Influence of Thyroid State Upon Optically Evoked Potentials in the Midbrain of Goldfish.* (31165)

TOSHIAKI J. HARA, AUBREY GORBMAN, AND KAZUO UEDA†
Department of Zoology, University of Washington, Seattle.

We have reported previously that exogenous thyroxine exerts a sensitizing, or augmenting action upon optically evoked potentials in the optic tectum of goldfish(3). In the course of further experiments, we found that if successive optic stimuli are given, midbrain potentials in deep layers (350-400 μ below the mid-dorsal surface of the tectum) evoked by the second, and later, stimuli vary in several ways, depending upon the intervals between the successive light flashes. This finding permitted us to test the influence of thyroid hormone upon the excitability cycle of the optic system, with the expectation that the information obtained might lead to a better understanding of the nature of hormone action upon this system. Bradley *et al*(1) found that photic driving was absent or difficult to induce in rats thyroidectomized at birth. Further, Lansing *et al*(5) found in man a reduction of the brain's response to high frequency flickering light during thyroid deficiency. Thus, the few investigations directed to this question already have shown that thyroid hormone influences this phase of central nervous function in mammals. Goldfish were used in these studies not only because they are adaptable for neurophysiologic experiments, but also because there is so little known of the action of thyroid hormone in adult teleosts(9).

Materials and methods. Common goldfish, *Carassius auratus* L., were obtained from a commercial breeder in Martinsville, Indiana; they ranged from 9 to 12 cm in length, and were of both sexes. The methods and techniques for eliciting or recording evoked tectal potentials were generally similar to those described previously(3).

L-thyroxine (sodium salt) and thiourea, freshly dissolved in physiological saline solution for fresh water teleosts, were injected daily intraperitoneally into the goldfish at the dosages of 50 μg or 100 μg in a volume of 0.05 ml. In other goldfish, the same volume of physiological saline solution was injected into the animals in the same manner. Observation and recording of evoked potentials were carried out after 3 weeks of daily injections.

The flash stimuli were produced with a Grass PS-2 photostimulator delivering blue-white, 10 microsecond flashes, at approximately 1.5×10^6 horizontal candle power (intensity setting 16, and at a distance of 2 feet). The flash lamp (#PST-2) was positioned 100 cm from the eye. By combined use of a Grass S-4 stimulator with the photostimulator, paired flashes and trains of flashes having time intervals variable between 20 to 1000 milliseconds were presented to the eye.

Potentials from the tungsten microelectrodes on the mid-dorsal surface of the contralateral optic tectum, or inserted into it, were led into the cathode-follower probe of a Grass P5 A.C. preamplifier, then displayed on a Tektronix 502 oscilloscope and recorded with a kymograph camera. Half amplitude responses of this over-all recording system were 0.1 cycle at a low, and 2 kilocycle at a high frequency. All the experiments were carried out in a darkened and quiet room at temperatures varying between 23.8° and 24.5°C.

Results. Single flash stimulation. The potentials evoked at the surface of the optic tectum by a single flash stimulation consisted of a negative spike of 2 peaks (Fig. 1) When the microelectrode was inserted gradually, an inversion of polarity was found, in goldfish of this size, at the depth between 200 to 300 μ. The amplitude of the deep positive potential reached a maximum at a depth of about 350 to 400 μ. In all animals studied, the potentials evoked at this depth, with a given intensity of stimulus, were relatively

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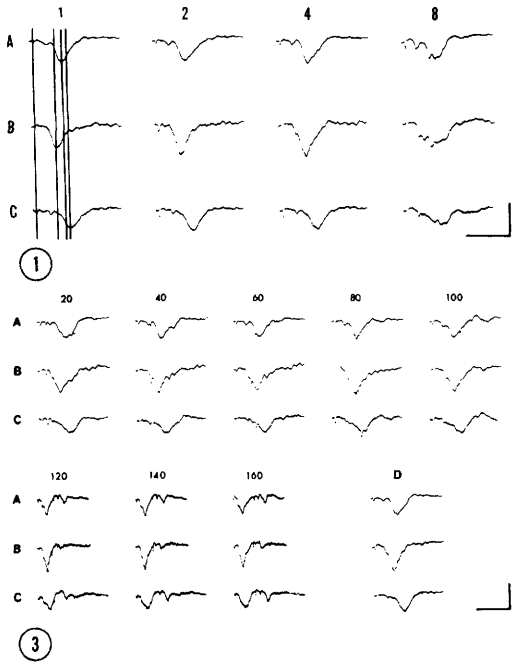
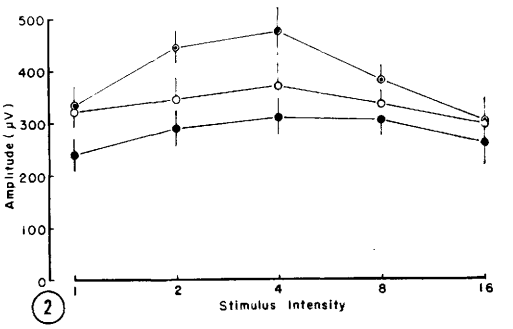
† Present address: Zoological Inst., Faculty of Science, Univ. of Tokyo, Japan.

