

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**
 - 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**
 - 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](#)
 - The Allen mouse brain in situ hybridization database (<https://mouse.brain-map.org/>)
- **Horizontal mouse brain images**
 - Yetman, M., Lillehaug, S., Bjaalie, J. G., Leergaard, T. B., & Jankowsky, J. (2018). Neuropsin tetracycline-transactivator expression: horizontal sections (case 2877) [Data set]. Human Brain Project Neuroinformatics Platform. <https://doi.org/10.25493/AYBB-BXV> | [View original dataset](#)
 - 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](#)
 - 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>
Granular layers of the olfactory bulb	The most anterior section where the granular layer of the olfactory bulb is last seen (Figure 1, red)
Anterior corpus callosum	The most anterior section where the corpus callosum is visible (Figure 1, green)
Anterior nucleus accumbens	The most anterior section where the nucleus accumbens is visible (Figure 2, red)
Anterior striatum	The most anterior section where the striatum is visible (Figure 2, green)
Genu corpus callosum	The most anterior section in which the corpus callosum is continuous across the midline (Figure 3)
Merging of anterior commissure	The most anterior section in which the anterior commissure is continuous across the midline (Figure 4)
Anterior hippocampus	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
Amygdalar nuclei	A triangle shaped nuclei compilation that can be seen through many sections medially in the brain going anterior to posterior (Figure 6)
Optic chiasm/Optic tract	The development of the optic chiasm into optic tract, and the change in shape of optic tract when moving posteriorly (Figure 7, red)
Habenular nuclei/habenular commissure	The most posterior section where habenular nuclei develops into habenular commissure (Figure 8, red)
Posterior commissure	Most anterior section where posterior commissure appears dorsally to 3rd ventricle (Figure 9)
Splenium corpus callosum	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
Ventral dentate gyrus	The most anterior section where ventral dentate gyrus is visible (Figure 11)

Posterior mammillary nucleus	The most anterior section where the nucleus is still visible (Figure 12)
Posterior end of thalamus	The most anterior section in which the thalamus is no longer visible (Figure 13)
Posterior superior colliculus	The most posterior section showing the posterior superior colliculus (Figure 14)
Posterior dentate gyrus	The most posterior section showing the dentate gyrus (Figure 15)
Posterior subiculum	The most posterior section showing the posterior subiculum (Figure 16)
Inferior colliculus	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)
Anterior pontine nuclei	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)
Separation of inferior colliculus and brainstem	The first section posteriorly where the inferior colliculus has separated from the brainstem and cerebellum in visible medially (Figure 19)
Posterior inferior colliculus	The most posterior section where the tissue of inferior colliculus is still visible (Figure 19)
Facial nerve	The section where facial nerve is seen across the whole brainstem (Figure 20)
Cerebellar nuclei	The most anterior and posterior sections after the separation of brainstem and cerebellum in which the nuclei are visible (Figure 21)
Separation of cerebellum and brainstem	The first section posteriorly where cerebellum and brainstem are no longer connected (Figure 22)
Posterior cerebellum	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.
Inferior olive, anterior and posterior end	The most anterior and the most posterior sections in which the inferior olive is visible (Figure 24 and 26, respectively)
Merging of tissue around central canal	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)

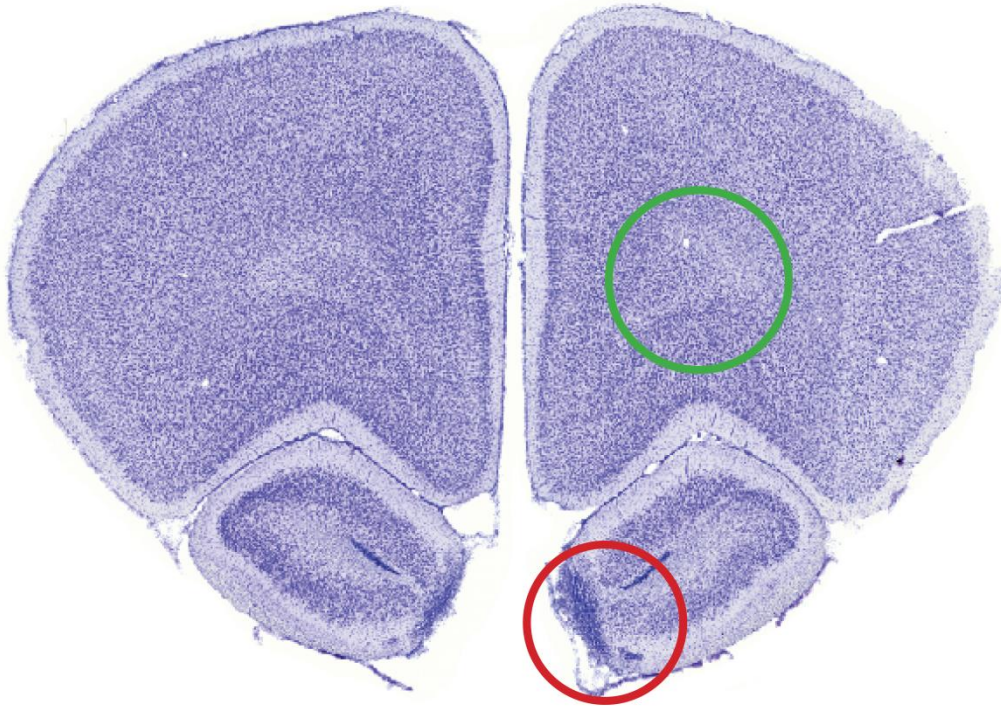


Figure 2: Anterior striatum (dorsal striatum in green, nucleus accumbens in red)

