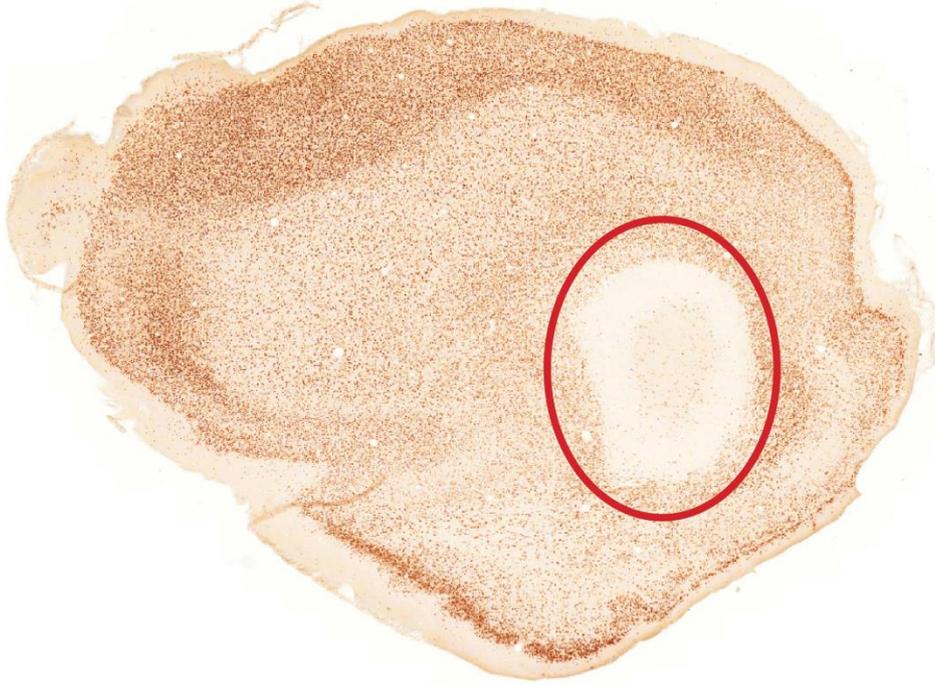
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|   |   |
|---|---|
| <b>Merging of anterior commissure</b>       | The section where the merging of anterior and posterior part of the anterior commissure is visible (Figure 15)    |
| <b>Lateral septal nucleus</b>               | The first section where the striatum is completely gone and all that is visible is the septal nucleus (Figure 16) |
| <b>Medial striatum</b>                      | The most medial section where the striatum is still visible (Figure 17)   |
| <b>Medial dorsal hippocampus</b>            | The most medial section where the end of the dorsal hippocampus is still visible (Figure 18)                      |
| <b>Dorsal cortex of inferior colliculus</b> | The most medial section where the tissue of the inferior colliculus is still visible (Figure 19)                  |
| <b>Medial habenular nucleus</b>             | The medial section where the nucleus becomes visible on top of the round shaped thalamic nuclei (Figure 20)       |

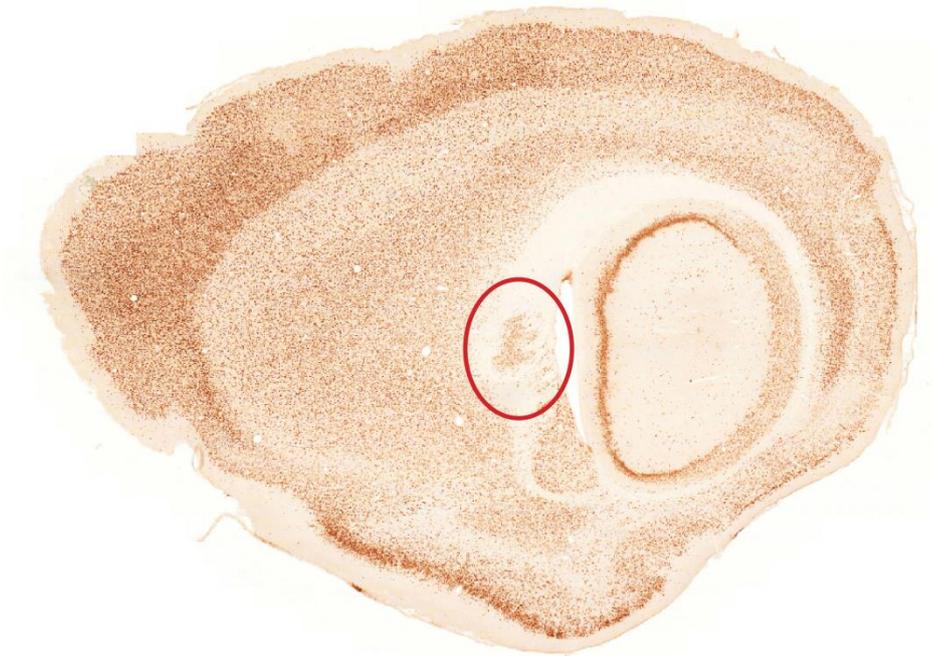
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**Illustration of landmarks**

