ELECTROENCEPHALOGRAPHIC PATTERNS OF THE GOLDFISH (*CARASSIUS AURATUS* L.)*

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(*Received* 5 *August* 1958)

INTRODUCTION

The first recordings of electrical potentials from the teleost brain were made by Adrian and Buytendijk (1931), who used isolated brain stem preparations from *Carassius auratus* L. Slow potential changes (1–3 cyc./sec.) were recorded from the medulla oblongata, corresponding to the rhythm of the goldfish respiratory activity. Patterns of higher frequency and low amplitude were also recorded from the optic lobes.

Enger (1957) has used the codfish (*Gadus callarias*) for implanted-electrode recordings, which were somewhat obscured by artifacts due to breathing movements and to the pressure of the electrodes on the brain surface. He obtained, however, records of the spontaneous electrical activity in the brain of an unanaesthetized fish, as well as mesencephalic responses to stimulation by light flashes. Waves of a frequency of 8–13 cyc./sec. dominated the electrical pattern of the midbrain; these were compared by Enger to the mammalian alpha rhythm. He found evidence for an arousal reaction in the mesencephalon following sudden photic stimulation, but none following auditory stimulation.

In a series of studies of the catfish (*Ameiurus nebulosus*) mesencephalon, Buser (1949*a*, *b*, 1950, 1951, 1955), Buser & Dussardier (1953) and Buser & Scherrer (1950) analysed tectal responses to electrical stimuli applied to the cut end of the optic nerve; some initial trials were also made using photic stimulation of the intact eye. A complex response to electrical stimulation was recorded from the surface of the optic tectum with monopolar electrodes; it consisted of two groups of components: (1) an initial rapid complex, consisting of one or several rapid diphasic oscillations (duration 2 msec.); followed by (2) a slower component, consisting of one or two larger, slower waves, 20–25 msec. in duration. Upon photic stimulation the first component lasted about 50 msec., the second 100–150 msec. The two components were dissociable; for example, nembutal applied to the surface of the optic tectum abolished the slow response without affecting the rapid component; the slow response was found only on the optic tectum, never along the optic nerve. Buser therefore concluded that this larger, slower component is postsynaptic in origin.

* This investigation was supported in part by Research grants from the National Science Foundation, no. C 4029 (J. P. S.) and no. 37085 (I. J. W.), and The Netherlands Organization for Pure Research (Z. W. O.), no. 96-44-66 (J. P. S.).

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In the present study, the electrical activity of the major brain divisions of the goldfish (*Carassius auratus* L.) was investigated. Effects of anaesthetic, responses to optic stimulation, and pathways of optic impulses within the brain were studied, in the following groups of experiments:

(1) Patterns of spontaneous electrical activity were recorded from various parts of the brain, in curarized and unanaesthetized preparations. Surface recordings were made from telencephalon, mesencephalon, cerebellum and medulla oblongata (Fig. 1). The effect of the level of anaesthesia upon these patterns was studied, using different concentrations of urethane (ethyl carbamate).

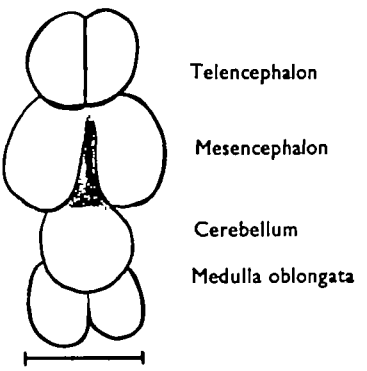

Fig. 1. Dorsal view of exposed goldfish brain (camera lucida drawing). Horizontal line indicates 0·5 cm. Bipolar leads were made between: 1, left and right telencephalon; 2, two points of left or right telencephalon; 3, left and right mesencephalon; 4, various points of left and right mesencephalon; 5, two points of cerebellum; 6, left and right medulla oblongata; 7, two points of left or right medulla.

(2) Repetitive light flashes were used to elicit an arousal reaction.

(3) The eye was stimulated by single light flashes and responses were recorded in the four parts of the brain listed above. The optic nerve was then cut on one side, and spontaneous electrical activity and responses to single photic stimuli were subsequently recorded from the same brain divisions.

(4) Spontaneous electrical activity and responses to single light flashes were recorded locally from small areas on the surface of the optic tectum. Following this, one-quarter to three-quarters of the retina was destroyed by electrocautery, and responses were again recorded from the same tectal areas, in an attempt to describe the organization of retinal projection on the midbrain.

(5) Microelectrodes were used to record the response to photic stimulation from deeper layers of the optic rectum.

MATERIALS AND METHODS

Operations

Seventy goldfish, averaging 13 cm. in length, were used in these experiments. Each fish was wrapped in wet gauze and held firmly in a screw clamp just behind the gills, with the lower jaw resting in a curved plastic trough. The animal was usually

anaesthetized with urethane (0·5–8% in different experiments); the anaesthetic solution was continuously dripped from an elevated vessel, through a tube, into the mouth and over the gills. When potentials were to be recorded from an unanaesthetized subject, the fish were immobilized by intramuscular injection of D-tubocurarine (Squibb's 'Intocostrin'), using 0·4 mg./100 g. body weight.

To expose the brain for recording, the skin was peeled away and the bony brain case was gently removed in small pieces, with a pair of jewellers' forceps. Operations were performed under a binocular dissecting microscope with adjustable magnification.

