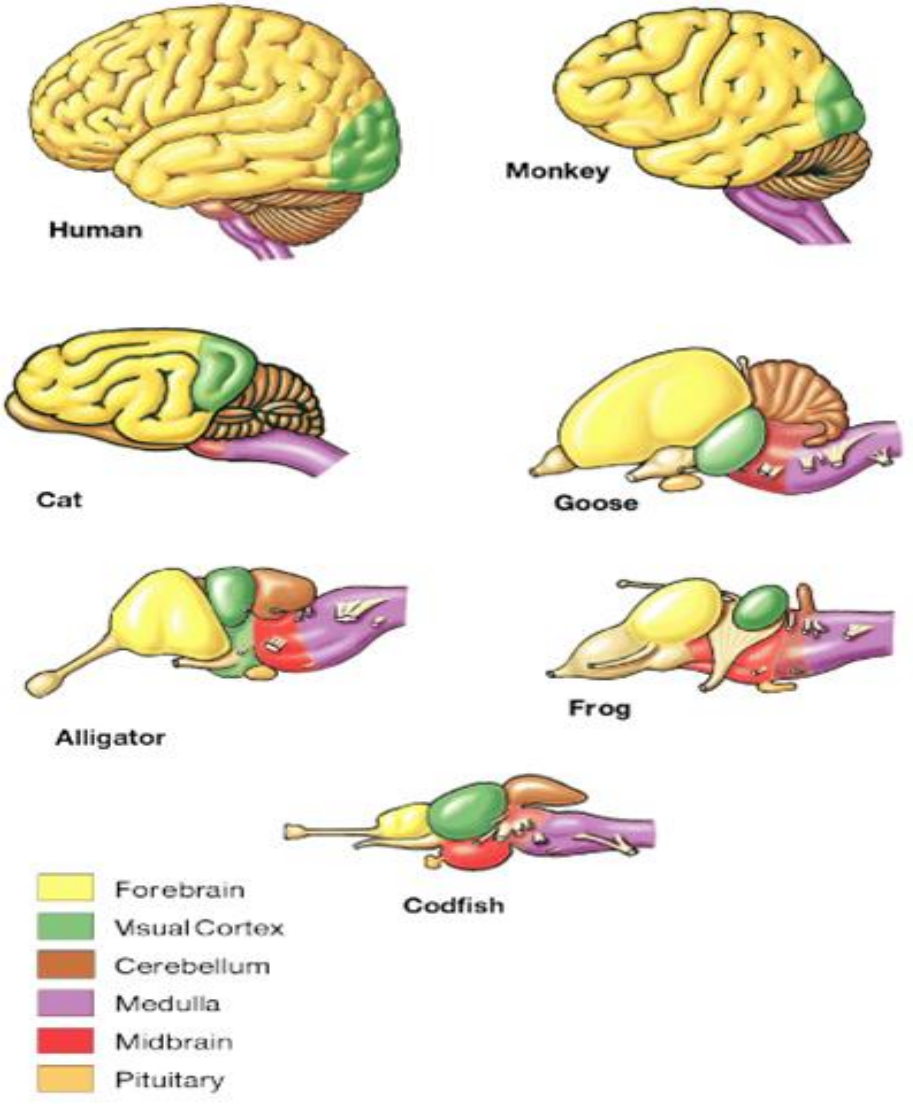
# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

1. Comparative study of brain of lower and higher vertebrates.
2. Dissection of Chicken Brain
3. Dissection of Goat Brain
4. Histology and sectioning of major areas in the brain.
5. Brief introduction of the staining techniques and stains used in brain Histology.
6. Anatomical mapping of major hypothalamic centers.
7. Study of pituitary and pineal cell types through prepared slides.
8. Hands on training in Electrophysiology:
9. Some important behavioural techniques in neuroscience:
    (a). Rotarod
    (b). Morris water maze
    (c). 8 Arm radial maze or T – Maze
10. Study of MRI and CT-SCAN images for diagnosis of various neurological conditions

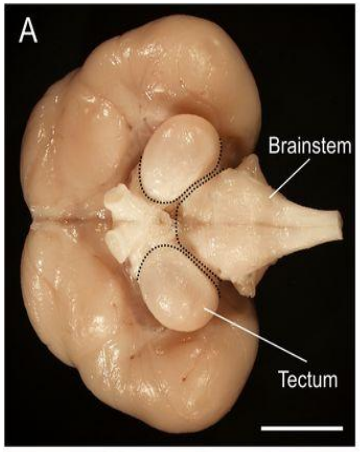
# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

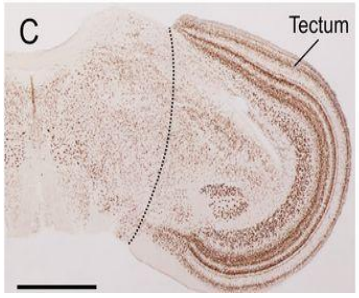
## Practical no 1: Comparative study of brain of lower and higher vertebrates.

