STUDIES ON THE ROLE OF ARTIFICIALLY INDUCED EEG CHANGES IN THE REGULATION OF ANTERIOR PITUITARY FUNCTIONS

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ABSTRACT OF THE THESIS

Studies on the Role
of Artificially Induced EEG Changes
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The electroencephalogram (EEG) of the chicken hypothalamus was recorded under the influence of repetitive photic stimulation, while thyroid activity was assessed by determining blood protein bound ^{131}I levels in sequential samples. EEG waves in regions examined were distinctly entrained with a flicker frequency. The blood PB^{131}I level was increased by 20 min photic stimulation of 3 cps and was decreased by 12 cps flicker, while 24 cps flicker exerted no effect on thyroid function. Blood PB^{131}I level was increased by TSH and TRH treatments. These results indicate that the frequency-specific effects are mediated by changes in the hypothalamo-pituitary function.

Blood PB^{131}I was determined in chickens which were exposed to cold and photic stimulation. Slight increase in thyroid activity was resulted from 9 cps

flicker in chickens at a room temperature. When the birds were exposed to cold only, a slow but significant elevation in $PB^{131}I$ levels was resulted. On the other hand, the birds exposed to cold did not indicate any sign of increase in $PB^{131}I$ when 9 cps flicker was delivered to them. The results suggest that this photic stimulation modifies the responsiveness of the hypothalamo-pituitary function to cold exposure.

In the third experiment concentrations of corticosterone, immunoreactive thyrotropin (TSH) and growth hormone (GH) in plasma were measured 25 min after repetitive photic stimulation of 3, 9, 15 and 24 cps in rats. Photically evoked responses were recorded from midbrain reticular formation, amygdala, hippocampus and medial basal hypothalamus. The patterns of evoked responses at all the points examined were altered by the changes in stimulation frequency, forming the specific rhythmic patterns. Mean corticosterone concentration was increased but no significant change was demonstrable in mean TSH and GH levels. The results suggest that corticosterone concentration is dependent on the electrophysiological state of the CNS which is characterized by the specific rhythmic evoked responses.

ACKNOWLEDGMENTS

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I also wish to express my deepest appreciation to Drs. Jiro Goto, Koji Sato, Shunzo Oshima and all my colleagues at the department of animal physiology for their participation in discussing research problems during my Ph.D. course.

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INTRODUCTION

Homeostasis is an essential characteristic of vertebrates to preserve a constant and optimal internal environment as a necessity of normal function. Homeostatic mechanisms involve the two primary integrating systems, the nervous system and the endocrine system. The former sends electrical impulses via neurons as information, while the latter transmits information by means of chemical messengers, the hormones, throughout the circulatory system. Because their roles are similar, it is not surprising that they are closely related.

During last decades, the role of the central nervous system (CNS) in regulating the anterior pituitary function has received attention from many investigators. Due to unique anatomic arrangements between the hypothalamus and the anterior pituitary, this axis has been extensively explored with respect to its physiological implications. It is now generally considered concerning the mode of information transmission that the control of anterior pituitary hormones is manipulated by means of neurohumors which are elavorated in the hypothalamus, transported to the hypophysial portal veins and then carried to the anterior pituitary. These hypothalamic

neurohumors which stimulate synthesis and release of the anterior pituitary hormones such as ACTH, TSH, GH, LH and FSH are called corticotropin releasing factor (CRF), thyrotropin releasing hormone (TRH), GH releasing hormone (GRH), LH releasing hormone (LRH) and FSH releasing hormone (FRH), respectively. TRH has been identified as a tripeptide (Folkers et al., 1969; Mitnick and Reichlin, 1971; Bassiri and Utiger, 1974). LRH and FRH seem to be a decapeptide (Baba et al., 1971; Redding et al., 1971; Schally et al., 1971). Purification, isolation and identification of other releasing hormones are in progress. On the other hand, the hypothalamic neurohumor which inhibits synthesis and release of prolactin from the anterior pituitary is known as prolactin inhibiting factor (PIF). Studies of growth hormone inhibiting factor are also now under a way of investigation.

The pathways of information transmission between the CNS and endocrine systems are illustrated in Figure 1. Environmental stimuli cause release of hypothalamic releasing factors via neurons. Pituitary hormones released by releasing factors stimulate tissue, or the target organs to secrete hormones. On the other hand, excess amount of peripheral and pituitary hormone exerts inhibitory effects on the anterior pituitary and hypothalamus as well as other structures of the CNS such as

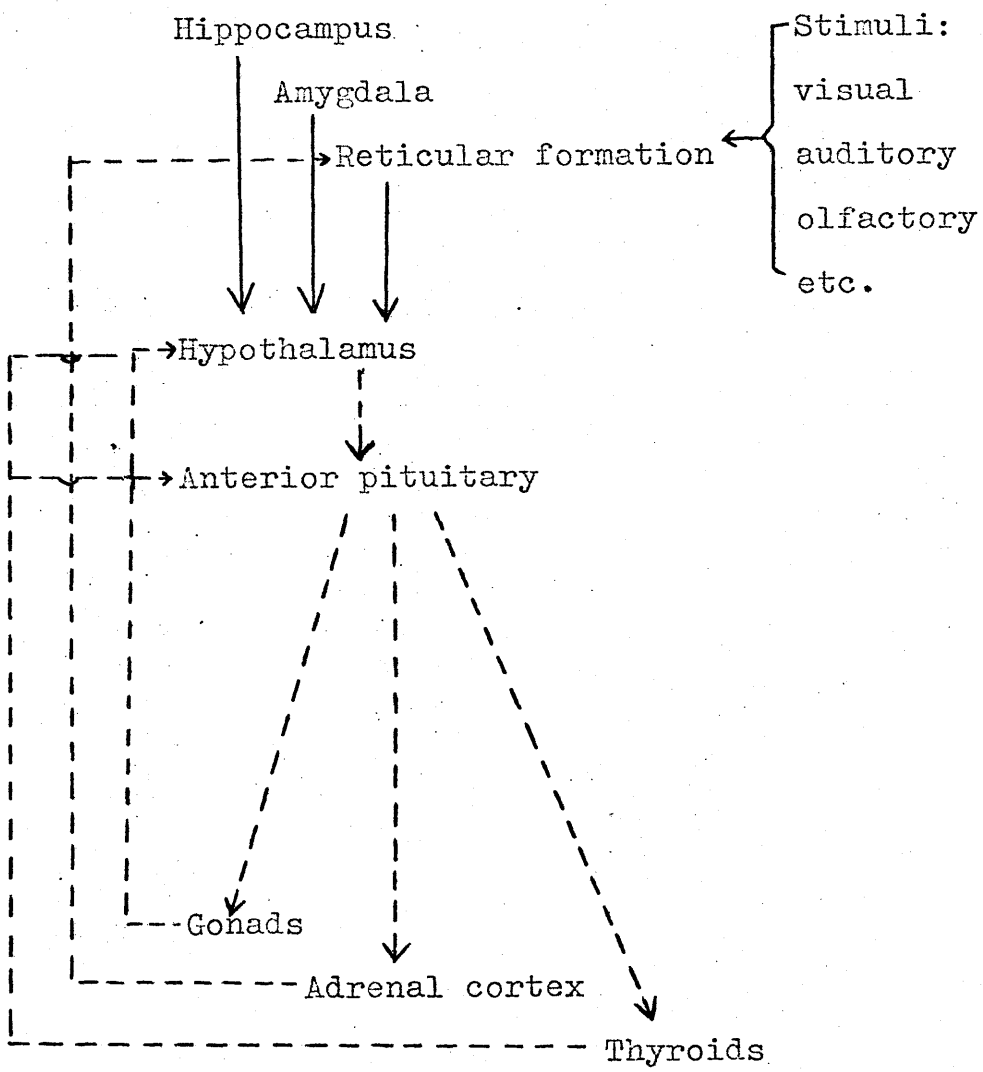

Figure 1. The pathway of information transmission between the central nervous system and the endocrine system. (------, hormonal; ———— , neural)

amygdala, hippocampus and reticular formation. The sites of negative feedback actions vary depending upon the hormones. The hypothalamo-anterior pituitary system thus plays an important role in homeostasis.

