

Chapter 2

Dissection of Rodent Brain Regions

Sabine Spijker

Abstract

Dissection of brain tissue is an important step in sample preparation for (subcellular) proteomics studies. In this chapter, brain removal and separate dissection of multiple brain regions from a single brain are described in step-by-step protocol. This concerns dissection from fresh or frozen tissue of cerebellum, hippocampus, prefrontal cortex, and striatum.

Key words: Dissection, Mouse, Brain, Hippocampus, Cerebellum, Cortex

1. Introduction

The mammalian brain is structurally organized into distinct anatomical regions (Fig. 1) mostly exerting dedicated control over specific physiological and behavioral functions. Various brain structures of interest to neuroscientists are the olfactory bulb, which is involved in sensing odors, the prefrontal cortex, which is important for executive control together with the striatum, the latter of which is also involved in integration of movement and reward processing. The hippocampus controls formation and retrieval of associative and episodic memories, the cerebellum is involved in motor learning and coordination, and controls voluntary learned physical movements. The pons contains a white matter tract of cranial nerves that among others relays signals between the cerebrum and cerebellum, and the medulla controlling autonomic functions, and relaying information between the brain and spinal cord.

At the cellular level, synaptic strength and neuroplasticity show clear brain region-specific features, as is evident from different plasticity mechanisms related to learning in cerebellum and hippocampus (1–3). Accordingly, the synapses across these brain regions show overlapping yet distinct proteomes (4, 5).

The majority of quantitative neuroproteomics studies today focus on the indicated brain regions (Fig. 1). In this chapter, I will discuss methods to dissect multiple brain regions from a single brain based on existing atlases (6, 7), as a way to allow region-specific (subcellular) proteomics analyses.

1.1. Fresh vs. Frozen Dissection

Fresh dissection of neuronal tissue has the advantage that particular brain regions can easily be dissected based on visual information, such as differences in color of adjacent tissues, and on the natural anatomical boundaries of certain regions present in the brain. Examples of these are the cerebellum that can be easily taken off from the medulla and pons (Figs. 1a and 2a), and are distinct in color, and the hippocampus that differs from the occipital cortex by color and because it lies loosely on the thalamus, basically only connected by the fornix (Fig. 1b). For other tissues, such as medial prefrontal cortex (mPFC) and striatum, dissection in both fresh and frozen tissue could be carried out, with frozen tissue likely yielding a slightly more accurate dissection, because it allows thinner sections to be made. In fresh tissue, dissection of these structures is more challenging as these regions are highly

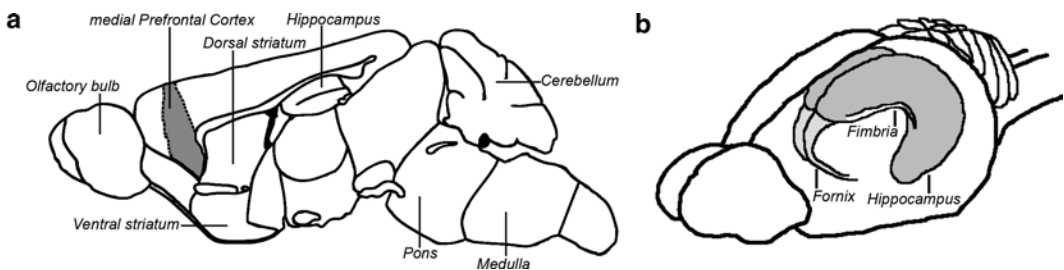


Fig. 1. (a) Schematic representation of a sagittal section of the mouse brain. Indicated are specific brain regions. Dissection of the cerebellum, the hippocampus, the medial prefrontal cortex (mPFC; gray), and striatum (dorsal and ventral) will be discussed. (b) Schematic representation of the hippocampus and fornix in the brain.

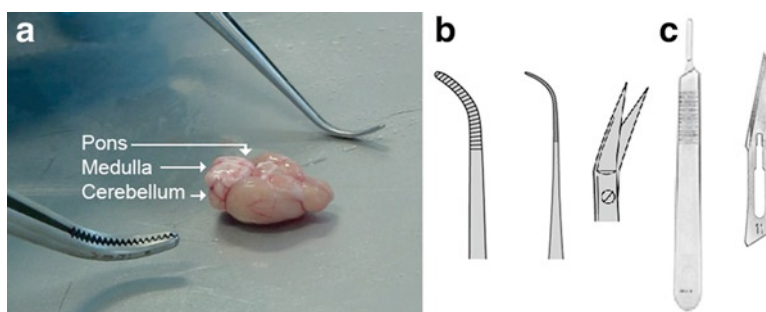


Fig. 2. Dissection tools. (a) For mouse brain dissection, a large curved serrated (*front*) and a small curved serrated (*back*) forceps are used. (b) Examples (Fine Science tools (FST) 11003-12, FST 11152-10) are shown in comparison to a mouse brain. (c) Scalpel with holder for subsequent dissection of the mPFC and striatum.

interconnected, and change shape going from rostral to caudal. However, it should be noticed that with today's biochemical methods for isolation of subcellular structures, brain slices should not be thinner than ~300–400 μm as they will float in the lysis buffer and hence it becomes hard to homogenate these properly.

2. Materials

The products used are listed below. Comparable products from other suppliers should also be effective. Underlined is equipment visible on the photographical and schematic representation of the dissections (Figs. 3–14).

