NUCLEUS ISTHMUS OF TOAD IS SECONDARY VISUAL CENTER

WANG SHURONG (WANG SHUJUNG 王书荣), YAN KUN (颜 坤)
AND WANG YINTING (王荫亭)
(*Institute of Biophysics, Academia Sinica, Beijing*)

Received December 8, 1981; revised May 5, 1982.

ABSTRACT

Visual responses of 148 units have been recorded with micropipettes from the left nucleus isthmus areas of toads (*Bufo bufo gargarizans*), their auditory and tactile responses also examined, and 122 recording sites marked with cobalt sulphide. This study indicates that: (i) 118 units responding solely to visual stimuli are found within the nucleus, and 4 multisensory units outside, (ii) there exist a visual field map and binocular units in the nucleus, and (iii) response latencies of the isthmic units to a spot of light range from 120 to 540 msee, with no correlation with recording sites.

I. INTRODUCTION

In anuran amphibians the midbrain nucleus isthmus (NI) is a highly developed neural structure, but little is known about its functional significance[1,2]. Larsell[3] suggested that this nucleus is related to the well developed auditory and optic apparatus. Recent studies have shown that electrical stimulation of the optic nerve[4] or visual stimulation to the eye[5-9] can elicit neuronal discharge from the contralateral NI, with minimum latencies of 20—110 msec. This nucleus is also a relay station for information from the eye to the ipsilateral tectum[7-10]. Vinogradova and Manteifel[8] reported that some visual-auditory units may be recorded from NI. Therefore, it is needed to clarify whether NI is only a visual center, or, instead, a multimodal integration center.

Furthermore, it appears that anuran NIs differ in their developmental levels from genus to genus. For example, NI is more developed in the frog *Rana esculenta* than in the clawed toad *Xenopus laevis*. This might be correlated with the difference between their retino-techal systems[11]. Frog has a larger frontal and dorsal binocular field, while toad possesses a larger frontal and anterior-lower binocular field[12,13]. The difference may be reflected on the physiology of NI of frog and toad.

Previous studies[14,15] have reported that in the NI of a frog there is a visual map and binocular units. This study shows that the NI of a toad appears to be a "pure" visual center, and there are some differences in anatomy and physiology between the NI in a frog and that in a toad.

II. METHODS

Adult toads *Bufo bufo gargarizans* (6.0—8.5 cm from snout to vent) were used in all experiments. Surgical operation, stereotaxic fixaion, recording electrodes and visual stimulation were the same as described previously[14,15]. Under operation microscope, the point at which the cerebellum and tecta meet was taken as the origin of the coordinates[16]. Visual units were explored throughout the isthmic area following the stereotaxic coordinates of the toad's NI ($X = 0.55$—1.00, $Y = -0.25$—0.20, $Z = 0.75$—1.50 mm). The location and extent of the receptive field (RF) of the isolated units were plotted on a perimeter chart before the response properties of the units to visual stimulation were tested.

Responses of the recorded units to acoustic (clicks, whistles and handclaps) and tactile stimulation were also examined. Tactual stimuli were delivered to the flank and limbs by means of light stroking with a tube brush while the two eyes were occluded.

In order to measure the response latencies of isthmic units, a spot of light was projected from a photostimulator on RF centers. Impulses controlling the electromechanical shutter triggered a storage oscilloscope, on which the time between the take-off point of sweeps and the first spike elicited was considered as latency.

After marking the recording sites with CoS and completing histological procedures[14,15], NI sections and the marked spots were drawn with the aid of a camera lucida to verify recording sites and reconstruct a visuotopic map in the nucleus.