Studies attempting to elucidate regulatory mechanisms of the CNS in controlling anterior pituitary functions have been based on the techniques of stimulation and lesion at the hypothalamic points, and pituitary transplantation. It is not too much to say that recent progress in neuroendocrinology is largely dependent upon them as well as precise determination of hormones by radioimmunoassay which requires only minute volume of plasma sample. However, these studies have still certain limitation for depicting dynamic activity of neurons in the CNS responsible for hypothalamic hypophysiotropic activity. There have been many studies in attempt to correlate the endocrinological phenomena with electrical activity in the CNS. Pioneer studies have been performed at first by group of Sawyer and Kawakami. EEG after-reaction is a unique pattern of the EEG, since it represents patterns analogous to those in arousal state in animals assuming a state similar to that during sleep. They reported that appearance of such phenomenon was influenced by copulation and vaginal stimulation (Sawyer and Kawakami, 1959; Kawakami and Sawyer, 1959). Since

their studies have visualized real phenomena occurring in the brain (electrical activity) associated with changes in endocrine activity, they stimulated investigators to carry out many other works demonstrating relationship between the CNS activity and endocrine functions. The electrical activities used as indexes of the CNS activity are classified as three categories: EEG, multi-unit activity (MUA) and single unit activity. In most studies effects of hormones are demonstrated on the basis of changes in electrical activity of the CNS before and after hormone treatment: i.e. changes in the amount of paradoxical sleep (kawakami et al., 1964), certain frequency components of the EEG pattern (Kawakami et al., 1964; Shimada, 1974), integrated value of MUA (Terasawa et al., 1969; Michal, 1974) or responsiveness of single neurons in the CNS to hormone treatment (Slusher et al., 1966; Kawakami and Saito, 1967; Kawakami and Kubo, 1971; Pfaff et al., 1971; Ondo and Kitay, 1972; Van Deleft and Kitay, 1972; Nagler et al., 1973). These studies might show both acute and chronic effect of hormones on the CNS activity by continuous recordings of electrical activity. These are conventional manners of experiments attempting to find some relationship between hormone action and neural functioning. That EEG frequency components or individual neuronal activity is

affected by hormone administration does not substantially mean that they reflect the specific actions of the CNS in regulating hormone release in question. It seems extremely difficult to advance the study further to reveal regulatory mechanisms of the CNS in controlling anterior pituitary functions by the approach employed in the previous studies.

Increase or decrease in discharge frequency observed in the peripheral nerves is generally considered as a mode of information transmission from the peripheral receptors to the CNS in response to stimuli. These informations are transmitted to many different neurons in the CNS and a consequence of interaction of neuron-to-neuron conveyed to the effector organs.

There are many reports in respect to regulatory mechanisms of the CNS controlling blood glucose levels. Depending upon blood glucose levels, changes in firing rate of single neurons of the vetromedial hypothalamus and the lateral hypothalamus are observed. It is generally considered that these specific neurons function in the regulation of the blood glucose levels by increasing or decreasing firing rate. However, these studies do not refer to links between activity of these neurons and anterior pituitary functions. Also, most of these studies have been performed in animals under anesthesia

which largely alters normal function of the anterior pituitary such as GH (Schalch and Reichlin, 1966), ACTH (Cook et al., 1973) and TSH (Brown and Hedge, 1972) secretion. To my knowledge, there has been no electrophysiological study which has successfully demonstrated specific neuronal actions controlling the anterior pituitary activity. It seems reasonable to assume that the anterior pituitary may be controlled not merely by increasing or decreasing the frequency in discharge of a set of neurons with a fixed function, but rather by reactions of interrelated neurons with stochastic properties. It is estimated by Schade (1970) that there are 400 to 3,000 synapses for a single hypothalamic neuron and 125,000 to 150,000 neurons at the hypothalamus. It is generally accepted that neurons which produce releasing factors are localized at the basal medial hypothalamus (hypophysiotropic area). However, one might imagine a difficulty to specify hypothalamic neuron which produces specific releasing factor and to record the electrical activity of this neuron. Group of Feldman and Dafny (1970) who demonstrated changes in responsiveness of hypothalamic neurons but could not reveal fixed direction of the responsiveness to sensory stimuli or glucocorticoid treatment in their extensive study using statistical analyses on the bases of firing rate, fre-

quency- and interval-histograms. Their results led the author to leave out tentatively the view to deduce possible integrating model of the CNS function from individual neuronal activity showing different directions of responsiveness to the same stimulus. There is no reason to deny hypothesizing that hypothalamic regulation of anterior pituitary function might be performed by forming a certain pattern of neural activity among different members of neurons in the brain. EEG might reflect such a mass activity of the CNS. In their recent review, Rubin et al. (1974) described a correlate of the episodic release patterns of anterior pituitary hormones to sleep stages which are defined by descriminating EEG patterns.

If it is possible to maintain a constant state in the brain specifically associated with the anterior pituitary function, one might suggest that the electrical activity of the CNS in such a state might be related with a control of endocrine functions. Repetitive photic stimulation has been known to entrain a constant pattern of the EEG. This phenomenon, EEG driving, seems to provide a constant state of the CNS.

Accordingly, the present study was undertaken in attempt to elucidate a possible role of the rhythmic EEG waves which may affect excitability of hypothalamic

neurons in the regulation of the anterior pituitary function. This dissertation consists of the three experiments as follows. (1) The rhythm of the CNS activity was induced by photic stimulation and then, corresponding alterations in thyroid activity were assessed in the chicken. (2) The second experiment was undertaken to study the effect of photic stimulation on the responsiveness of hypothalamo-pituitary-thyroid axis of the chicken to cold exposure. (3) The third experiment was carried out in the rat to study the relationship between the CNS and anterior pituitary function, because the rat has been the subject of extensive neuroendocrine studies. In this species, plasma corticosterone was assessed after photic stimulation in the wakeful and anesthetized states. Plasma TSH and GH levels were also determined and effect of photic stimulation on EEG waves and anterior pituitary functions were discussed.