Recordings

Recordings were made with an Offner electroencephalograph; for surface recordings, silver-silver chloride wires were used initially; later, fine-pointed cotton wicks moistened with physiological saline were employed for more precise localization. Microelectrodes for deep recordings were made from tungsten wire 125 μ in diameter, electrolytically sharpened. A modification of the method of Hubel (1957) was used. The wire was suspended between two hooks; it passed through a hole 5 mm. in diameter in a nickel plate, in which was suspended a drop of 2M-KOH; the nickel plate and one hook were connected with a 6 V. a.c. source. Electrolytic erosion of the wire in the KOH solution resulted in a pair of electrodes which separated in the drop. Tips electrolytically sharpened in this manner are 1–5 μ in diameter.

The tungsten wires were then soldered to a thin copper wire for connexion with the apparatus. The assembly was coated by immersion in Glyptal 1201 Enamel (General Electric), and baked at 125° C. for 1–2 hr. A second coat was applied if necessary. Insulation was tested by immersing the electrode in a 1% NaCl solution and passing direct current (9 V., electrode negative) through the solution. Hydrogen bubbling was observed only from the uninsulated tip, under the dissecting microscope, if insulation was adequate. The completed electrodes were mounted on a plastic rod and secured to a micromanipulator.

For photic stimulation, a Grass photostimulator (PS2) was employed. Flash duration was 10 μsec., intensity range between 6 and 100×10^4 f.c. Frequency of stimulation could be varied between 1 and 100 flashes per second.

Responses were also recorded with a Dumont dual-beam oscilloscope; the flash of the lamp was synchronized with the start of the sweep of one beam, the other beam was used as a time signal. Recordings of the oscilloscope picture were made with a Dumont kymograph camera on photosensitive paper.

RESULTS

(1) *General characteristics of spontaneous electrical activity of the goldfish brain*

The spontaneous electrical activity of various parts of the goldfish brain was investigated in the following stages of activity:

(*a*) Without anaesthetic: fish immobilized by injection of Intocostrin, or allowed to recover from light anaesthesia by leading fresh water over the gills for 15 min.

In the latter case, spontaneous tail movements were a sign of recovery from narcosis.

(*b*) 'Stage I' anaesthesia: a light stage of anaesthesia, using ½–2 % urethane. The fish did not react to touch, but did react to more severe stimuli such as introduction of a hypodermic needle into the abdominal wall.

(*c*) 'Stage II' anaesthesia: a deep stage of anaesthesia, using 3–5 % urethane. The fish reacted to neither touch nor injury (such as needle prick).

(*d*) 'Stage III' anaesthesia: very deep anaesthesia; 6–8 % urethane. Recordings were made both in a darkened, quiet room and in a lighted room with normal laboratory activity.

Although the patterns obtained varied somewhat in different fish, and in the same fish during the course of a single recording, a general pattern of activity can be described for each part of the brain.

Telencephalon (Fig. 2 A, B). The dominant rhythm from the telencephalon of an unanaesthetized fish in a quiet, dark room had a frequency of 4–8 cyc./sec., with an amplitude of 40–70 μV. The patterns of the two halves of the telencephalon were synchronous. A frequency of 9–14 cyc./sec. was also present, becoming relatively more prominent in a lighted room with normal background noise level. Occasionally a very regular, faster rhythm of 35–40 cyc./sec. dominated the whole pattern of activity; it tended to appear during the later part of the recording periods.

Under stage I anaesthesia, the dominant frequency continued to be 4–8 cyc./sec. although faster, lower-amplitude frequencies began to be more noticeable. Under stage II, the pattern slowed to 4–6 cyc./sec., while the amplitude decreased, in general falling from 50 to 20 μV. within a 10 min. period.

Mesencephalon (Fig. 2 C, D). A dominant frequency of 7–14 cyc./sec. prevailed on the surface of the optic tectum in a quiet, dark room. The amplitude was higher than in the telencephalon (60–180 μV.). The pattern was synchronized over the two halves of the mesencephalon. This dominant frequency was mixed with higher frequency waves (18–24 cyc./sec.) of lower amplitude. Again, if the room was light and not perfectly quiet, the higher frequency rhythm became more noticeable, although the 7–14 cyc./sec. pattern continued to dominate the picture.

In stage I anaesthesia, the faster frequency component became less noticeable; the 7–14 cyc./sec. rhythm continued to be dominant but its amplitude was lowered. Then, with increasing depth of narcosis, there was an initial decrease in frequency with enhancement of amplitude, followed by a period of constant frequency with decreasing amplitude.

Cerebellum (Fig. 2 E). In either light or dark room, regardless of noise level, there was a constant 25–35 cyc./sec. frequency in the cerebellum, of a low amplitude (20–50 μV.). This was not influenced by light anaesthesia. With the oscilloscope, it was also possible to detect an even faster rhythm of 120–180 cyc./sec., with an amplitude of 5–15 μV. Under deeper anaesthesia (stages II, III), the pattern became more irregular, however, with an increasing amount of low-frequency activity.

Medulla oblongata (Fig. 2 F). Activity in the vagal lobes of the medulla oblongata was characterized by slow (0·5–2 cyc./sec.) potentials of a low amplitude. Two groups of higher frequency waves were superimposed on this slow rhythm: a pattern of 8–11 cyc./sec., dominant in the dark; and an activity of 20–35 cyc./sec., observed during light narcosis and in a lighted room. This regular, fast activity disappeared under stage II anaesthesia.

