STEREOTAXIC APPARATUS FOR FROG BRAIN

T. RAMAKRISHNA AND K. N. SHARMA

Department of Physiology, St. John's Medical College and Department of Biophysics, All India Institute of Mental Health, Bangalore.

The stereotaxic apparata, designed for the studies on brain function in human beings (19), buffalo (13), goat (21), dog (2, 3, 12), cat (1, 7, 14), rabbit (17) and rat (10, 20) have greatly enriched our knowledge in neurophysiology.

Although several studies have been made on the histology of the central nervous system (CNS) in anurans (4, 5, 6, 8, 11, 22), frog is still not being used widely by the neurophysiologists. There are obviously many advantages of using this amphibian as an experimental animal such as the easy maintenance in the laboratory throughout the year and the extreme amenability of its CNS to the experimental procedures. In recent years, we have been interested in several studies involving the neural mechanisms of hunger and satiety for which we have made use of frog (15, 16,18). During the course of these studies, it was felt that a simple apparatus and method for stereotaxic localisation of the structures in CNS of frog would greatly facilitate such studies. *Atlas of the frog's brain* published by Kemali and Braiternberg (9) has given further impetus to this attempt of designing a stereotaxic apparatus for frog brain. This communication describes such an apparatus.

THE PRINCIPLE.

In the absence of a well defined head region, the conventional principles that govern a stereotaxic appartus for the mammalian head can not be relied upon in the case of frog. Hence the question of using ear pins to orientate and stabilize the skull does not arise. The instrument, described below provides for fixing the frog in such a way that the brain-stem longitudinally schematic as it is (Fig. 1) lies perpendicular to the electrode from above. The posterior meeting point of optic lobes in midline seen through the transleuscent dura is taken as the 0 point. Electrode movements in anteroposterior, lateral or in vertical dimensions with reference to this 0 point are expressed in millimetres.

DETAILS OF CONSTRUCTION:

Mild steel, wood and a good micromanipulator are essential materials. As is shown in the diagram (Fig. 2) the device consists of a heavy metal base (13x10'') on which a prealigned wooden board (9.5x4'') is mounted. There is a vertical metal carrier fixed to the anterior side of the metal base. This vertical carrier holds a horizontal plate. These vertical and hori-

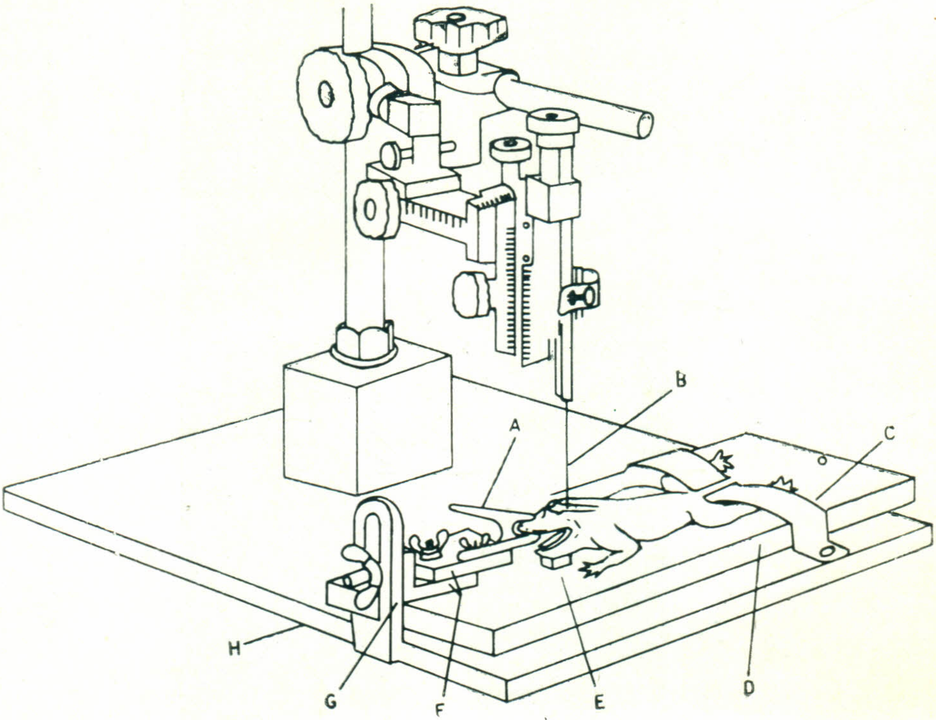

Fig. 1 : Stereotaxic apparatus for frog brain.
A — Jaw clip (The other in the pair is not shown in the diagram).
B — Electrode; C — Hind-limb holder; D — Wooden-board;
E — Lower-jaw supporter; F — Horizontal plates;
G — Vertical carrier; H — Metal base.