MATERIALS AND METHODS

Experiment I: Effects of Photic Stimulation on Hypothalamic EEG and Thyroid Activity in the Chicken

Young broiler chickens weighing from 800 to 1000 g (35-55 days) were used in this experiment. They were kept in cages at a room temperature of 25 ± 2°C and illuminated between 0500 and 1900. Food and water were supplied ad libitum but were withheld during experimental manipulations which were carried out between 0900 and 1400. As no sex differences were noted in the parameters of thyroid function and in the EEG examined, the data from male and female birds were combined.

Photic stimulation was administered in the dark by a photo-stimulator (Sanei Sokki) with a xenon lamp flash of 0.1 msec duration at various frequencies. The flash light was placed at the distance of 20 cm from the birds.

EEG driving

Silver EEG electrodes (0.2 mm diameter, insulated except for a 0.5 mm portion at the tip) were chronically implanted into the hypothalamus and on the dura mater, employing a stereotaxic instrument, and secured to the

skull with dental cement. For EEG recordings, a monopolar lead was used with the reference electrode fixed on the parietal bone. EEG recordings were made on a Sanei Sokki electroencephalograph 3 days after electrode implantation.

At the termination of the experiment a lesion was made at the tip of the electrode by passing a d.c. current, and the birds were killed by decapitation. The brain was removed, fixed in 10 % formalin and stored in 30 % sucrose solution for 3 days, respectively. It was then frozen, sectioned in 25 μm slice and stained with luxol fast blue and cresyl violet for histological verification of the electrode location.

Pituitary-thyroid function

Thyroid activity was assessed by counting the radioactivity of blood protein bound iodine of birds given an intravenous injection of $Na^{131}I$ (carrier free, 300 μCi/kg body weight) 48 hr prior to the first blood sampling. Following heparinization, the brachial vein was cannulated with a polyethylene tubing (ID 0.45 mm), and 0.5 ml blood samples were withdrawn at intervals of 30 or 60 min. After each collection of blood, the same amount of flushing saline was injected. Chickens were restrained in a normal position during the experimental procedures.

In order to avoid the escape of hormonal ^{131}I, 50 mg of human plasma protein was added to the 0.5 ml of blood sample, as chicken plasma protein has less affinity to thyroid hormones than that of mammals. $PB^{131}I$ was precipitated by addition of 10 % trichloroacetic acid and washed three times. The radioactivity of the precipitate was counted to more than 10,000 counts, using a well-type scintillation counter (Kobe Kogyo). Thyroid data were expressed as a $PB^{131}I$ response index according to Brown and Hedge (1972). Results from groups of birds were pooled for each experiment and are represented in the figures by means accompanied by standard errors. Student t-test was used to determine the change of the responsiveness of thyroid activity to different frequencies of photic stimulation. The TSH used was NIH-TSH-B5 (Bovine); synthetic TRH was supplied by the Institute of Protein Chemistry, Osaka University.

Experiment II: Effect of Photic Stimulation on Thyroid Activity in the Cold Exposed Chicken

In the foregoing experiment broiler chickens were used, but the present experiment was performed using White Leghorns since they are more responsive to cold exposure than broiler chickens. The number of the birds used was sixteen males weighing 900 to 1,000 g

(50-60 days). The preparation of animal with brachial catheters and the experimental protocol was the same as that of Experiment I. Briefly, this involves administration of 300 μCi/kg of $Na^{131}I$ 48 hr before the experiments and placement of brachial catheters just before experiments. The present experiments consist of two experiments. First, effect of 9 cps photic stimulation on thyroid activity was studied at a room temperature (25 ± 2°C). Secondary, cages separately containing two groups of birds to be exposed to cold were carried to a cold room (5°C) and left there for 3 hr. A 9 cps flicker was provided to one of the groups for the first 45 min of the cold exposure time while only cold exposure was given to the other group.

Thyroid activity was assessed by counting $PB^{131}I$ as described before. $PB^{131}I$ response index served as the basis of evaluating the pituitary-thyroid function. Synthetic TRH was intravenously injected to the control birds 3 hr after the first blood sampling. Each group consists of 4 birds.

Experiment III: Effects of Photic Stimulation on EEG Activity and Anterior Pituitary Functions in the Rat

A total of 98 young male rats (Wister strain, 8-10 weeks old) weighing 200-250 g were used in these

studies. They were housed for 2-4 weeks under conditions of controlled lighting (illumination from 0500-1900 alternating with 10 hr of darkness) and room temperature (22 ± 2°C). Food and tap water were available at all times.

Photic stimulation was delivered in the dark by a photo-stimulator as described before. The flash light was placed at the distance of 10 cm from the rat.

Evoked potentials

The rats were anesthetized by intraperitoneal injection of brietal-sodium (80 mg/kg). The surgical procedures consisted of exposing the skull and stereotaxically introducing silver bipolar electrodes, with inter-tip distances of 0.5 mm. Electrodes (0.2 mm in diameter) were insulated except for a tip. They were implanted into the medial basal hypothalamus, hippocampus, amygdala, and reticular formation.

The EEG recordings were carried out by an electroencephalograph (Sanei Sokki) in conscious, unrestrained rats placed in a glass beaker and in anesthetized state (Hexobarbital sodium, 250 mg/kg). Photic stimuli were delivered as aforementioned, and the electrical activity amplified by the electroencephalograph, was displayed on ATAC 250 (Nihon Koden) oscilloscope. Fifty sweeps of 200 msec, triggered with photic stimuli,

were added and the readout was made by the polygraph (Nihon Koden).

Electrode placement in the brain was verified by the atlas of König and Klippel (1963).

Plasma corticosterone, TSH and GH levels

Twenty three of the total rats were subjected to photic stimulation for 25 min at 1200 under unanesthetized, unrestrained conditions. Two or three rats were placed in small boxes with mirrors so that the flash light was diffusely provided to all rats throughout the experiment.

The effect of photic stimulation plasma corticosterone levels was also examined in another 37 anesthetized rats. Since anesthesia augments plasma corticosterone levels, relatively large dose of dexamethasone (200 μg/100 g body weight, Merck Co. Ltd) freshly dissolved in saline was injected subcutaneously at 0900, while controls received saline only; 3 hr later (at 1200), dexamethasone-treated rats were anesthetized by 25 mg/100 g body weight of hexobarbital sodium (HXB) and immediately subjected to photic stimulation for 25 min. At the termination of the experiment, rats were decapitated and trunk blood was collected within 2 min following onset of cage opening. Plasma was separated by centrifugation (2500 rpm, 15 min), frozen and stored at -20°C

until determination of hormone levels. Corticosterone concentrations were determined fluorometrically by the modified method of Guillemin et al. (1959) and were used as an index of ACTH secretion. Plasma TSH and GH levels of wakeful rats were also determined by radioimmunoassay. This assay was made by the help of Dr. M. Suzuki at the Institute of Endocrinology, Gunma University.