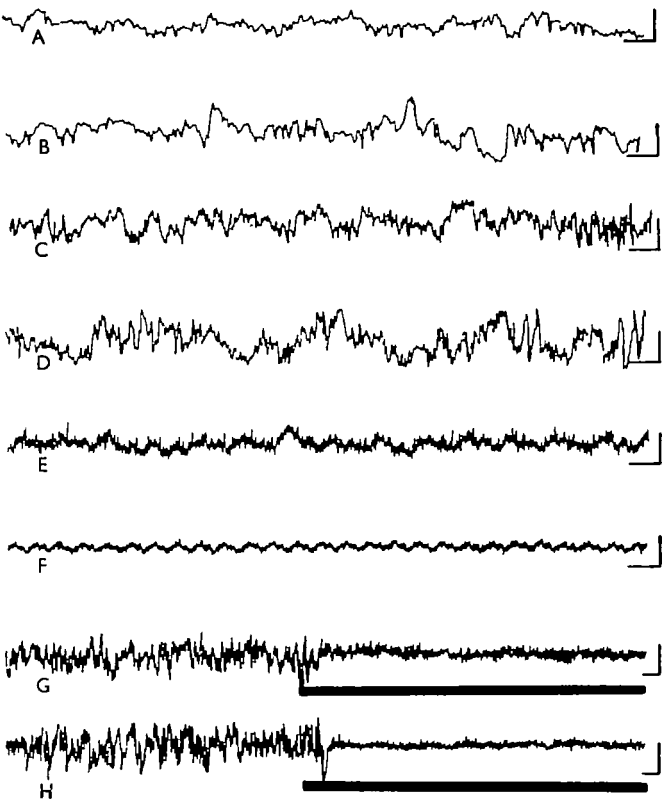

Fig. 2. Recordings from various parts on the brain. A, Recording from two points of right telencephalon; B, recording from left and right telencephalon; C, recording from two points of right mesencephalon; D, recording between left and right mesencephalon; E, recording from cerebellum; F, recording from medulla; G, recording from right mesencephalon; H, recording from left mesencephalon; G and H, horizontal black lines indicate rapid repetitive light flash (75 cyc./sec) with 20×10^4 f.c. light intensity. Calibration: vertical line indicates 150 μV.; horizontal line indicates 1 sec.

In general, it can be seen from the above results that light anaesthesia does not alter the normal electrical pattern of activity of the teleost brain markedly. For the last three groups of experiments, therefore, a light level of urethane anaesthesia was employed for technical reasons.

It appears that each division of the brain is characterized by a distinct and peculiar pattern of frequencies, probably based upon the cyto-architecture, anatomical connexions, and function of that section of the brain. The lowest frequency was

found in the telencephalon, followed by that in the mesencephalon and then the medulla; the cerebellum had the highest frequency. Amplitude of potentials was highest in the mesencephalon.

(2) *Arousal reaction on photic stimulation* (Fig. 2G, H)

An arousal reaction was obtained in unanaesthetized or lightly anaesthetized fish upon stimulation with repetitive light flashes at a frequency of 50–100 flashes per second. This reaction was most consistently found in the mesencephalon, where the 7–14 cyc./sec. rhythm was abruptly replaced by the 18–24 cyc./sec. frequency. Both rhythms are normally present in the spontaneous pattern of the optic tectum, as previously noted; during arousal, however, the faster frequency dominated the pattern. In some cases this arousal reaction was recorded from the telencephalon; here again, a faster component, normally present, became dominant in response to rapid repetitive photic stimulation. No arousal reactions were found in cerebellum or medulla oblongata under these conditions.

Although some high-amplitude activity sometimes occurred in the first 250 msec. of high-intensity stimulation, in general the amplitude of the high-frequency waves was lower than the amplitude of the spontaneous activity before arousal. This brief high-amplitude component seemed to be associated with a direct response to the high-intensity stimulus. Upon cessation of the repetitive light stimulus, the normal pattern replaced the arousal pattern in all fish, within a period of 1·5 min.; in most fish it occurred within 20 sec.

The level of anaesthesia of the fish had an important influence upon the arousal reaction. Under stage II anaesthesia, the frequency and amplitude of the response was reduced (to 10–18 cyc./sec.). Under the deepest anaesthesia, it was possible to elicit an arousal reaction in only one of eight fish.

(3) *Response to single light flashes*

The monopolar surface recording shown in Fig. 3B illustrates the general characteristics of the optic tectum response to single light flashes. Various areas of the tectum differed slightly with respect to amplitude and duration of the response, as will be shown later.

The latency of the response recorded from the mesencephalon of slightly anaesthetized fish was 30–40 msec. The first part of the response consisted of two to four rapid diphasic spikes, of 40–50 msec. total duration and about 100 μV. amplitude. This complex will be referred to as the 'A complex'. A second portion, the 'B complex', consisted of a large negative spike, with a duration of 90–110 msec. and amplitude 100–200 μV.; it was sometimes preceded by a small positive deflexion, and was followed by a slower positive deflexion of 30–40 msec. duration and 30–40 μV. amplitude. Finally, a slow negative after-potential followed, the 'C complex', with a duration of 50–60 msec. and amplitude of 30–50 μV. The total response lasted 250–325 msec. and was predominantly negative in character.

In the midbrain of a fish in stage II anaesthesia the A complex was not detectable against the background of spontaneous electrical activity; the B complex was

reduced in amplitude. In stage III, the amplitude of the B complex was still further reduced.

With a stimulus of low intensity (6–15 × 10^4 f.c.) no A complex could be detected, and the B complex was of lower amplitude. The effects of low-intensity stimulation were similar in appearance to the response under stage II anaesthesia. At a stimulus intensity of 40 × 10^4 f.c. or greater the complete response was recorded.