III. Results

1. *Response Properties of Isthmic Units*

Three-fourths of the recorded units were silent, the rest with spontaneous activity may be grouped into two kinds: one kind had spasmodic discharge while the other burst more or less regularly. For example, unit TP 90 was bursting at a considerably

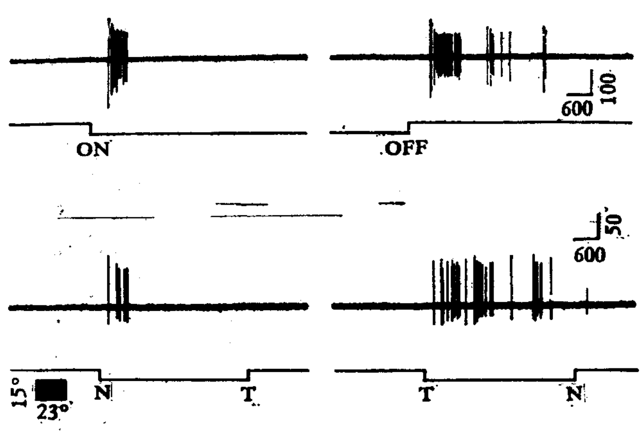

Fig. 1. Discharge pattern of isthmic units.

Upper: a unit producing ON-transient and OFF-sustained responses to a spot of light. Lower: responses of a unit to a 15° × 23° black rectangle moving in nasotemporal (NT) or temporonasal (TN) directions. Downward shift of lower trace indicates the start of stimulation; upward, end. Scale: time, msec; amplitude, μV.

regular interval of 15.4 ± 2.4 sec, each burst consisting of 55—56 spikes. Four multimodal units had stronger spontaneous activity, but all of them were beyond NI boundaries.

RFs of NI units were larger than 18°, the largest one of which was 167°. Units with RFs of elliptical shape accounted for 80%, and the others were round. One-third of the units had their RF centers in the lower visual field. No RF centers were found within 60° solid angle just above the toad's head though this space was included in the RF extent of some units. The majority of RF centers were located in the contralateral hemifield; only 9 RF centers were situated slightly over the midline (ipsilateral 2.3°).

These isthmic units produced either weak or no response to the changes of background illumination. Units accounting for 90% reacted strongly to the onset and offset of a spot of light. With respect to the lasting duration of discharge, these units could be divided into 3 groups: (i) ON-transient and OFF-sustained responses (25%, Fig. 1, upper), (ii) similar ON and OFF responses (55%), and (iii) ON-sustained and OFF-transient responses (10%). The rest were either ON or OFF units, with 1% refractory units. Maximum spike amplitude recorded was 360 μV. Comparative studies did not show significant effects of cobalt ions contained in electrodes on the spike amplitudes of NI units.

These units responded briskly to the movement of targets through their RFs. Units accounting for 90% ceased firing immediately after the targets stopped moving; others continued to discharge for 1—8 sec. Most units were directionally selective in response to the movement of a target, mainly in temporonasal (Fig. 1, lower) or ventrodorsal direction. For 10 units, the more vigorously they responded, the closer a visual stimulus was approching to the toad's frontal midsagittal plane.

Four binocular units were also recorded, whose RFs were in front of the toads' heads, with a visual space including 20° inferior and 90° superior to the horizontal

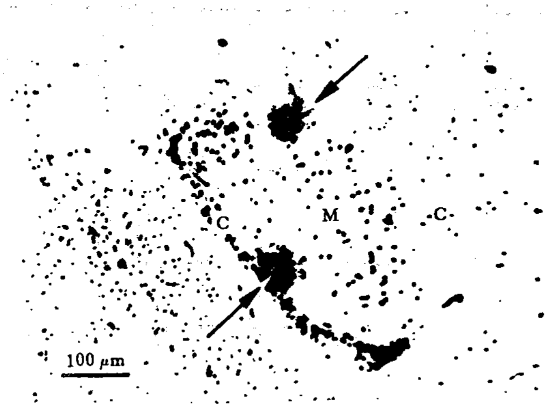

Fig. 2. Microphotographs of NI coronal sections showing two marked spots (arrowheads) in an electrode track. Their RF centers are represented by the ends of solid line 2 in Fig. 3B. C and M indicate the cortex and medulla of NI respectively. Dorsum is upward, medial side to the left, rostrum faces the observer.

meridian and 15° left and 40° right of the midline. They were predominantly excited by the contralateral eye and stimulation to the ipsilateral eye could only elicit weak responses. Three sites successfully marked were within the medullary mass of the rostral half of NI.