RESULTS

Experiment I: Effects of Photic Stimulation on Hypothalamic EEG and Thyroid Activity in the Chicken

This experiment was carried in 10 birds, with each undergoing two or three electrodes implants. Locations of electrodes were determined by the atlas of van Tienhoven and Juhasz (1962) and are shown in Figure 2. Unexpectedly, EEG recordings taken from the surface were not affected by any of the flash frequencies examined (Figure 3). On the other hand, those taken widely distributing points in the hypothalamus, extending ventromedial area to the anterior commissure, to the medial area to the caudal half of the nucleus rotundus (Figure 2), showed profound alterations elicited by photic stimulation (Figure 3 and 4). The frequency of EEG waves was almost identical with that of photic stimulation (Figure 4). The amplitude of the evoked response was high when the flicker frequency was low and tended to decrease with an increase in the frequency. Although monopolar electrodes were employed in the present study, essentially the same results were obtained in the pattern when evoked responses were recorded in

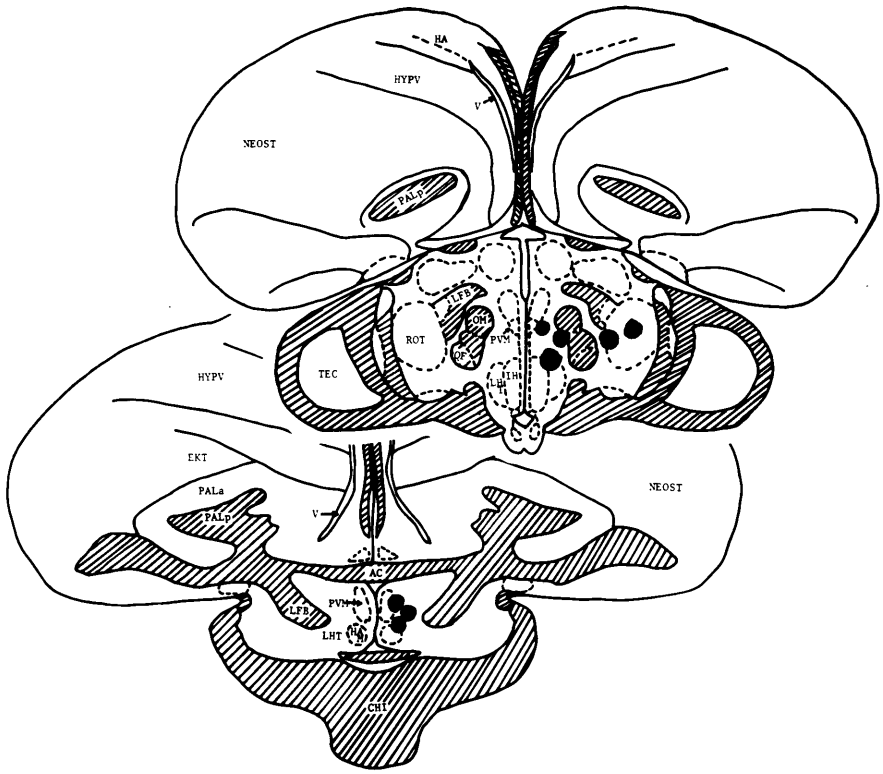

Figure 2. Position of recording electrodes in the anterior and posterior hypothalamus (indicated by dots). AC, anterior commissure; CHI, optic chiasma; HA, hyperstriatum accessorium; HAM, nucleus hypothalamicus lateralis anterior medialis; HYPV, hyperstriatum ventrale; LFB, lateral forebrain bundle; LH, Lamina hyperstriaticus; LHT, nucleus hypothalamicus lateralis; NEOST, neostriatum; Palp, paleostriatum primitivum; PVM, nucleus paraventricularis magnocelluralis; QF, quintofrontalis; ROT, nucleus rotundus; SM, tr. septomesencephalicus.

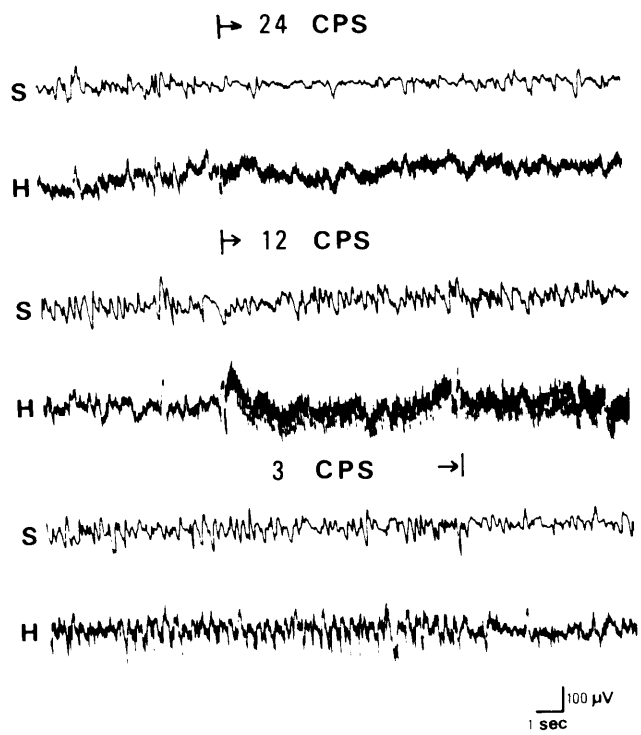

Figure 3. Effect of repetitive photic stimulation on EEG patterns in brain surface and hypothalamus (note clear EEG driving in hypothalamus but not in brain surface).

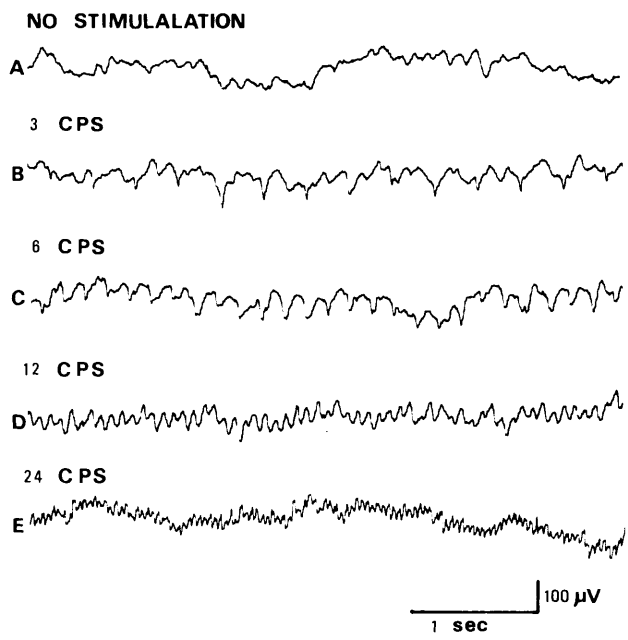

Figure 4. Effect of repetitive photic stimulation of 3, 6, 12 and 24 cps on EEG in hypothalamus (Ventrocaudal area to anterior commissure).

the hypothalamus and nucleus rotundus by using bipolar electrodes (Figure 5).

Figure 6 shows the blood $PB^{131}I$ levels of sequential samples in control birds over a 4 hr experimental period. A gradual linear decrease was observed. Intravenous injection of TSH (50 mU/kg body weight) or TRH (50 µg/kg body weight) caused a marked elevation of $PB^{131}I$, indicating a rapid increase in thyroid glands were responsive to their tropic hormones under the experimental conditions used.