2.1. Brain Removal

1. Surgical scissors – Straight sharp/blunt 12 cm (Fine Science tools (FST) 14001-12).
2. Narrow pattern forceps – Curved 12 cm, 2×1.25 mm (FST 11003-12; Fig. 2a, b).
3. Iris scissors – Large loops, angled (FST 14107-09; Fig. 2b).

2.2. Brain Dissection

1. Metal iron-free plate (favorably 10×10 cm; 5–30 mm thick).
2. For dissection of a mouse brain: One small curved blunt forceps with serrated tips for dissection (Graefe Forceps – 0.8 mm tips curved serrated, 0.8×0.7 mm (FST 11052-10), or Graefe Forceps – 0.5 mm tips curved serrated, 0.5×0.4 mm (FST 11152-10; Fig. 2a, b)) and one larger forceps with curved

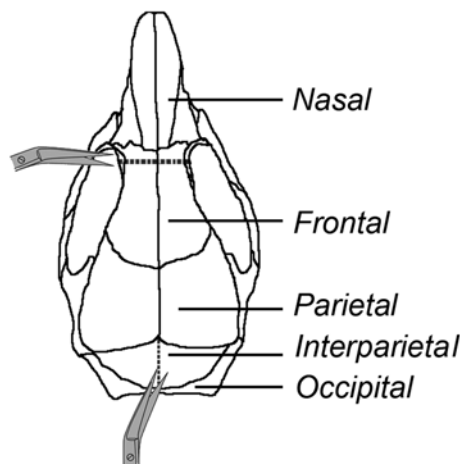


Fig. 3. Schematic representation of the mouse skull, showing the different bone plates. Indicated are the cuts to be made (*dark gray hatched line*) in the interparietal bone, as well as in the anterior part of the frontal bone.

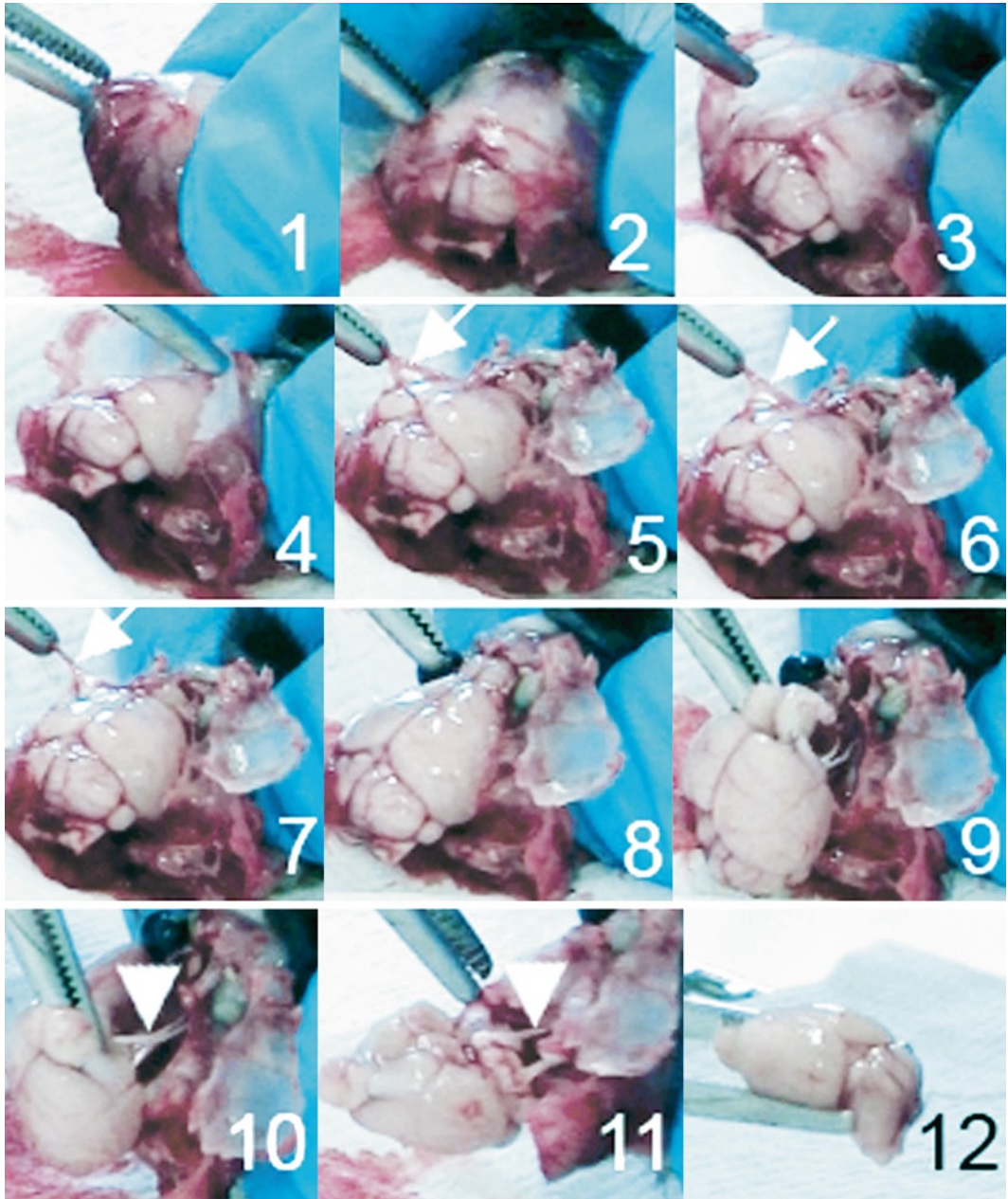


Fig. 4. Removal of the brain from the skull. Take care of the meninges (*arrows*) as these could rupture the brain while taking it out. Carefully cut the cranial nerves (*arrowheads*) upon taking out the brain.