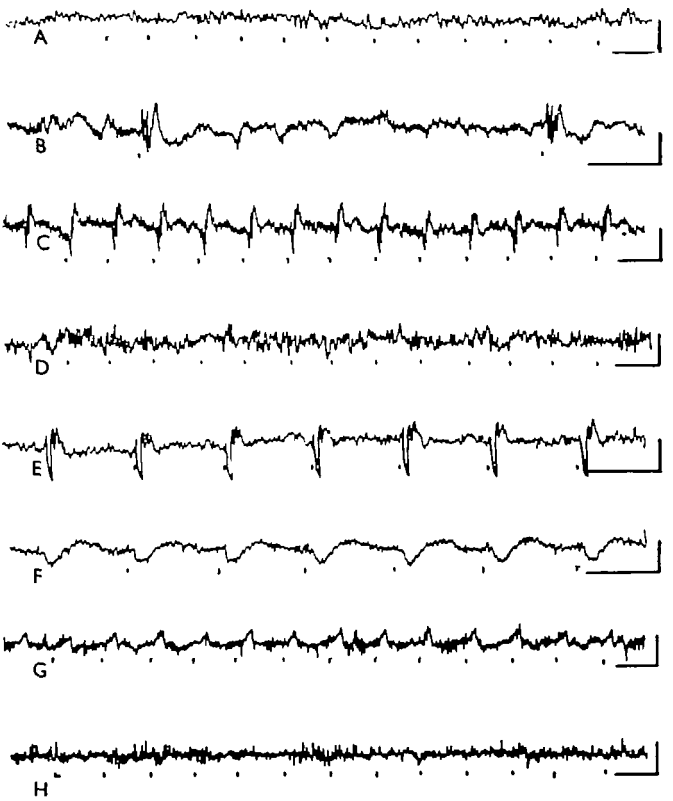

Fig. 3. Recordings from various parts on the brain. A, Bipolar recording from right telencephalon; B, monopolar recording from right mesencephalon; C, bipolar recording from left mesencephalon after cutting left optic nerve; D, bipolar recording from right mesencephalon after cutting left optic nerve; E and F, recording from left mesencephalon, anterolateral portion, before and after cautery of three quarters of the retina, leaving only the superior nasal quadrant intact; G, bipolar recording from medulla. Dots indicate single light flashes, duration 10 μsec., intensity 100 × 10^4 f.c. Calibration: vertical line indicates 150 μV., except in D where it represents 250 μV.; horizontal line indicates 1 sec.

If the optic nerve of one eye was severed the spontaneous electrical activity of the contralateral lobe was depressed, and no response to light flashes could be elicited (Fig. 3 C, D). Spontaneous activity and responses of the ipsilateral lobe remained unchanged. These findings are in agreement with those of Buser (1949*b*).

No response to a single light flash could be recorded from the telencephalon (Fig. 3A) or medulla oblongata (Fig. 3H). A response was recorded, however, from the cerebellum (Fig. 3G). This was a slow negative wave, with a latency of 60–80 msec., followed by a small positive deflexion. The duration of this response was 60–120 msec.; amplitude was 80–125 μV. (Fig. 6). There was no initial component corresponding to the A complex of the tectum. When one optic nerve was cut, this cerebellar response was usually undetectable; it disappeared also under stage II anaesthesia.

(4) *Localized responses of the optic tectum*

Using fine-pointed cotton-wick electrodes, spontaneous electrical activity and responses to single light flashes were recorded from small areas of the optic tectum. Following this, part of the retina was destroyed by electrocautery and recordings of spontaneous activity and of responses to flashes were made again from the same areas.

Two parts of the mesencephalon departed from the general pattern described above for response to photic stimulation. In the anterior part of the optic tectum the A complex was not detectable against the background activity; the B complex was always present here, however, and its amplitude was higher at the anterior pole than at any other point on the tectum. The lowest amplitude of the B complex—as little as 50% of the amplitude found in other tectal areas—was found at the posterior pole.

Destruction of a part of the retina had the following type of effect on the general photic response pattern (Fig. 3E, F): no A complex could be recorded from some region of the midbrain (in which it had previously been present), and amplitude of the B complex was reduced 40–50% in this region.

Fig. 4 summarizes diagrammatically the results of experiments in which there was destruction of three-quarters of the retina. The left optic tectum is shown, as viewed from above; below it is represented the contralateral (right) eye, with the destroyed portion blackened. On the tectum one circle represents placement of bipolar electrodes in a series of different experiments. Open circles indicate tectal areas where response was unchanged after retinal injury; black circles indicate areas of changed response (40–50% lowering of the amplitude of the B complex, and/or absence of the A complex).

After destruction of all but the superior nasal quadrant of the right retina, the response to single light flashes remained unchanged in part of the posterolateral portion of the contralateral tectum, and at the anterior pole.

After destruction of all except the inferior nasal quadrant, the response was unchanged only in a part of the posteromedial portion of the tectum, and the anterior pole.

After destruction of all except the inferior temporal quadrant, response was unchanged only in part of the anteromedial portion of the tectum.

After destruction of all except the superior temporal quadrant, response was unchanged only in part of the anterolateral portion of the tectum.

In a second group of experiments, only one-quarter of the retina was destroyed, and a search was made for changed response. Results are summarized diagrammatically in Fig. 5, with the left tectum and right eye represented as before.

After destruction of the superior nasal quadrant, the response was changed only in part of the posterolateral portion of the contralateral tectum.