With 24 cps flicker, no effect on thyroid activity was observed and the $PB^{131}I$ response index featured the same decline as the control. The decrease in $PB^{131}I$ caused by 12 cps flicker indicates that the situation in the hypothalamus under these conditions is associated with the inhibition of TSH and thyroid secretion. In contrast, the state in the CNS induced by 3 cps flicker resulted in an increase of thyroid function (Figure 7).

Experiment II : Effect of Photic Stimulation on Thyroid Activity in the Cold Exposed Chicken

EEG recordings were not made in White Leghorns, but similar distinct evoked potentials can be observed at the different sites of hypothalamus in response to

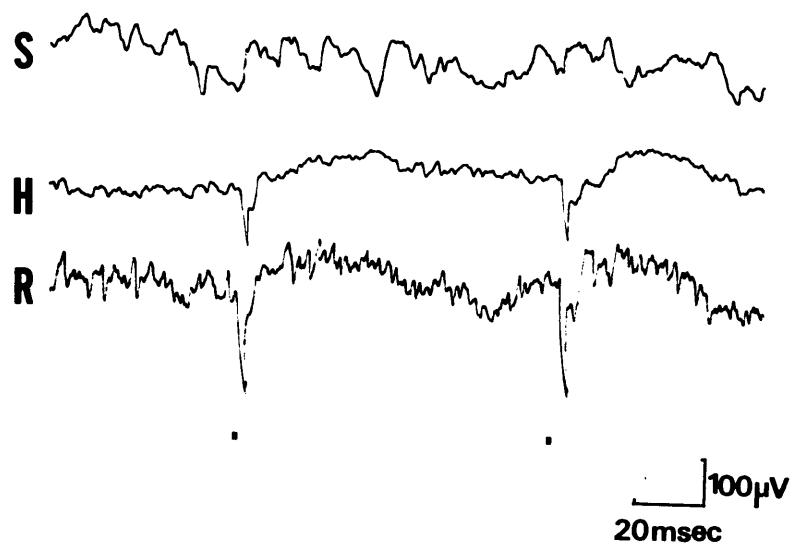

Figure 5. Bipolar recordings of the EEG in brain surface (S), hypothalamus (H) and nucleus rotundus (R) evoked by photic stimulation (dots).

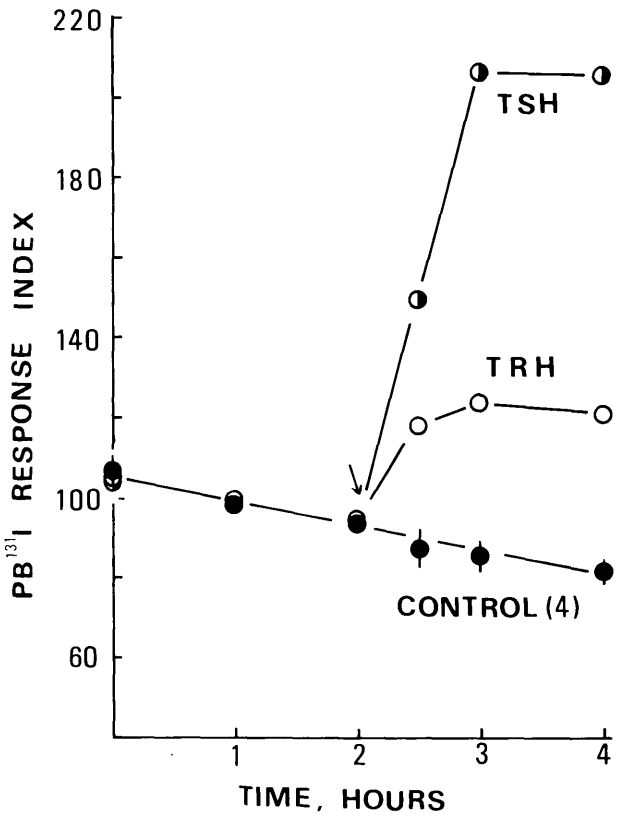

Figure 6. $PB^{131}I$ levels during 4 hr experimental period in untreated birds and effect of TSH or TRH injection; arrow indicates time of injection. Index is calculated by averaging cpm of first 3 samples taken prior to experimental manipulation, and then expressing cpm of all samples from that birds as a percentage of this average. Change in index from 1 to 2 and 3 hr in control birds: -4.3 ± 2.5 and -12.5 ± 3.9 (mean $\pm$ SE).

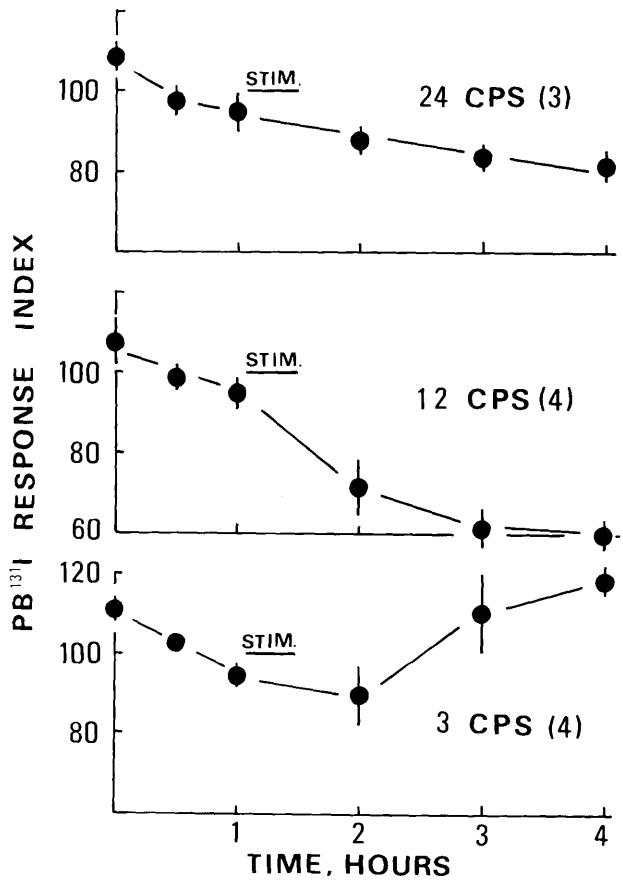

Figure 7. Thyroid response to repetitive photic stimulation of 3, 12, or 24 cps. Change in index from 1 to 2 and 3 hr: 24 cps, -7.1 ± 0.7 and -11.4 ± 1.8; 12 cps, -19.2* ± 2.5 and -42.4* ± 4.6; 3 cps, -4.4 ± 3.6 and +26.2** ± 4.0 (* and ** indicate difference from control value statistically significant at 5 and 1 % level, respectively).

photic stimulation (personal communication with J. Yano). Figure 8 shows the blood $PB^{131}I$ levels of sequential samples over a 3 hr experimental period in White Leghorns. The same linear decline was also observed in the control birds as seen in broiler chickens. Intravenous injection of TRH resulted in a sharp increase in $PB^{131}I$. This is suggestive of activation of TSH release and an immediate rise in thyroid secretion.