blunt tips to hold the brain, e.g., the narrow pattern forceps (FST 11003-12). For a rat brain, larger forceps are needed, like the Graefe Forceps – 1.0 mm Tips Curved Serrated, 1 × 0.9 mm (FST 11652-10), Semken forceps curved Serrated – 13 cm, 1.3 × 1 mm (FST 11009-13), or FST 11003-12 for

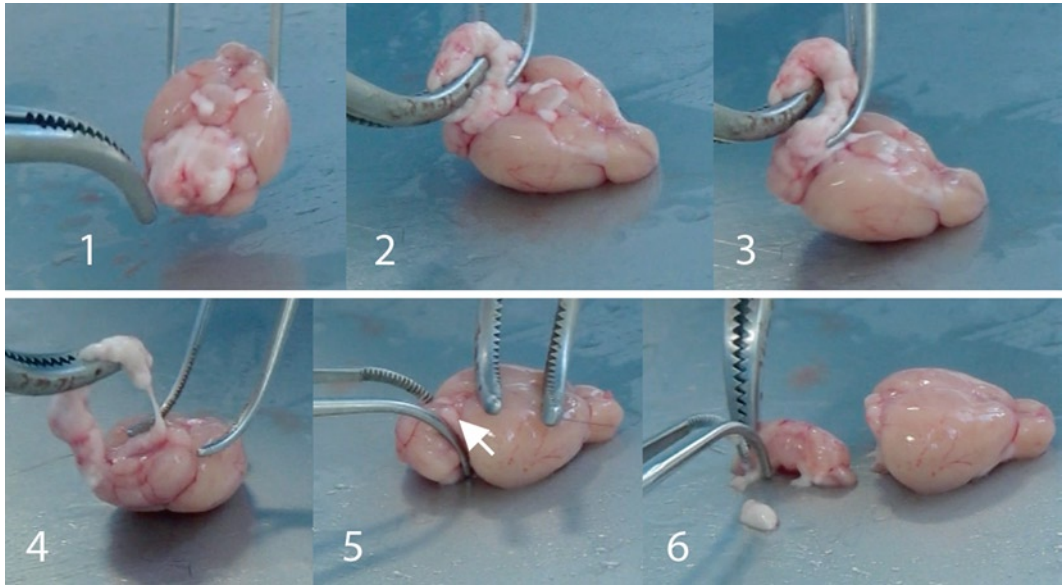


Fig. 5. Dissection of the cerebellum, freed from the medulla, and the pons. The *arrow* indicates the colliculus inferior.

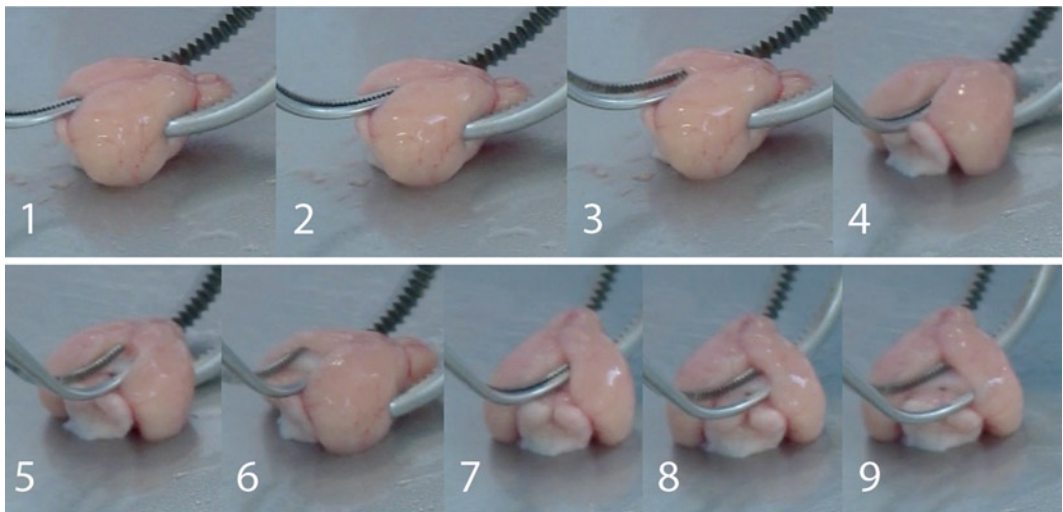


Fig. 6. Opening the cortex from the midline to free the hippocampus.

dissection and Narrow Pattern Forceps – Curved 14.5 cm, 2.25 × 1.45 mm (FST 11003-14) or Standard Pattern Forceps – Curved 12 cm, 2.5 × 1.35 mm (FST 11001-12) to hold the brain.

3. Razor blade (dissection of mPFC and striatum).

4. Scalpel handle (FST #10003-12 Scalpel Handle #3–12 cm) with scalpel (FST #10011-00) as illustrated in Fig. 2c (dissection of mPFC and striatum).