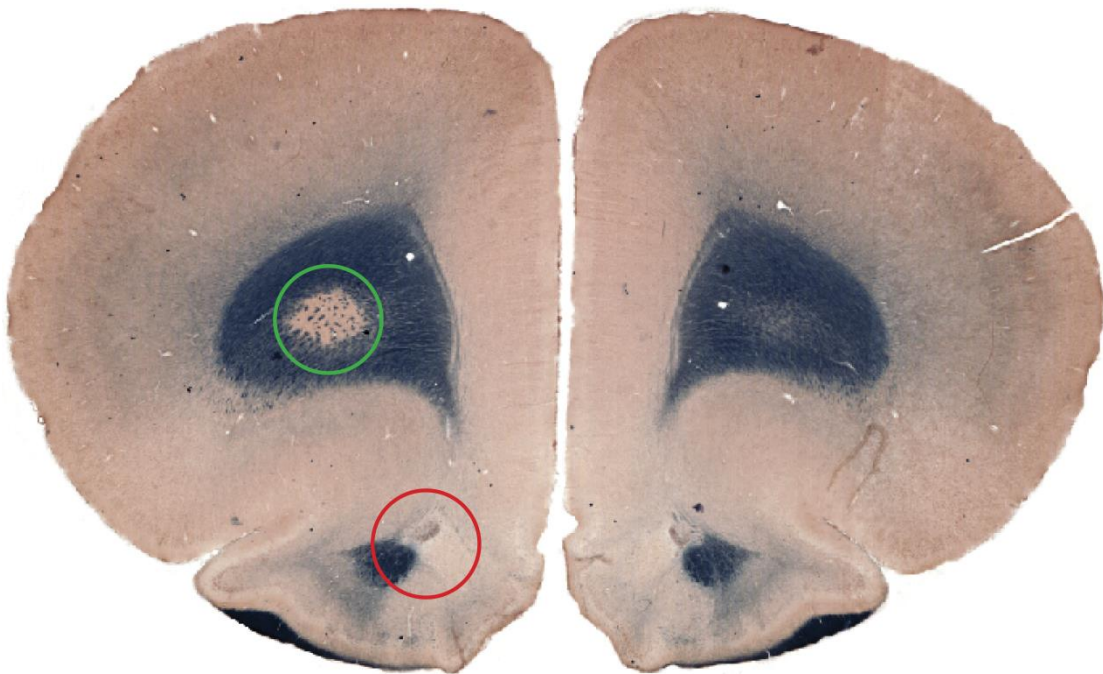


Figure 3: Genu of corpus callosum

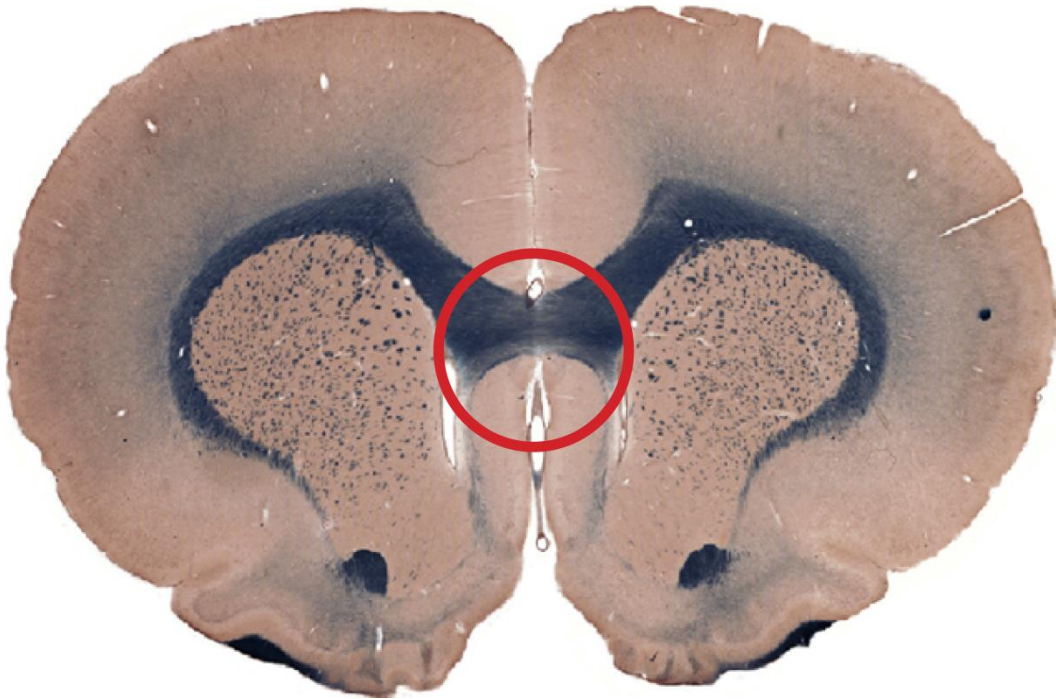


Figure 4: Merging of anterior commissure



Figure 5: Anterior hippocampus

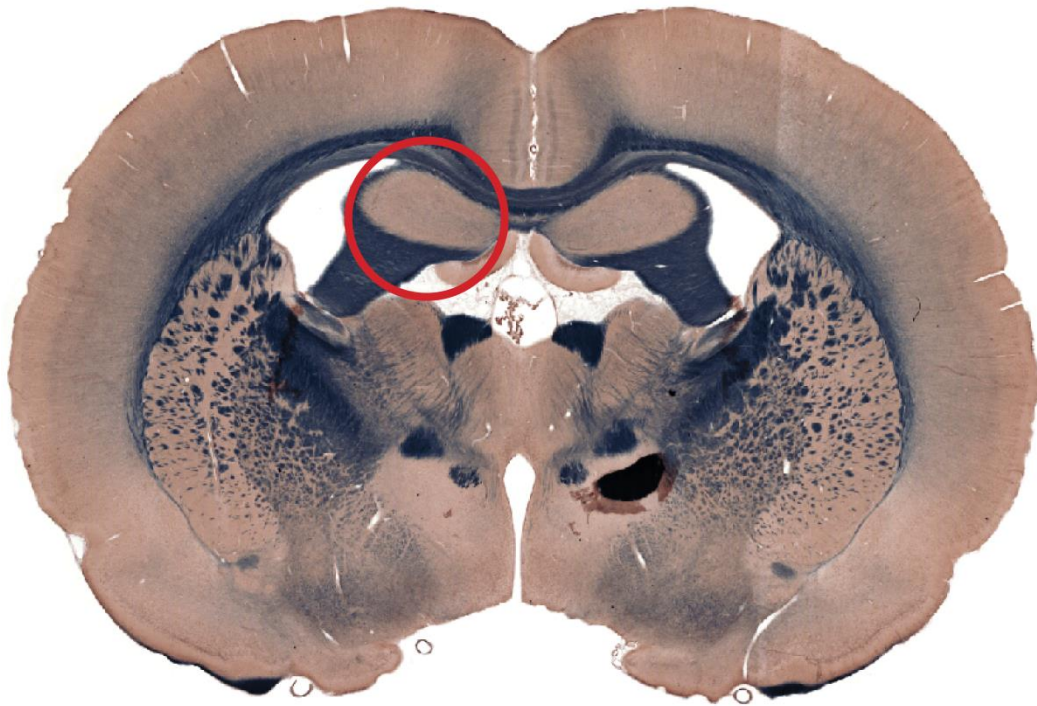


Figure 6: Amygdala

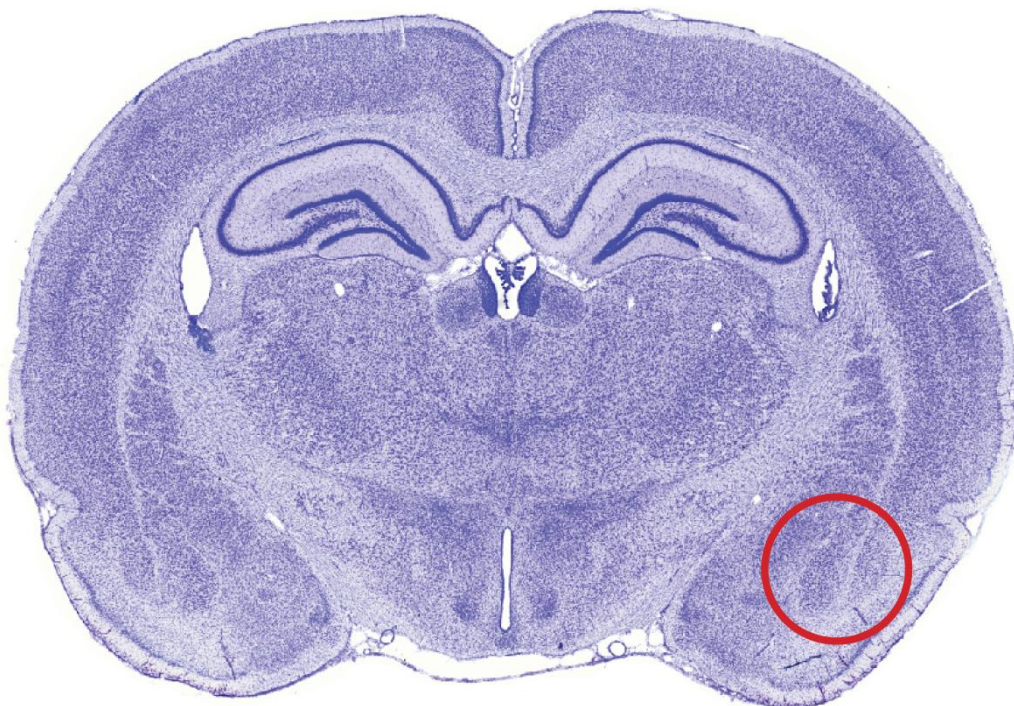


Figure 7: Optic tract in red, mid-level of the anterior hippocampus in green

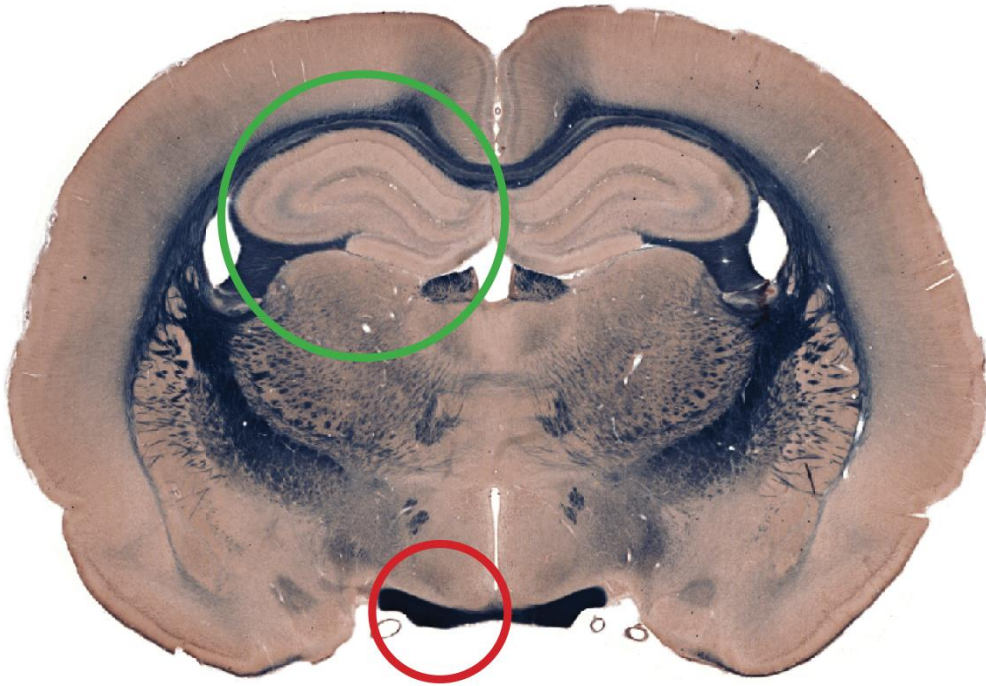


Figure 8: Habenular commissure



Figure 9: Posterior commissure



Figure 10: Splenium of the corpus callosum

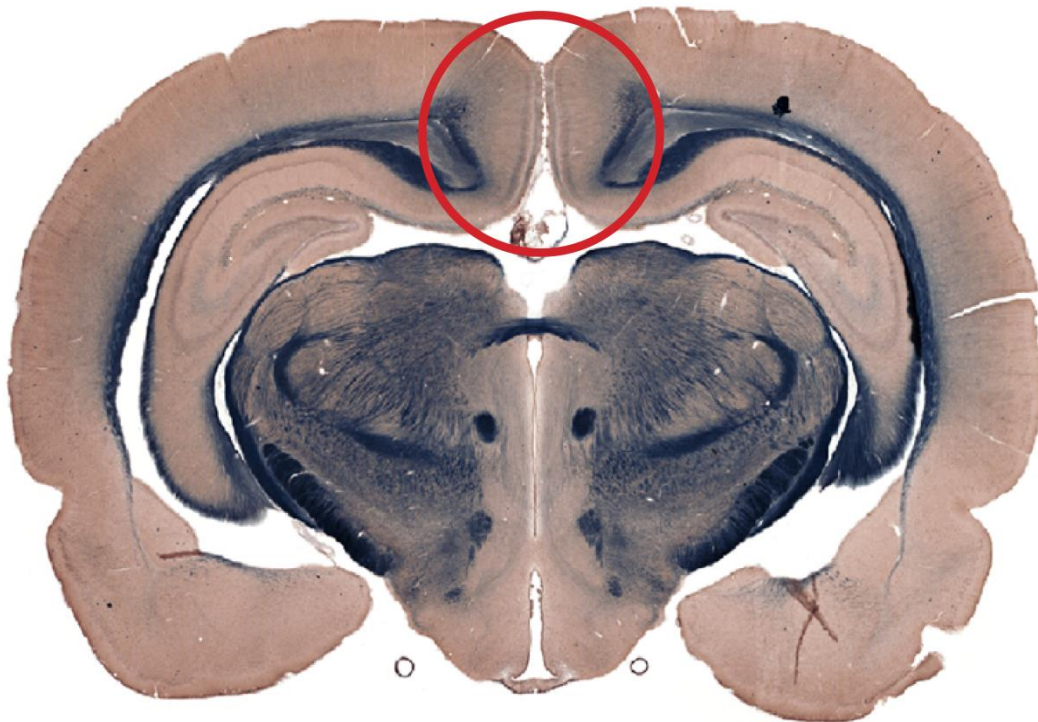


Figure 11: Ventral dentate gyrus