Slight increase in thyroid activity was elicited by 9 cps flicker in the chicken at a room temperature. When the birds were maintained in a cold room, a slow but significant increase in $PB^{131}I$ levels was resulted (Figure 8). On the other hand, photically stimulated birds did not indicate any sigh of augmentation in $PB^{131}I$, although they were exposed to cold. They showed the same decline of the $PB^{131}I$ levels as seen in birds in the room-temperature.

Experiment III : Effect of Photic Stimulation on EEG Activity and Anterior Pituitary Functions in the Rat

EEG activity

The locations of the EEG electrodes are illustrated in Figure 9. Patterns of the background activity of amygdala, reticular formation and anterior hypothalamus monitored before and after anesthesia are shown in

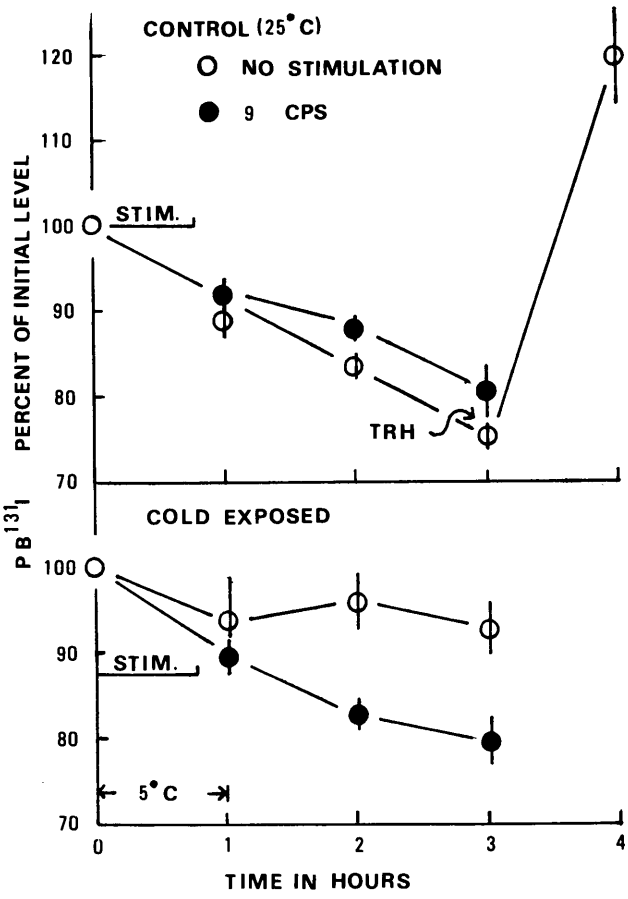

Figure 8. Thyroid response to repetitive photic stimulation of 9 cps and to cold exposure (N=4).

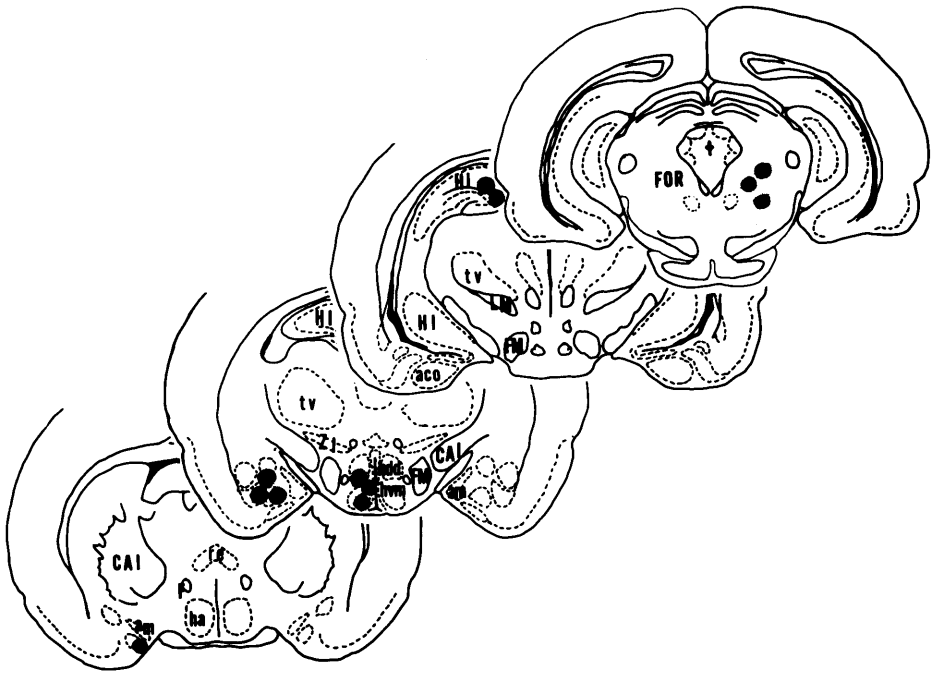

Figure 9. Position of recording electrodes in various points in rat brain. The dark points indicate the location of the tip of the electrode. (am, nu. amygdaloideus medialis; aco, nu. amygdaloideus corticalis; CAI, capsula interna; F, columna fornics; FM, fasciculus medialis prosencephali; FOR, formatio reticularis; ha, nu. anterior hypothalamus; hdd, nu. dorsomedialis hypothalamus; HI, hippocampus; hvm, nu. ventromedialis hypothalamus; LM, lemniscus medialis; re, nu. reuniens; tv, nu. ventralis thalamus; ZI, zona incerta).

Figure 10. EEG recordings from the unanesthetized, unrestrained animals showed low-amplitude, fast wave patterns. At the beginning of anesthesia (10-20 min), more slow-wave patterns appeared and an increase in the amplitude of EEG waves was seen in amygdala and midbrain reticular formation of most rats. In some instances the EEG showed a reduced amplitude soon after injection of the same dose of HXB. The EEG recording from anterior hypothalamus shown in Figure 10 was one of them; Arousal EEG waves were replaced by sharp spikes. The spikes slowly decreased in frequency. The extent of this slowing process in EEG activity varies with individual rats. In contrast to the clear photically evoked response in the chicken, in the rat the evoked response was hardly observable in the recording of the EEG background activity under both wakeful and anesthetized conditions. However, when the responses were summated, one could confirm that photic stimulation provoked a well defined response in all the areas of the brain examined (Figure 11, 12, 13 and 14). Since the photically evoked response occurred with a long lasting latency, the basic patterns of the evoked potential was first recorded at lower frequencies such as 0.25 and 0.5 cps. Alterations in the pattern of evoked responses by increasing flicker frequency were observed. Waves forms