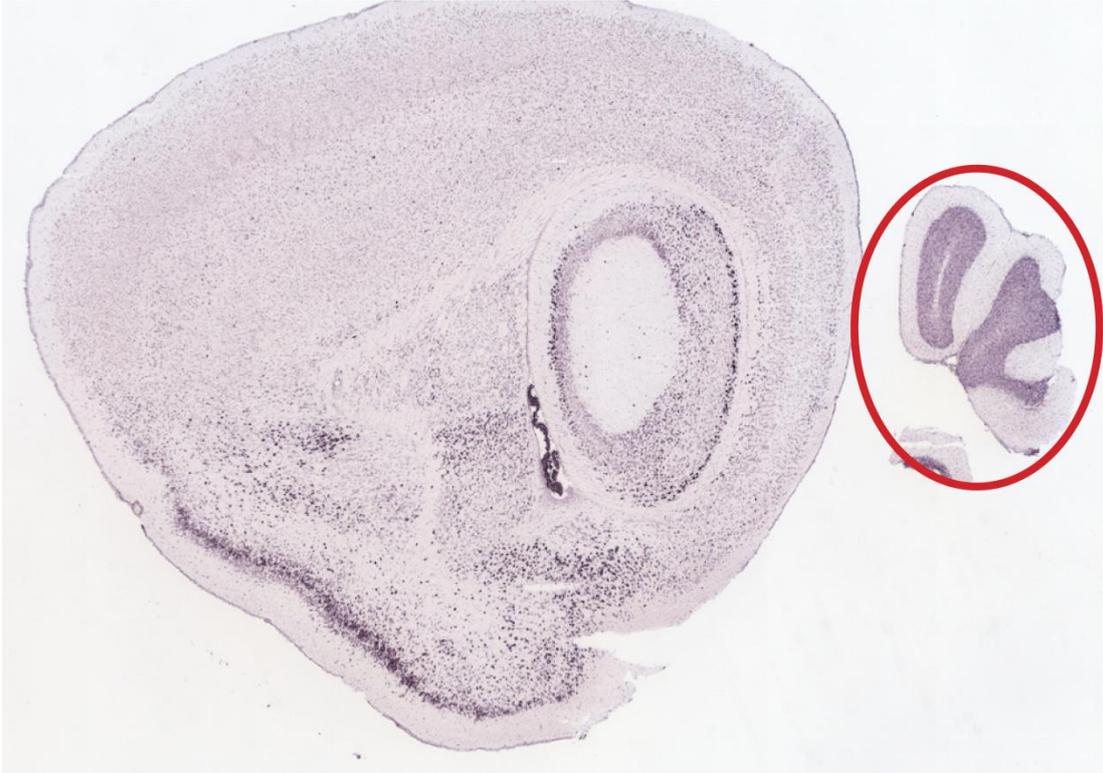
**Figure 1: Lateral hippocampus**



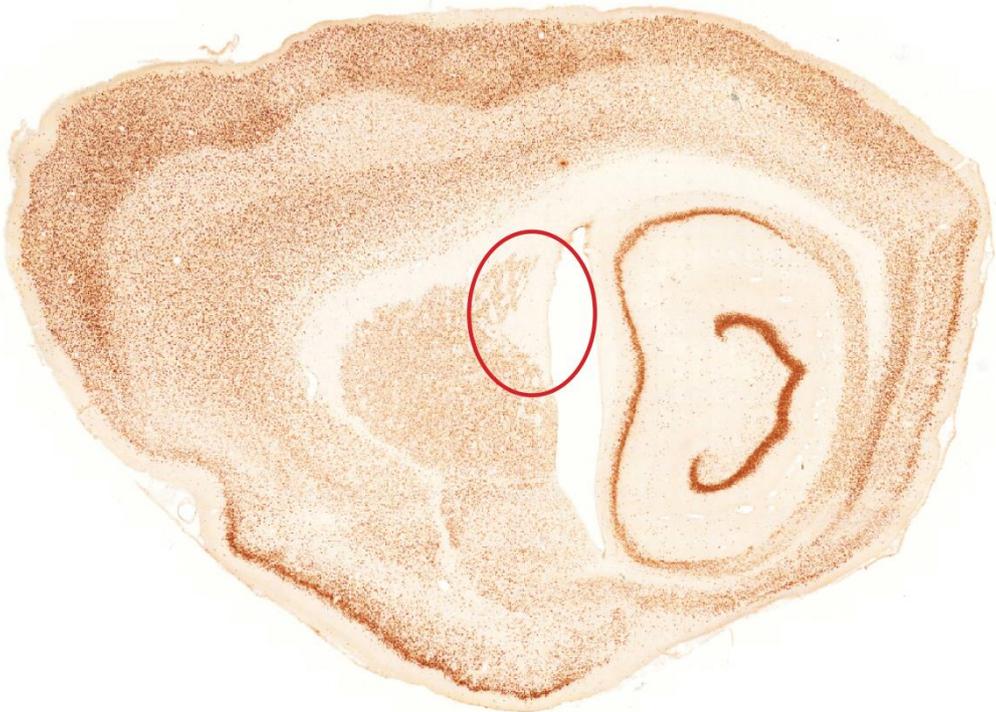
**Figure 2: Lateral striatum**



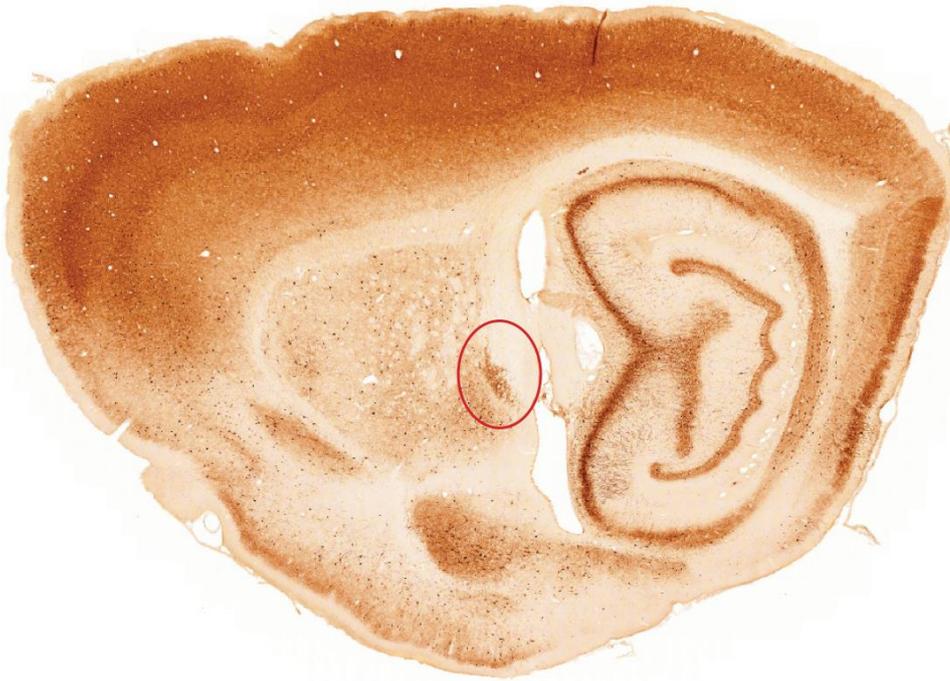
**Figure 3: Lateral cerebellum**



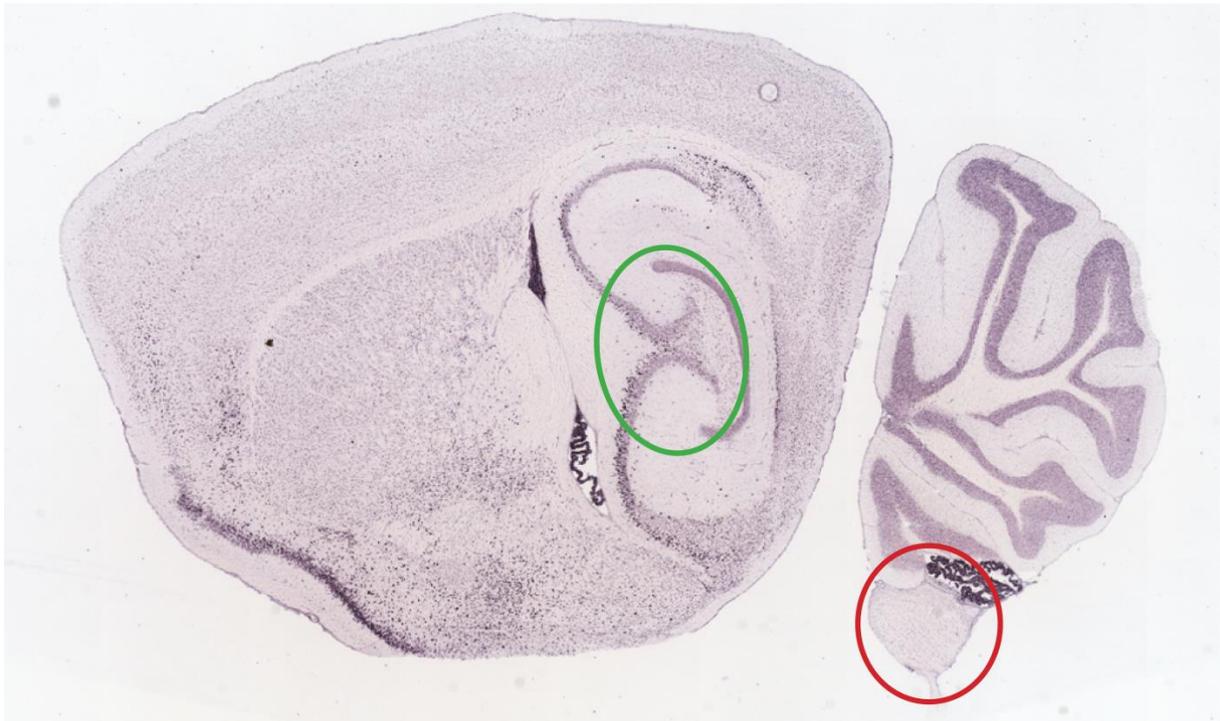
**Figure 4: Internal capsule**



**Figure 5: Lateral globus pallidus**



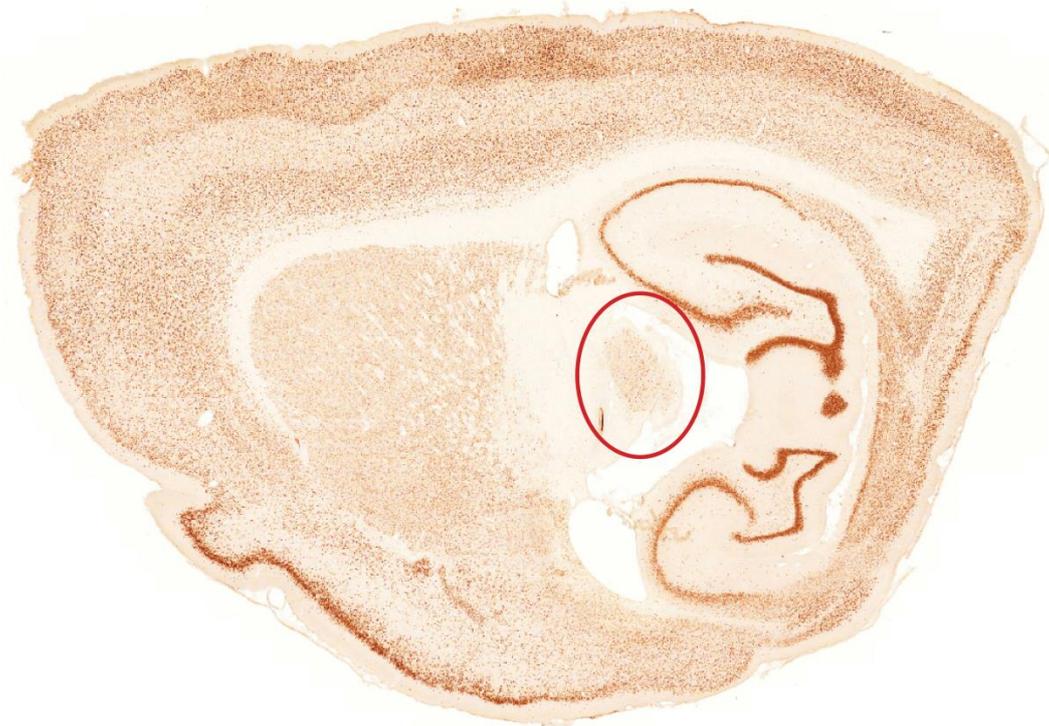
**Figure 6: The brainstem (red) and the x shaped CA3 (green)**