consistent. In general, the time to peak was gradually shortened, while the amplitude of the potential increased, as the stimulus intensity was increased.

As illustrated in Fig. 1, the mean time to reach the maximum response measurement from the point of the stimulus artifact to the point of maximum response amplitude in thyroxine-treated fish is 48.8 msec (55.5 $\pm$ 1.7); in control fish it is 67.5 msec (69.8 $\pm$ 3.0) and in thiourea-treated animals it is 78.0 msec (76.5 $\pm$ 3.6). It is observable also that there is an increase in amplitude of the potentials in thyroxine-treated animals, and a decrease in thiourea-treated goldfish, compared to the saline-injected controls (Fig. 2). The increase in amplitude above the control value in the thyroxine-treated animals reached mixiumum with the stimulus strength 2 and 4. On the other hand, the relative lowering of amplitude of the response in thiourea-treated fish was greater at the lowest stimulus strength rather than at the higher.

Paired flash stimulation. To test the influence of thyroxine and thiourea upon the sequence of excitability changes in the optic





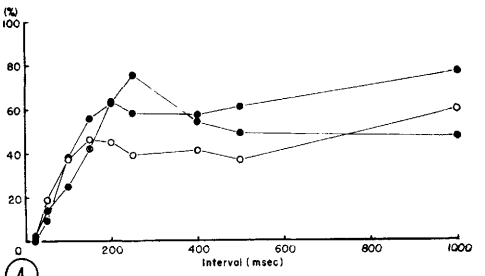


FIG. 1. Potentials evoked by single flash light stimuli of 4 different increasing intensities (1, 2, 4 and 8) in the optic tectum in 3 goldfish: control (A), thyroxine-injected (B), and thiourea-treated (C). In column 1, the first vertical line indicates time of stimulation for all 3 animals. The following 3 lines indicate time to reach maximum response in thyroxine-treated, control, and thiourea-treated animals, respectively. Calibrations: vertical, 500 μV; horizontal, 100 msec.

FIG. 2. Effects of thyroxine and thiourea treatments upon maximum amplitude of the tectal evoked potential at different stimulus strengths. Thyroxine increased it (double circle), while thiourea had opposite effects (closed circle). Open circle indicates maximum amplitudes in control goldfish.

FIG. 3. Tectal responses to paired light flash stimuli in the control (A and A'), in the fishes injected with thyroxine (B and B') and with thiourea (C and C'). Numbers at top of each column indicate the separation, in msec, between first and second stimulus. D shows control response to a single flash stimulation in the 3 states mentioned above. Calibration: vertical, 500 μV, horizontal, 100 msec.

FIG. 4. Excitability cycle of the optic tectum in control (open circle), thyroxine- (double circle) and thiourea-treated animals (closed circle). Each point represents percentage (ordinate) of maximum amplitude of second (test) response to maximum amplitude of first (conditioning) response at a given time separation of the stimuli (abscissa).

tectum, paired flash stimuli and trains of repeated stimuli were used. Following the first, or conditioning stimulus, the optic tectum passes through an excitability cycle, demonstrable in changes in the subsequent responses to a second test stimulus applied at different intervals after the first. Amplitude of potentials in the second, or test responses varied, depending upon the length of the time interval between conditioning and testing. For intervals less than 40 msec, no clear test potential was observed (Fig. 3 and 4), although complications in the patterns of response were produced. Complete recovery, as judged by production of a test response exactly like that after the conditioning stimulus, required approximately 5 seconds. Between these 2 extremes, the optic tectal excitability cycle could be followed by using as criterion the variation in simple amplitude of the test response; the cycle could be divided arbitrarily into 3 phases: (1) an early recovering phase (40-150 msec), (2) a reducing phase (150 to 250 msec or more), and (3) a late recovering phase (after 250 to 450 msec). In saline-injected control fish, increase of the conditioning to test time interval up to 150 msec resulted in a rise in amplitude of the test response to about one-half of the conditioning one. With further prolonging of the interval, a decrease in the test response amplitude occurred, until about 250 msec, and then a plateauing. The test response thereafter slowly augmented as the intervals were further increased.