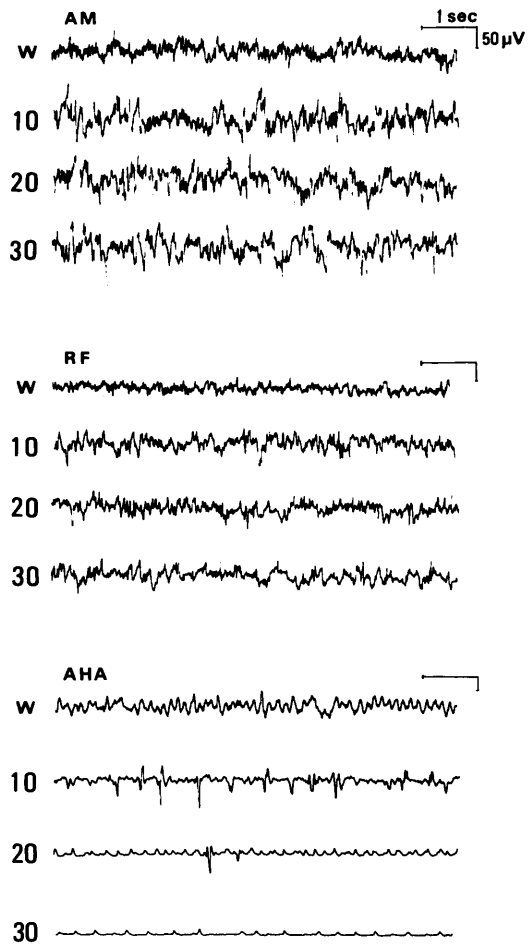

Figure 10. EEG patterns in amygdala (AM), reticular formation (RF) and anterior hypothalamus (AHA) in waking (W) and anesthetized states (10, 20 and 30 min after hexobarbital injection). These recordings were taken in different rats.

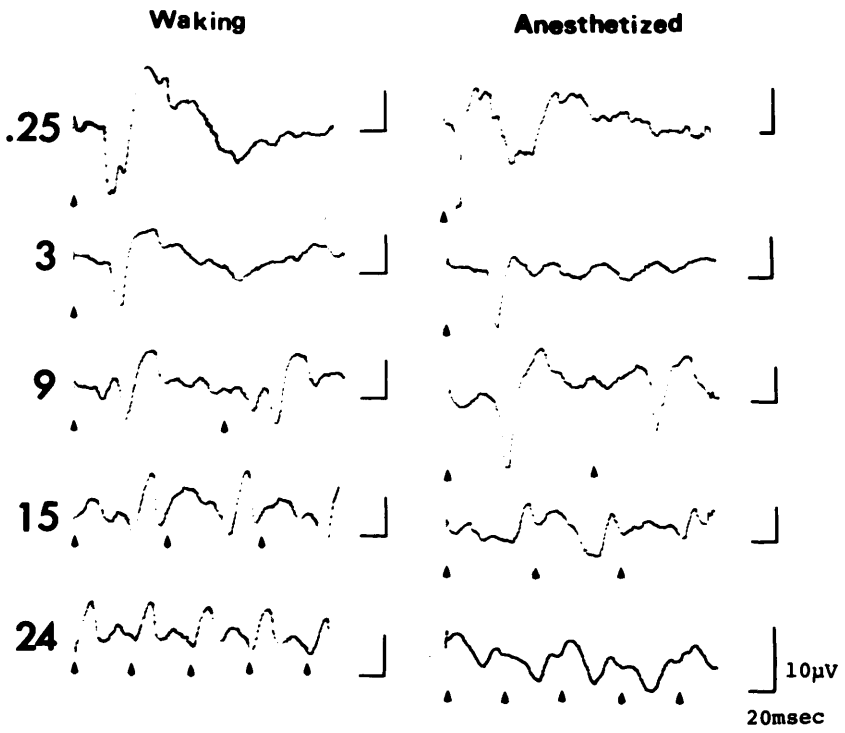

Figure 11. Average photically evoked responses in reticular formation in waking and anesthetized states. Figures in left denote frequency of photic stimulation (cps). Dots indicate the stimulation.

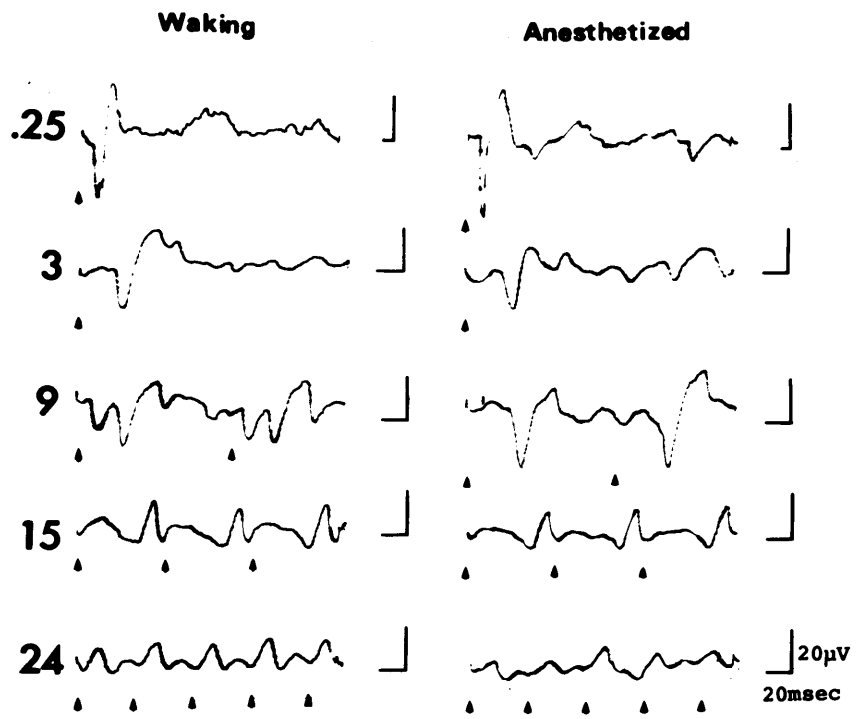

Figure 12. Average photically evoked responses in amygdala in waking and anesthetized states. Legend same as for Figure 11.

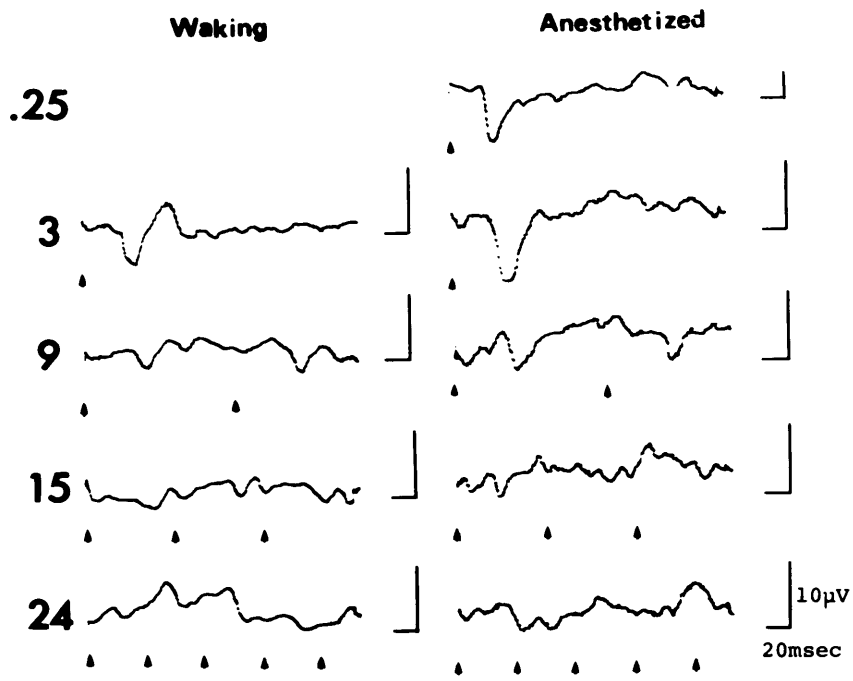

Figure 13. Average photically evoked responses in hippocampus in waking and anesthetized states. Legend same as for Figure 11.