2. *Projection of Visual Field on Nucleus Isthmus*

Although the toad's NI consists of a cell-dense cortex and a cell-sparse medulla, there is no prominent cell-free band internal to the medioventral cortex (Fig. 2).

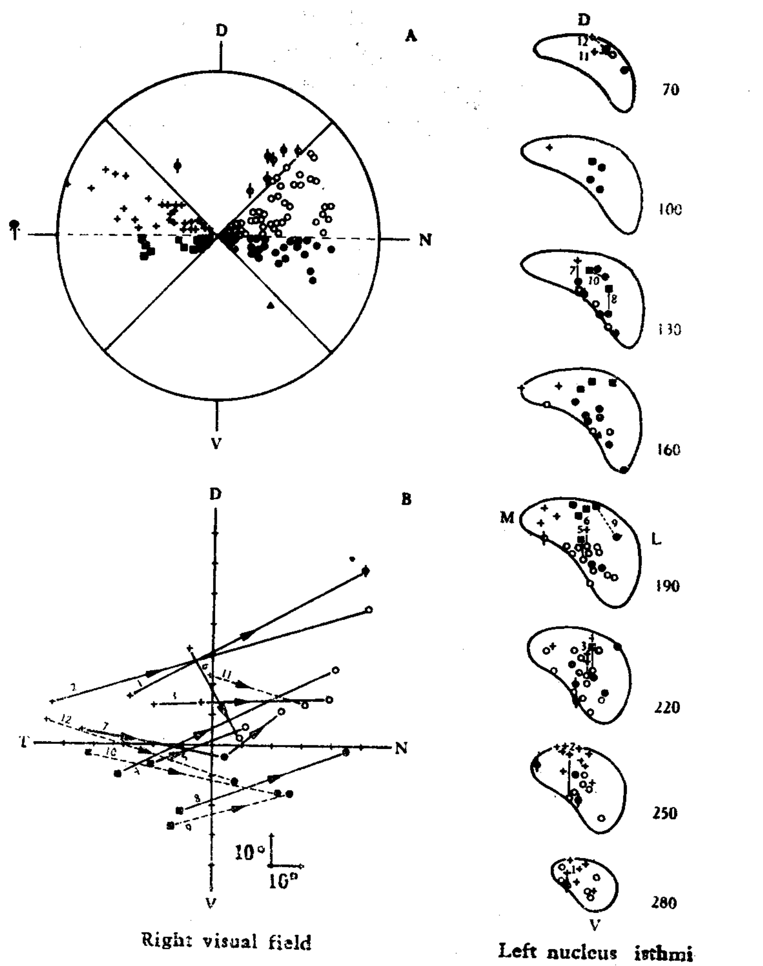

Fig. 3. A: distribution of the recorded units in the left NI and of their RF centers in the right visual field, which extends outwards 90° from its center. Symbols used in the field are as those in NI drawings. Two oblique diameters rotating 45° from the horizontal meridian divide the visual field into nasal (N), temporal (T), dorsal (D) and ventral (V) quadrants. The crossing represents the optic axis. The figures on the right indicate distance (μm) from the caudal pole of the ipsilateral tectum. D, L, M and V symbolize the dorsal, lateral, medial and ventral sides respectively. B: RF center shift whose directions are shown by arrows. Solid lines signify downward advancement of an electrode in the same track, and dashed lines lateralward movement. Symbols and numerical order correspond to those used in section drawings.

The toad's NI measures 260 (rostrocaudal) × 470 (dorsoventral) × 500 μm (mediolateral), with cell counts of 4,800.

The recording sites marked with CoS show that 118 units responding exclusively to visual stimuli were localized within NI; 3 visual-auditory units 45—120 μm directly in front of the rostral pole of NI; 1 visual-auditory-tactile unit 220 μm just beneath the ventral pole of NI.