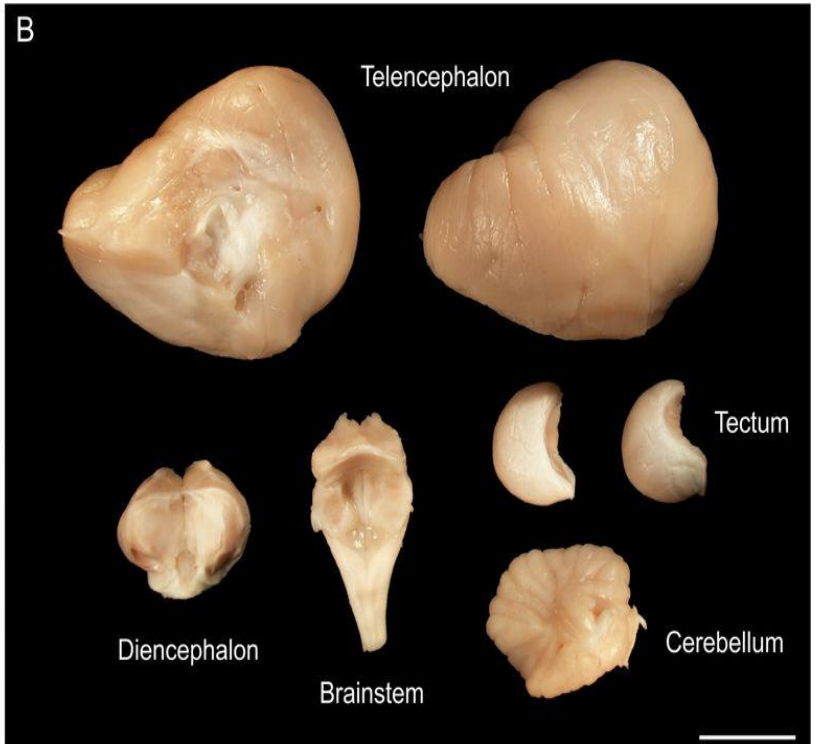


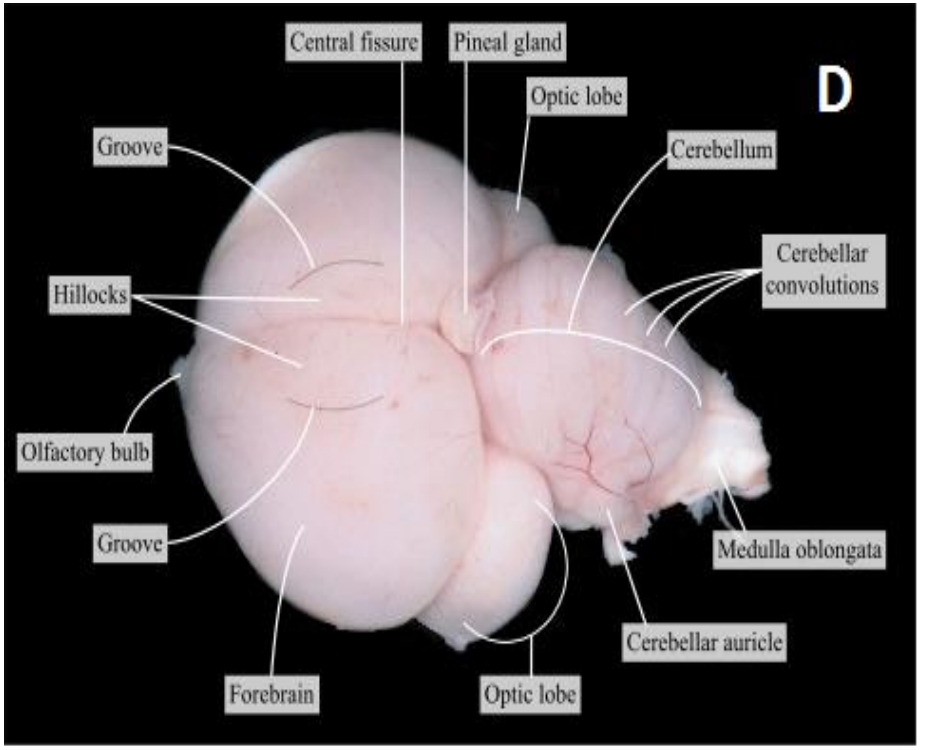
# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 2: Dissection of Chicken Brain




*(A) Ventral side of the brain showing approximate lines of dissection of the brainstem and tectum.*