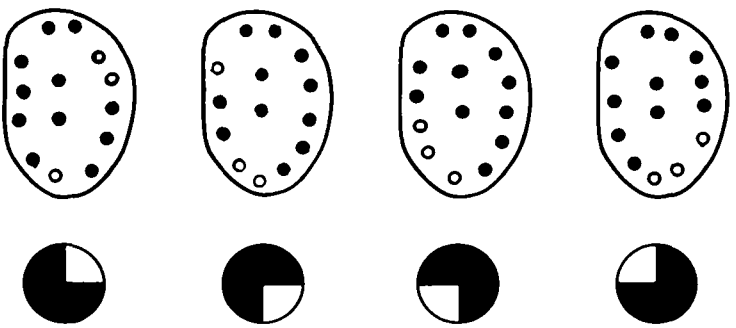

Fig. 4. Schematic representation of results obtained by local bipolar recording before and after cautery of three-quarters of the retina. In the upper row are represented views of the left optic tectum, with circles to indicate electrode placement. Each circle indicates one bipolar electrode placement. Open circles indicate unchanged response; black circles indicate alteration of response. In the lower row, the contralateral (right) eye is shown as viewed from the side of the fish. The blackened segments are those destroyed by cautery.

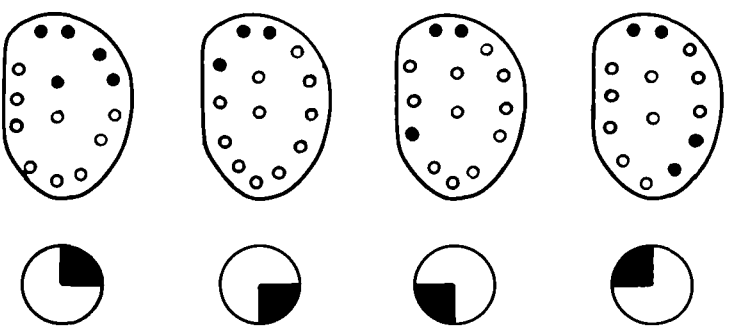

Fig. 5. Schematic representation of results obtained by local bipolar recordings before and after cautery of one-quarter of the retina. Left tectum and right eye as in Fig. 4.

After destruction of the inferior temporal quadrant, the response was changed in part of the anteromedial portion of the tectum, and at the posterior pole.

After destruction of the superior temporal quadrant, the response was changed in part of the anterolateral portion of the tectum, and at the posterior pole.

The results obtained in the two sets of experiments, summarized in Figs. 4 and 5, show localizations of changed or unchanged response which are approximately complementary.

The anterior and posterior portions, and the middle portion of each lobe, were affected in the same way by destruction of any portion of the quadrant, in contrast to the localized changes found at the margins of the tectum. Possible reasons for this will be discussed later.

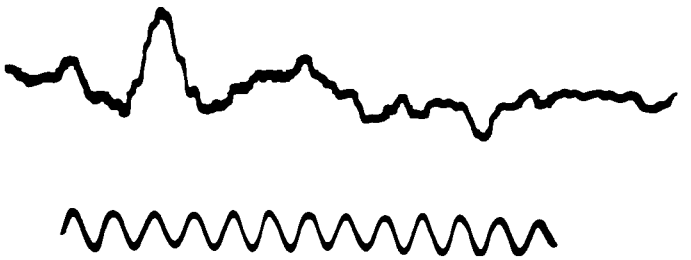

Fig. 6. Bipolar recording from the surface of the left cerebellum. Photograph with Dumont kymograph. (Upwards: negative.) Time signal: 40 cyc./sec., 40 μV. Single light flash at the beginning of the recording.

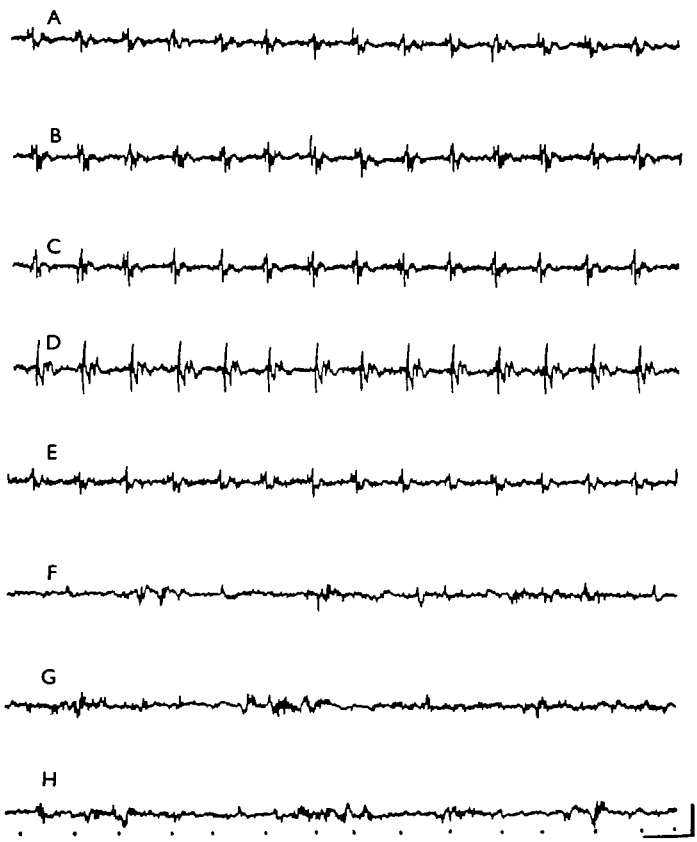

Fig. 7. Bipolar microelectrode recordings from the posterior part of the tectum opticum. A, 40; B, 80; C, 120; D, 160; E, 200; F, 240; G, 280; H, 320 μ under the surface of the tectum opticum. Dots indicate single light flashes, duration 10 μsec., intensity 100×10^4 f.c. (for all records). Calibration: vertical line indicates 150 μV.; horizontal line indicates 1 sec.