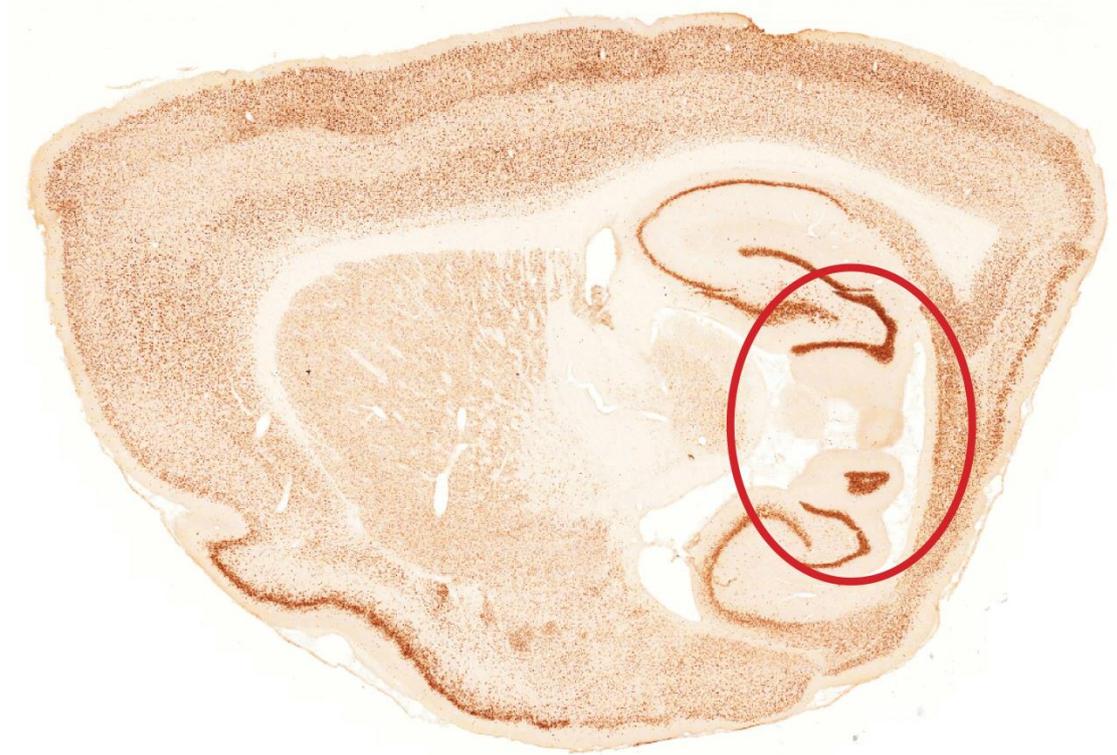
**Figure 7: Cerebellar nuclei**



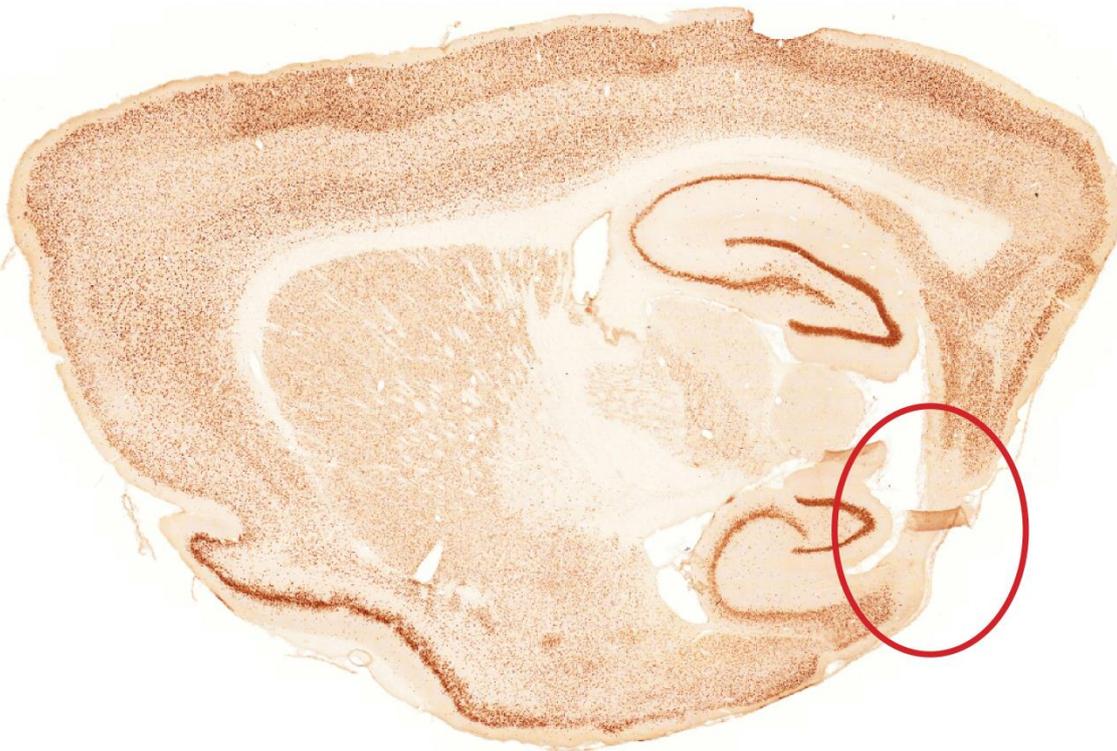
**Figure 8: Ventral geniculate nucleus**



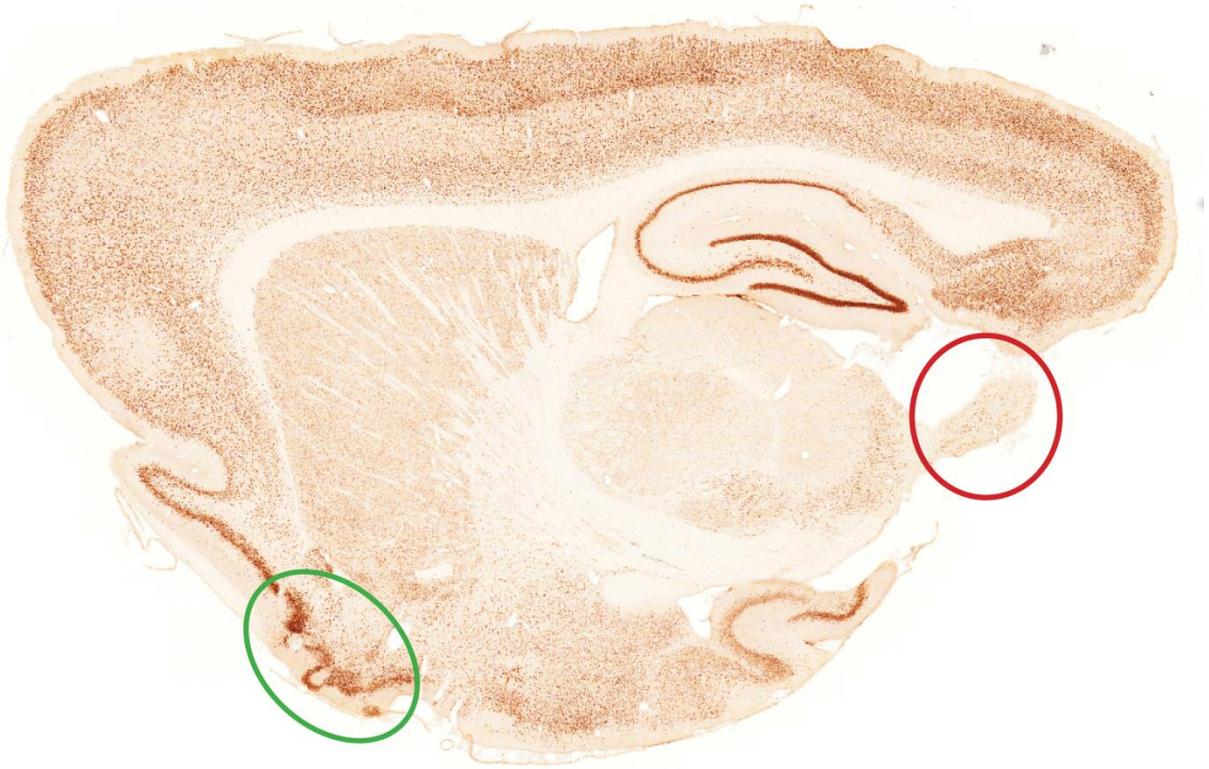
**Figure 9: Separation of dorsal and ventral hippocampus**



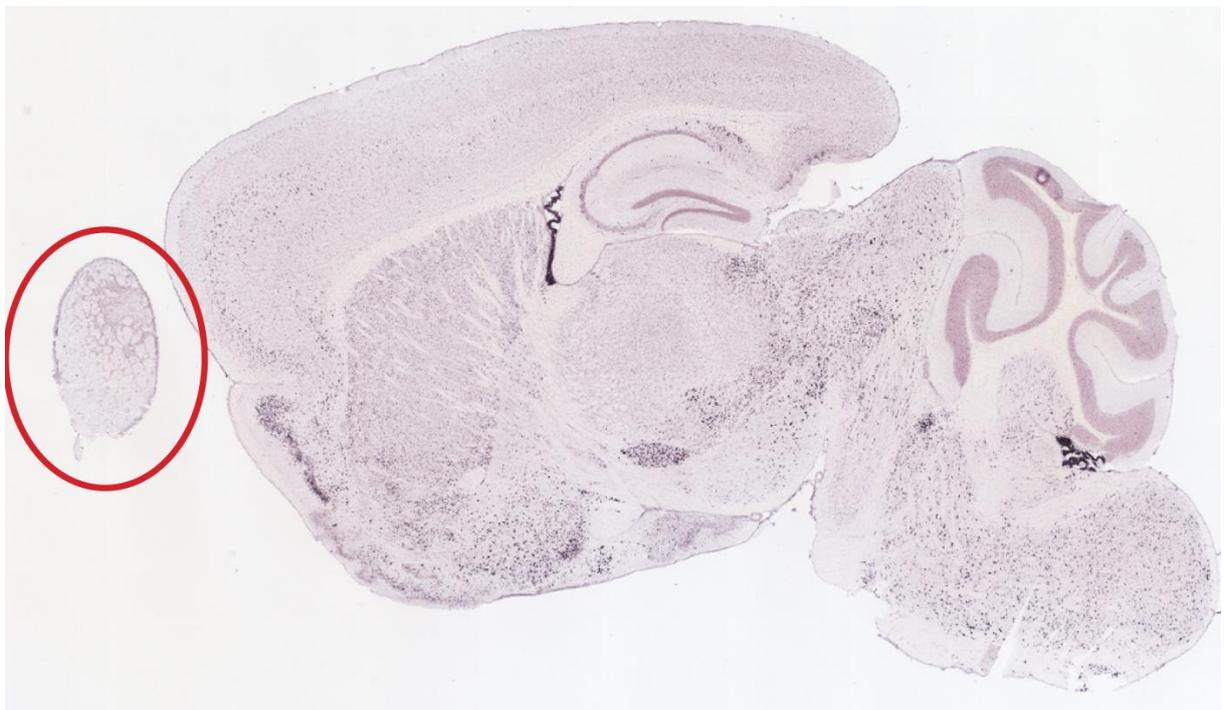
**Figure 10: Separation of dorsal and ventral parahippocampal region**