There is a good correspondence between RF locations of visual units in the visual field and positions of their recording sites marked inside the nucleus (Fig. 3). Most of the visual field including the contralateral hemifield and the nasal 40° of the ipsilateral hemifield projects on NI topographically. The upper field projects on the rostral half and medioventral part of the caudal half of NI; while the lower field is represented on the caudal half as well as the central and dorsal part of the rostral half.

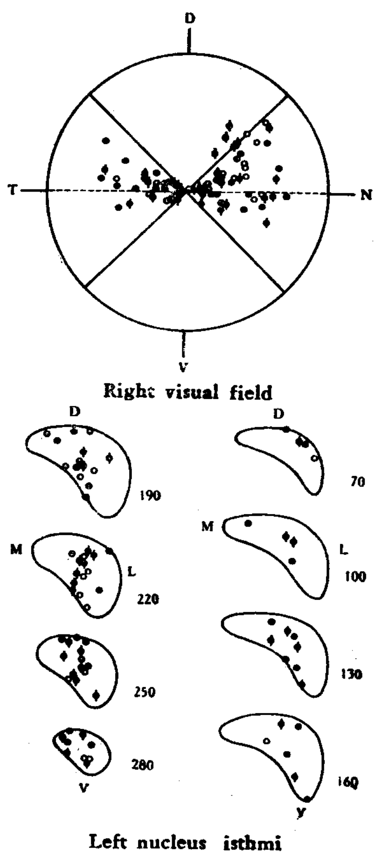

Fig. 4. Distribution of 74 units measured in the left NI and of their RF centers in the right visual field. Symbols used in the field correspond to those in sections: open circles represent units whose ON-latency is longer than OFF-latency; filled circles represent units whose OFF-latency is longer than ON-latency; speared circles represent units whose ON-latency is about equal to OFF-latency.

Generally speaking, the RF centers of units shifted nasodorsalward with the downward moving of an electrode (Fig. 3B, solid lines). They were moved 40°±7.1° horizontally toward the nose and 14° ± 9.9° dorsally upward when an electrode was advancing 100 μm downward. In most cases the RF centers of the recorded units were shifted in nasoventral direction with the lateralward moving of an electrode (Fig. 3B, dashed lines).

3. *Response Latencies of Isthmic Units*

The ON-OFF response latencies of 74 units to a stationary spot of light on their RF centers were measured and their recording sites marked with CoS. Their ON- and OFF-latencies ranged 120—450 msec and 125—540 msec respectively. According to the average latency the measured units may be categorized into three types: (i) ON-latency Lon (240 ± 53 msec) is about equal to OFF-latency Loff (247 ± 60 msec), 30 units; (ii) Lon (252 ± 65 msec) is longer than Loff (191 ± 61 msec), 18 units; and (iii) Lon (221 ± 60 msec) is shorter than Loff (280 ± 84 msec), 26 units. No correlation between latencies of these units and their recording sites was found (Fig. 4).

IV. Discussion

Some investigators indicate that NI is a multisensory center of anurans[3,5] and teleosteans[17]. It is also suggested that the frog's NI is a relay station for the pathway between the tectum and/or the visual region of the thalamus and the cerebellum[6], or the special somatic sensory zone of the midbrain is connected, via NI, with that of the rhombencephalon[18]. However, Gruberg and Lettvin[9] have recently indicated that the anuran NI is exclusively connected with the tectum, though NI of a teleost *Navodon modestus* also receives its input from the nucleus pretectalis[19] and there is a tecto-pretecto-isthmic circuit[20]. Le Gros Clark's suggestion[21] is now widely accepted that the anuran NI is a homologue of the mammalian parabigeminal nucleus (see [7]). Apart from the superior colliculus, no other projection sources to the cat's parabigeminal nucleus were found[22]. Harting et al.[23] insist that the tree shrew's parabigeminal nucleus is a multisensory center; nevertheless, this has not yet been verified electrophysiologically.