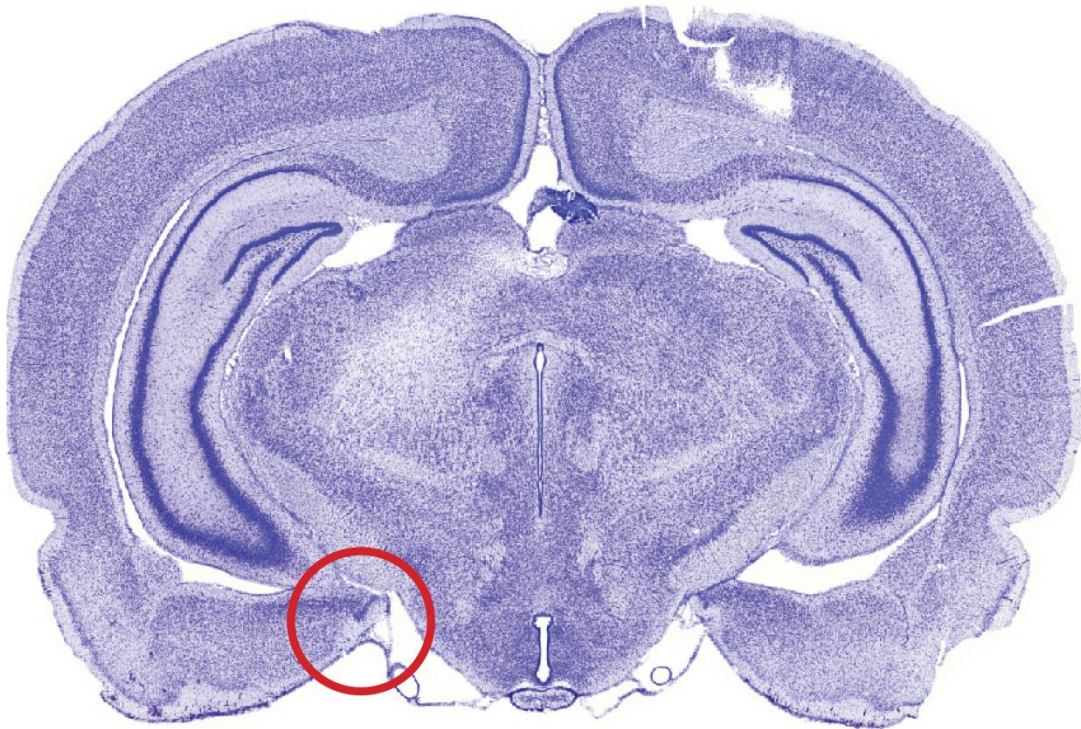


Figure 12: Posterior end of mammillary nucleus

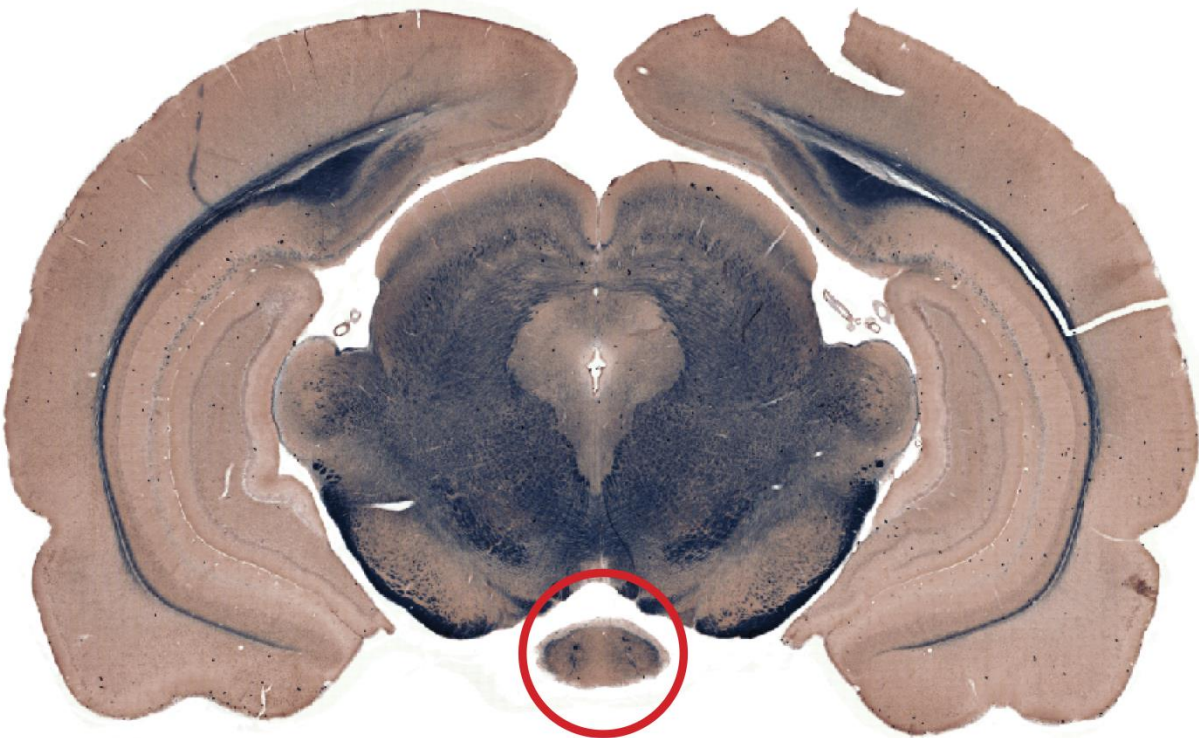


Figure 13: Posterior end of thalamus

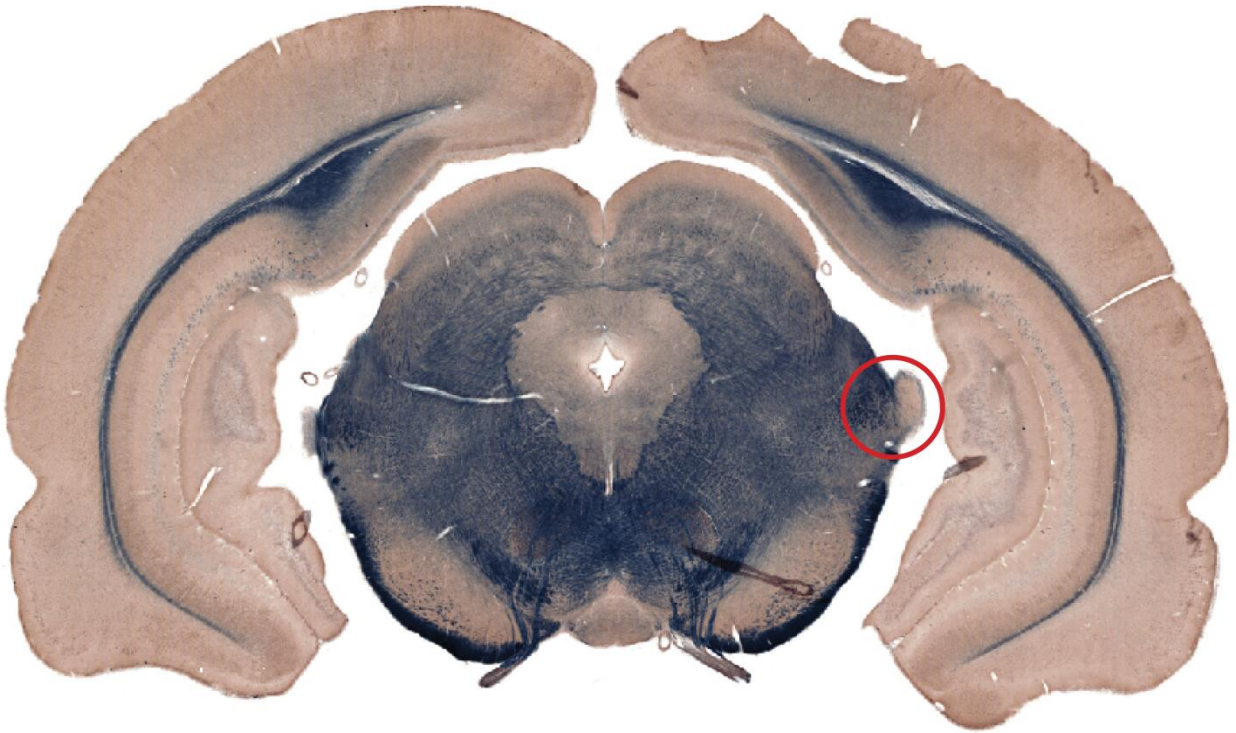


Figure 14: Posterior end of superior colliculus



Figure 15: Posterior end of dentate gyrus

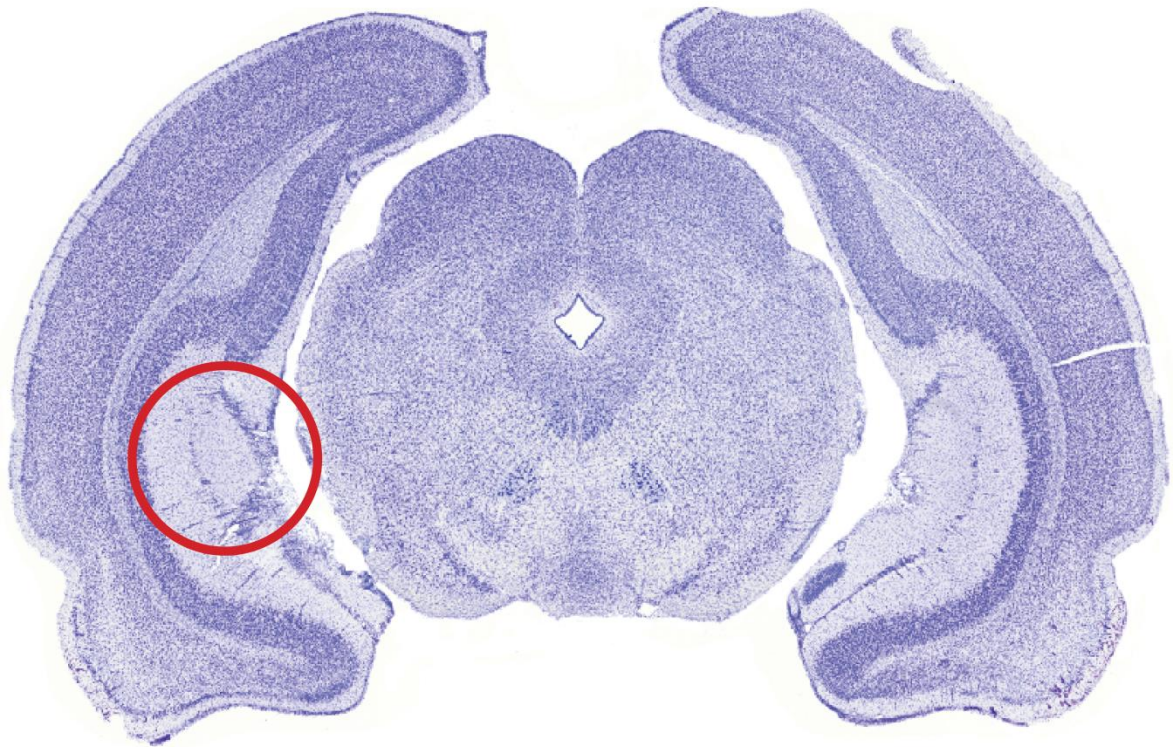


Figure 16: Posterior end of subiculum

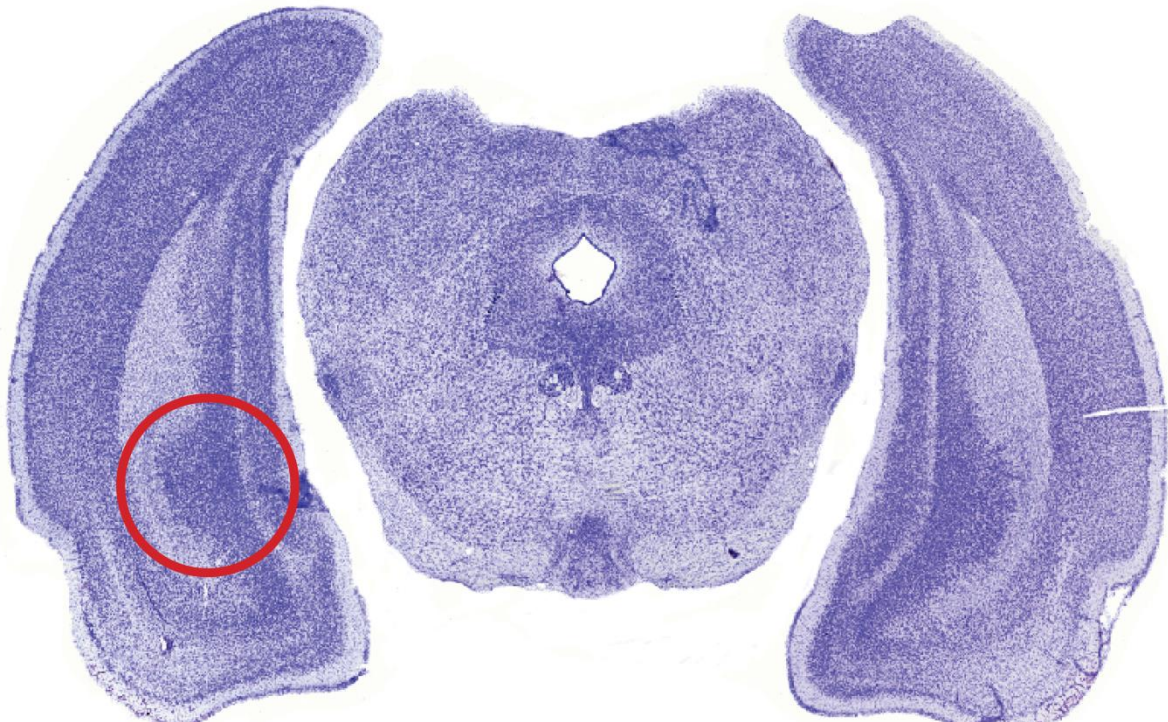


Figure 17: Inferior colliculus

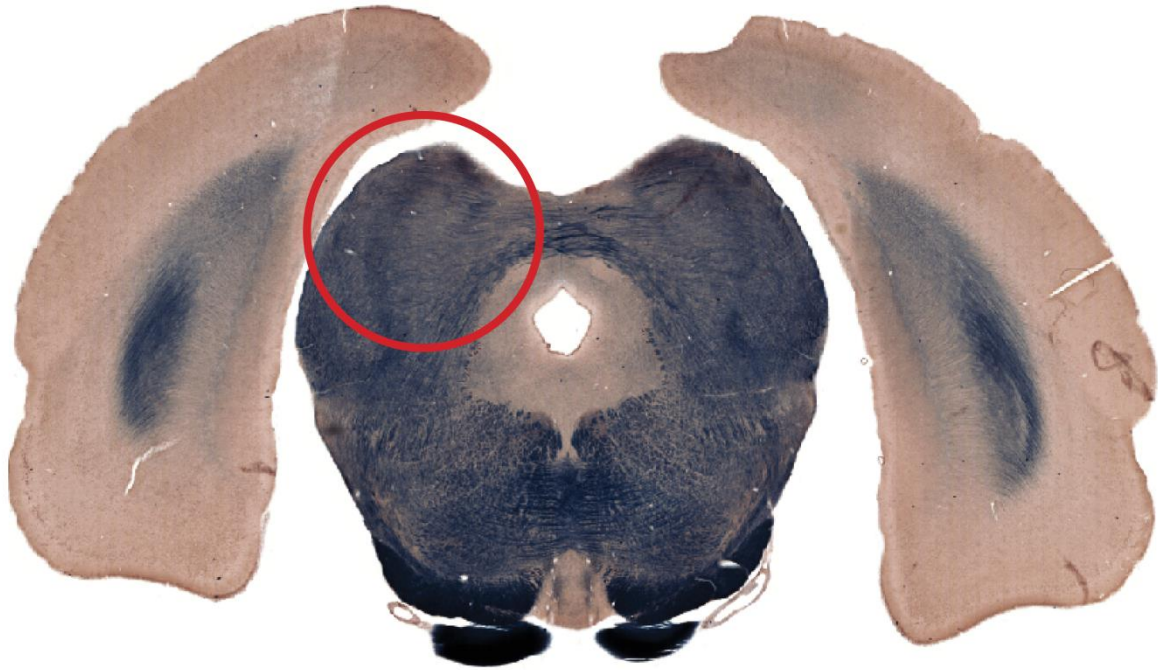


Figure 18: Anterior pons



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

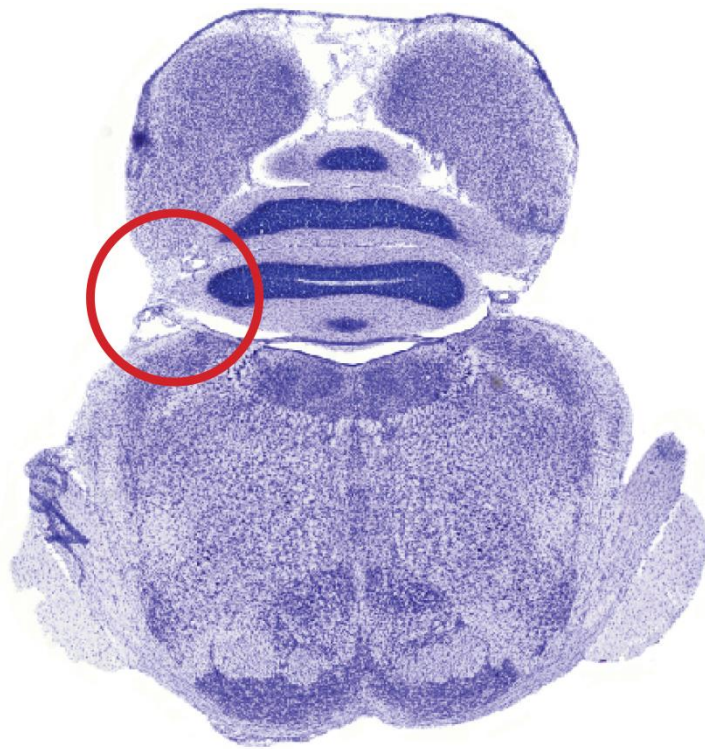