(5) *Recordings with microelectrodes*

The response to photic stimulation, as described in general form above, was also obtained from deeper layers of the optic tectum. A maximum (especially of the B complex) response was recorded between 100 and 200 μ, when electrodes

were placed in the middle and posterior part of the tectum (Fig. 7); deeper than this there was no recognizable response. The response was recorded as deep as 2000 μ when electrodes were inserted at the anterior pole of the tectum (Fig. 8); this reflected the fact that vertically inserted microelectrodes at this point passed on a tangent to the ventricle, through the grey matter of the curved anterior margin of the optic tectum.

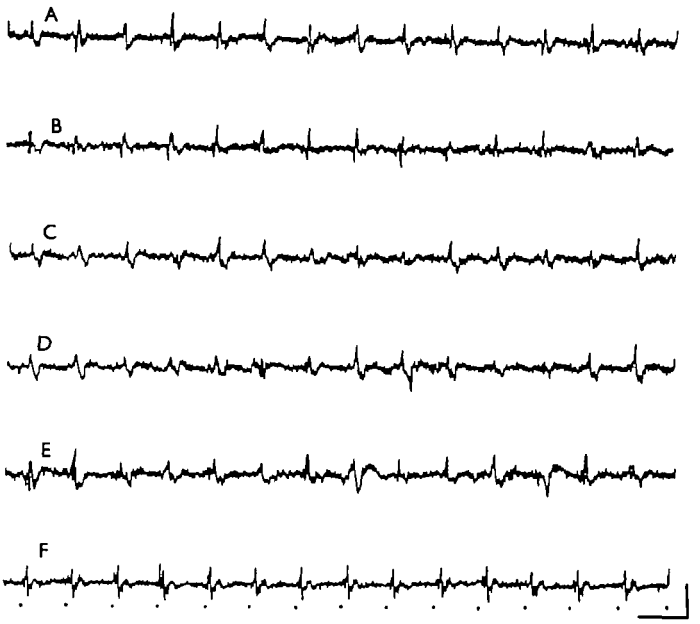

Fig. 8. Bipolar microelectrode recordings from the anterior parts of the tectum opticum. A, 250; B, 500; C, 750; D, 1000; E, 1250; F, 1500 μ under the surface of the tectum opticum. Dots indicate single light flashes, duration 10 μsec., intensity 100×10^4 f.c. (for all records).

DISCUSSION

(1) It was noted in the results that each part of the brain is characterized by a particular pattern of electrical activity. The medulla oblongata was characterized by a slow, low-amplitude rhythm corresponding to the respiratory activity. The highest frequencies (25–35 cyc./sec. and 120–200 cyc./sec. were found in the cerebellum. The telencephalon and mesencephalon were characterized by considerably lower frequencies, of 4–8 and 7–14 cyc./sec., respectively.

The electrical activity of the medulla and cerebellum was not noticeably influenced by light or noise. In the telencephalon and diencephalon, higher frequency waves (9–14 and 18–24 cyc./sec.), which are of low amplitude in a quiet, dark room, were accentuated when the recording room was light and moderately noisy.

Light anaesthesia did not influence the electrical activity of the medulla or cerebellum. Under deep anaesthesia the frequency of the cerebellar activity decreased. In both telencephalon and diencephalon the higher frequency waves became less noticeable under light urethane anaesthesia, and the amplitude of the

dominant lower frequency waves was lowered. As anaesthesia was further deepened there was an initial lowering of frequency with enhancement of amplitude of the dominant waves, followed by a period of constant frequency with decreasing amplitude. This pattern follows the sequence of changes in the cortex of the rat during the progression of urethane narcosis, described by Schadé (1957).

Enger (1957), who recorded a dominant frequency of 8–13 cyc./sec. from the codfish mesencephalon, compared this pattern with the mammalian alpha rhythm. He pointed out that the 8–13 cyc./sec. rhythm recorded in the codfish alternated with spindles, and that it was accentuated in quiet and dark but suppressed in noise and light. It was also more pronounced under light anaesthesia. The mammalian activity shows similar features. He pointed out that the little differentiated paleothalamus of fish may be homologous with the thalamo-reticular system of mammals, and speculated that the presence of the 8–13 cyc./sec. rhythm in the codfish mesencephalon may be associated with the thalamo-reticular system of fish, as the alpha rhythm is believed to be associated with the thalamus of mammals, through a reverberating cortico-thalamic circuit.

The patterns of electrical activity recorded in the two halves of the optic tectum were synchronous. Furthermore, the two halves of the telencephalon produced synchronous patterns. Since the commissural system between the halves of the mesencephalon did not conduct evoked potentials from one half of the mesencephalon to the other, in the experiments on monocular blinding, it seems unlikely that these commissures are involved in the synchronization of the two halves. Another possibility is that the electrical activity of both halves is governed by a more ventral structure in the brain stem, which is either anatomically or functionally unpaired.

(2) Rapid repetitive flashing consistently caused the appearance of a mesencephalic pattern which resembled the typical mammalian arousal pattern (Moruzzi & Magoun, 1949). There was a suppression of slower activity and accentuation of high-frequency low-amplitude activity; this pattern disappeared within 1·5 min. after cessation of stimulation, and was replaced by the normal pattern of activity. Since the fish has in its paleothalamus a structure which is homologous with the thalamo-reticular system of mammals, a similar thalamo-reticular activating system may be responsible for the arousal in fish as well as mammals.

(3) A response to single-flash stimulation was described from the mesencephalon and cerebellum. The cerebellar response, with a latency of 60–80 msec., can be expected from the anatomical connexions between tectum and cerebellum. According to Kappers (1936), Goldstein (1905), Brickner (1929), Charlton (1933), Leghissa (1955) and other authors, there is a well-developed projection system from tectum to cerebellum consisting of both crossed and uncrossed fibres. Although this cerebellar response has not been noted previously in teleosts, it was shown in cats recently by Fadiga, Pupilli & von Berger (1957).