*(B) Brain dissected into parts used for isotropic fractionation.*

*(C) Tectum*

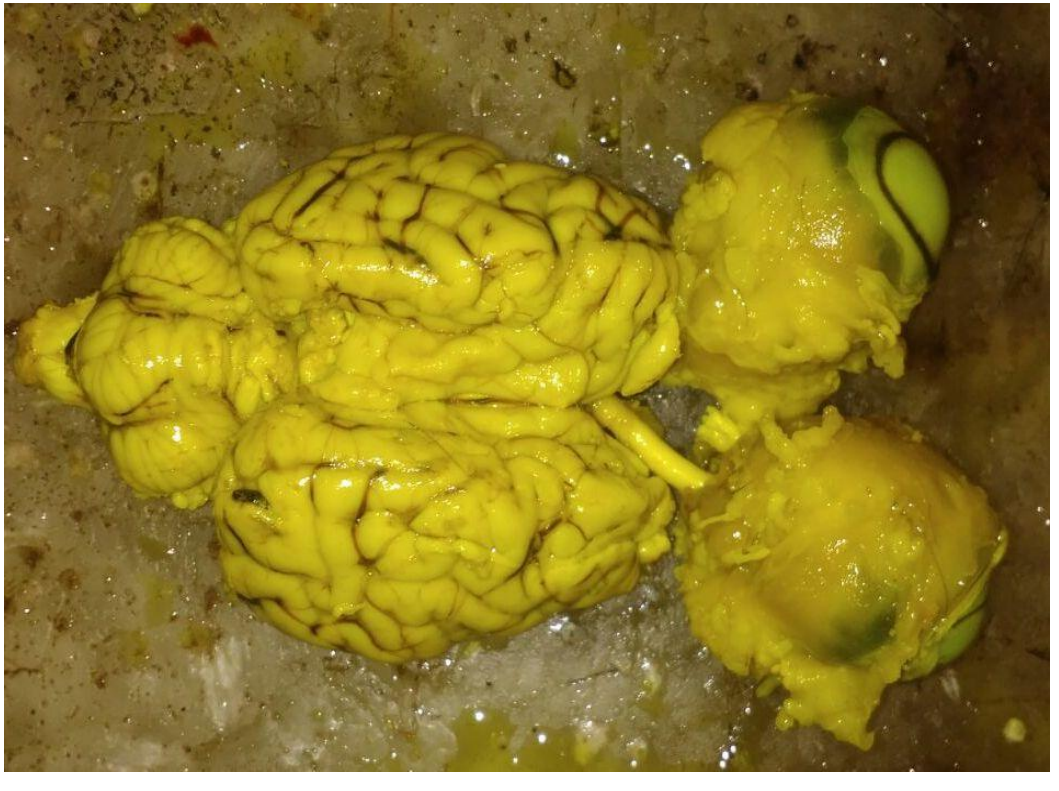
*(D) Various regions of brain*

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 2: Dissection of Chicken Brain

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 3: Dissection of Goat Brain



Dorsal view of Goat brain

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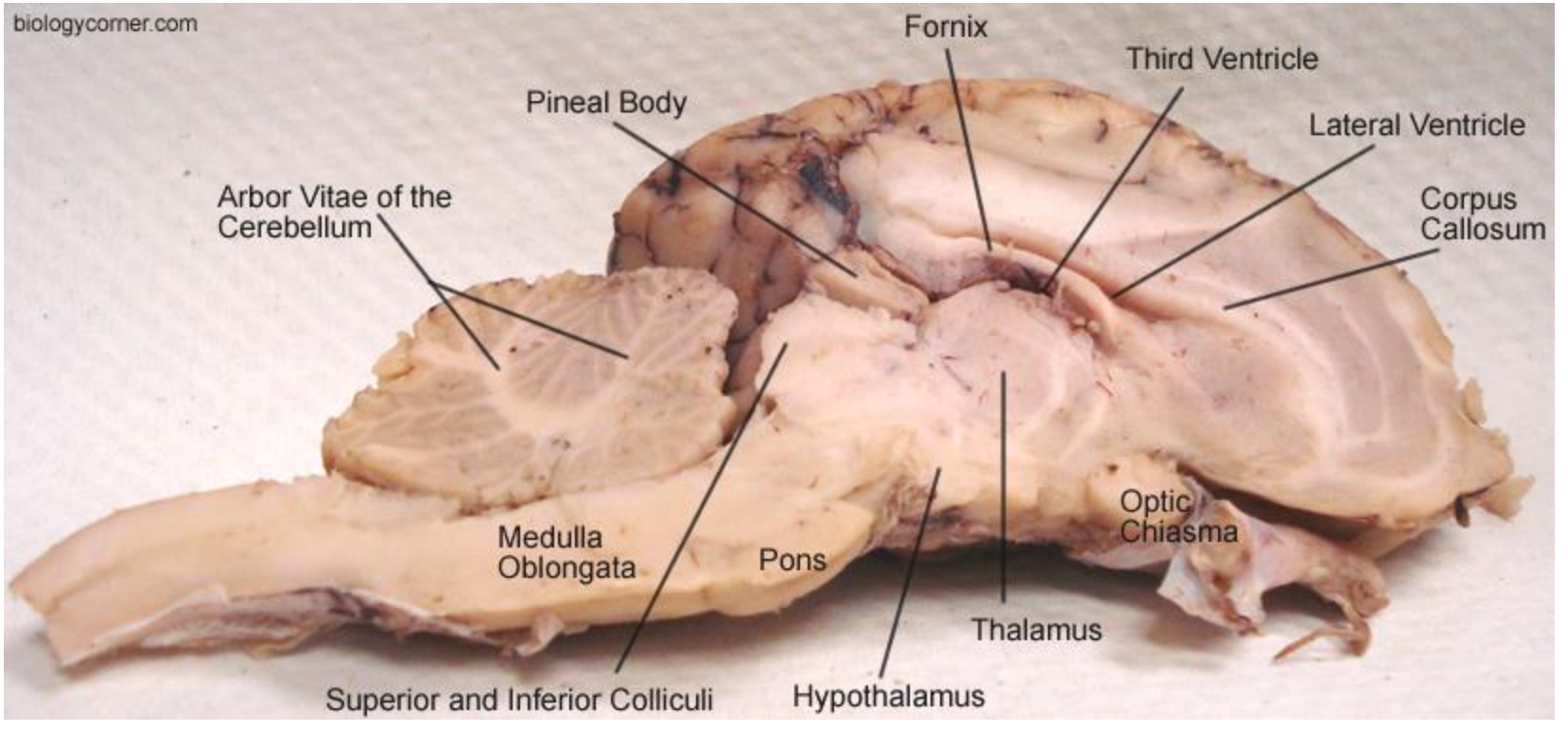
## Practical no 3: Dissection of Goat Brain