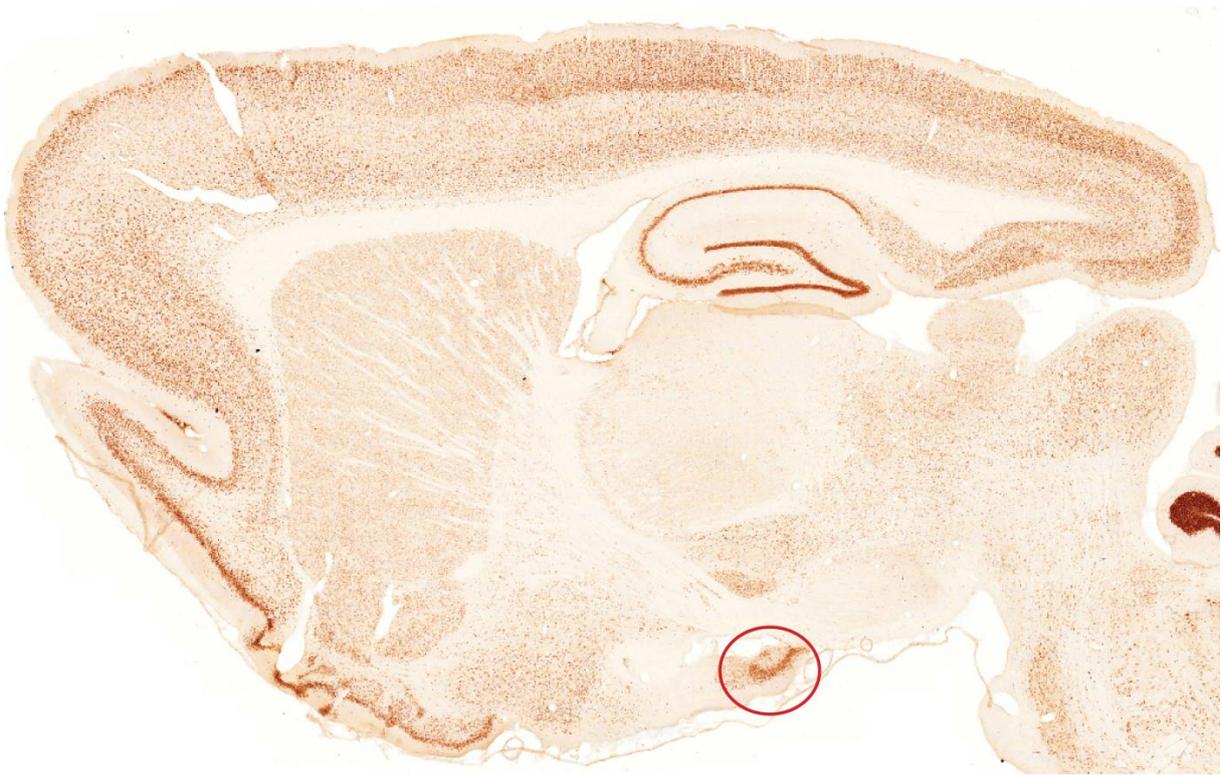
**Figure 11: Brachium of inferior colliculus (red) and olfactory tubercle (green)**



**Figure 12: Olfactory bulb**



**Figure 13: Medial end of ventral hippocampus**



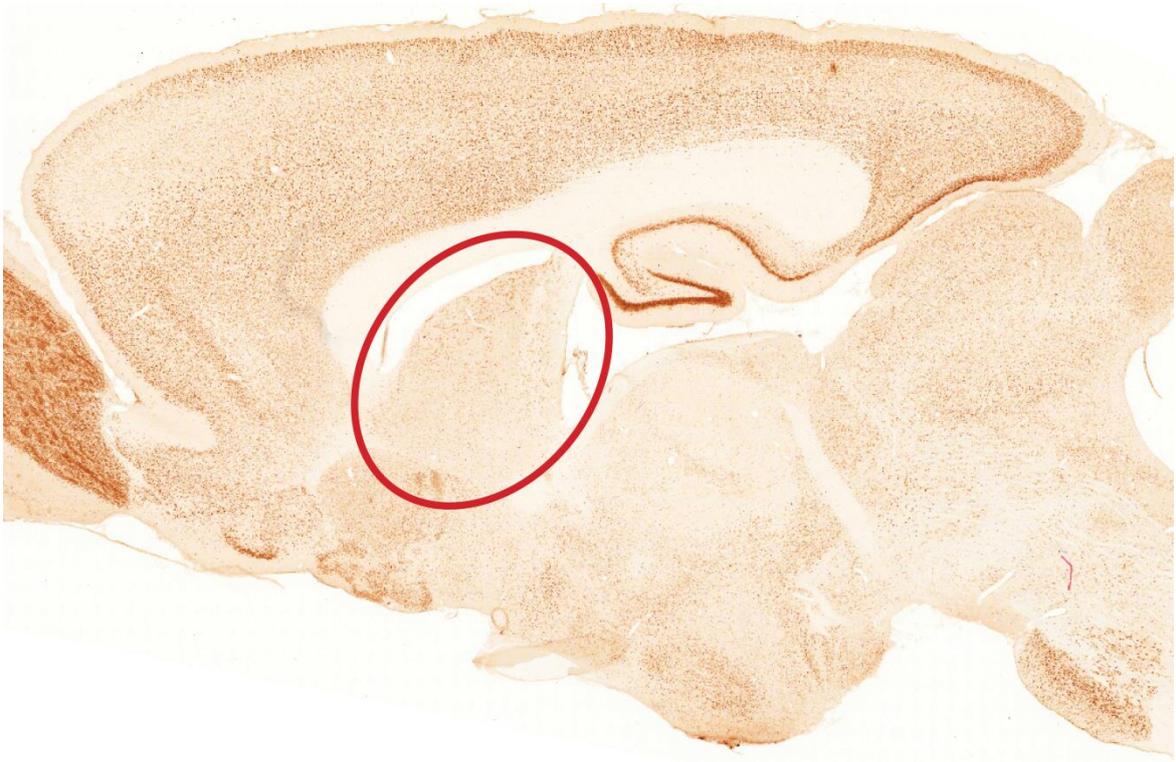
**Figure 14: Pontine nuclei**



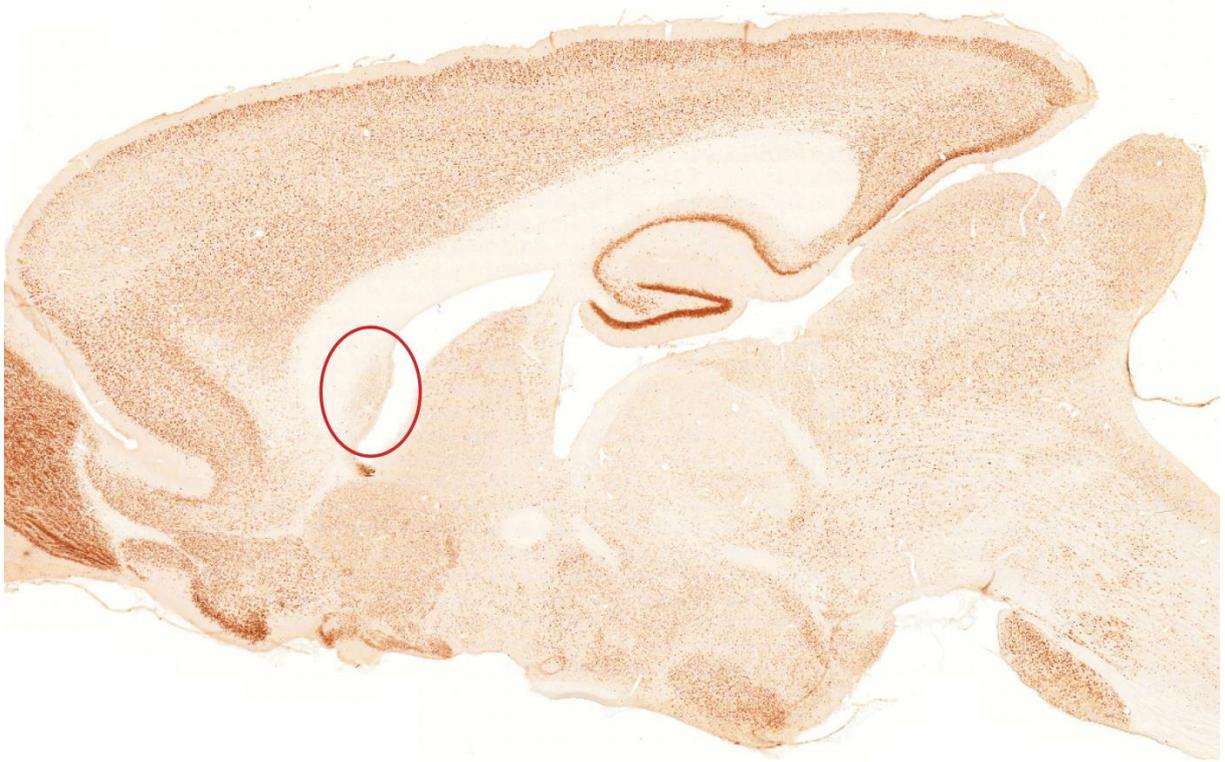
**Figure 15: Merging of anterior commissure**