zontal plates have a sliding arrangement with respect to each other and can be fixed at desired heights or in different loci of A P plane by means of wing nuts. A pair of clips are screwed at the end of the bifurcated horizontal plate to hold the upper jaw of the frog. The hind limbs are held firm by means of a pair of clamps provided at the posterior end of the wooden board. In order that the brain region may lie perpendicular to the electrode a wooden piece of the dimensions (1.0"x0.7"x0.4") is fixed in front of the jaw holders. The lower jaw of the anaesthetized or pithed frog is made to rest on this supporter.

The micromanipulator with movement capability in three planes is fixed on the metal frame at right angles to the length of the wooden board. The wooden board has shallow grooves, cut at a gradient on the surface and leading to an outlet provided at the posterior end. This helps in draining the solutions that might be used during the experimentation.

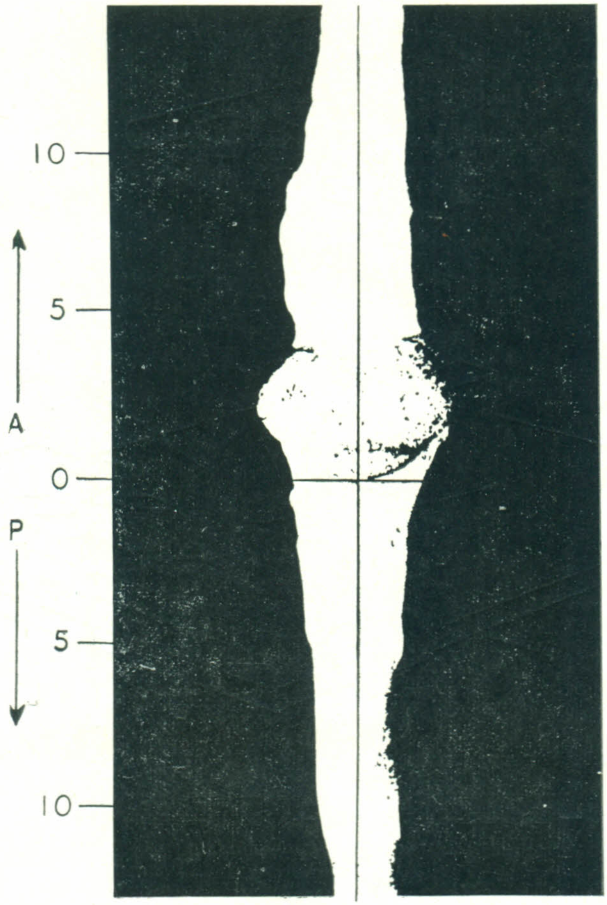

Fig. 2 : Frog — brain in stereotaxic coordinates.
A-Anterior & P-Posterior to the stereotaxic zero in millimeters.

PROCEDURE TO ARRIVE AT 0 POINT:

After removing the skin, the skull is chipped off with a driller head, held laterally leaving only the dura, when the inner segments of the brain can be seen. The posterior meeting point of the optic lobes in midline is taken as the 0 point. To make sure of the same, a few more guidelines can be relied upon. By means of a needle fixed in the electrode holder of the micromanipulator, the distance between the junctions of the upper and lower jaw on either side i.e., the interquadrate distance can be measured. The midline is equidistant from the two quadrates. After fixing the midline this way, the electrode can be moved posteriorly towards the meeting point of the optic lobes and the '0' position.

CONCLUSIONS

We have used frog both male and female ranging in the weight between 55-65 *g*. Although the wieght range of adult frog is 100-110 *g* as we were primarily interested in the neurophysiological study, we found the younger ones more suitable for two reasons. Bleeding was much less in this group and the response more marked. Hence the device, described above mostly conforms to the dimensions of this group. However, it is hoped that it would not be difficult to adapt and modify the same to any size group. Fig. 3 indicates the coordinates in the A P plane, where the fasciculus solitarious can be seen in different weight ranges.

