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**Study Protocol**

# Rapid Extraction of Prefrontal Cortex and Hippocampus for Molecular Analysis in Rats

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**Abstract**
Memory functions in laboratory animals are a crucial area of research in neuroscience, with a particular focus on identifying drugs that enhance various aspects of memory, particularly long-term declarative memory. This field involves studying cognition and memory from behavioral and molecular perspectives. Researchers evaluate changes in genes, enzymes, and proteins to discover the neurobiological mechanisms underlying memory formation and retrieval. The hippocampus and prefrontal cortex are key brain regions associated with memory and cognition, undergoing synaptogenesis following the acquisition of new information or skills. The production of specific proteins plays a crucial role in consolidating and storing memories, necessitating precise analysis of these brain regions to understand the molecular mechanisms involved in memory processes fully. However, the rapid degradation of these proteins by cellular enzymatic activities highlights the need for efficient and timely extraction techniques to ensure precise and reliable analysis. This methodological study aimed to introduce a rapid and accurate method for extracting the prefrontal cortex and hippocampus in rats.
**Keywords:** Brain extraction, Rat, Hippocampus, Prefrontal cortex

## Introduction

The investigation of memory processes in laboratory animals is one of the most extensively researched topics in the field of neuroscience. This area of study has concentrated on the search for drugs that enhance various aspects of memory, particularly long-term declarative memory, which can be assessed in both humans and animals.[1,2] Cognition and memory in animals are studied from both behavioral and molecular perspectives. At the behavioral level, several well-established tests such as the Morris water maze, Barnes maze, radial arm maze, and novel object recognition are commonly employed to assess cognitive functions in animal models. These tests provide valuable insights into animals' spatial learning, memory, and problem-solving abilities.[3] Furthermore, at the molecular level, researchers evaluate changes in genes, enzymes, and proteins that correlate with observed cognitive and behavioral alterations, providing a deeper understanding of the underlying neurobiological mechanisms governing memory formation and retrieval.[4] The hippocampus and prefrontal cortex are of particular interest among the key brain regions associated with memory and cognition. These areas are known to undergo synaptogenesis and the formation of new synaptic connections, following the acquisition of new information or skills.[5,6]

In the memory formation process, the production of specific proteins is crucial in the consolidation and storage of memories. Given the importance of these proteins in memory formation, it is essential to accurately separate and analyze the hippocampus and prefrontal cortex to meticulously examine their gene expression patterns and protein profiles. This targeted approach enables researchers to gain a comprehensive understanding of the molecular mechanisms involved in memory processes.[6,7] However, due to the rapid degradation of these proteins by cellular enzymatic activities, their half-life becomes a critical factor to consider.[8] The short half-life of memory-related proteins highlights the need for efficient and timely extraction techniques to ensure accurate and reliable analysis. This study aimed to introduce a rapid and accurate method for extracting the prefrontal cortex and hippocampus in rats (Supplementary file 1, Movie, Chapter 1).

## Protocol

### *Equipment and Apparatus*

The tools and supplies required to remove the prefrontal cortex and hippocampal tissue of the rats are listed in Table 1.

**Table 1.** List of Equipment and Chemical Materials for Brain Extraction

| Equipment | | Chemical | |
|---|---|---|---|
| Bohler Bone Cutter | Aesculap, Germany | Alcohol (70%) | Kimia Alcohol Zanjan, Iran |
| Cold Plate | Did Sabz, Iran | Ketamine and Xylazine | Alfasan, The Netherlands |
| Double Edge Razor Blades | Lord, Egypt | Normal saline (0.9% saline) | Samen, Iran |
| Fine Scissor | Aesculap, Germany | PBS | Sigma, Germany |
| Forceps, Fine Tip, and Curved | Aesculap, Germany | | |
| Laboratory Spatula | Cole Parmer, France | | |
| Petri Dish | AHN Biotechnologie GmbH, Germany | | |
| Pointed Curved Tweezer | Aesculap, Germany | | |
| Rodent Skull Awl-Dilator | Arman Poshtiban Teb, Iran | | |

*Note.* PBS: Phosphate-buffered saline.

## Prefrontal Cortex Extraction

1. Sedate the male rat by injecting ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally to achieve deep anesthesia. Then, euthanize the rats according to the protocol described by Norouzi-Bonab et al and extract their brain.[9]
2. Rinse the brain with phosphate-buffered saline (PBS) solution, then separate the hemispheres and place them on a brain matrix on a cold plate.
3. According to the Paxinos atlas, the rat PFC spans a range of about 8-9 mm in the anterior-posterior axis, from around +5.64 mm to -3.00 mm relative to bregma. In order to facilitate and increase the accuracy of sampling, the range of AP = + 2.8 to AP = + 4.8 is suitable and accurate.
4. Start by cutting the anterior border of the prefrontal cortex, removing a portion of the frontal cortex along with the olfactory bulb.
5. Then, cut the posterior border of the prefrontal cortex, carefully separate the prefrontal cortex, and transfer it to a Petri dish containing PBS for washing.
6. Finally, separate the slice from the middle and gently and carefully isolate the cortical part from the central area using a sharp blade (Supplementary file 1, Movie, Chapter 2).

## *Hippocampus Extraction*

Sedate the male rat by injecting ketamine (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally to achieve deep anesthesia. Then, euthanize the rats according to the protocol described by Norouzi-Bonab et al and extract their brain.[9]

1. Rinse the brain with PBS solution in a Petri dish, then separate the hemispheres and place them on the lateral side on a cold plate using a sharp blade.
2. Identify the hippocampus, located in the upper cortex of the Corpus Callosum area, and separate it from the inside of the lateral ventricle at coordinates between AP = -2 mm and AP = -7 mm.[10] A banana-shaped area with a pale color different from the color of the cerebral cortex can be observed.
3. Gently dissect the banana-shaped hippocampus area and remove any excess tissue using a sharp blade (Supplementary file 1, Movie, Chapter 3).

## Conclusion

This methodical approach to brain extraction ensures the accurate isolation of specific brain regions, including the prefrontal cortex and hippocampus, while maintaining the integrity of the tissues throughout the process (Supplementary file 1, Movie, Chapter 4).

### Authors' Contribution

**Conceptualization:** Seyed Zanyar Athari.
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**Funding acquisition:** Seyed Zanyar Athari.
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### Competing Interests

None.

### Ethical Approval

This study was approved by Research Ethics Committee of Vice-Chancellor in Research Affairs - Tabriz University of Medical Sciences (Code: IR.TBZMED.VCR.REC.1397.053).

### Funding

None.

### Supplementary Files

Movie: Rapid Extraction of Prefrontal Cortex and Hippocampus for Molecular Analysis in Rats

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