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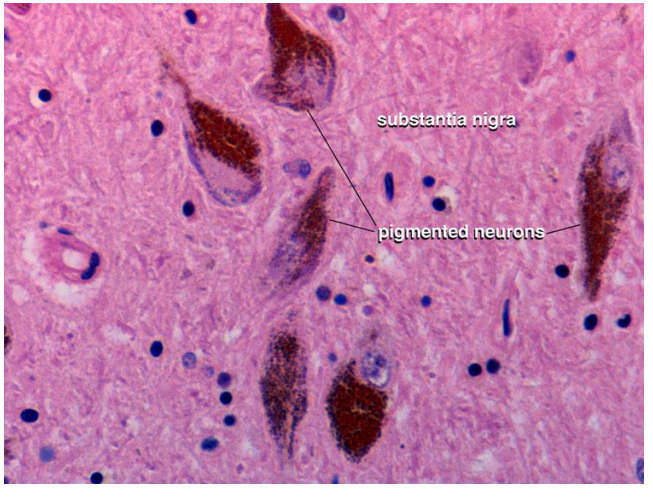
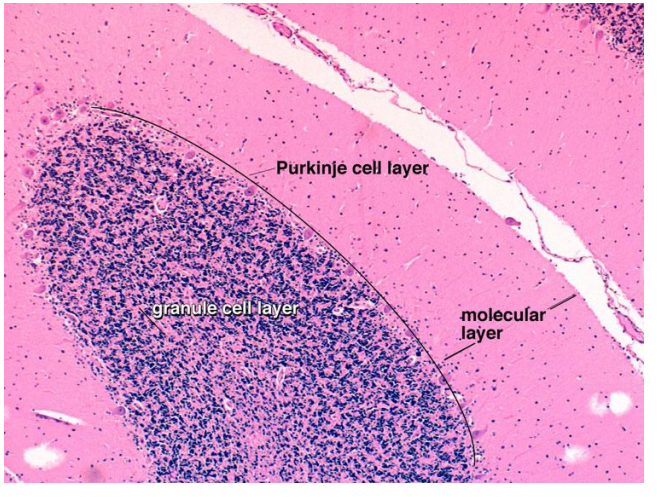
## Practical no 4: Histology and sectioning of major areas in the brain



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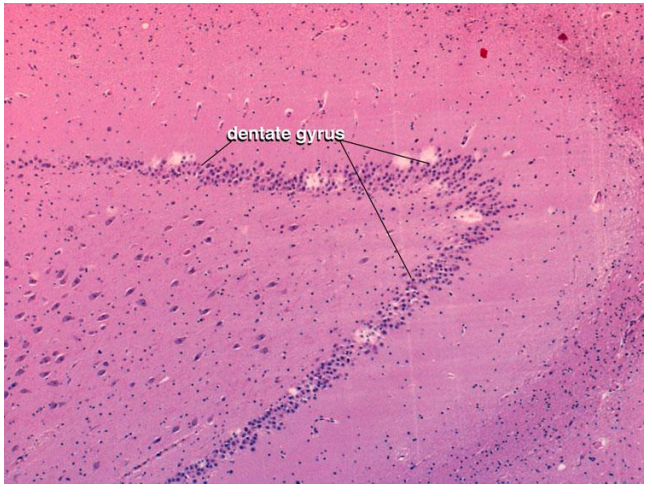
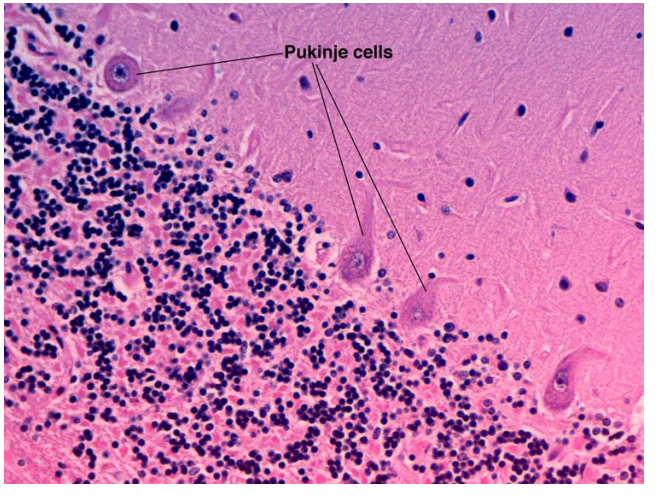


# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 5: Brief introduction of the staining techniques and stains used in brain histology

**H&E staining**

This is a standard staining method used in pathology.

Typically, the cytoplasm of cells is eosinophilic (acidophilic) and is stained red, whereas the nuclei and nucleoli are "hematoxylinophilic" (basophilic) and are stained blue.

Nissl bodies, which are the rough endoplasmic reticulum of neurons, are basophilic, while other cytoplasmic structures are mostly eosinophilic.

Axons and dendrites cannot be distinguished unless there are swelling-related changes.

Astrocytes lack an eosinophilic cytoplasm and have nuclei that appear large and quite clear.

When astrocytes react to tissue damage, they appear eosinophilic because their cytoplasm becomes more abundant as a result of an increase in fibrous components, which also accumulate in the nerve processes.

Oligodendroglia are smaller than astrocytes, with basophilic densely staining nuclei and a barely visible cytoplasm.

The nuclei of microglia present with basophilic club-shaped terminations and are thus easily distinguished.

**Nissl staining**

Neuronal Nissl bodies (the rough endoplastic reticulum) are stained purple using cresyl violet.

The stained Nissl bodies appear aggregated and brindled.

Nissl staining is convenient for measuring the density of neurons because stained cells are clearly defined and easily measured.

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 5: Brief introduction of the staining techniques and stains used in brain histology

**Luxol-fast blue (LFB) staining**

LFB is a myelin sheath stain that stains phospholipids (constituents of myelin sheath) blue.
Luxor is the name of a city in Egypt where the blue dye is collected.
The myelin-rich cerebral white matter is stained blue.
In demyelinating diseases, where myelin sheath is broken down, distribution of lesions can be clearly identified.

**Kluver–Barrera (KB) staining**

KB is the most common nervous system-specific stain used.
It involves double staining with Nissl and LFB stains, and it simultaneously stains both neurons and the surrounding myelin sheath.
Magnified images of stained specimens resemble those observed in LFB staining; however, because the neurons are also Nissl stained, the gray matter turns slightly blue.