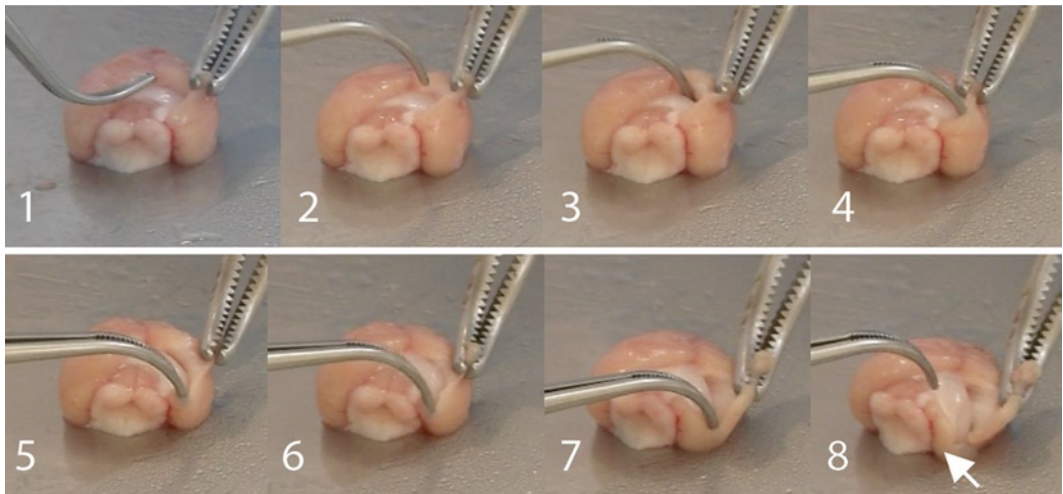


Fig. 7. Removal of cortex from the right hippocampus. The *arrow* indicates a piece of cortex left behind on the hippocampus. Note the difference in color between the two structures.

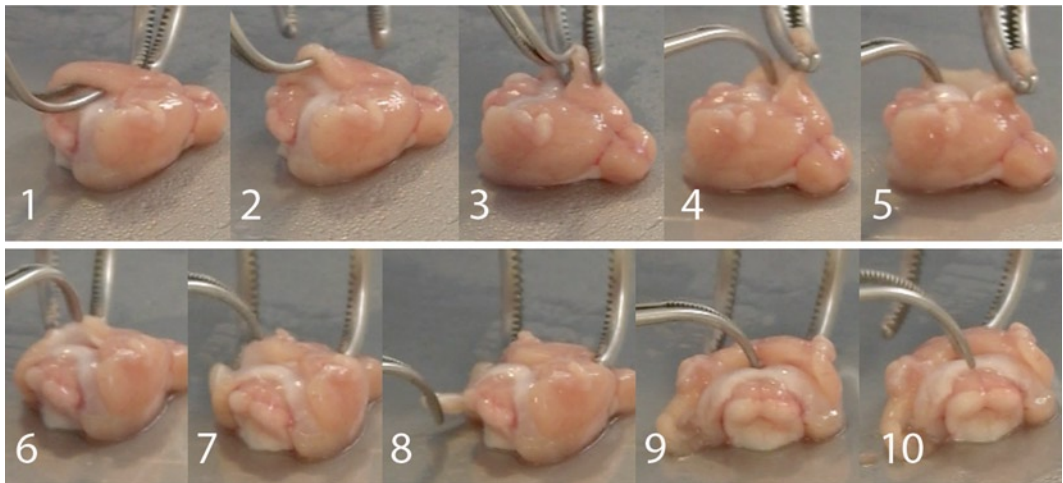


Fig. 8. Removal of cortex from the left hippocampus.

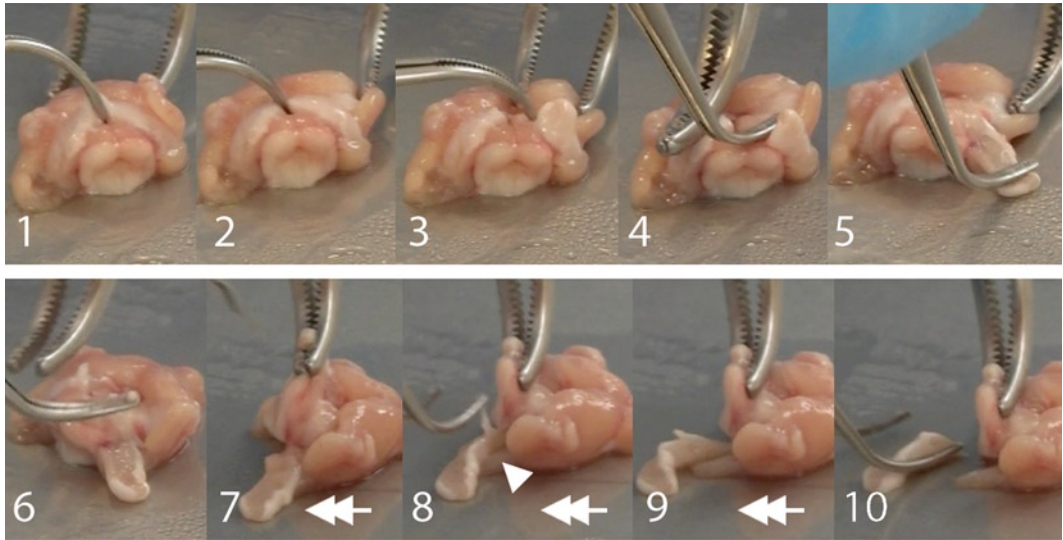


Fig. 9. Dissection of the right hippocampus. *Arrowhead* indicates a piece of cortex. The *double arrow* indicates the direction in which the hippocampus is rolled to free it from the cortex.



Fig. 10. Removal of a piece of cortex left behind on the hippocampus (*arrowhead*). Note the difference in color between the two structures.