The early recovering phases of the excitability cycles in both thyroxine- and thiourea-treated animals were prolonged, compared with the controls; at the same time the amplitude of the test response recovered up to about 65% (thyroxine), or 78% (thiourea) of the conditioning response contrasted with 50% in the controls. On further separation of conditioning and test stimuli, the test response amplitude not only decreased less than the others in thyroxine-treated animals; it also recovered more rapidly than the control. At a separation of one second, the test response in thyroxine-treated fish was about 80% as large as the conditioning response; at this time the controls had recovered 60%

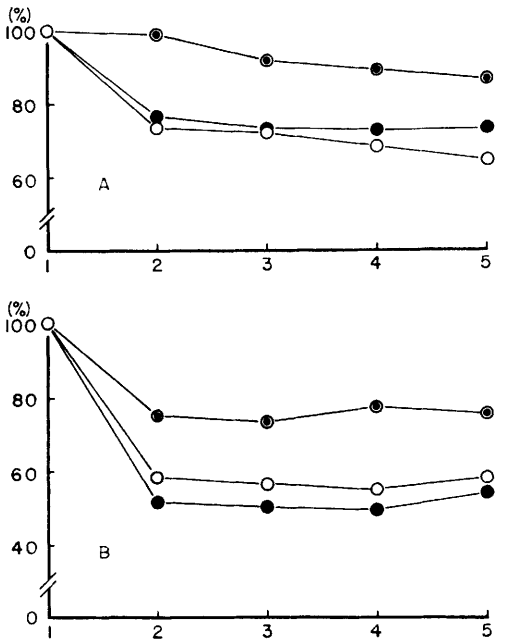


FIG. 5. Response of optic tectum to repetitive flash stimuli (½ cycle per second in A, and 1 cycle per second in B). Each dot represents percentage (ordinate) of amplitude of each numbered response (abscissa) to amplitude of first response. Control, open circle; thyroxine-treated, double circle; thiourea-treated, filled circle.

amplitude; thiourea animals had recovered only 45% amplitude (Fig. 4).

Repetitive flash stimulation. Since complete recovery occurred approximately 5 seconds after termination of the conditioning response, each potential obtained with repetitive stimulation at rates slower than 1/5 sec yielded responses equal to the first response. As the frequency of repetitive stimulation increased, the second and subsequent responses progressively were reduced in amplitude. However, the amplitudes of individual responses obtained at a given continuous stimulus frequency remained almost unchanged during periods of repeated stimulation. In thyroxine-treated animals, the potential amplitude in responses to repetitive stimulation at any frequency tested, was consistently higher than the control, but no significant difference was observed between controls and fish injected with thiourea (Fig. 5). In 3 of 11 fishes injected with thyroxine, supramaximal responses were observed at low stimulus frequencies (1/10 to 1/3 flashes per second).

Discussion. Whether or not potentials evoked in the optic tectum of the goldfish by light stimuli are post-synaptic is not clear. Furthermore, the precise site(s) of action of thyroxine in the visual system is not known. The fact that more than 9 days are required before one may measure hormone action upon this system suggests(3) that the action of thyroxine may be related to some structural changes, possibly at the synaptic regions in the visual system, rather than to general metabolic effects. Morphological actions by thyroid hormone on the vertebrate central nervous system are well known (see review by Gorbman(2) and Bradley *et al* (1)).

In man, there is evidence that responsiveness to photic stimulation is increased after thyroid hormone administration(4,6,7). In our experiments with goldfish, although thyroid hormone was clearly stimulatory, inhibition of thyroid function by injected thiourea had little influence upon the midbrain response to single, or repeated flash stimulation. This may suggest a normal low level of function of thyroid hormone in the optic system of goldfish.

Although a number of investigators have described the excitability cycle in the visual cortex in mammals(8), few such studies have utilized lower vertebrates. In the response elicited in the cat's visual cortex by 2 brief flashes, the second response reaches half the amplitude of the first one, when the 2 flashes are 150 milliseconds apart(6). It has been recognized that the visual cortex excitability cycle of rats and rabbits(8) has a supramaximal phase which occurs 100 to 150 msec after application of a first stimulus. Thus a second stimulus applied 100-150 msec after the test stimulus evokes a larger primary negative potential than did the test stimulus (8).