Figure 20: Facial nerve

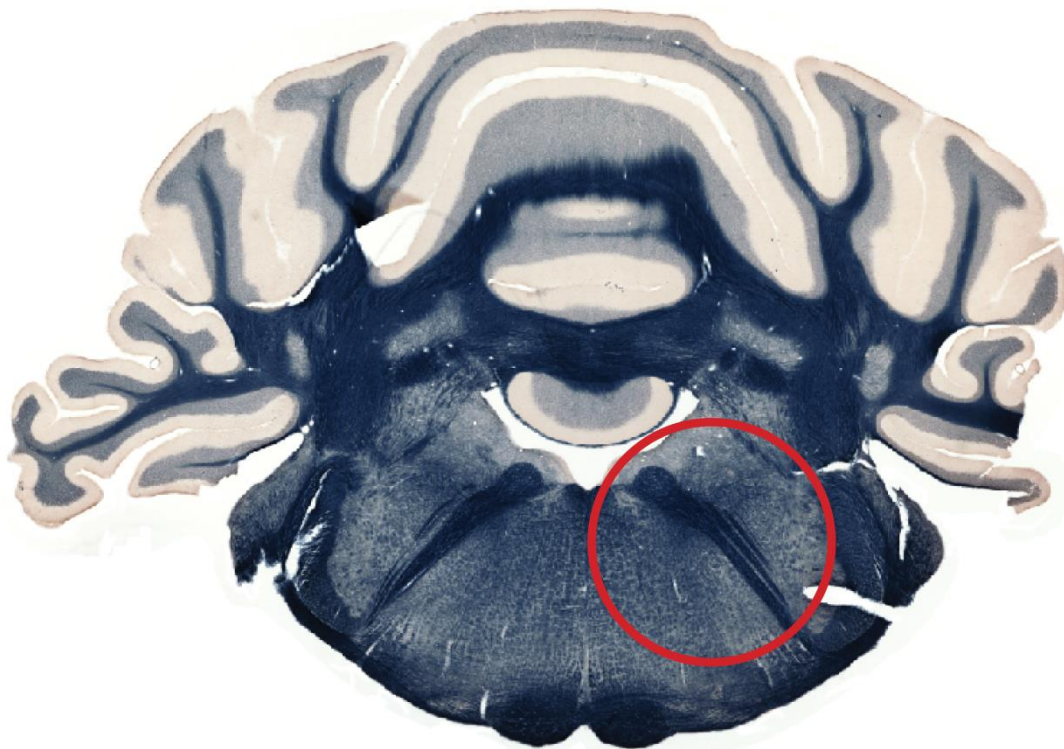


Figure 21: Anterior part of cerebellar nuclei



Figure 22: Separation of cerebellum and brainstem

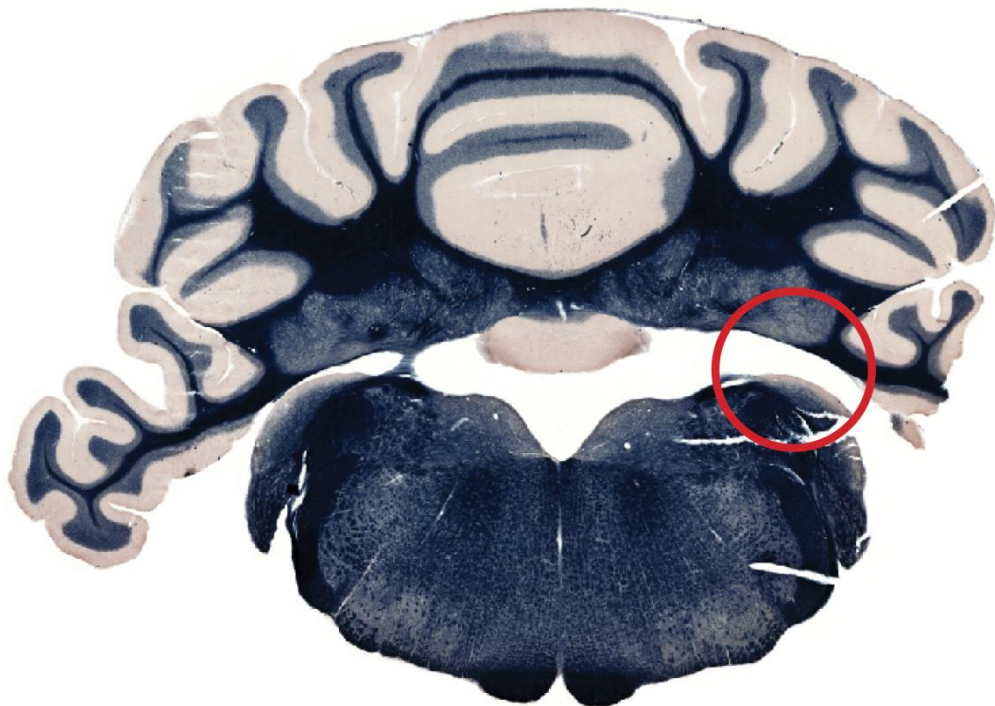


Figure 23: Posterior part of the cerebellar nuclei

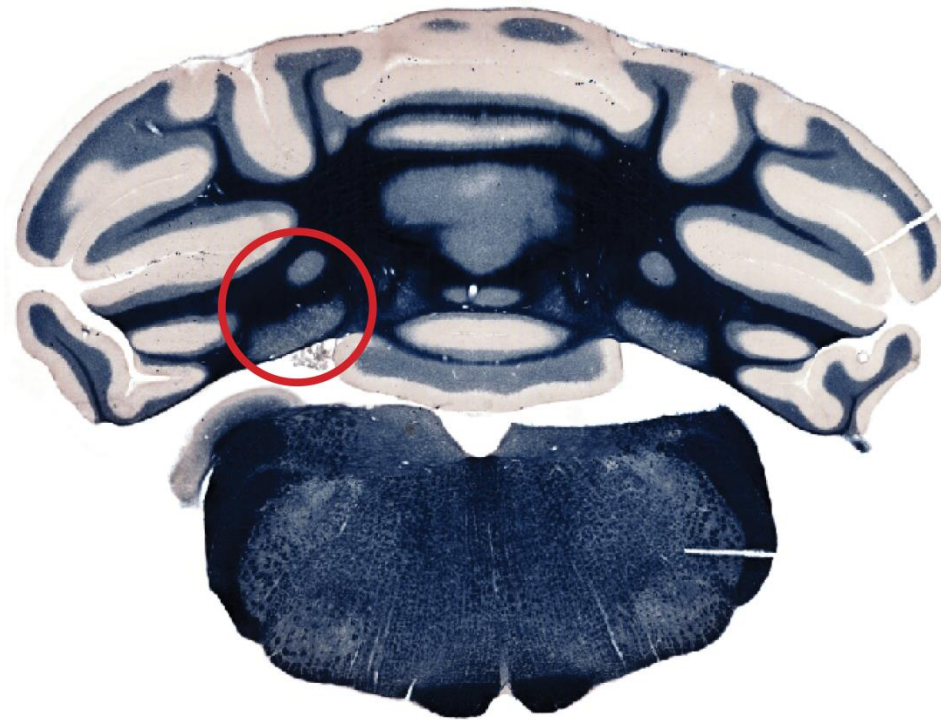


Figure 24: Anterior inferior olive

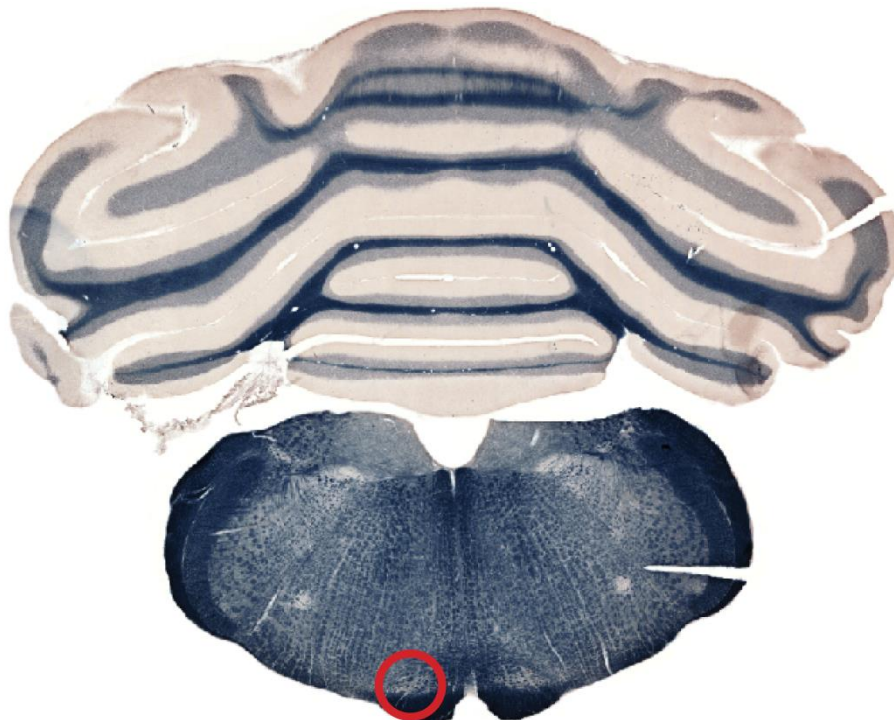


Figure 25: Merging of the tissue around the central canal

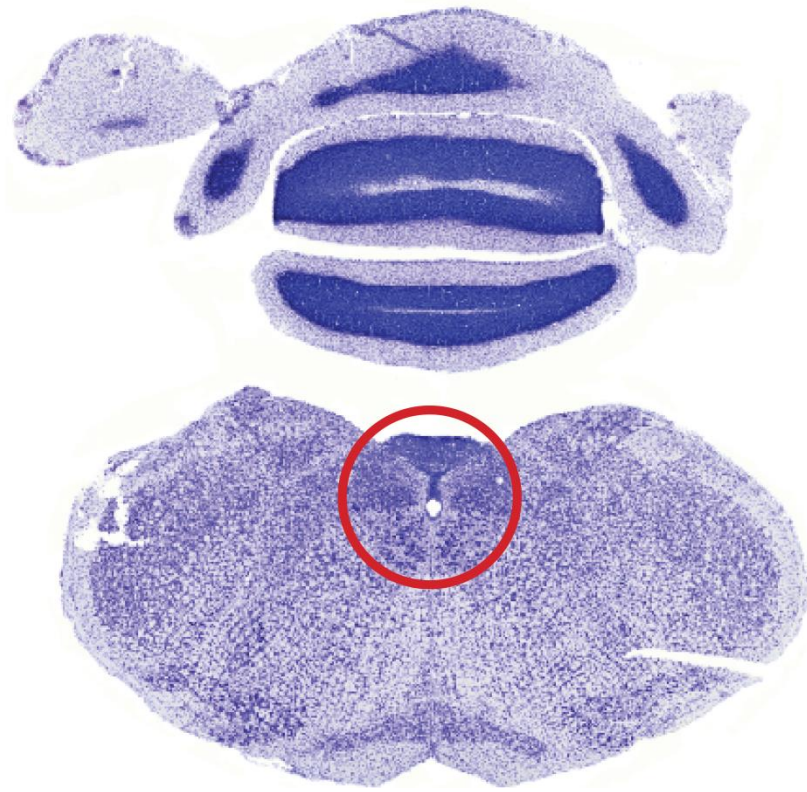
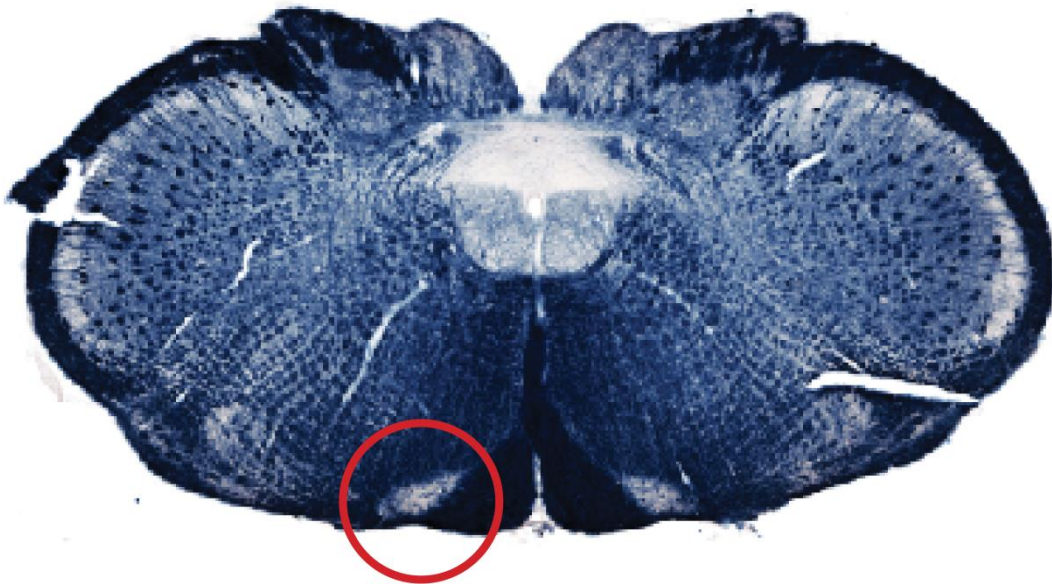


Figure 26: Posterior inferior olive



Landmarks in the sagittal plane (lateral to medial)