**Bodian silver staining**

Bodian staining uses silver proteins, copper, and gold chloride to stain neuronal cell bodies (soma) and nerve processes dark brown.
Normal and abnormal structures formed by abnormal fiber components are also stained.
Argentaffin parts include areas of localized axonal swelling (spheroid), dendritic lesions, and Alzheimer's neurofibrillary degeneration [neurofibrillary tangles (NFTs)].

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 5: Brief introduction of the staining techniques and stains used in brain histology

**Other silver stains used for nerve tissue**

Other silver staining (Bielschowsky and methenamine silver staining) are only performed as required.
Bielschowsky staining clearly stains nerve fibers.
Methenamine silver is an argyrophilic stain that enables clear visualization of amyloid components of senile plaques; immunostained images of β-amyloid proteins show similar results.

**Holzer staining**

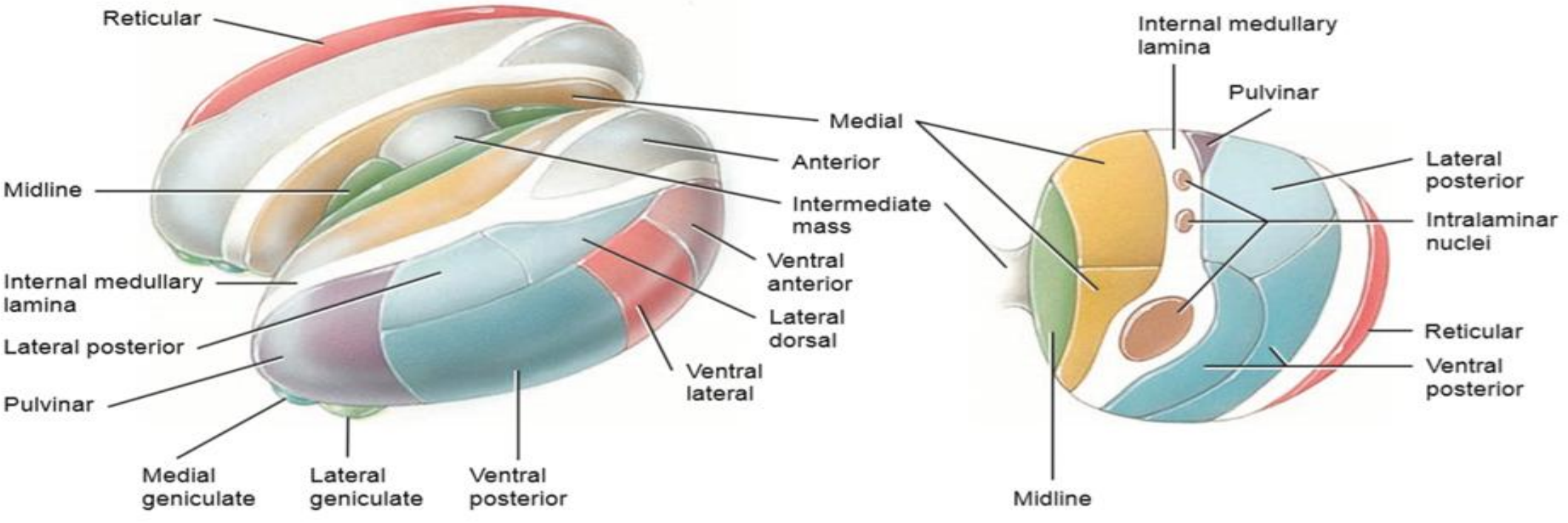
The fibrous components of astrocytes are stained purple with Holzer's crystal violet.
In particular, gliosis, which is secondary scarring following nerve damage, is clearly stained.
Therefore, sites that have been damaged can be macroscopically detected.
Because the aniline in the stain solution is harmful, staining must be performed in a laboratory draft chamber (fume hood).
Holzer's staining in addition to phosphotungstic acid hematoxylin (PTAH) staining can be used to detect gliosis.

**Gallyas–Braak (GB) staining**

GB staining gained rapid popularity in the field of neuropathy.
It is a type of argyrophilic stain, it does not stain the normal existing tissue, thereby enabling clear identification of deposits partly consisting of abnormal tau and other pathological structures.
While these can be detected with H&E and Bodian staining, they were first visualized with GB staining.
Nonetheless, the normal background tissue is not stained, whereas only abnormal structures are stained black; therefore, GB is convenient when examining the distribution of abnormalities.