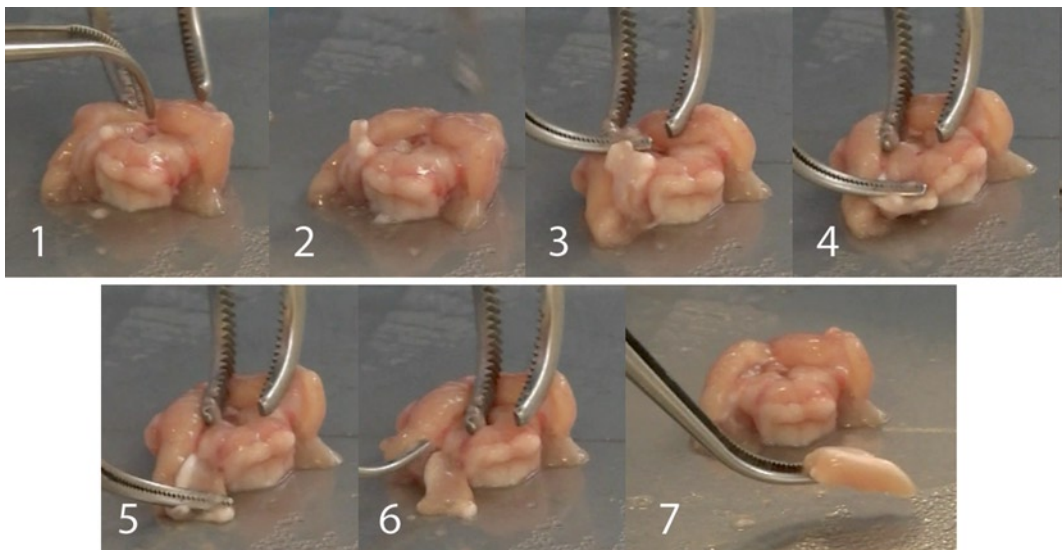


Fig. 11. Dissection of the left hippocampus.

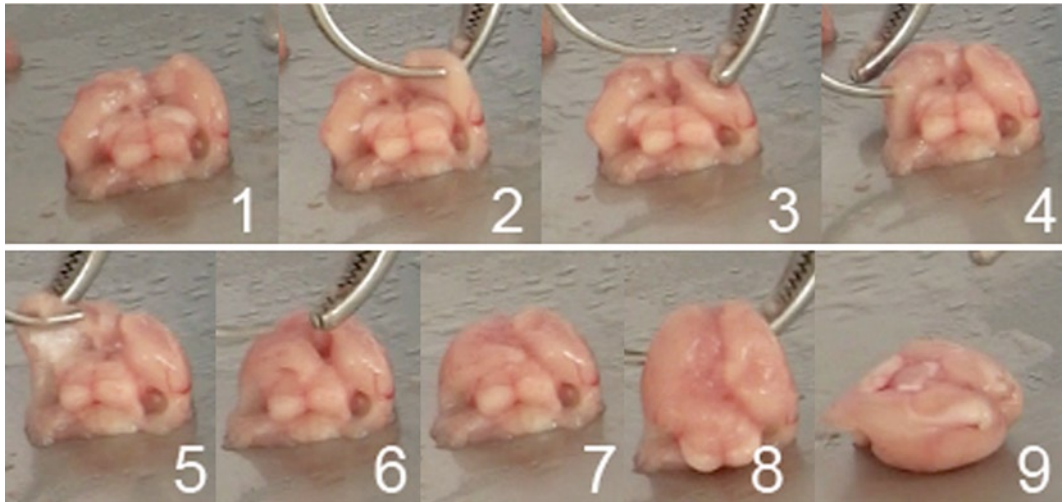


Fig. 12. Placing back the cortex after hippocampal dissection in preparation of subsequent dissections. The brain could be snap-frozen or used for dissection of fresh tissue as presented in Figs. 13 and 14.