**Figure 16: Lateral septal nucleus**



**Figure 17: Medial striatum**



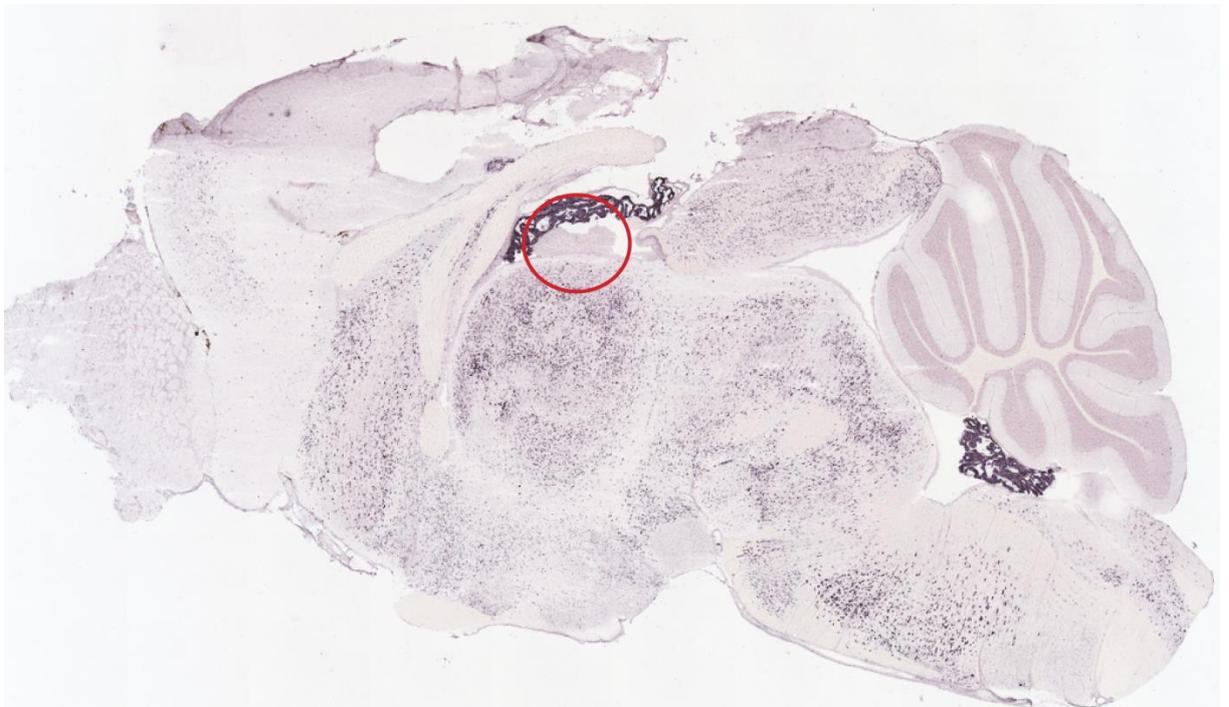
**Figure 18: Medial dorsal hippocampus**



**Figure 19: Dorsal cortex of inferior colliculus**



**Figure 20: Medial habenular nucleus**



## Landmarks in the horizontal plane (dorsal to ventral)

| <u>Landmark</u>   | <u>Description</u>   |
|---|--|
| <b>Dorsal superior colliculus</b>                       | The most dorsal section where the superior colliculus is visible (Figure 1, red)   |
| <b>Dorsal inferior colliculus</b>                       | The most dorsal section where the inferior colliculus is visible (Figure 1, green)   |
| <b>Dorsal hippocampus</b>                               | The most dorsal section where the hippocampus is visible (Figure 2)  |
| <b>Dorsal cerebellum</b>                                | The most dorsal section where the cerebellar tissue is visible (Figure 3)  |
| <b>Corpus callosum</b>                                  | The most dorsal section where the corpus callosum merges across the middle (Figure 4). Also, more laterally, the shape of the anterior part of the corpus callosum may assist in the anchoring process |
| <b>Dorsal striatum</b>                                  | The most dorsal section where the striatum is visible (Figure 5)   |
| <b>Fasciola cinereum</b>                                | The most dorsal section where the fasciola cinereum is visible as two long parallel bands of cells medially in the brain (Figure 6)  |
| <b>Lateral septal nuclei</b>                            | The first section where dorsal fornix is replaced with the septal nuclei (Figure 7)  |
| <b>Separation of medial and lateral dentate gyrus</b>   | The most dorsal section where the dentate gyrus separates into a medial and lateral part (Figure 8)  |
| <b>Thalamic nuclei</b>                                  | The most dorsal section where the thalamic nuclei becomes visible between the medial and lateral dentate (Figure 8)  |
| <b>Dorsal olfactory bulb</b>                            | The most dorsal section where the tissue of the olfactory bulb is visible (Figure 9)   |
| <b>Merging of 3rd ventricle and lateral ventricle</b>   | The most dorsal section where the two ventricles merge (Figure 10)   |
| <b>Dorsal cerebellar nuclei</b>                         | The most dorsal section where the cerebellar nuclei are visible in the deep cerebral white matter of the cerebellum (Figure 11)  |
| <b>Globus pallidus</b>                                  | The most dorsal section where the globus pallidus is visible (Figure 12)   |
| <b>Anterior commissure</b>                              | The most dorsal section where the anterior commissure is conjoined across the midline in the anterior region of the brain (Figure 13)  |
| <b>Separation of the midline of anterior commissure</b> | The most dorsal section where the anterior commissure is divided into left and right parts (Figure 14)   |
| <b>Dorsal brainstem</b>                                 | The most dorsal section where the cerebellar tissue is replaced by brainstem tissue and the 4th ventricle is prominent (Figure 15)   |

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|   |   |
|---|---|
| <b>Ventral striatum</b>                           | The most ventral section where the striatum is clearly visible (Figure 16)  |
| <b>Ventral cerebellum</b>                         | The most ventral section where the cerebellar tissue is visible (Figure 17)   |
| <b>Piriform cortex</b>                            | The most dorsal section where a clear band of cells of the piriform cortex is visible along the lateral part of each hemisphere (Figure 18) |
| <b>Separation of brainstem and ventral cortex</b> | The most dorsal section where the brainstem and the ventral part of the cortex separate (Figure 19)   |
| <b>Ventral olfactory bulb</b>                     | The most ventral section where the layers of the olfactory bulb are still visible (Figure 20)   |
| <b>Ventral hippocampus</b>                        | The most ventral section where the hippocampal formation is still visible (Figure 21)   |
| <b>Optic chiasm</b>                               | The most dorsal section where the optic chiasm becomes a band across the anterior part of the brain (Figure 22)                             |
| <b>Pontine nuclei</b>                             | The most dorsal section where the nuclei is seen anteriorly in the brain stem (Figure 23)   |

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**Illustration of landmarks**