The response from the optic tectum was described in terms of an initial A complex and a later B complex. The A complex, being the first event in the sequence of the evoked potential, might be thought of as an action potential of the

optic nerve. This initial part of the response pattern was not elicited at the anterior pole of the optic tectum, although optic tract fibres are concentrated in that region. Furthermore, the A complex has a relatively long latency (30–40 msec.). Therefore, the first response recorded can hardly be due to the impulses conducted by the optic fibres, but must be considered as a synaptic potential.

Buser (1950), in his study of the complex mesencephalic potentials caused by electrical stimulation of the cut optic nerve, proposed that synchronized activity of the deeper cell layers is responsible for the B complex. In the present study the B complex was found to increase in amplitude, with respect to the A complex, as microelectrodes were pushed to greater depths of the mesencephalon. A maximum of the B complex was recorded at a depth of about 100–200 μ, which roughly corresponds to the deep cell layer.

According to Leghissa (1955) the thickness of the deeper cellular layer of the tectum, composed of motor and horizontal associative neurons, is greatest anteriorly. This is the region of the highest amplitude of the B complex. The B complex has a reduced amplitude posteriorly, where this layer is thinnest. The greater amplitude of the B complex in the anterior region of the tectum may be an expression of the greater thickness of the deeper cell layers in this area.

When the optic nerve of the goldfish was cut on one side, all responses to photic stimulation were abolished in the contralateral tectum, which is explained by the complete decussation of the optic nerves (Kappers, Huber & Crosby, 1936). There is a well-developed commissural system between the deeper cell layers of the two halves of the optic tectum. However, the B complex, which as discussed above probably develops in the deeper layers of the unaffected side, apparently did not spread through the intertectal commissural system to the affected half.

After the optic nerve is cut the spontaneous electrical activity of the contralateral half of the tectum is reduced; in particular, there is a striking reduction in the low-frequency waves. In the normal fish the amplitude of the spontaneous activity is maintained and even increased in the dark. It can be expected that in the dark afferent excitation is reduced. Therefore, the reduction of spontaneous activity following monocular blinding probably is not due simply to cessation of nerve impulses from the optic fibres. It seems possible that the lowered amplitude of activity observed is due to a spontaneous discharge from the injured ends of the nerve fibres. Unsynchronized bombardment from this source might cause a decrease in amplitude of the spontaneous activity of the tectum and suppression of low-frequency activity. Although the pattern somewhat resembles arousal, it probably is not due to activation of the reticulo-thalamic system since the ipsilateral half of the tectum is not affected.

A continuous flow of impulses from severed nerve fibres in fish, like that suggested here, was postulated by Parker (1934, 1936) to explain the following experiment. If a small tail area of the killifish (*Fundulus*) is denervated by a superficial transverse cut, the denervated area will at first darken, then fade after a few days. If a new transverse cut is then made distal to the original cut, a new dark band will appear between this cut and the edge of the tail, within the already denervated area. The

formation of the dark band in each case is interpreted by Parker as being due to a prolonged discharge of the nerve fibres innervating the melanophores. A cold block stops these injury-caused impulses and causes fading of the darkened area.

The cerebellar response to optic stimulation is abolished by monocular blinding. This might be attributed in part to a 50% reduction in the number of impulses arriving at the cerebellum via tecto-cerebellar paths. If considerable facilitation were needed for this response, this might explain the great effect of a 50% reduction in afferent impulses. Furthermore, this response was found only in fresh preparations and was easily abolished by aging or injury. This may also explain why Buser (1949 *a*) failed to find a cerebellar response to electrical stimulation of the cut optic nerve in catfish.

(4) It was shown that the retinal projection on to the contralateral optic tectum could be roughly localized by the use of methods of electrical recording. Talbot & Marshall (1941) have reported a point-to-point projection of the retina on the visual cortex of the cat and monkey, using electrical recording. An indirect method of mapping the superior colliculus, based on reflex motor responses, was used for cats by Apter (1945, 1946), and for fish by Chauchard & Chauchard (1927 *a*, *b*) and Akert (1947, 1949).

Buser & Dussardier (1953), using electrical recording, have shown that each retinal quadrant of certain teleost fishes sends fibres to a different part of the optic lobe. Their findings were confirmed in a recent study by Gaze (1958) who used a microelectrode to explore the surface of the frog mesencephalon. He mapped points of maximal response to a small (15″) flashing light source. Maximal responses were used for mapping because responses could be evoked from a single point on the optic lobe by stimulation of an area of 5° to 60° radius in the visual field. Despite the overlapping of receptive fields he was able to show a coarse mapping of the retinal projection, such that points on the naso-inferior quadrant of the retina projected to the posteromedial part of the lobe. Points on the temporo-inferior retina projected to the anterior part of the lobe near the midline. Points on the naso-superior retina projected to the posterior part of the lobe near its lateral edge, while those on the temporo-superior retina projected to the anterior part of the lobe near its lateral edge. Recordings made along the midline of one optic lobe corresponded roughly to light stimuli in the central regions of the contralateral visual field.

A direct method of anatomical localization is based on partial destruction of the retina, followed by Marchi staining of degenerated nerve fibres. This method was used by Lubsen (quoted in Ströer, 1940), by Ströer (1940) for *Salmo salar* and *Clupea harengus*, by Akert (1950) for *Salmo irideus*, and by Leghissa (1955) for *Carassius auratus*. By this method a coarse projection of the retinal quadrants on the tectum has been demonstrated. The optic nerve divides into two fascicles. A medial fascicle, derived from the inferior part of the retina, spreads from the anterior pole of the contralateral tectum on to the dorsomedial surface. A lateral fascicle carries fibres from the rest of the retina. Fibres (contained in the lateral fascicle) from the superior part of the retina radiate from the anterior pole to the lateral

surface of the tectum. The nasal retinal afferents terminate on the posterior pole of the tectum, and the temporal afferents on the anterolateral portion of the tectum.