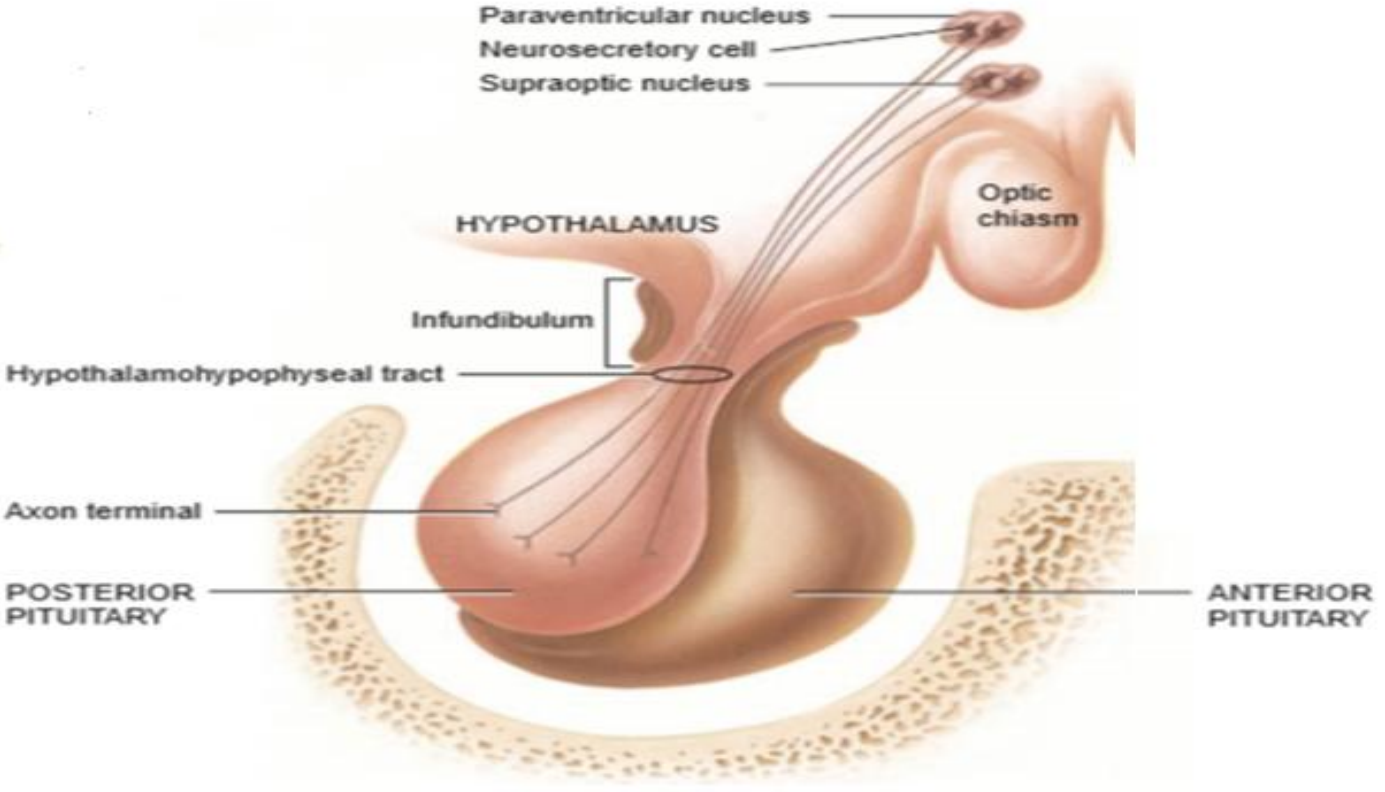
# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 6: Anatomical mapping of major thalamic centers.



# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 6: Anatomical mapping of major hypothalamic centers.



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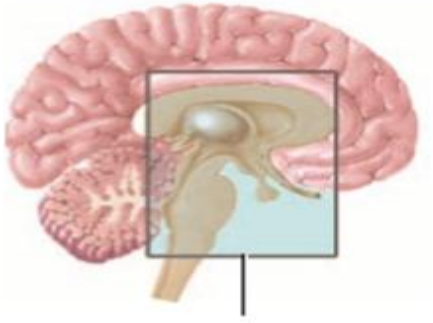
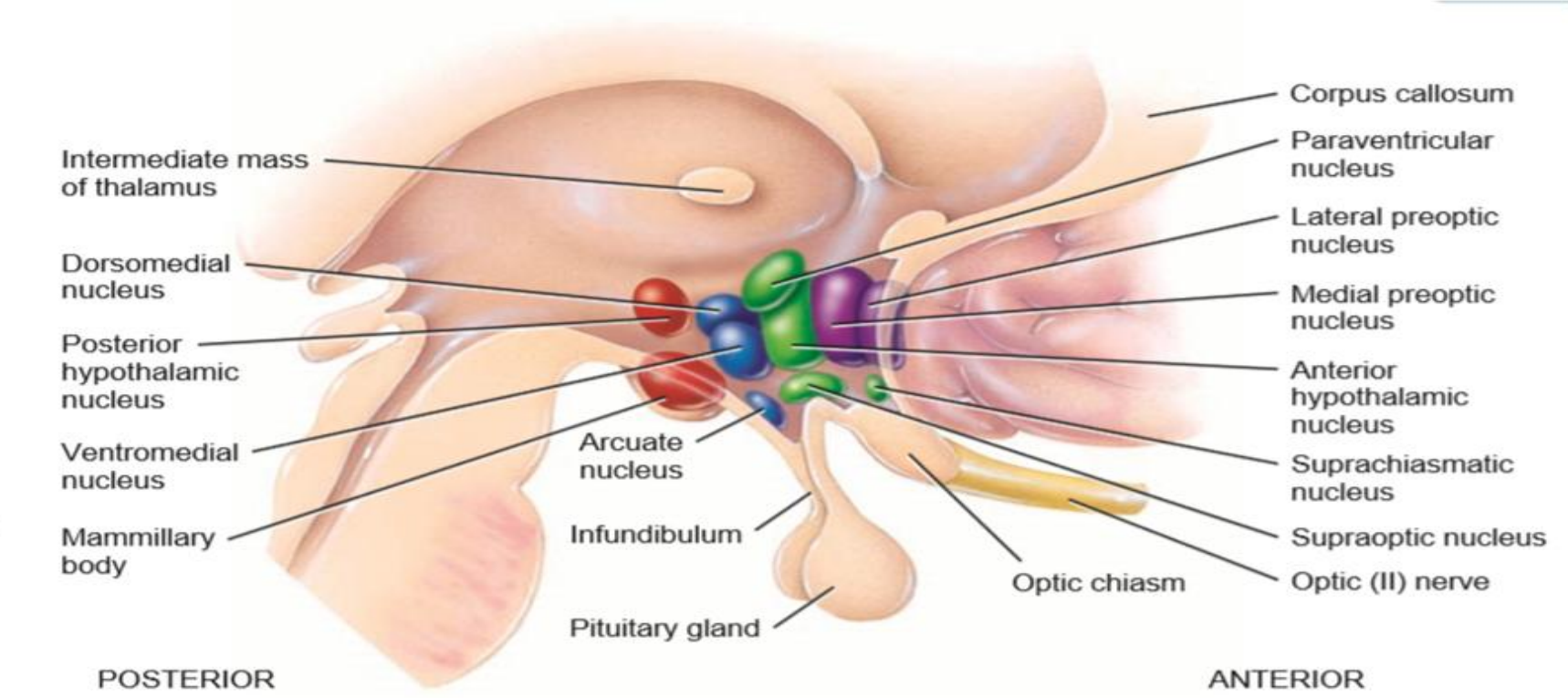