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

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

Illustration of landmarks

Figure 1: Lateral hippocampus

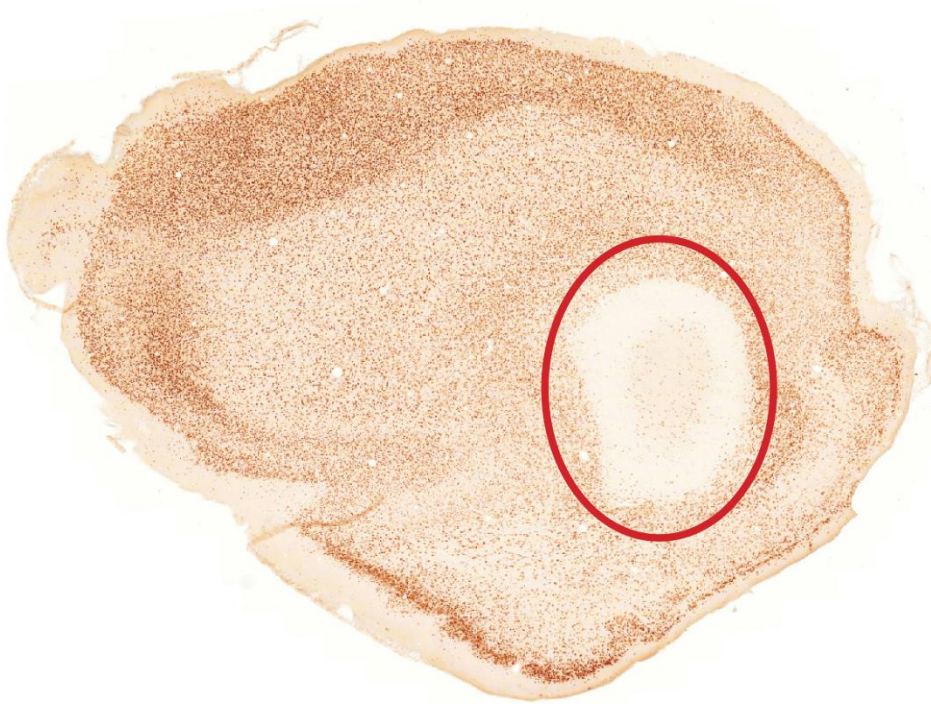


Figure 2: Lateral striatum

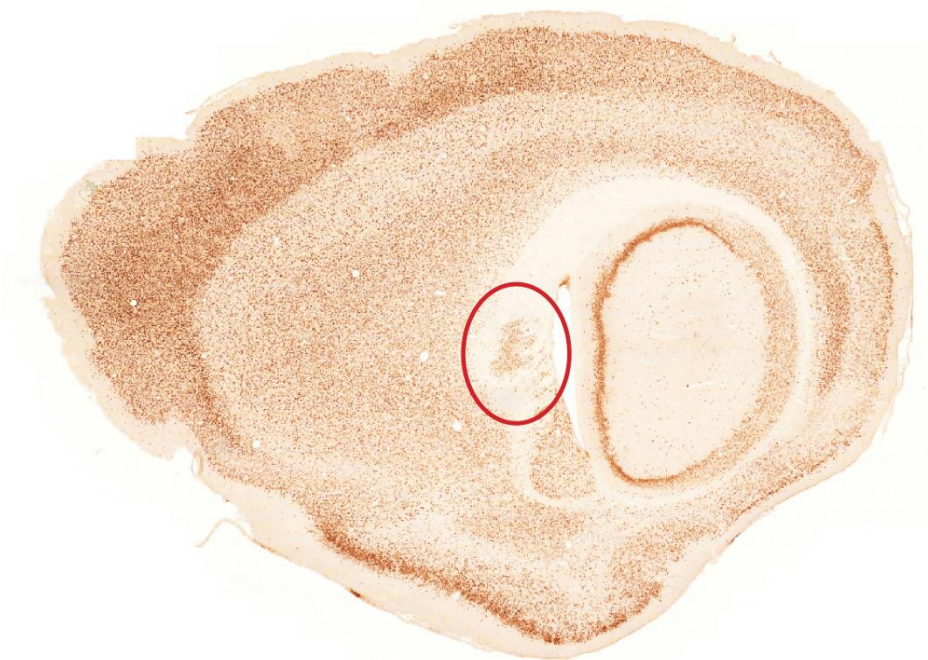


Figure 3: Lateral cerebellum

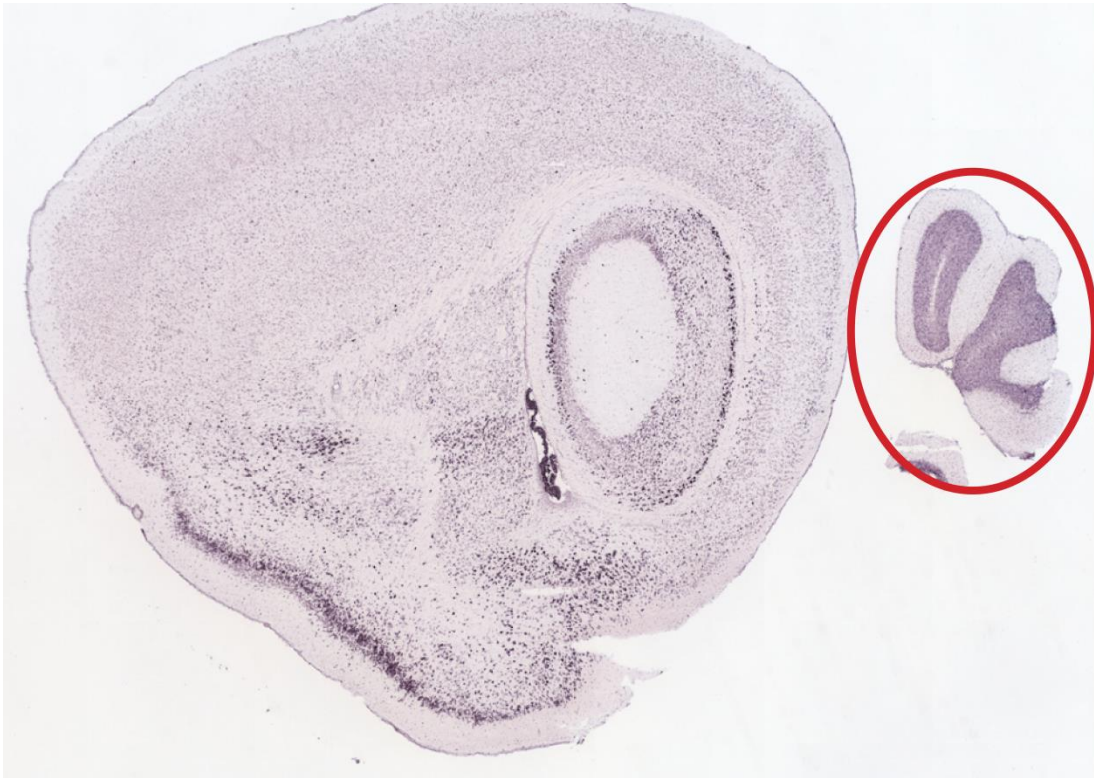


Figure 4: Internal capsule

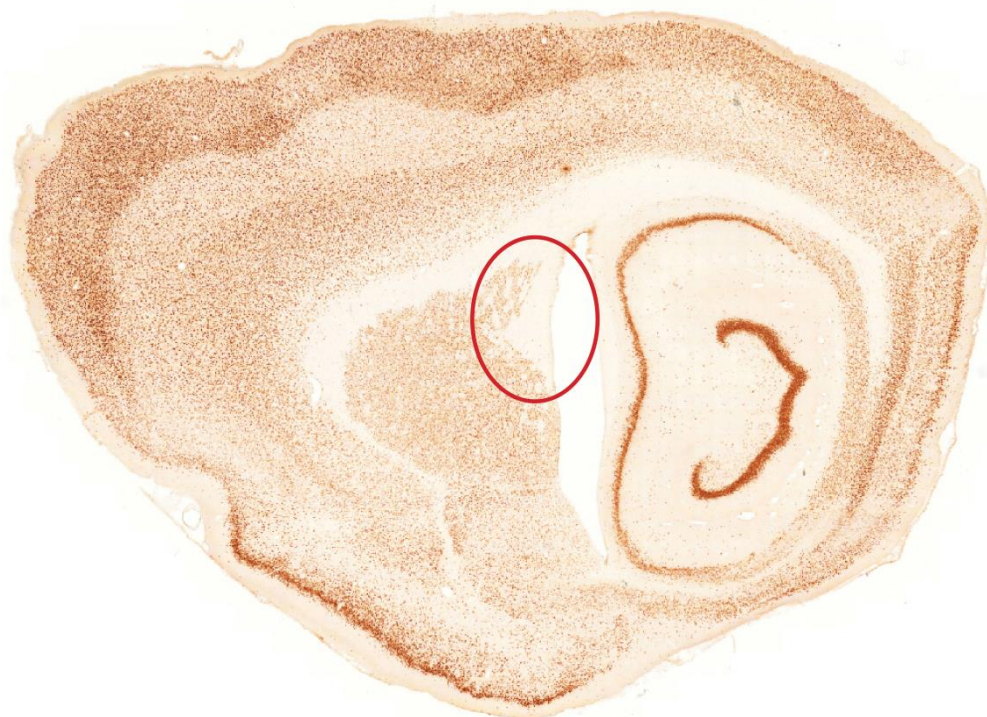


Figure 5: Lateral globus pallidus



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



Figure 7: Cerebellar nuclei

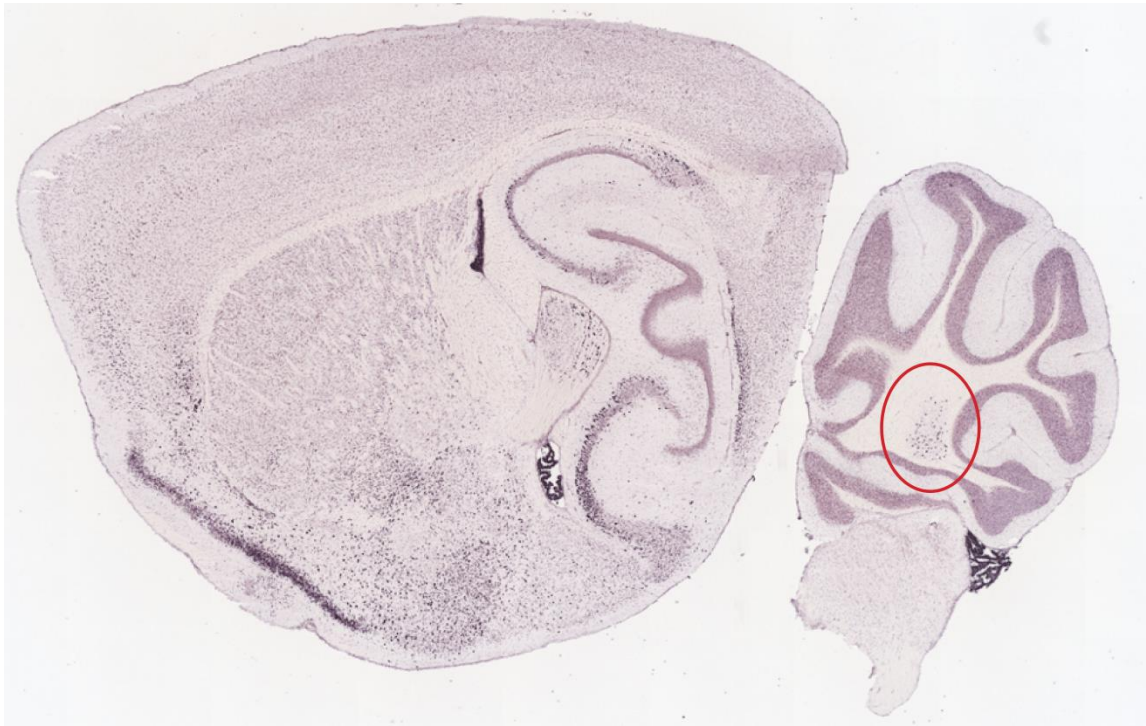


Figure 8: Ventral geniculate nucleus

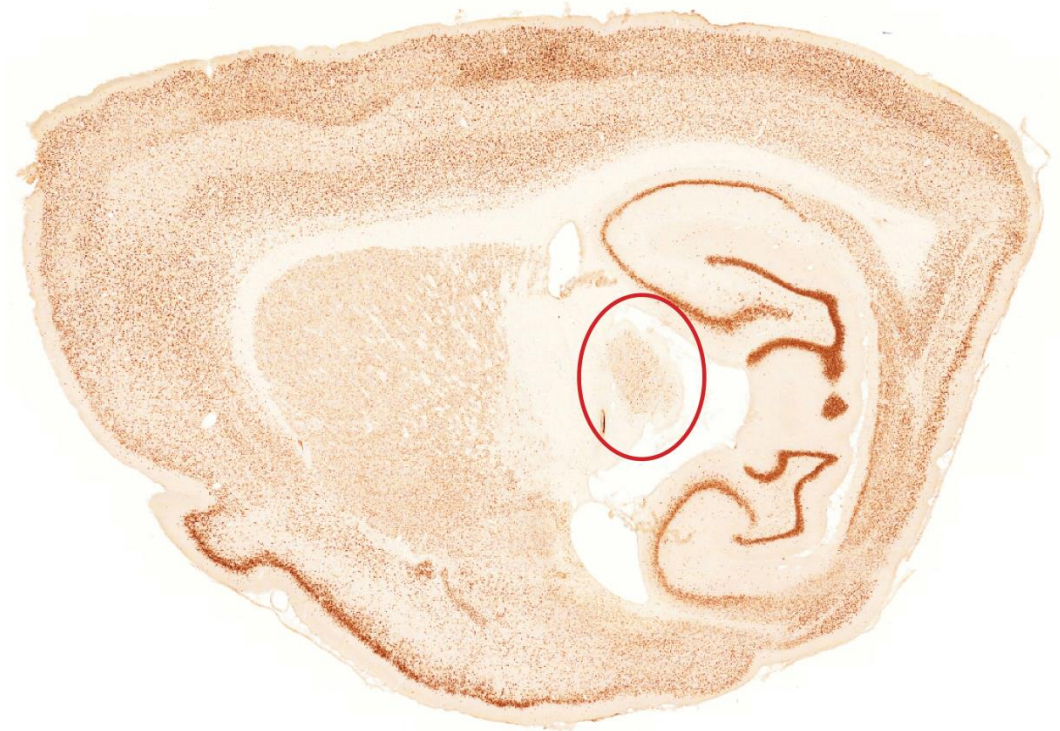


Figure 9: Separation of dorsal and ventral hippocampus

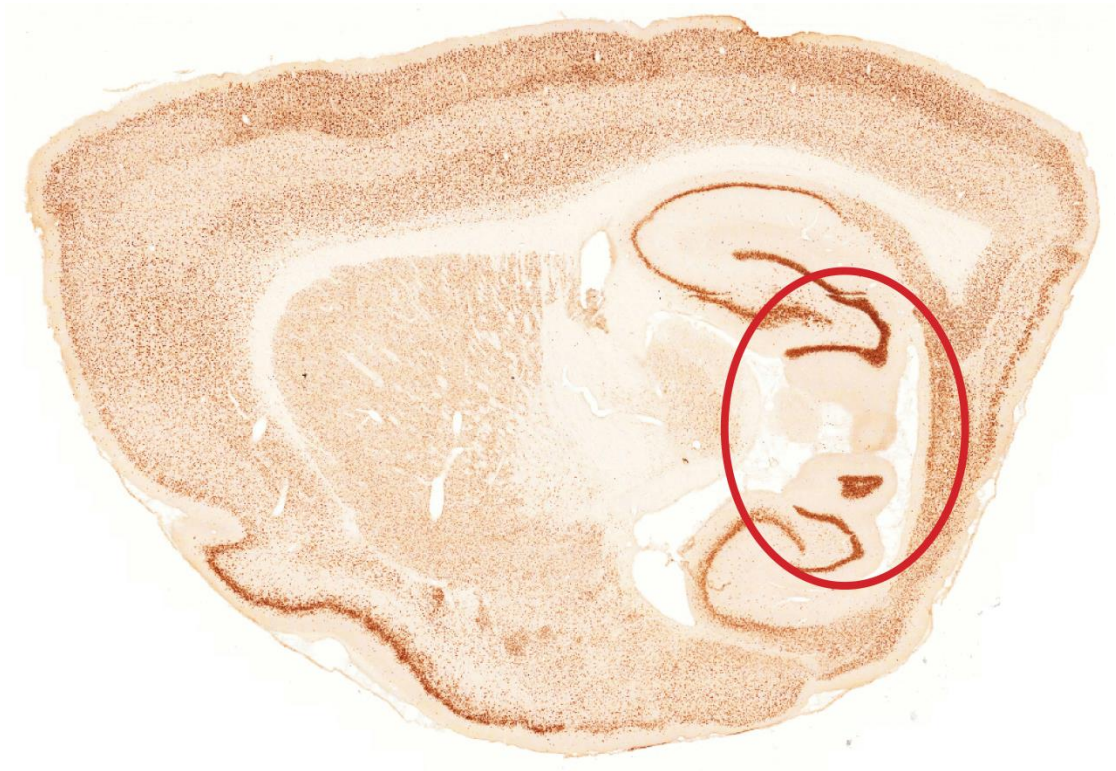


Figure 10: Separation of dorsal and ventral parahippocampal region

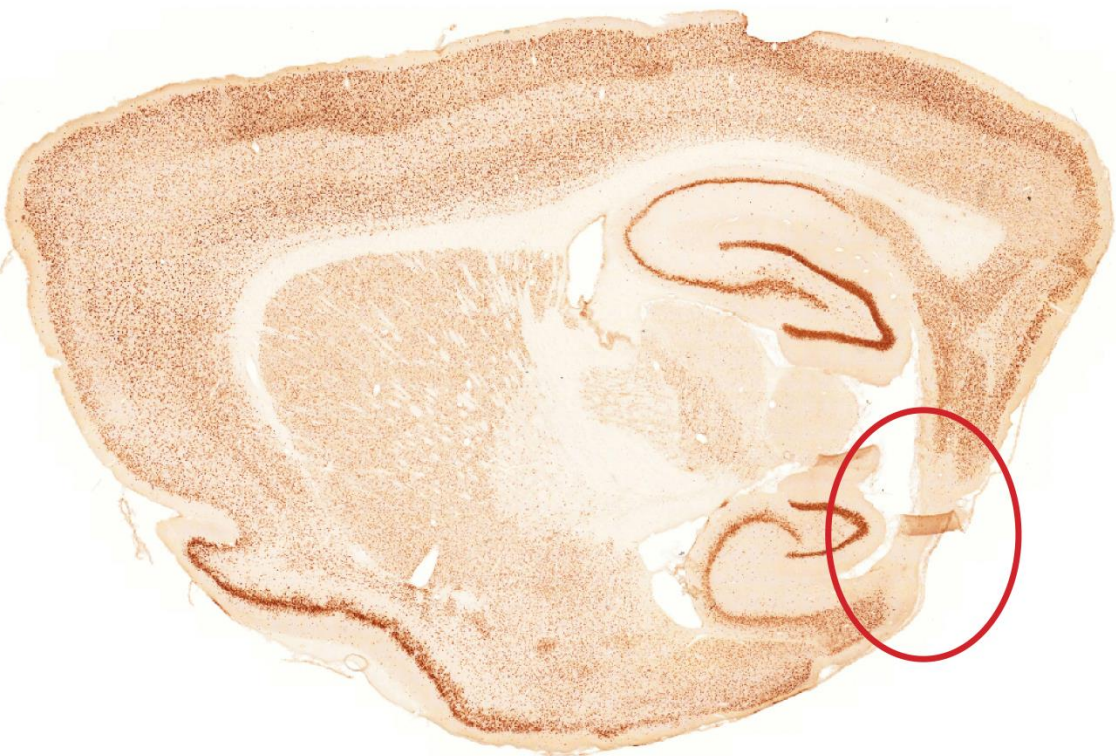


Figure 11: Brachium of inferior colliculus (red) and olfactory tubercle (green)