**Figure 1: Superior colliculus (red) / Inferior colliculus (green)**



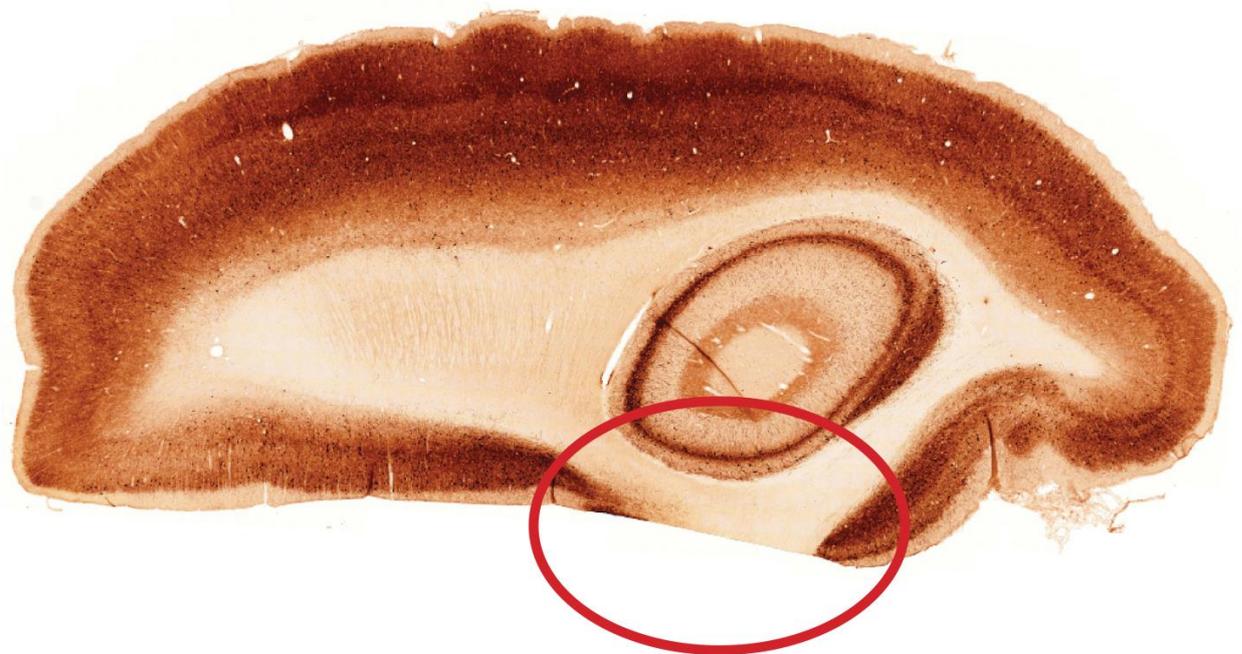
**Figure 2: Dorsal hippocampus**



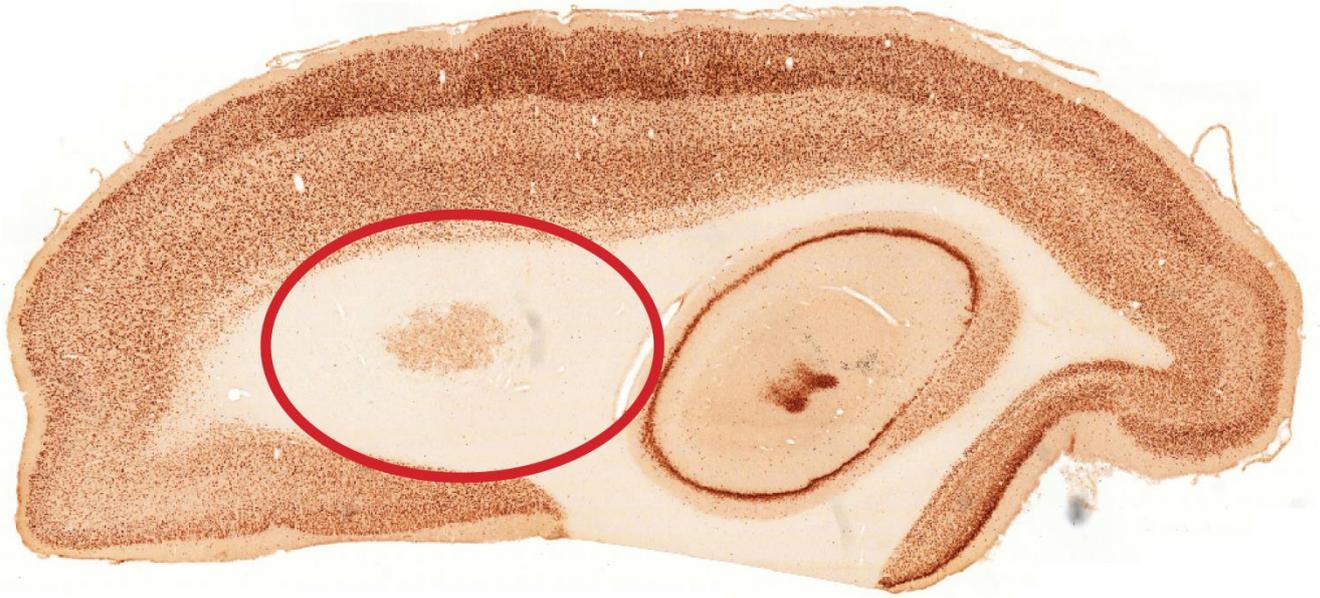
**Figure 3: Dorsal cerebellum**



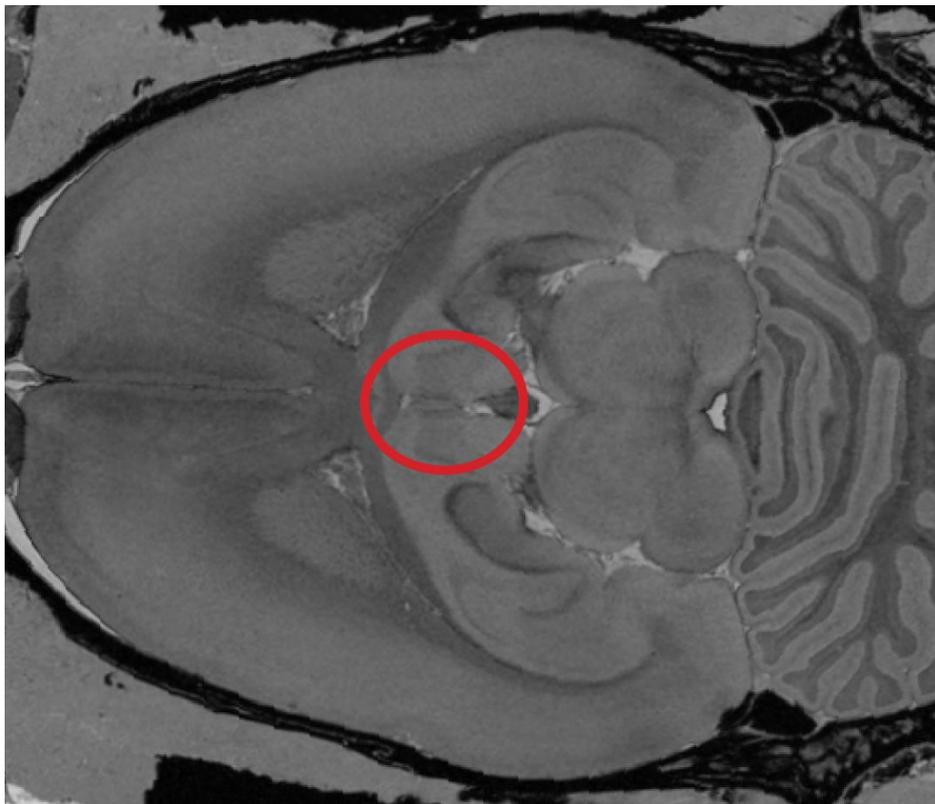
**Figure 4: Corpus callosum**



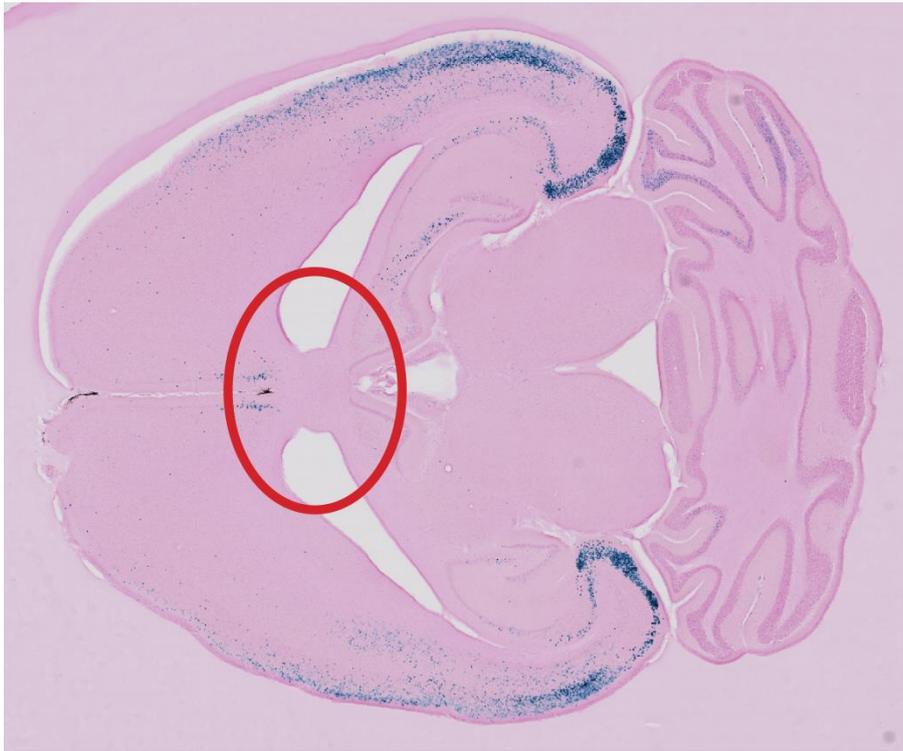