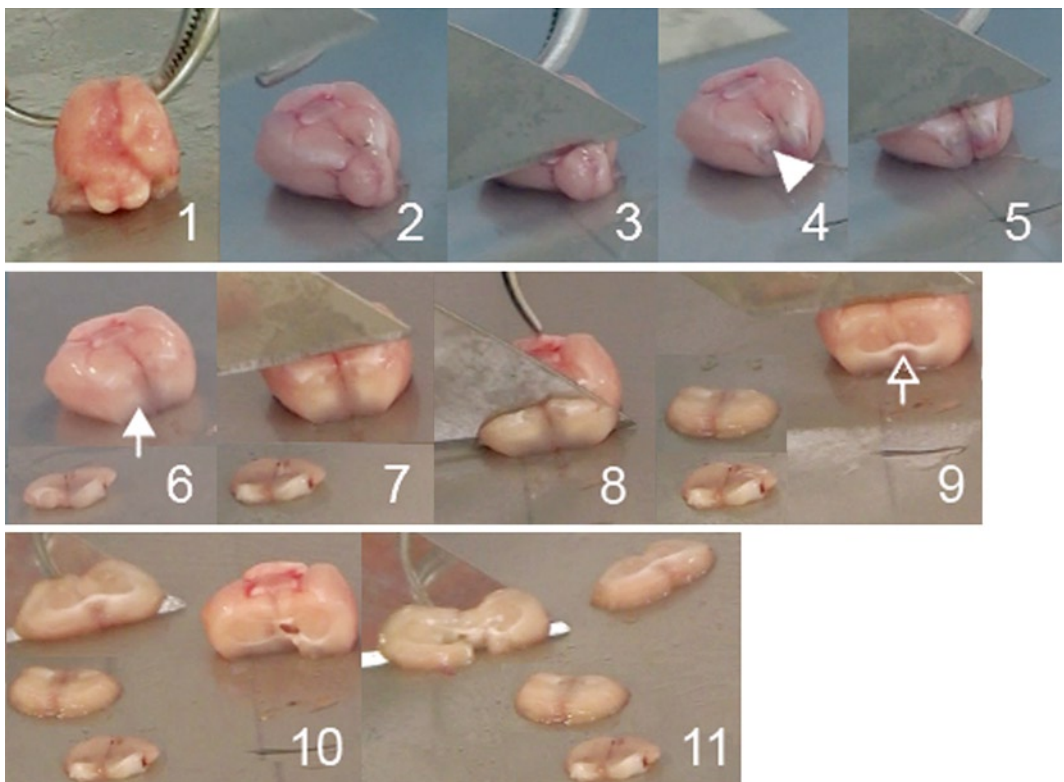


Fig. 13. Coronal slices are made in preparation of subsequent dissections of, e.g., the mPFC and striatum, as presented in Fig. 14. *Arrowhead* in 4 shows the anterior commissure. Anterior forceps of the corpus callosum (AFCC), indicated by an *arrow*, shine through the section from which the mPFC will be taken (see Fig. 14.1). *Open arrow* in 9 shows the genu corpus callosum (GCC).

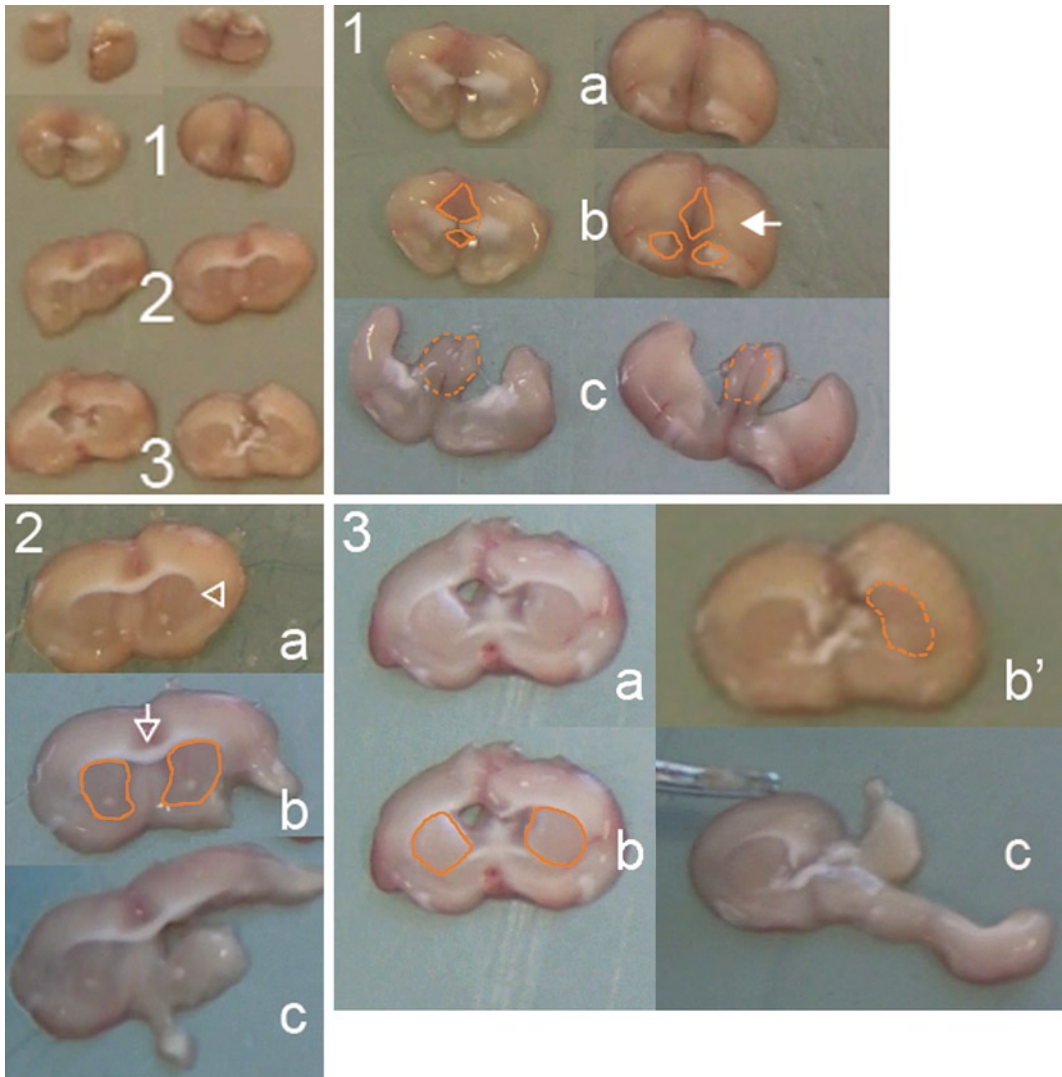


Fig. 14. From coronal sections #1–3 (left), the mPFC could be dissected from section #1, and the striatum could be dissected from sections #2–3. b and b' indicate the outline for dissection (orange line). c shows the dissected parts. AFCC, indicated by an arrow in 1b, shine through the section from which the mPFC will be taken. Open arrow in 2b shows the GCC. Open arrowhead in 2a indicates the boundary of the striatum and the cortex by the capsula externa.