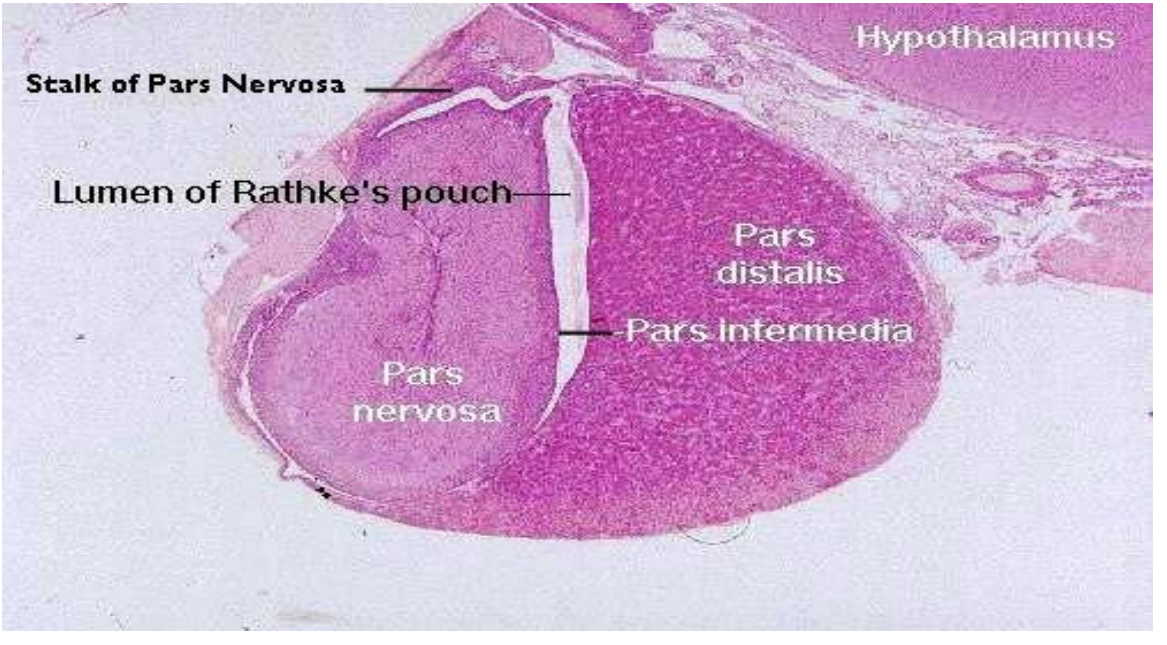

Key:

- Mammillary region
- Tuberal region
- Supraoptic region
- Preoptic region

Sagittal section of brain showing hypothalamic nuclei

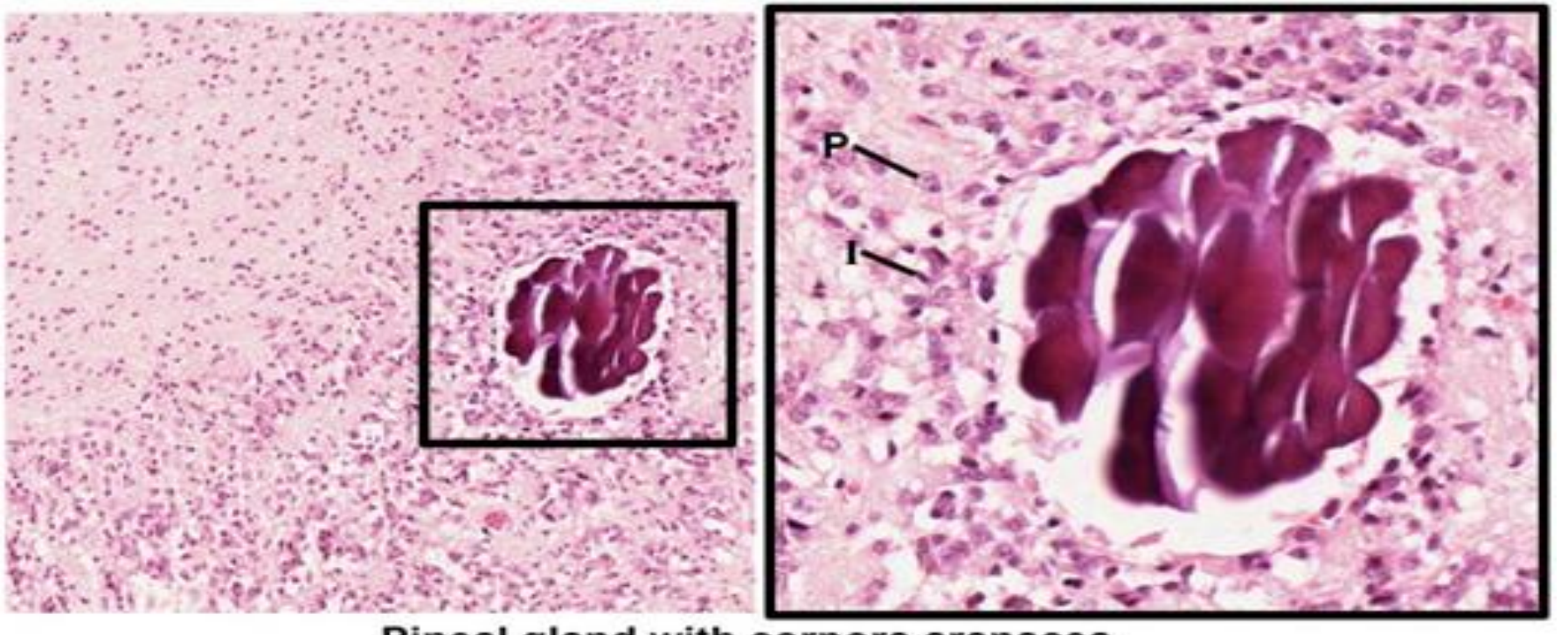
# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 7: Study of pituitary through prepared slides.



# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 7: Study of pineal prepared slides.




Pineal gland with corpora aranacea

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 8: Rotarod


Rotarod test is a common procedure used in neuroscience and behavioural research to assess motor coordination and balance in rodents.

**Familiarization:**

Allow the rodents (typically mice or rats) to acclimate to the testing environment for a certain period before the actual experiment.

This helps reduce stress and anxiety.

Place the rodents on the Rotarod apparatus at a stationary position for a short duration to familiarize them with the equipment.

**Training Session:**

Conduct a training session to teach the rodents how to walk on the rotating rod.

Start the rod at a low speed (e.g., 4-6 revolutions per minute) and gradually increase the speed over the course of the training session.

Allow the rodents to walk on the rotating rod until they become proficient in maintaining their balance.

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 8: Rotarod

**Pre-Test Handling:**

Randomly assign the rodents to different experimental groups if applicable, and record relevant information such as age, weight, and any pre-existing conditions.

**Test Session:**

Set the Rotarod to the desired speed or use a protocol appropriate for your specific experimental design.
Place the rodents on the rotating rod one at a time and record the time each animal is able to stay on the rod before falling or rotating off.
Repeat the test multiple times with sufficient resting periods between trials to prevent fatigue.

**Data Analysis:**

Analyze the collected data, which typically includes the latency to fall (the time the rodent remains on the rotating rod) for each trial.
Remember to follow ethical guidelines and institutional regulations for the care and use of animals in research.

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 9: Morris water maze

The Morris water maze is a widely used behavioural test in neuroscience to assess spatial learning and memory in rodents.

**Apparatus Setup:**

Set up a large circular pool filled with opaque water.

Divide the pool into four quadrants, and mark these quadrants as different zones (e.g., North, South, East, and West).

Place a hidden platform just below the water surface in one of the quadrants.

**Training:**

Conduct a series of training trials, during which the rodent is placed in the water at various starting points.

The rodent must learn to navigate and find the hidden platform using spatial cues within the testing environment.

Each trial should have a time limit, and if the rodent fails to find the platform within that time, it can be gently guided to the platform.

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 9: Morris water maze

**Testing:**

After the training period, perform a probe trial without the hidden platform to assess spatial memory. Allow the rodent to swim freely in the pool for a specific duration.

Record and analyze the time spent in each quadrant, with the expectation that the rodent spends more time in the quadrant where the platform was previously located.

**Retention Tests:**

Conduct additional retention tests on subsequent days to evaluate the long-term memory of the rodent. The rodent should remember the platform location and continue to spend more time in the target quadrant.

**Data Analysis:**

Analyze the data collected during the testing and retention trials. Use parameters such as escape latency (time to find the platform), swim speed, and time spent in each quadrant to assess spatial learning and memory.

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 10: 8 Arm radial maze or T – Maze

The radial arm maze is a behavioural test used to assess spatial learning and memory in rodents.

**Apparatus Setup:**

Prepare a radial arm maze, which typically consists of a central platform with a number of arms radiating out from it.

The number of arms can vary (e.g., 8 or 12 arms), and each arm should have a food reward or goal at the end.

**Habituation:**

Allow rodents to habituate to the maze environment without any rewards.

This helps them become familiar with the maze structure.

During habituation, let the rodents explore the maze freely for a short period without any food rewards.

# ZOPCLD1: BRAIN FUNCTION AND MENTAL AWAREMESS

## Practical no 10: 8 Arm radial maze or T – Maze

**Training:**

Begin the training phase by placing a food reward at the end of each arm.
Allow the rodents to explore the maze and find the food rewards.
This encourages spatial learning as they associate specific arms with rewards.
Set a criterion for the number of correct choices or time spent in the maze to proceed to the next phase.

**Testing:**

During testing, some arms are baited with rewards, while others are left empty.
The goal is for the rodents to remember and selectively choose the arms with rewards.
Record the number of correct and incorrect choices made by the rodents.
Limit the duration of the test to prevent habituation to the reward locations.

**Data Analysis:**

Analyze the data collected during testing, focusing on parameters such as the number of correct choices, errors, and the time taken to complete the maze.
Assess the rodents' ability to remember and navigate to the rewarded arms, which provides insights into their spatial memory and learning capabilities.
As with any behavioural test, researchers should consider ethical guidelines and ensure the proper care and handling of the animals throughout the experiment.