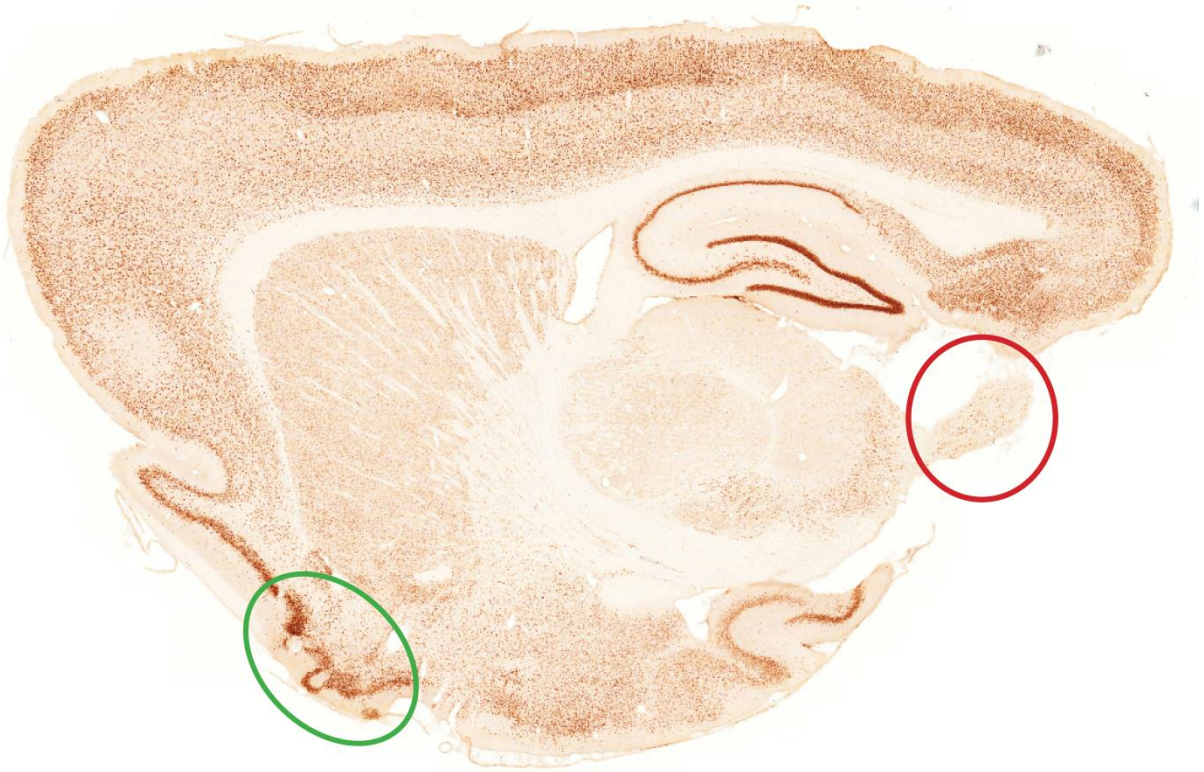


Figure 12: Olfactory bulb

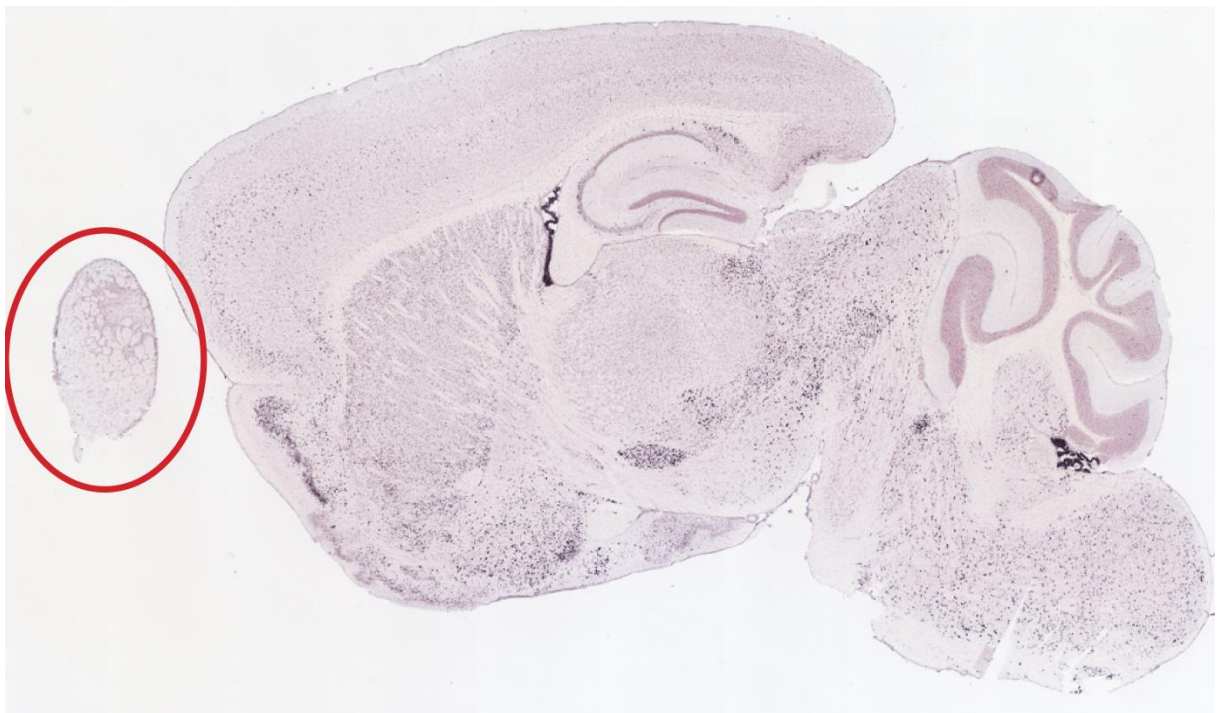


Figure 13: Medial end of ventral hippocampus



Figure 14: Pontine nuclei

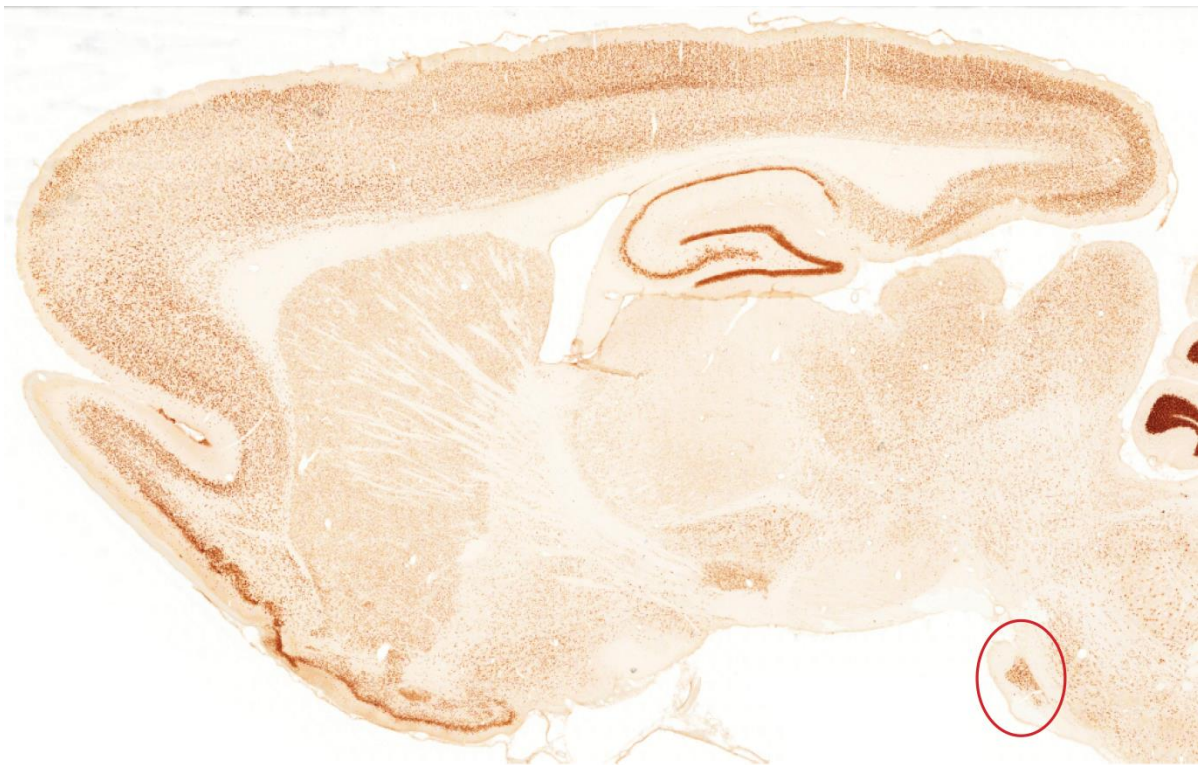


Figure 15: Merging of anterior commissure



Figure 16: Lateral septal nucleus

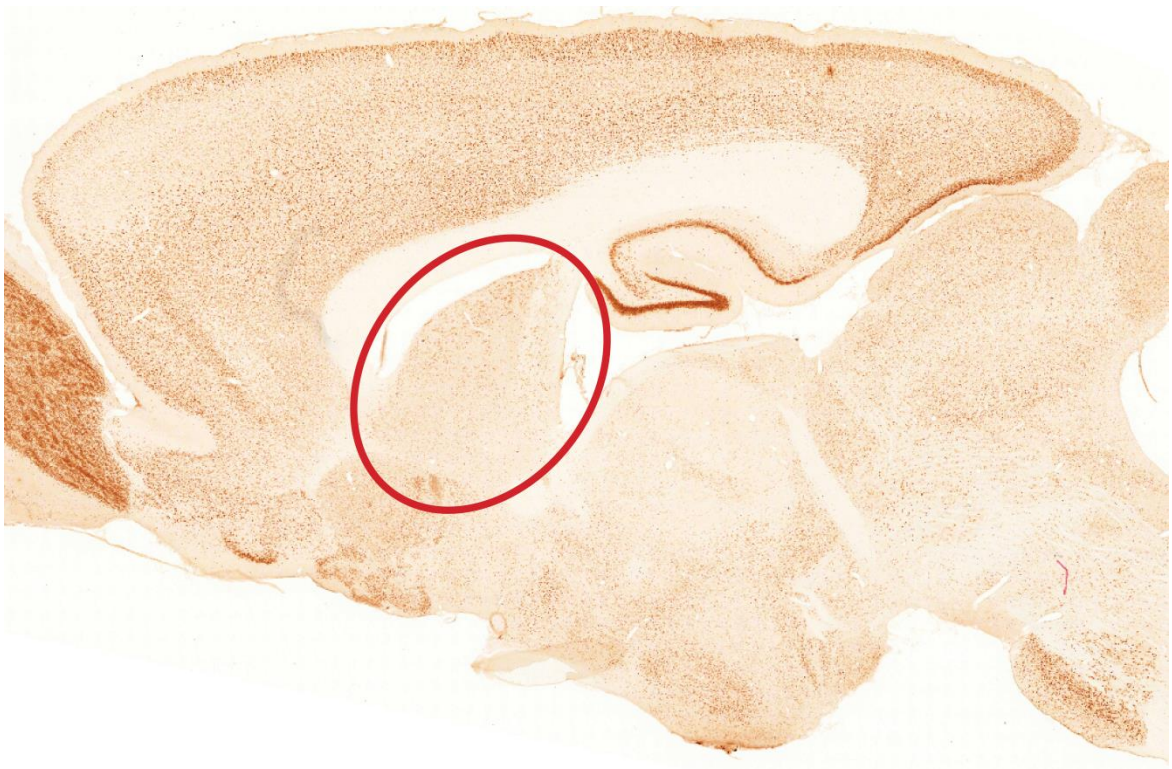


