CHAPTER III
MATERIALS AND METHODS

The Experimental Animals

All experimentals were performed on 38 adult common tree shrew (*Tupaia glis*) of both sexes weighing between 120-145 grams. Animals were housed in individual cages under controlled environmental conditions (12 : 12 light-dark cycle, room temperature). Animals were give food and tap water ad libitum for at least two weeks prior to the experiments

Animal Preparations and Measurements

Animal were anaesthetized by intraperitoneal injection of sodium pentobarbital (Sagatal) at the initial dose of 25 mg/kg body weight, then,theyweremaintainedatan aesthetic condition by interval Corneal small dosesreflex, swallowing movement, rapid and forceful breathing and rigid abdomen were observed for the assesment of level of anaesthesia.

1. cannulation of the Femoral Artery

Femoral artery was exposed and polyethylylene tube (PE-50 outer diameter = 0.945 mm.) filled with heparinized saline (20 unit/ml) was inserted through a small incission until its tip reach abdominal aorta. The animal was then placed in a stereotaxic apparatus for rat (Narishige SR-6) with the bite bar set 5 mm. below the ear bars.

2. Data Measurement

Arterial blood pressure (ABP) and heart rate (HR) were detected through pressure transducer (Elcomatic EM 751 A) and

cardiotachometer (Harvard) and were recorded on the fourth channel oscillograph (Harvard). Rectal temperature of the animal was keep at 37 C by temperature controller. ABP and HR were measured from experimental animals in control, electrical stimulation and lesion groups. Control measurements were made immediately before stimulation orlesion with electrode placed in the position. Results were expressed in percentage change of ABP and HR. A positive percentage change indicates the increase, while the negative is decrease.

Experimental Protocal

1. Electrical Stimulation

1.1 Preparation of Microelectrodes

Stimulation of specific brain areas in this study was performed through monopolar microelectrode. The stimulating microelectrode were facbricated from tungsten wire (outer diameter 150 um.) insulated with insulating varnish. The tip of microelectrode was sharpened by electrochemical ething until the diameter of the fine tip of the electrode had a diameter between 1-2 um. Prior to the experiment, the tip of electrode was exposed for 5-20 um in length to allow the direct contact between the electrode and brain tissue. The electrical impedance of the electrode is between 0.8-2.5 MegOhms.

1.2 Electrical Stimulation of Specific Brain Areas

After fixing the animal in the stereotaxic apparatus, the skin and connective tissues overlying the posterior occipital bone were cut and retracted laterally to expose the skull, then all attached muscles were separated away. The estimated position of fastigial nucleus (FN) in the cerebellum was assessed from the landmarks on the skull and dura surface following the previous work of Ware (1979) and from serial cresyl violet-stained sections of the

tree shrew brain. The craniotomy was performed over area in the posterior part of skull by a drill and occipital bone was removed to expose the cerebellum by bone cutting. The electrode was mounted on a stereotaxic micromanipulator and lowered to the cerebellum at a 0 angle from the vertical (coordinates relative to apex : 2.65-3.85 mm. posterior to, and 0.5-1.5 lateral to the stereotaxic zero reference point and 6.2-7.2 depth from the surface of dura matter).

The microelectrode was sterotaxically placed in the area and clectrical stimulation was performed through monopolar electrodes as the cathodes, the anode was a clip attached to a scalp muscle. Stimulating pulse were generated by a square-wave stimulator (Nihon-Kohden SEN-3201) and passed through a photoelectric stimulus isolation unit (Nihon-Kohden SS-201 J). Stimulus current intensity was calculated from the voltage drop across a 10 KOhms resister connected in series with the active pole of the stimulating electrode; the voltage drop was displayed on an oscilloscope (Leader LBO - 522). To localized a fastigial pressor active site, the electrode was move drownward at 0.5 mm. interval with stimulation (40 - Strain of negative rectangular 0.1 mS. pulse, 0.15 mA strengh). When a fastigial pressor response was elicited, the electrode was moved up and down in a step of 0.1 mm. interval to locate the site with highest responses. Density of current was limited between 0.01-0.8 mA. A diagram for electrophysiological investigation was shown in (Fig. 1.).

2. Electrolytic Lesion

Unilateral lesions were made by DC current (0.35 mA. D.C.) in two areas, white matter anterior to rostral pole of FN (W.ant.rFN), and rostral portion of FN (rFN) for 30-S. through electrodes similar to those used for stimulation. Electrical stimulation was performed in ipsilateral rFN of W.ant.rFN-lesioned animal. Conversely, electrical stimulation was performed in ipsilateral W.ant.rFN of rFN - lesioned animals.