**Figure 5: Dorsal striatum**



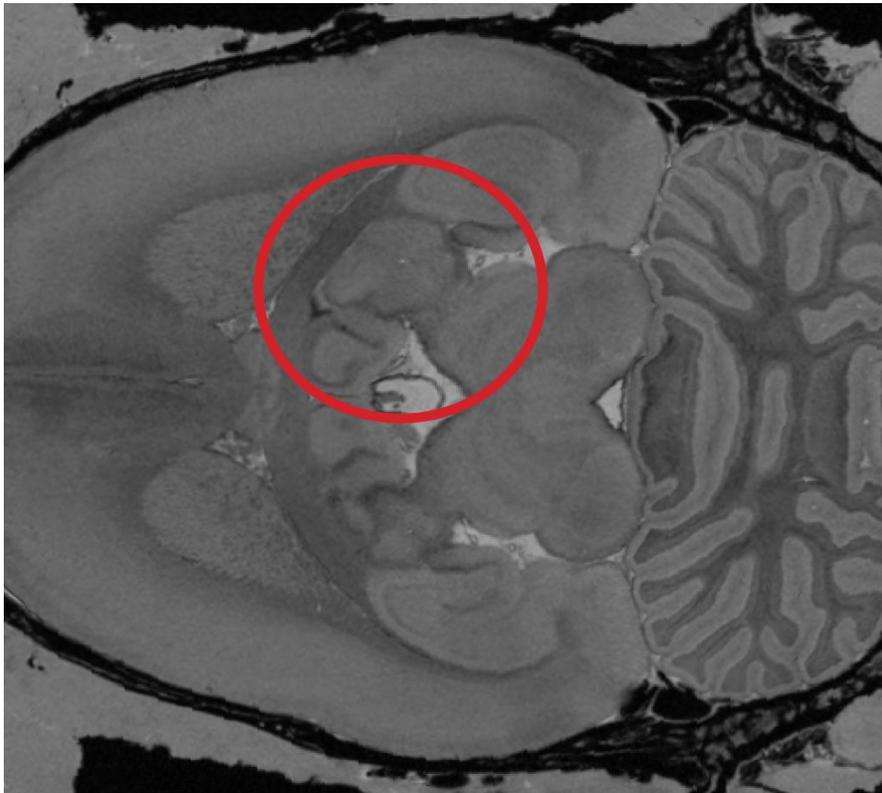
**Figure 6: Fasciola cinereum**



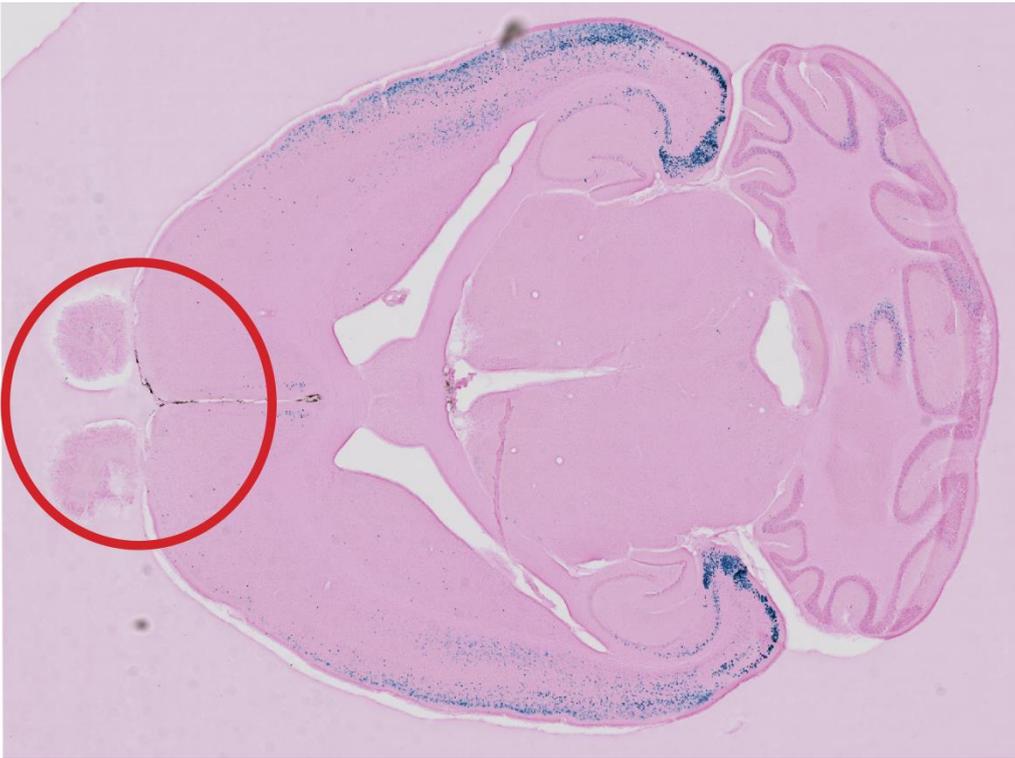
**Figure 7: Lateral septal nuclei**



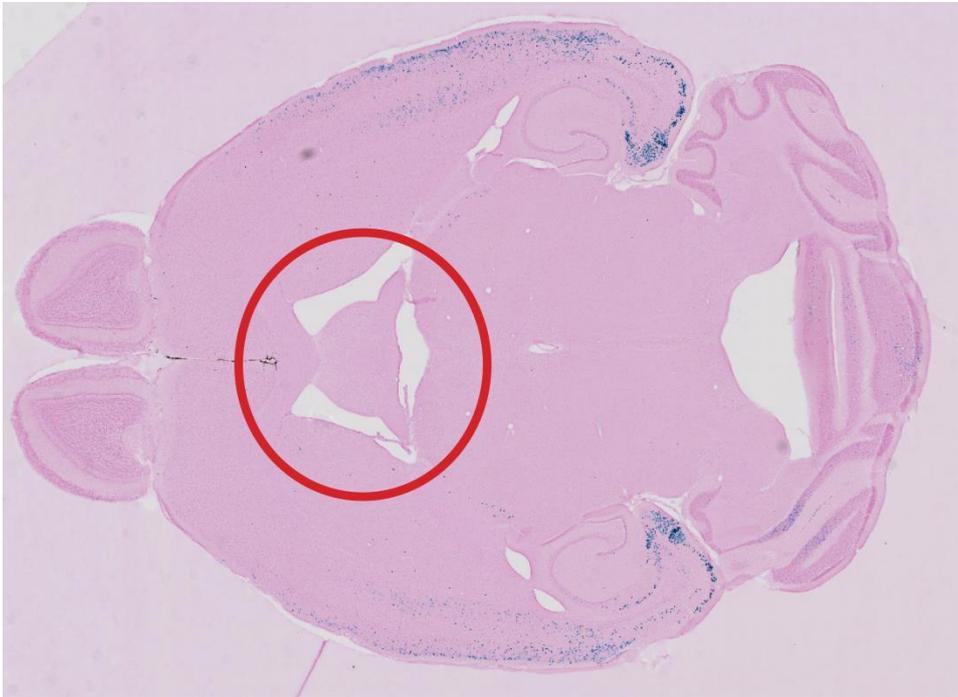
**Figure 8: Thalamic nuclei and separation of the dentate gyrus**