Figure 17: Medial striatum

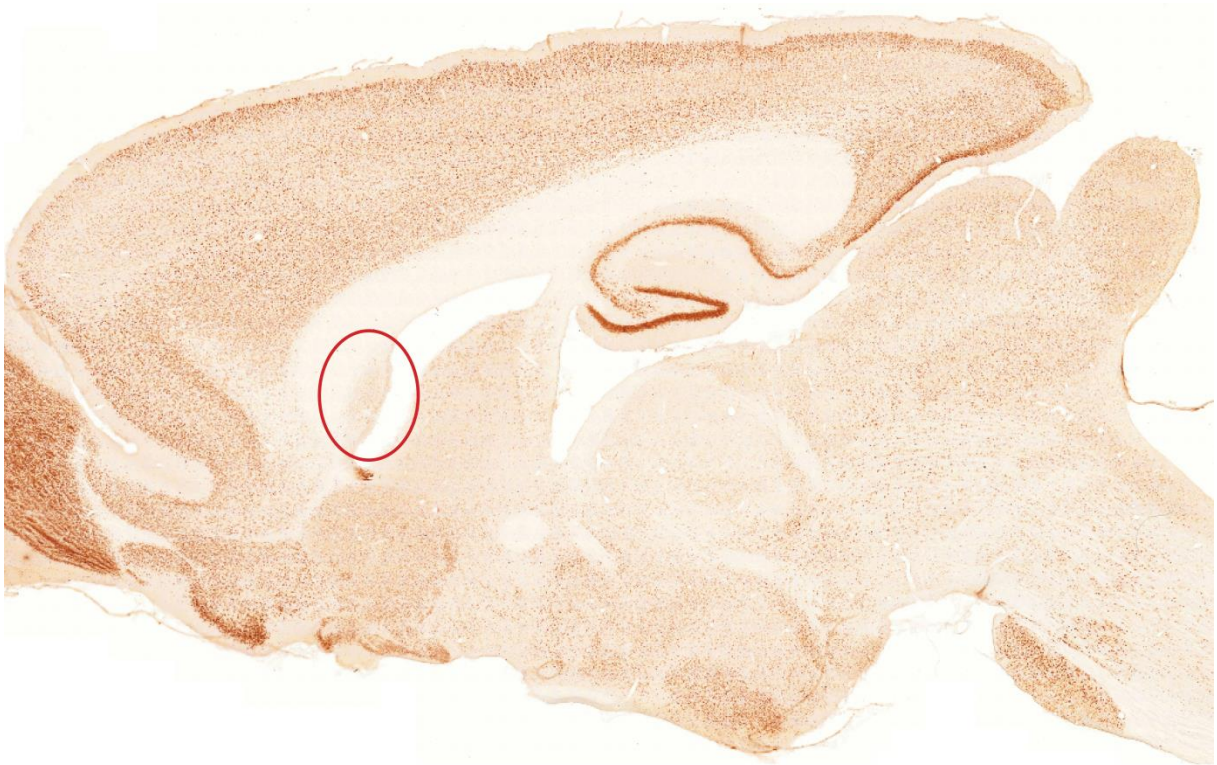


Figure 18: Medial dorsal hippocampus



Figure 19: Dorsal cortex of inferior colliculus

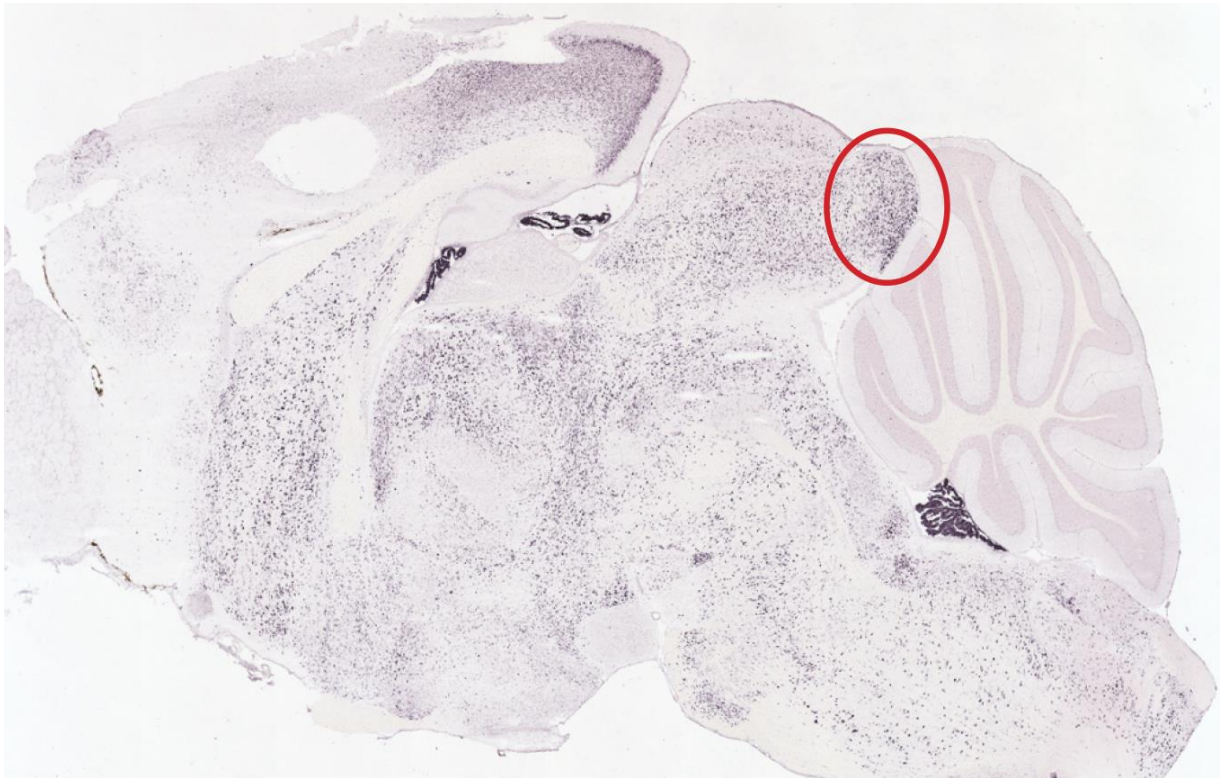
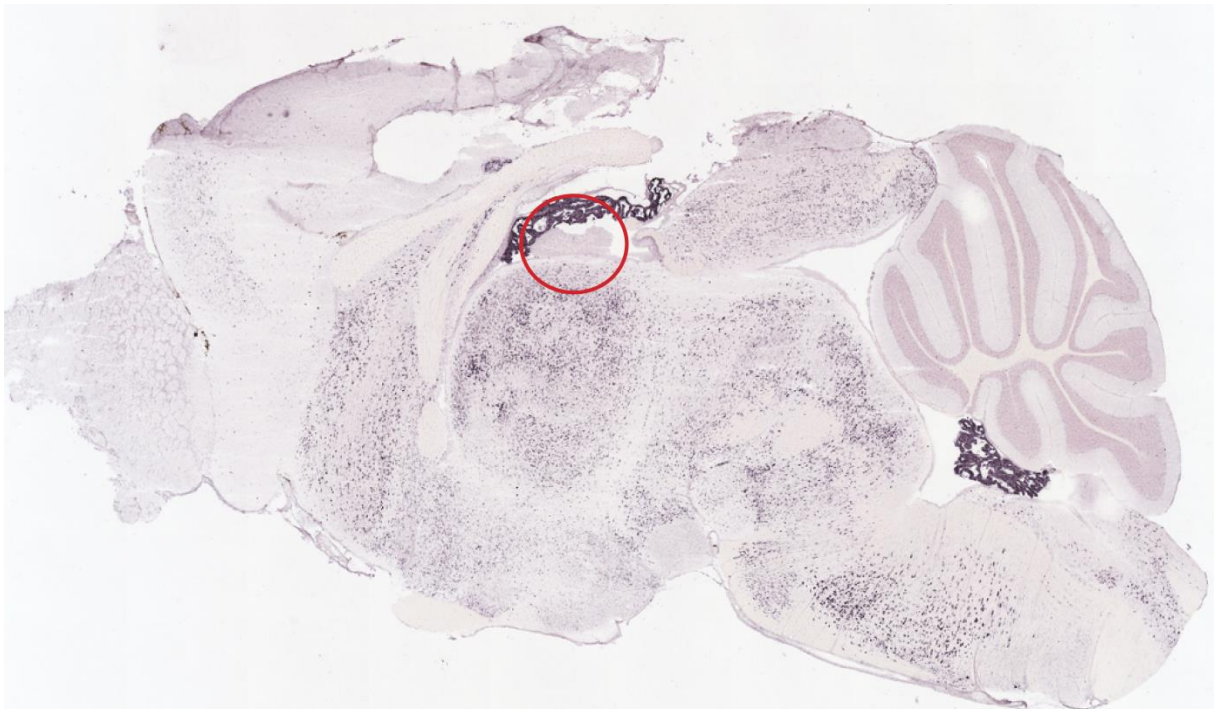


Figure 20: Medial habenular nucleus



Landmarks in the horizontal plane (dorsal to ventral)