3. Methods

3.1. Brain Removal

1. Use cervical dislocation to prevent pre- and postsynaptic effects of anesthesia (e.g., with protein abundance and phosphorylation (8–11)), and a surgical scissor to remove the head with a cut posterior from the ears. Using the scissors, make a midline incision in the skin. Flip the skin over the eyes to free

the skull. Make a small incision (Iris scissors) on the top of the skull starting from the caudal part at the point of the (inter) parietal bone (Fig. 3), be careful not to cut through the brain. Make a firm cut through the most anterior part of the skull, between the eyes (frontal bone, Fig. 3). This enables to remove the brain more easily.

2. Tilt one side of the parietal bone with the curved narrow pattern forceps and break it off (Fig. 4.1–3). Do the same with the other side (Fig. 4.4). Most likely the frontal bone will remain. In that case, make a small incision that enables tilting and breaking off this bone plate. Be careful of the meninges that are surrounding the brain and that are between the brain and the skull; they could rupture the brain while breaking off the skull (arrow; Fig. 4.5–7).
3. When the brain is freed from meninges (Fig. 4.8), slide the curved narrow pattern forceps (closed) under anterior part of the brain (olfactory bulb) and tilt the brain gently upward (Fig. 4.9). Slide the forceps further down to break the optic nerves and other cranial nerves (arrowheads; Fig. 4.10, 11) and gently lift the brain out of the skull (Fig. 4.12).
4. Transfer brain to metal plate placed on ice to cool down the brain immediately. Wipe off excess blood. Note that these steps should be performed within 2–3 min.

3.2. Cerebellum Dissection

1. Place the brain with the dorsal side facing the metal plate (Fig. 5.1).
2. Lift with the curved narrow pattern forceps the medulla/pons upward (Fig. 5.2).
3. Using the small curved forceps (Graefe Forceps – 0.5 mm tip), cut through the pons by closing the forceps around the tissue (Fig. 5.2, 3).
4. When the majority of the white tissue is removed (Fig. 5.4), turn the brain around with the ventral side facing the metal plate (Fig. 5.5).
5. Place the small forceps between the cortical lobes and the cerebellum (Fig. 5.5), and snap the cerebellum off from the colliculus inferior (arrow, Fig. 5.5).
6. Finally, remove possible remaining parts of the pons (Fig. 5.6). Note that all previous steps should be performed within 1 min.

3.3. Hippocampus Dissection

1. Place the brain with the ventral side facing the metal plate (Fig. 6.1).
2. Place the small curved forceps between the cerebral halves in a closed position. Gently hold the brain in position with the large curved forceps (Fig. 6.1, 2).

3. Gently open the forceps (Fig. 6.3), thereby slowing the opening of the cortical halves.
4. Repeat this process of placing the closed forceps in between the cortical halves, and opening the forceps (Fig. 6.4, 5). The initial white-colored part encountered is most likely the corpus callosum, under it is the hippocampus.
5. Once an opening is obtained for 60% along the midline, direct the forceps (closed position) 30–40° counterclockwise (Fig. 6.5) to open up the left cortex from the hippocampus by repeatedly opening the forceps. Thereafter repeat the same for the right cortex by pointing the forceps in a 30–40° clockwise direction (Fig. 6.6).
6. Repeat this movement on either side until the upper part of the hippocampus is visible (Fig. 6.7–9).
7. Using the large forceps, gently pick up the cortex (Fig. 7.1).
8. Turn the small forceps (point downward; Fig. 7.2) to free the hippocampus from the cortex without damaging the cortex. Remember to enter the tissue always with closed forceps.
9. Again repeat the process of opening and closing the small forceps while moving them to the caudal part of the hippocampus/cortex boundary (Fig. 7.3–5).
10. Once at the most caudal part of the hippocampus/cortex boundary, move the small forceps through the cortex (Fig. 7.5–8). Possible remainders of cortex (arrow, Fig. 7.8), visible from a more pink/yellow color than the hippocampus (gray, translucent), can be removed at that moment by snapping it off using the small forceps, or can be removed later.
11. Repeat steps 7–10 to remove the left cortex from the hippocampus (Fig. 8.1–8). Figure 8.8 presents the removal of a cortical piece from the hippocampus as explained in step 10.
12. Move the cortical halves anterior from the cortex to reveal the fornix (see Fig. 1b). Using the small forceps cut the hippocampus separate from the fornix (Fig. 8.9).
13. In addition, separate the two halves of the hippocampus (Figs. 8.10 and 9.1, 2).
14. Gently push with closed forceps the hippocampal halve to the side (Fig. 9.3), while keeping the brain in position with the larger forceps (Fig. 9.4).
15. Continue with step 14, until the hippocampus is lying sideways of the brain (Fig. 9.5, 6).
16. Using the small forceps roll the hippocampus out of the brain (direction of arrow, Fig. 9.7–10) to remove it from the cortex that was still adhered (arrowhead, Fig. 9.8).

17. Inspect the hippocampus for pieces of cortex (yellow/pink), as visible in Fig. 10.1, 2 (arrowhead), and remove them (Fig. 10.3).
18. Repeat steps 14–17 for the left halve of the hippocampus (Fig. 11.1–6) to finally obtain both halves (Fig. 11.7). Note that together these steps should be performed within 2–3 min.
19. When the posterior part of the cortex is not needed as tissue sample, one could at step 6 instead of folding away the cortex, also dissect the cortex away from the medial to the lateral side using the small forceps. In this way, the hippocampus is easily accessible. This will also speed up the process of dissection.