Vinogradova and Manteifel[5] recorded 26 visual-auditory units from the frog's NI area; however, they did not identify the recording sites histologically. Our results show that units responding to visual and auditory or tactile stimulation are outside NI, and the recorded NI units respond solely to visual stimuli. The visual-auditory units were marked directly in front of the rostral pole of NI, presumably in the anterodorsal (Ad) or posterodorsal (Pd) tegmental nucleus. The visual-auditory-tactile unit was located in the lateral part of Pd. It seems likely from these that the toad's NI is a "pure" secondary visual center, but not a multisensory integration center.

The toad's NI units possess larger RF, indicating that they receive larger visual convergence. This is in agreement with the fact that isthmic cells receive their input from tectal neurons in the deeper layers[9,24], and have a larger dendritic field[1,24]. In regard to NI binocular units, as previously suggested[14,15], they receive their input from tectal binocular cells. However, on the whole, the specific functions that binocularity plays in visual capacities of frogs and toads remain an open question[12].

In comparison with frogs, the RF centers of toads are less distributed in the upper field and more in the lower field. This is probably associated with the differences in configuration of binocular visual fields, orientation of the eye and visually guided behavior of these species. It is suggested that anuran NI is mediated in some visuomotor responses[5]; however, it is puzzling that NI is solely connected with the tectum[9]. Therefore, the functional significance of NI in visual-guided behavior needs to be explored further.

References

[1] Khalil, S. H. & Lázár, G., *Acta Morph. Acad. Sci. Hung.*, **25** (1977), 51—59.
[2] Nieuwenhuys, R. & Opdam, P., In *Frog Neurobiology*, (Eds. Llinas, R. & Precht, W.), Springer, Berlin-Heidelberg-New York, 1976, 811—855.
[3] Larsell, O., *J. Comp. Neurol.*, **60** (1934), 473—527.
[4] Виноградова, В. М. и Мантейфель, Ю. Б., *Нейрофизиология*, **9** (19977), 33—40.
[5] ————, *Ж. Эвол. Биохим. Физиол.*, **15** (1979), 172—178.
[6] Ansorge, K. & Grüsser-Cornehls, U., *Exp. Brain Res.*, **29** (1977), 445—465.
[7] Gruberg, E. R. & Udin, S. B., *J. Comp. Neurol.*, **179** (1978), 487—500.
[8] Glasser, S. & Ingle, D., *Brain Research*, **159** (1978), 214—218.
[9] Gruberg, E. R. & Lettvin, J. Y., *ibid.*, **192**(1980), 313—325.
[10] Grobstein, P. et al., *ibid.*, **156** (1978), 117—123.
[11] Мантейфель, Ю. Б., *Зрительная Система и Поведение Бесхвостых Амфибий*, Изд. «Наука», Москва, 1977.
[12] Fite, K. V., *Behav. Biol.*, **9** (1973), 707—718.
[13] Grobstein, P. et al., *J. Comp. Neurol.*, **190** (1980), 175—185.
[14] Wang, S. J. et al., *Neurosci. Letters*, **23** (1981), 37—41.
[15] ————, *Scientia Sinica*, **24** (1981), 1292—1301.
[16] Kemali, M. & Braitenberg, V., *Atlas of the Frog's Brain*, Springer, Berlin-Heidelberg-New York, 1969.
[17] Sligar, C. M. & Voneida, T. J., *J. Comp. Neurol.*, **165** (1976), 107—124.
[18] Opdam, P. et al., *ibid.*, **165** (1976), 307—332.
[19] Ito, H. et al., *Brain Research*, **207** (1981), 163—169.
[20] Sakamoto, N. et al., *ibid.*, **224** (1981), 225—234.
[21] Le Gros Clark, W. E., *J. Anat.*, **67** (1933), 536—548.
[22] Sherk, H., *J. Neurophysiol.*, **42** (1979), 1656—1668.
[23] Harting, J. K. et al., *J. Comp. Neurol.*, **148** (1973), 361—386.
[24] Wang, Y. T. et al., *Kexue Tongbao*, in press.