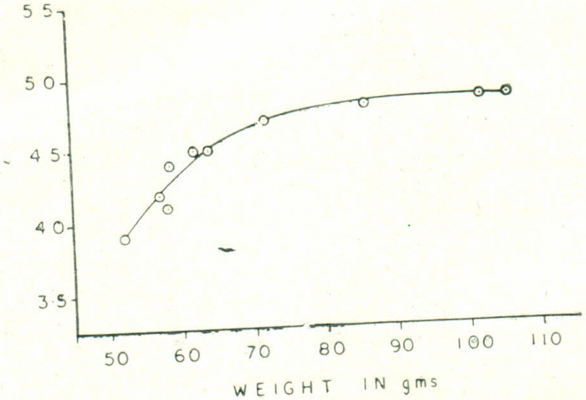

Fig. 3: Position of *fasciculus solitarius* in different weight-groups of frogs.
P — Posterior to the stereotaxic zero in millimetres.

REFERENCES

1. Blum, B. and B. Feldman. A mountable stereotaxic apparatus for the cat. *Electroenceph. Clin. Neurophysiol.*, **16** : 403-408, 1964.

2. Domino, E.F. A simple dog head holder for use with the labtronics stereotaxic instrument. *Electroenceph. Clin. Neurophysiol.*, **12** : 521-522, 1960.

3. Dua-Sharma, S., K.N. Sharma and H.L. Jacobs. *The canine brain in stereotaxic coordinates.* M.I.T. Press, Cambridge Mass., USA, 1970.

4. Frontera, J.G. A study of the anuran diencephalon. *J. Comp. Neurol.*, **96** : 1-69, 1952.

5. Hillman, D.E. Morphological organisation of frog cerebellar cortex : A light and electron microscopic study. *J. Neurophysiol.*, **6** : 818-846, 1969.

6. Hoffman, H.H. The olfactory bulb, accessory olfactory bulb and hemisphere of some anurans. *J. Comp. Neurol.*, **120** : 317-368, 1963.

7. Jasper, H.H. and C. Ajmone - Marsan. *A sterotaxic atlas of the Diencephalon of the cat.* Ottawa: National Research Council of Canada, 1960.

8. Kennard, D.W. The anatomical organisation of neurons in the lumbar region of the spinal cord of the frog· (Rana temporaria) *J. Comp. Neurol.*, **III**, 447-467, 1959.

9. Kemali, M. and V. Braitenberg. *Atlas of the frog's brain* Springer-Verlag, Berlin. 1969.

10. Krieg, W.J.S. Accurate placement of minute lesions in the brain of the albino rat. *Q. Bull. N.W. Univ. Med. Sch.* **20** : 199-208, 1964.

11. Larsell, O. The cerebellum of the frog. *J. Comp. Neurol.* **36** : 89-112, 1923.

12. Lim, R.K. Chan Nao Liu and R.L. Moffit. *A stereotaxic Atlas of the Dog's Brain.* Springfield : C. S. Thomas, 1960.

13. Manchanda, S.K., K. Singh and B.K. Soni. Stereotaxic apparatus for the Buffalo (Bubalus bubalis) Brain. *Physiol. Behav.*, **6** : 727-728, 1971.

14. Olszewski, J. *The Thalamus of the Macaca mulatta ; An Atlas for use with the stereotaxic instrument.* Basel : S. Karger, 1952.

15. Ramakrishna, T. and K.N. Sharma. Organization of gastric chemoceptive projections in frog brain stem. *Ind. J. Physiol. Pharmacol.*, **15** : 38, 1971.

16. Ramakrishna, T., K.N. Sharma and S. Dua-Sharma. Response characteristics of chemoceptive neurons in frog brain-stem. *Ind. J. Physiol. Pharmacol.*, **16** : 256, 1972.

17. Sawyer, C.H., J.W. Everett and J.D. Green. The rabbit diencephalon in stereotaxic coordinates. *J. Comp. Neurol.*, **101** : 801-824, 1954.

18. Sharma, K.N., H.L. Jacobs, V. Gopal and S. Dua-Sharma. Vagosympathetic modulation of gastric mechanoreceptors : effect of distention and nutritional state. *J. Neural Transmission.* **33** : 113-154, 1972.

19. Spiegel, E.A., H.T. Wycis, M. Marks and A.S. Lee. Stereotaxic apparatus for operations on the human brain. *Science*, **106** : 349-350, 1947.

20. Stellar, E. and N.P. Krause. New Stereotaxic instrument for use with the rat. *Science.* **120** : 664-667, 1954

21. Tindal, J.S., G.S. Knaggs and A. Turney. The forebrain of the goat in stereotaxic coordinates. *J. Anat.*, **103** : 457-469, 1968.

22. Wilson, L.D., J.A. Weinberg and H.A. Bern. The hypothalamic neurosecretory system of the tree frog Hyla regilla. *J. Comp Neurol.*, **107** : 253-267, 1957.