3.4. Prefrontal Cortex and Striatum Dissection (Fresh)

1. After removal of the hippocampus, use the large forceps to fold back the cortex into the original position (Fig. 12.1–7).
2. Then, place the brain with the dorsal side facing the metal plate (Fig. 12.8, 9). We will now start to make coronal sections in which the prefrontal cortex and striatum are visible at different levels (Figs. 14 and 15).
3. Take a sharp razor blade and make sections, the first one being to cut off the *olfactory bulb* (Fig. 13.1–3).
4. The anterior commissure is well visible at this point (arrowhead, Fig. 13.4).
5. The first section (Fig. 13.5, 6) contains mainly motor cortex.
6. Be aware that the subsequent section contains the anterior forceps of the corpus callosum (AFCC) shining through (arrow, Figs. 13.6 and 14.1b), with a darker area in the middle that represents the mPFC.
7. Cut the section containing the mPFC (Fig. 13.7–9).

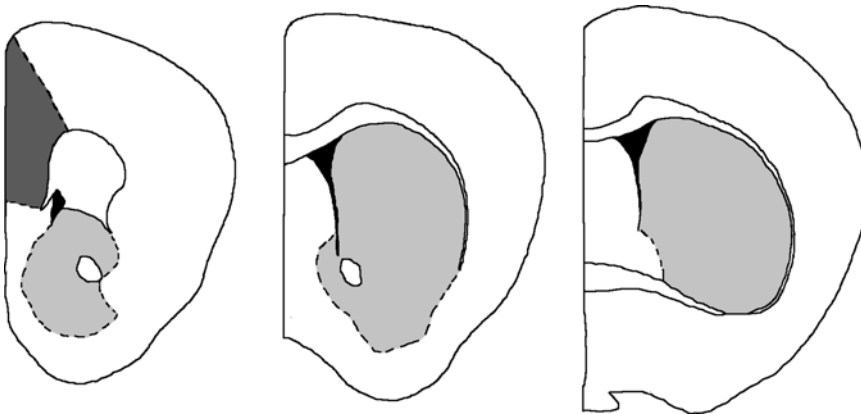


Fig. 15. Schematic representation of sections #1–3 from Fig. 14. Indicated are striatum (light gray) and mPFC (dark gray) for dissection. Ventricle is indicated in black.

8. In the remaining brain, the genu corpus callosum (GCC; open arrow, Figs. 13.9 and 14.2b) is now well visible, as well as the dorsal and ventral striatum that are separated from the adjacent cortex by the capsula externa (open arrowhead, Figs. 14.2a and 15).
9. After cutting this section (Fig. 13.10), the brain should now show the joining of the anterior commissure that is best visible in Fig. 14.3a. This marks the absence of the ventral striatum in this section.
10. The last section therefore contains only dorsal striatum (Fig. 13.11).
11. From each brain, the last three sections are used for dissection of the mPFC and striatum (Fig. 14, left, mPFC, section #1; striatum, sections #1–3).
12. For the *mPFC*, take section #1. The mPFC, containing the prelimbic and infralimbic cortex, is visible as a darker area between the AFCC (Fig. 14.1a, b, left section). Note that the infralimbic cortex ends when the GCC is present (Fig. 14.1a, b, right section).
13. Cut through the GCC to dissect the mPFC in a diamond-like shape (Fig. 14.1b, c). Be careful not to take along any material from the AFCC.
14. For the *striatum*, take sections #1–3. From section #1, the ventral striatum is visible as a darker structure surrounded by the somewhat lighter and less translucent cortex, as well as the AFCC (Fig. 14.1b).
15. From section #2, both the dorsal and ventral striatum have a darker appearance than the surrounding cortex (Fig. 14.2a). Around the midline, the septum, a structure similar in color as the cortex, separates the two striatal halves.
16. Dissect the striatum from the GCC and adjacent capsula externa (caudal and lateral), as well as from the ventricle and septum (medial), and cortex (ventral), as indicated by the natural borders (Fig. 14.2b, c).
17. In section #3, only the dorsal striatum is present, as the anterior commissure now connects both hemispheres.
18. Dissect the dorsal striatum from the corpus callosum and adjacent capsula externa (caudal and lateral), as well as from the ventricle and septum (medial), and cortex (ventral), as indicated by the natural borders (Fig. 14.3b, c).

3.5. Prefrontal Cortex and Striatum Dissection (Frozen)

1. After replacing the cortex halves into the original position (Fig. 12.1–7, see step 1 in Sect. 3.4), immediately snap-freeze the brain. This can be done in liquid nitrogen, or in isopentane cooled on dry ice. Be careful with the latter method, as isopentane is toxic and should be discarded properly.

2. Store the tissue (-80°C) in aluminum foil to prevent freeze-drying.
3. Prior to dissection, place the brain for at least 1 h at -20°C , preferably on a metal plate in a cryostat.
4. After the tissue reached a stable temperature, take a razor blade to make manual coronal sections. Because the slices are thinner than when sectioning fresh tissue, more precision can be obtained in this way. However, fresh tissue yields a higher degree of visual information on the natural boundaries of brain areas that is partially lost upon freezing.
5. Based on the same visual cues as described above, cut out the regions of interest with a scalpel. Collect the tissue in an eppendorf tube, and store at -80°C until further use.
6. Note that putting plastic tubing around the scalpel holder will reduce the transfer of body heat to the scalpel, and thereby prevents the tissue from being thawed. In addition, wearing double gloves better insulates from cold and prevents heat being carried over to the tissue.

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