**Figure 9: Dorsal olfactory bulb**



**Figure 10: Merging of the 3<sup>rd</sup> ventricle and lateral ventricle**



**Figure 11: Dorsal cerebellar nuclei**



**Figure 12: Globus pallidus**



**Figure 13: Anterior commissure**



**Figure 14: Separation of anterior commissure**



**Figure 15: Dorsal brainstem**



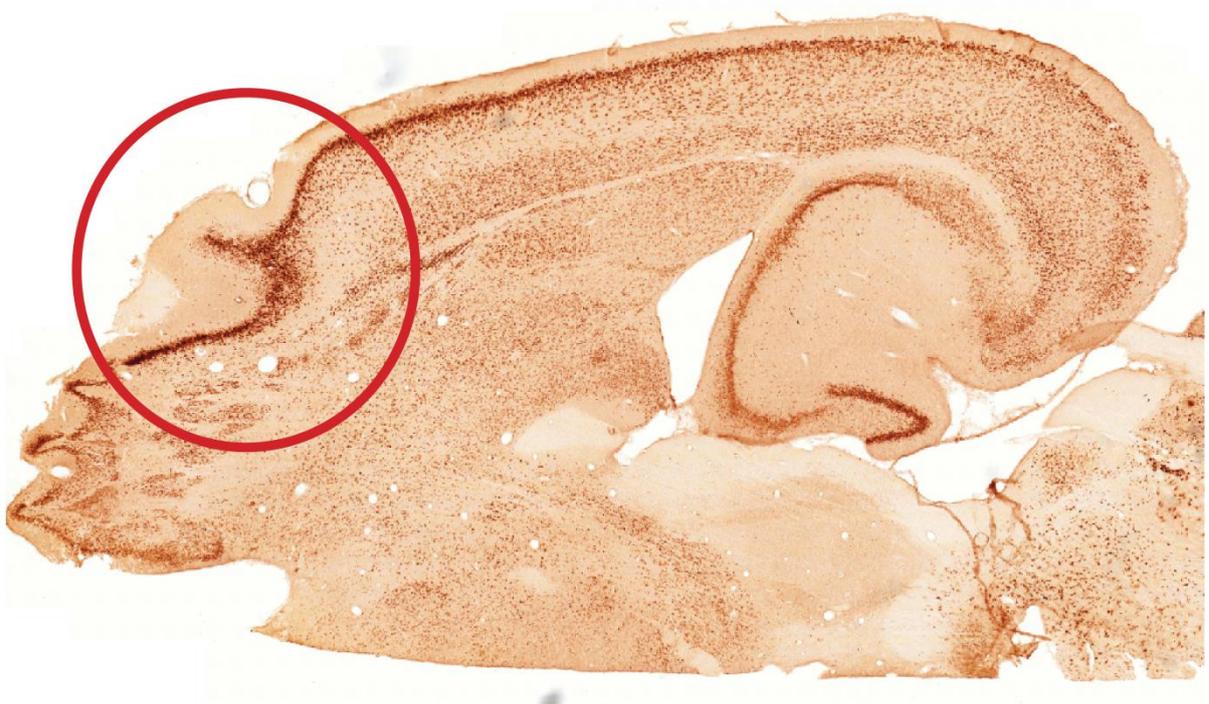
**Figure 16: Ventral striatum**



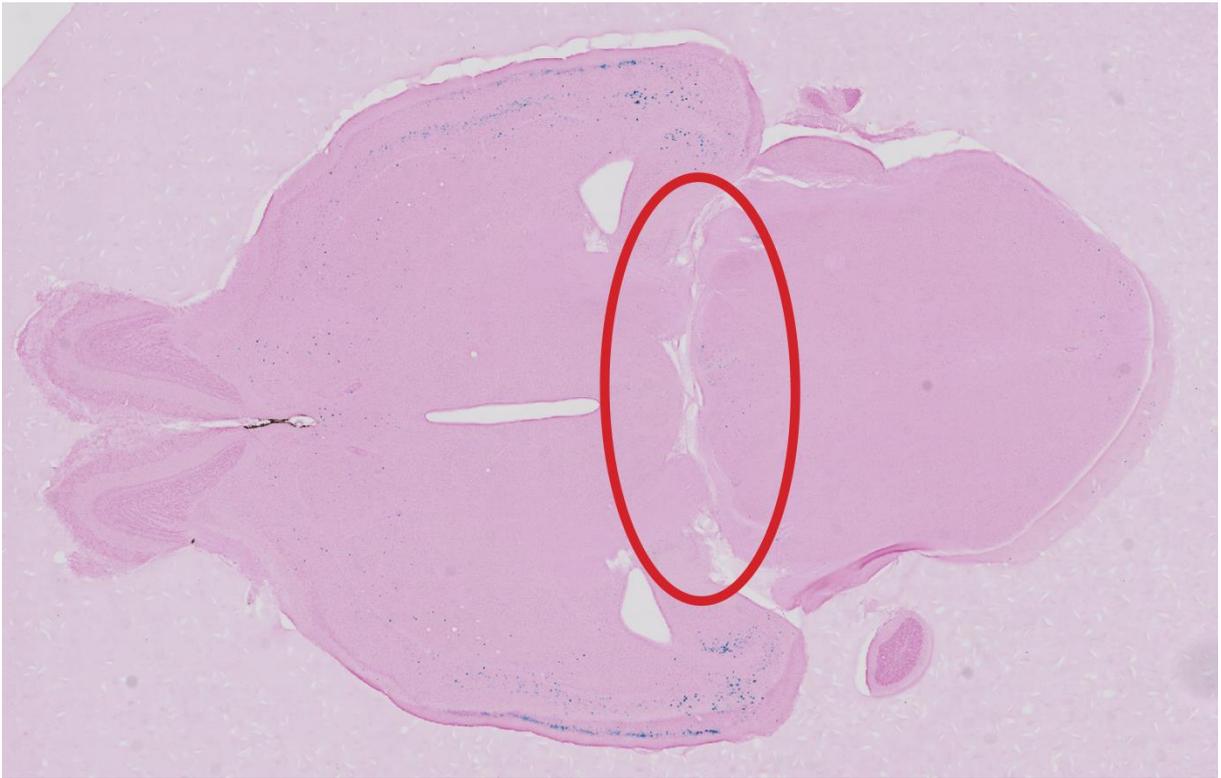
**Figure 17: Ventral cerebellum**



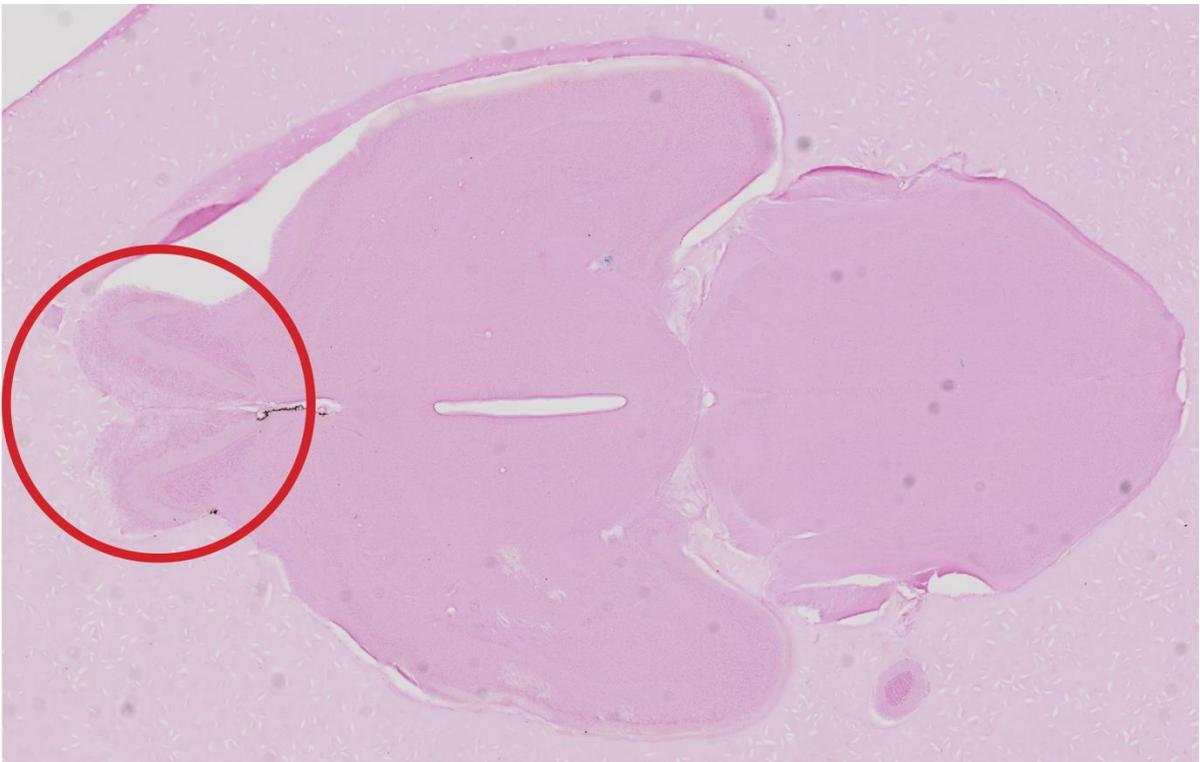
**Figure 18: Piriform cortex (anterior part marked)**



**Figure 19: Separation of brainstem and ventral cortex**



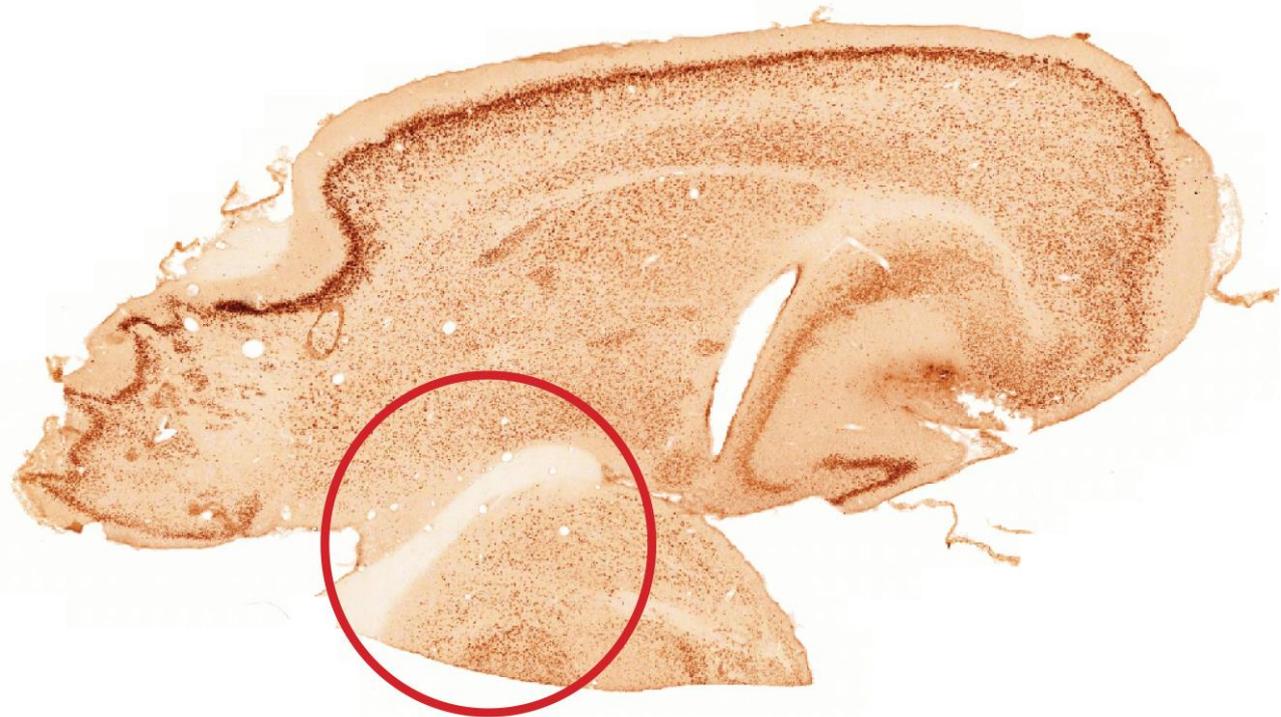
**Figure 20: Ventral olfactory bulb**



**Figure 21: Ventral hippocampus**



**Figure 22: Optic chiasm**



**Figure 23: Pontine nuclei**