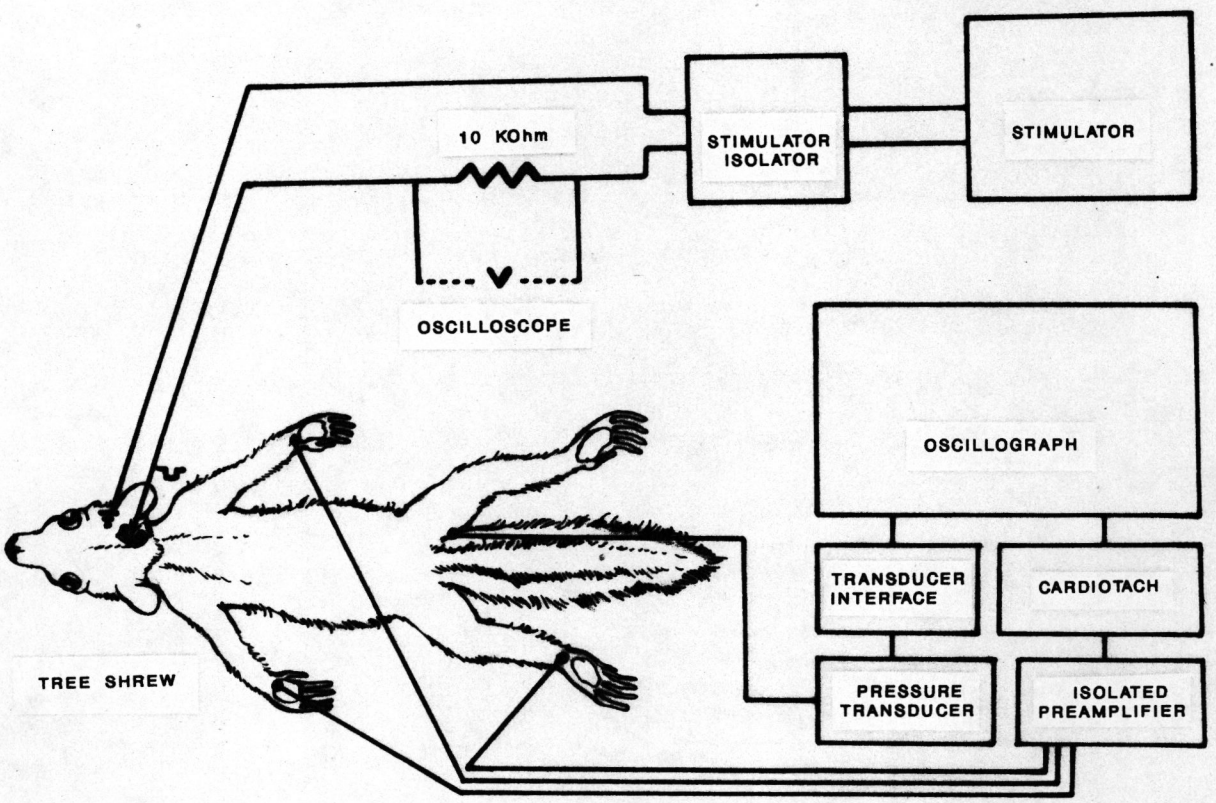

Fig.1. Diagrammatic picture of experimental arrangements routinely employed in experiments involving electrophysiological invertigation

3. Verification of Electrode Position and lesion

3.1 Stimulating Electrode Marking

For histology confirmation, at the end of each experiment, the site of stimulation were routinely marked by small electrolytic lesions through the same electrodes. The lesion were produced by passing a current of 750 mA.,12 V. for 60-S. from lesion marker (Grass LM 4 lesion marker).

3.2 Fixing the Brain Tissue

After the experiment, the animal was deeply anaesthetized with sodium pentobarbital and was perfused through femoral polythyline cannulation with 150 ml. of physiological saline and followed by the solution of fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 approximately (150 ml.). The brain was then removed from the skull immediately and the left side of the brain was always nicked for the identification of the left-right posistion in the sections. The brain was left in the same fixative for additional 72 hours before transfered into a solution of 30% Sucrose in the same phosphate buffer for 48 hours prior to sectioning.

3.3 Histological Techniques and Electrode Position Vertification

After complete fixation, serial coronal sections (20 um.) were cut on eryostat (American optical). The sections were collected in phosphate buffer and were then picked up onto microscopic glass slides. Identification of the brain structures was fascilitied by stainning the sections with cresyl violet (Sigma). After the staining, the sections were dehydrated through graded series of ethyl alcohol and xylene and mounted under a cover-glass. Microscopic investigations and photography of each serial coronal section were made to verify

stimulating and lesion sites with reference to the barin structures. Drawings of serial brain sections were performed in both control and lesion animals.

Statistical Analysis

Data obtained from the physiological experments, which included the following parameter : the arterial blood pressure, and heart rate measurement during control and experiment of each FN stimulation and lesion were compared by a paired *t* - test. Effects of stimulation in the various regions of brain were compared by the ANOVA, and lesion in the difference regions of the brain were compared by a unpaired *t* - test. P values of < 0.05 were considered to indicate statistical significance. Values are expressed as mean $\pm$ S.D.