In the recordings of responses to photic stimulation after partial destruction of the retina a localization of responses from the various retinal quadrants, around the margin of the tectum, was observed. This localization agreed with that found by anatomical methods. It was confined to the margins of the tectum, and responses from the anterior, middle, and posterior portions of the tectum were either not altered (anterior and middle) or always altered (posterior) by partial destruction of the retina.

The following working hypothesis of the retinal projection is proposed, based on the anatomical and electrophysiological findings.

Relevant considerations:

(*a*) The optic tract arrives at the medio-anterior pole of the contralateral lobe of the optic tectum, and its fibres can be seen to spread in a fan-like fashion over the tectal surface, on gross inspection.

(*b*) The A complex, as discussed above, is associated with the synaptic connexions made between entering optic nerve fibres and the tectal neurons.

(*c*) The B complex is a sign of subsequent activity in the deeper cell layers.

We assume, for this hypothesis, that soon after reaching the anterior part of the tectum some of the optic nerve fibres, or their branches, make synaptic connexions with central neurons; they continue to form synapses all along the course of their spread across the optic tectum. This is suggested by the finding that both A and B complexes can be recorded everywhere on the tectum except from the anterior pole. The majority of fibres gradually leave the main optic bundles and end at the margin of the tectum on either side of the bundle, establishing the projection described above. Although some degree of overlapping of this projection can be expected, the gross localization achieved by this anatomical arrangement is detected by electrophysiological methods. The tectal area corresponding with a quadrant mapped anatomically is larger than that mapped by electrical methods, which is a measure of the overlap of the projections.

Since no A complex was led off from the anterior pole of the tectum, there are probably few if any synaptic endings of the optic axons in this area. A large B complex is found here, however, which may be due to a spread of activity into the deeper grey matter from adjacent areas. This same spread of activity in the deeper cell layers, via horizontal associative neurons, also explains why the B complex is reduced, but not abolished, in the tectal areas which are affected by partial retinal destruction.

The middle areas of the tectum, according to this hypothesis, will be synaptically connected with afferents from the central areas of the retina, including all four quadrants. This is supported by the results, since both A and B complexes are found in this middle region when any portion of the retina remains intact. The posterior pole of the tectum, which showed a changed response after any retinal destruction, may receive fibres from all areas of the retina.

A quantitative consideration may be used to resolve the apparent contradiction in stating that the ending of fibres from all retinal quadrants is responsible for the presence of not noticeably changed A and B responses in the middle portion of the tectum, while the same anatomical arrangement is also the basis of a change in the evoked response of the posterior pole after destruction of any quadrant of the retina. If one assumes that the connexions between the thick bundles of optic fibres running over the centre of the tectum and the underlying grey matter are plentiful, then the loss of even a considerable percentage of the afferent impulses may cause changes so slight that they cannot be detected by the relatively coarse electrophysiological methods. On the posterior pole the fibre layer has thinned out considerably, however, and the grey matter is least well developed in this area. A reduction of these more sparse connexions may well result in a change in the evoked potential. If considerable spatial summation were involved in the elaboration of the evoked potential, the effect of even a slight reduction in the number of active nerve fibres could have a major effect at low levels of innervation. A similar reasoning was used to explain the absence of a cerebellar response to light flashes in unilaterally blinded fish.

The coarse, overlapping, localized projection of retinal areas provided by this working hypothesis seems to be in agreement with both anatomical and electrical results.

SUMMARY

1. Bipolar surface electrodes were used to record electrical potentials from the brain of the goldfish, *Carassius auratus* L. Characteristic patterns of spontaneous electrical activity were described for telencephalon, mesencephalon, cerebellum and medulla oblongata. Changes in these patterns under deepening urethane narcosis were noted.

2. Rapid repetitive flashing light caused a change in the normal pattern of the mesencephalon which resembled the mammalian arousal. High-frequency (18–24 cyc./sec.) low-amplitude activity replaced the characteristic low-frequency waves (7–14 cyc./sec.); the lower-frequency activity returned 1·5 min. after cessation of stimulation.

3. Responses to single light flashes were described from the mesencephalon and cerebellum. An 'A complex' from the mesencephalon consisted of two to four rapid diphasic spikes, with a latency of 30–40 msec., duration of 40–50 msec., and amplitude of about 100 μV. A 'B complex' consisted of a large negative wave, duration 90–110 msec. and amplitude 100–200 μV., followed by a small positive deflexion, and a slow negative after-potential. The cerebellar response had a latency of 60–80 msec., duration of 100–120 msec., amplitude of 80–125 μV. The nature of these two components was discussed.

Monocular blinding abolished the response to light in the contralateral half of the optic tectum and in the cerebellum. The amplitude of the low-frequency spontaneous activity characteristic of the mesencephalon was reduced in the contralateral tectum.

4. Regional differences in the tectal response to single light flashes were described. Microelectrodes were used to record the photic response from deeper tectal layers.

After destruction of parts of the retina by electrocautery, a disappearance of the A complex and 40–50% reduction of the B complex was observed in restricted tectal areas. The distribution of the areas of changed response was shown to correspond to a coarse overlapping projection of retinal quadrants of the fish eye on to the contralateral optic tectum. A possible basis for the localization of this projection, in agreement with anatomical and electrophysiological findings, was described.

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