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

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

Illustration of landmarks

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



Figure 2: Dorsal hippocampus



Figure 3: Dorsal cerebellum

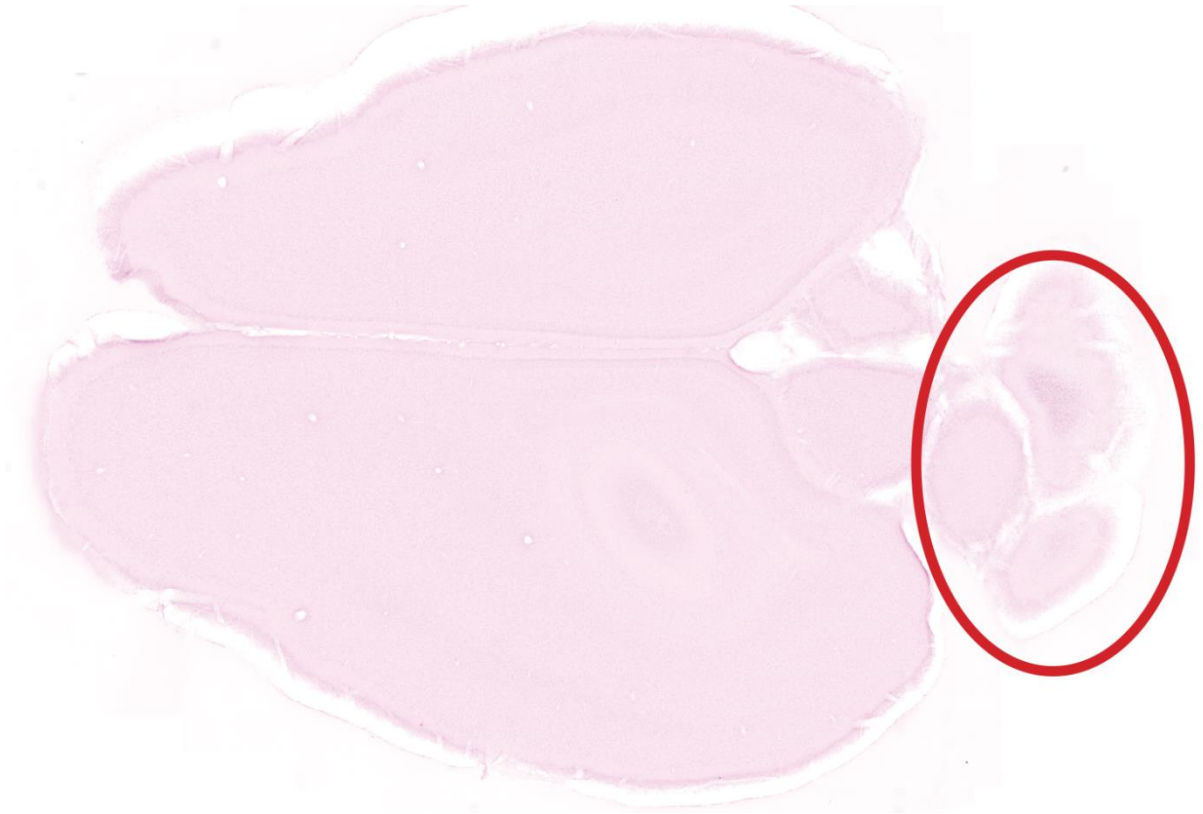


Figure 4: Corpus callosum

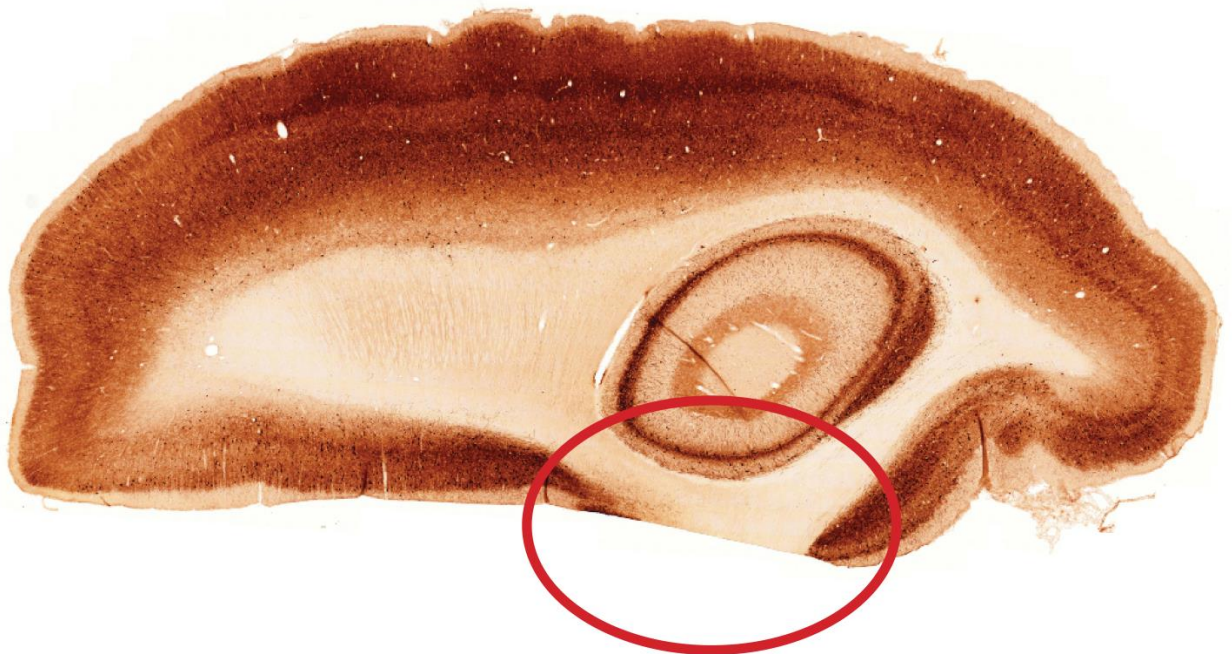


Figure 5: Dorsal striatum

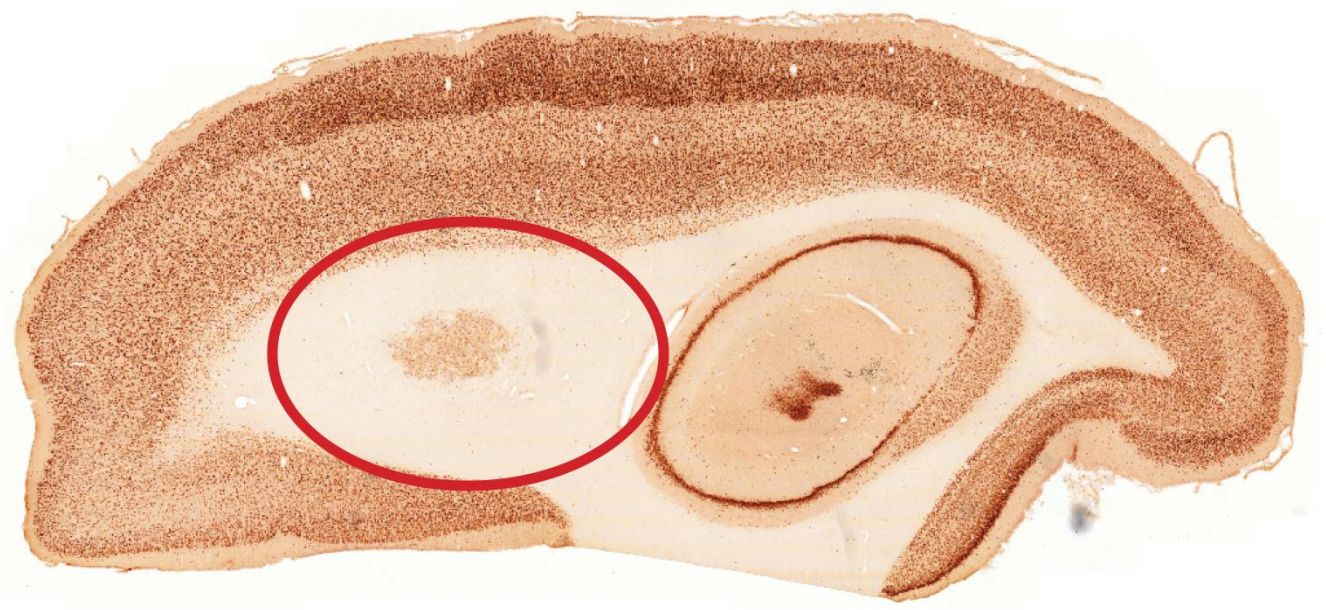


Figure 6: Fasciola cinereum



Figure 7: Lateral septal nuclei

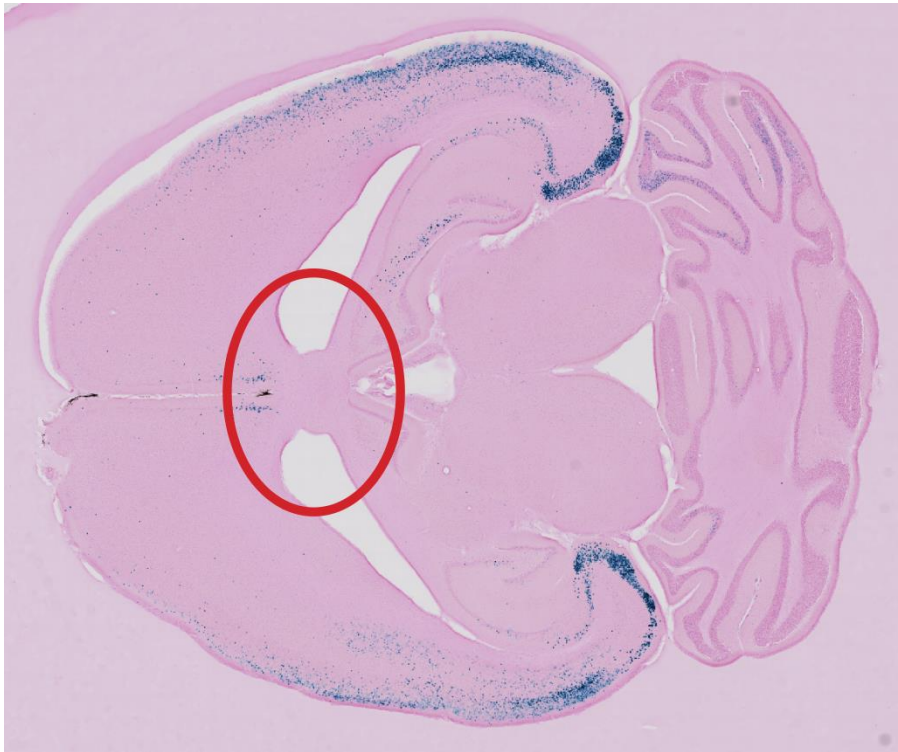


Figure 8: Thalamic nuclei and separation of the dentate gyrus

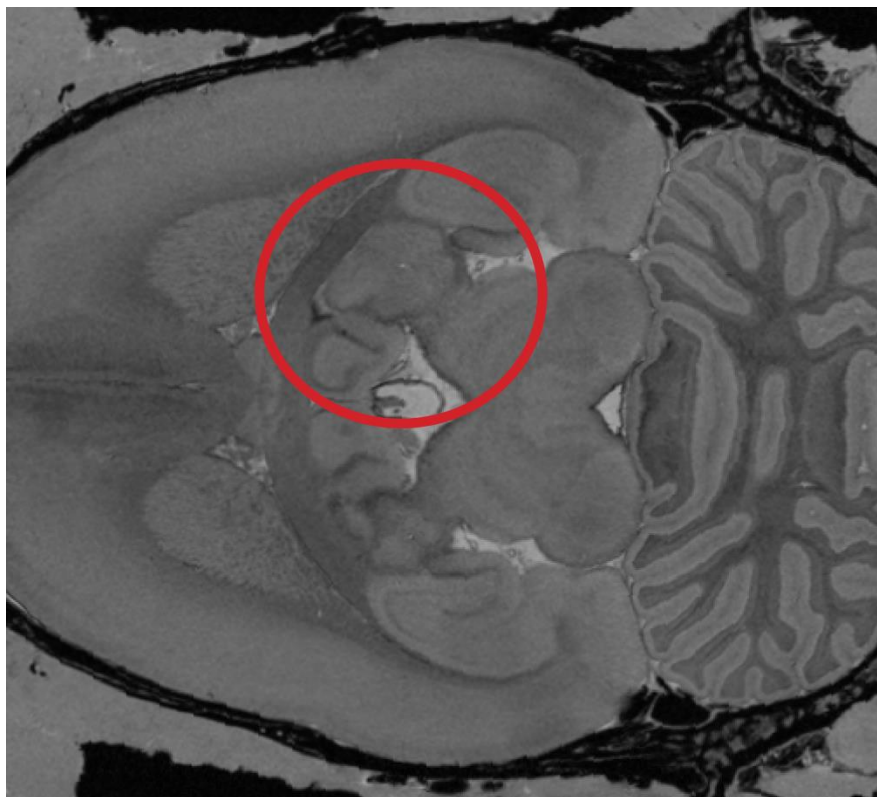


Figure 9: Dorsal olfactory bulb



Figure 10: Merging of the 3rd ventricle and lateral ventricle

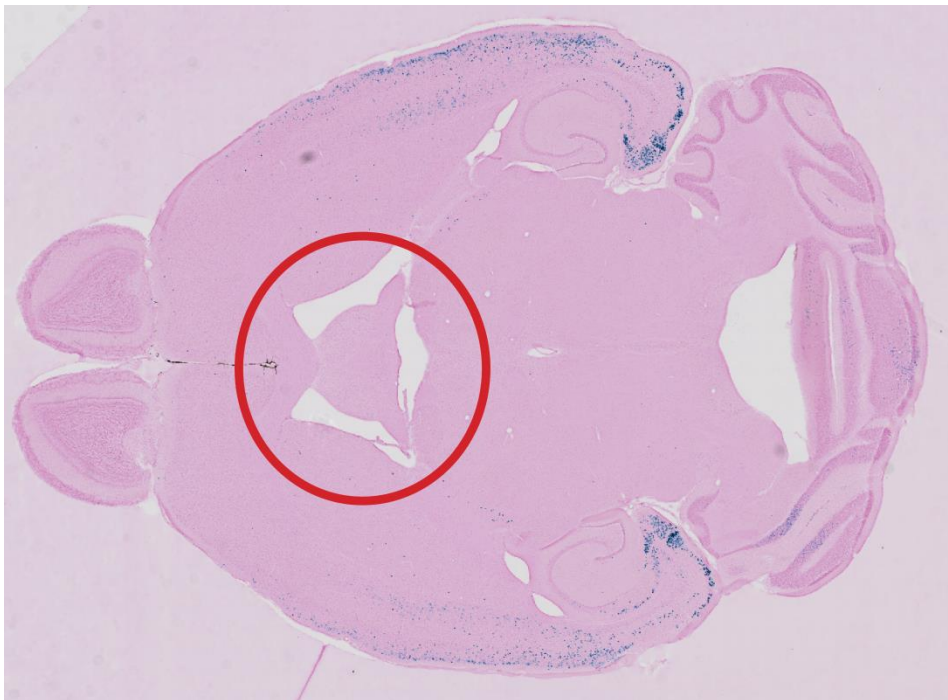


Figure 11: Dorsal cerebellar nuclei



Figure 12: Globus pallidus



Figure 13: Anterior commissure



Figure 14: Separation of anterior commissure

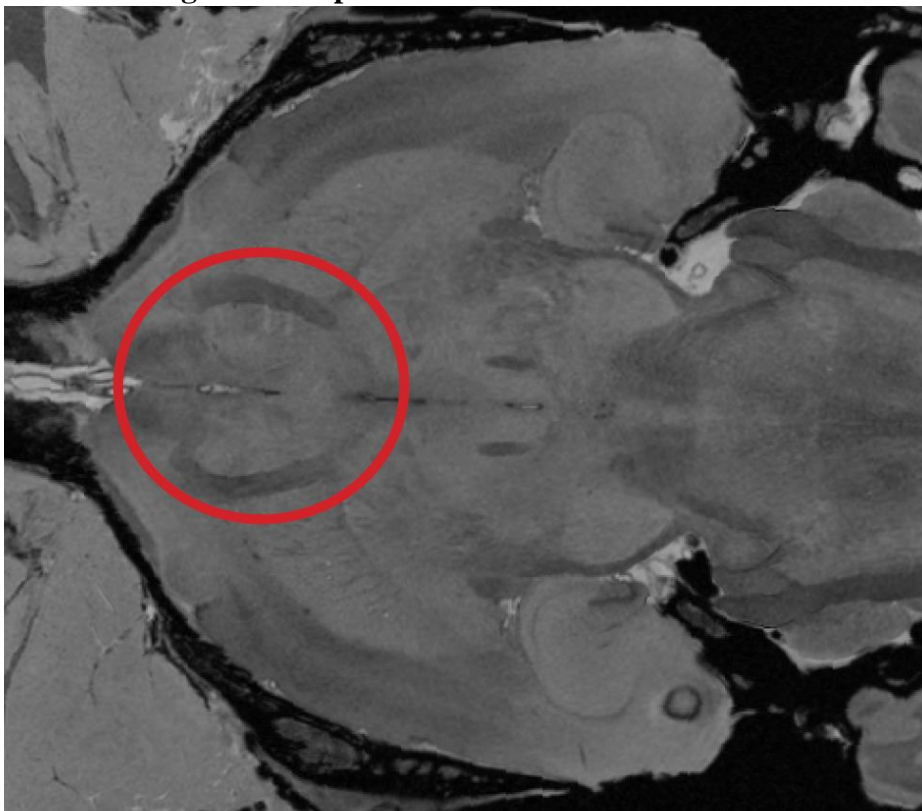


Figure 15: Dorsal brainstem



Figure 16: Ventral striatum

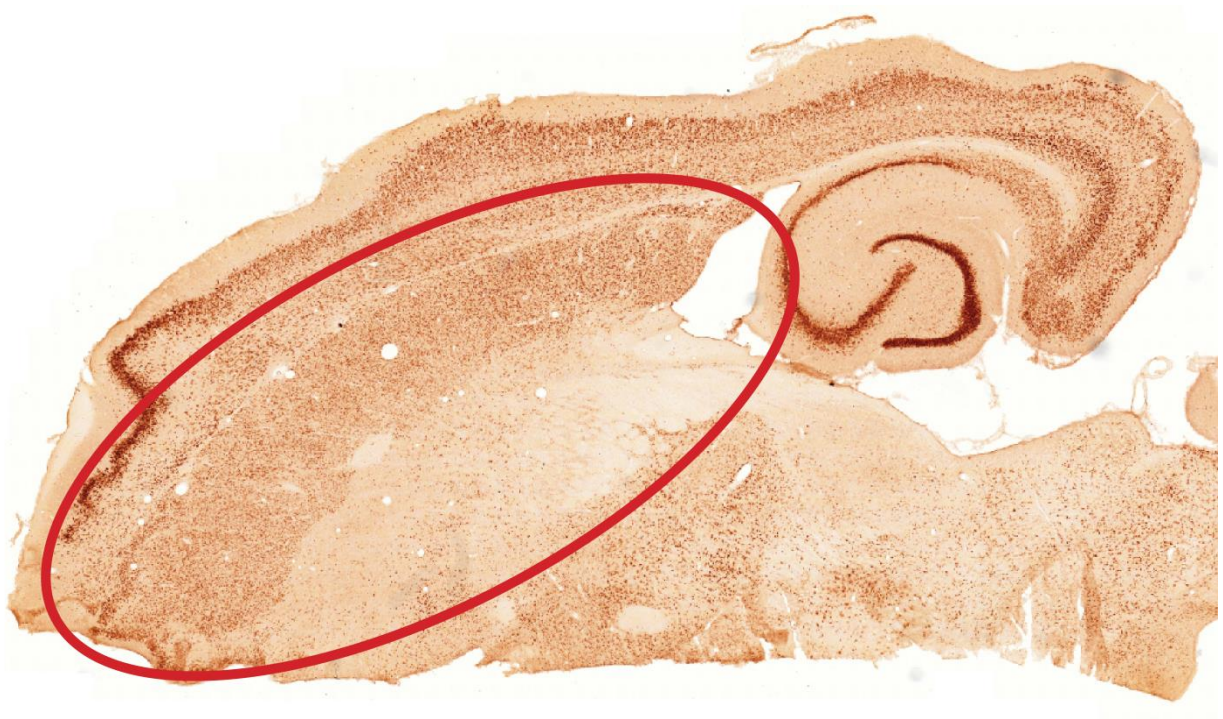


Figure 17: Ventral cerebellum



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