The 3 phases in the excitability cycle of the tectum in the goldfish reported here are qualitatively very similar to those obtained by us in the rainbow trout (unpublished experiments), although recovery occurred much more rapidly in the latter. The small peak appearing at 100 to 200 msec in the excitability cycle in the goldfish seems to be sequentially similar to the supramaximal negative phase of rats and rabbits. The fact that the early recovering phases were delayed both in the thyroxine- and thiourea-treated fish is difficult to understand in view of the general stimulatory action of thyroxine and the general oppositeness of action of thyroxine and thiourea. The effect of thyroxine on the excitability cycle of the trout optic tectum was similar to its effect on goldfish, indicating a definite and consistent effect of this hormone in the early phase. Following a reducing phase, recovery of excitability in the thyroxine-treated was much more rapid than that in the control, and in the thiourea-treated fish recovery was significantly delayed. These effects may be attributed to shortening or lengthening in latency times and in time to peak amplitude in each potential in thyroxine- or thiourea-treated specimens.

Konishi(4) observed that the second response is decreased in amplitude, but that excitability was partially recovered after the third response in tests using repeated stimulation of the optic nerve. However, in our experiments with goldfish, the second responses were reduced, and succeeding responses to repeated light stimuli remained relatively stable in size of evoked amplitude. In goldfish injected with thyroxine, the reduction in amplitude in responses to repetitive stimulation was much less than that of those in the control; no significant change was found in this respect in the thiourea-treated fish. Of special interest were supramaximal second responses observed during repetitive stimulation in some of the animals which were treated with thyroxine. Since the supramaximal phase in the cat's cortical evoked response seems to be independent of the retina, and the geniculate excitability cycle does not have a supramaximal phase(8), it is possible that the supramaximal phase in goldfish is a result of local tectal events. It could be formed by either recruitment of previously unresponsive elements, or by increase in amplitude of the elements concerned in the first response. It may be significant that in bullfrogs(7) thyroxine accelerated neuromuscular transmission both by increasing resting muscle membrane resistance, and by enhancing the

sensitivity of the receptor membrane (slow muscle fiber) to released transmitter substance from the nerve endings.

Summary. Effects of thyroxine and thiourea upon optically evoked potentials in the midbrain of goldfish were tested. Thyroid hormone treatment reduced latency, time to reach maximum response, and time to complete the response, and it increased response amplitude. Thiourea had the opposite effects. Thyroxine shortened the "recovery" time between successive stimuli, and thiourea prolonged it. Supramaximal second responses were found when light flash stimuli were repeated at 1/5 to 1/10 second intervals in some of the thyroxine-treated fishes. The data suggest that thyroxine has a facilitating action upon tectal synaptic events.

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Serial Cultivation of Human Leukemic Cells.* (31166)

DONALD ARMSTRONG† (Introduced by Werner Henle)
Virus Laboratories, Children's Hospital of Philadelphia, and School of Medicine, University of Pennsylvania, Philadelphia

The use of leukemic cells in studies of avian and murine leukemia has illuminated virus-cell relationships and antigenic properties of the cells(1,2). Long term serial cultures of leukemic cells have afforded convenient tools for study of the chicken and mouse diseases(1,3,4). The first human neoplasm of lymphocytic origin grown as a continuous suspended cell culture was the lymphoma of Burkitt(5,6). More recently, serial cultivation of human leukemic leukocytes has been achieved(7,8,9). This report concerns 3 serial cultures of human leukemic cells which have been initiated from pediatric patients, 2 with acute lymphoblastic leukemia and one with acute myeloblastic leukemia. The cultivation of the cells in suspension and observations on the cultures are described.

Materials and methods. Medium. Medium RPMI1629(7), a variation of McCoy's medium(10) was used as the growth medium in the development of all 3 serial cultures. The medium was supplemented with 25% inactivated (56°C, 30 minutes) fetal calf serum, 100 units of penicillin and 100 μg of streptomycin sulphate per ml. After cells were multiplying, they were grown also on Eagle's basal medium (BME) and Eagle's minimum essential medium (with glutamine 2 mM per ml and non-essential amino acids 0.2 mM per ml) supplemented with inactivated fetal calf serum, 10 to 25%, and the antibiotics in the concentrations mentioned.

Preparation of cells for culture. Blood (10 to 30 ml) was drawn from peripheral veins of leukemic patients into plastic 30 ml syringes containing 0.5 ml of heparin. After

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† Special Fellow (5-F3-CA-13,562) of Nat. Inst. Health. Present address: Memorial Sloan-Kettering Cancer Center, New York.