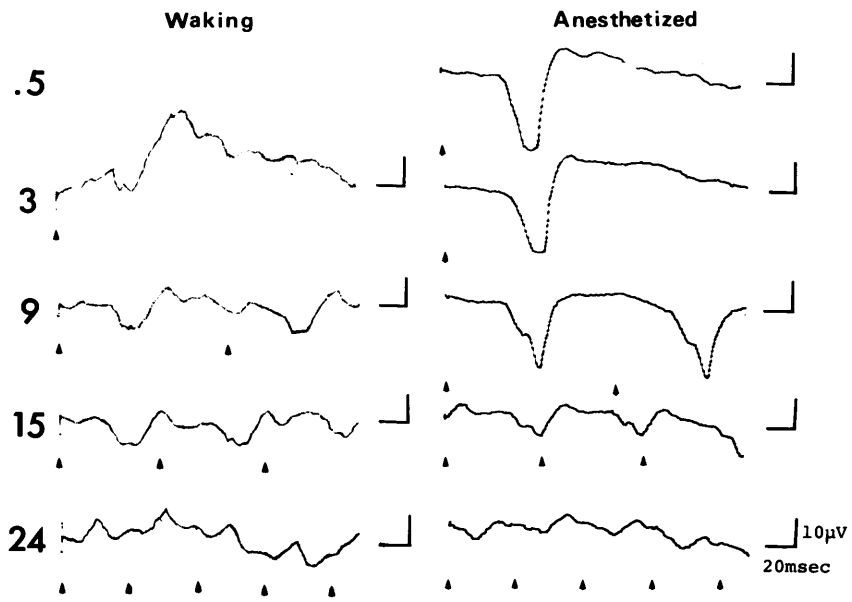

Figure 14. Average photically evoked responses in medial basal hypothalamus in waking and anesthetized states. Legend same as for Figure 11.

of evoked potentials in response to 0.25 and 3 cps photic stimulation are essentially the same and these potentials consist in most cases of an initial negative large, positive and long latency negative responses. The peak-latency of the initial negative potentials in the wakeful rat was longer than that in anesthetized rat. In the range of 0.25 and 9 cps, the peak-latency tended to prolong with an increase in flicker frequency in all the points under both wakeful and anesthetized states. At higher frequencies such as 15 and 24 cps, the initial negative potential disappeared because the time intervals of repetitive photic stimuli were shorter than the recovery time of the response. Therefore, the peak-latency and amplitude of the initial negative potentials were undetected.

Plasma corticosterone levels

Effect of photic stimulation on plasma corticosterone levels was investigated in dexamethasone- and HXB-treated rat (DHT rat). The result is shown in Figure 15. As was expected, plasma corticosterone was markedly elevated with HXB injection alone whereas it was suppressed 3 hr after subcutaneous injection of dexamethasone ($p<0.01$). When the rats were treated with both dexamethasone and HXB, the corticosterone levels were about the same as the control level, indicating

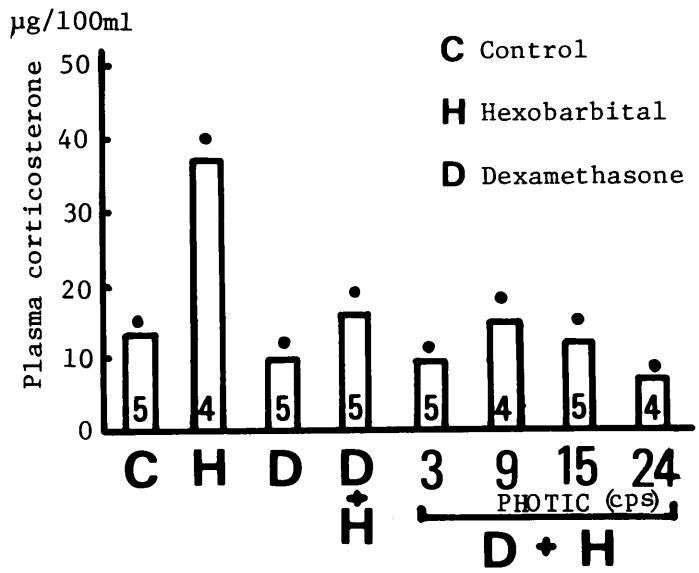

Figure 15. Plasma corticosterone levels in response to repetitive photic stimulation of 3, 9, 15 and 24 cps in anesthetized state. Figures in columns denote number of rats; dots indicate standard errors.

that the increase in corticosterone levels elicited by HXB was blocked by dexamethasone treatment. No significant difference was found between corticosterone levels in DHT rats and those in DHT rats which received 9 cps photic stimulation. Unexpectedly, plasma corticosterone levels in DHT rats were significantly decreased by 3 ($p < 0.01$), 15 ($p < 0.05$) and 24 ($p < 0.01$) cps photic stimulation.

In wakeful rats, the repetitive photic stimulation caused marked elevation in the corticosterone levels as seen in Figure 16. The increasedlevels of corticosterone affected by all the flash frequencies were significantly different from the control level ($p < 0.001$). A maximum increment in plasma corticosterone was produced with 15 cps flicker. This increased level was significantly different from those of 3 and 9 cps groups ($p < 0.01$) but not from that of 24 cps group. The level of 9 cps group (30.2 ± 4.3 µg/100 ml plasma) was significantly different from those of 15 and 24 cps groups ($p < 0.01$) but not from that of 3 cps group. There was the largest difference in the corticosterone level between 9 and 15 cps groups. This difference was 14.5 µg/100 ml plasma which was 47.3 % of the level of 9 cps group.

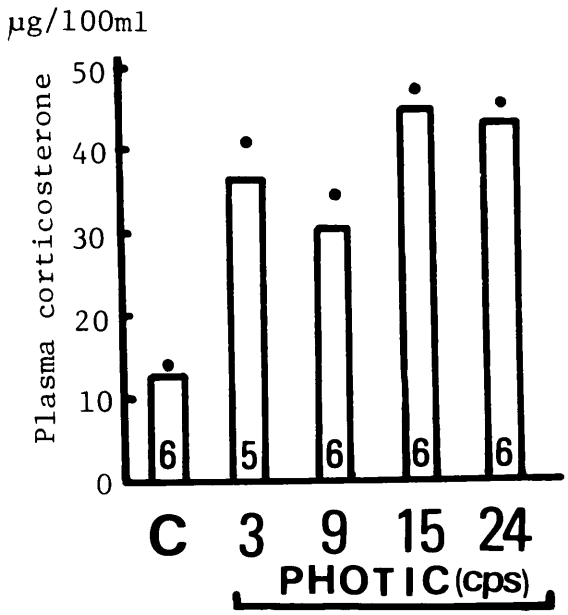

Figure 16. Plasma corticosterone levels in response to repetitive photic stimulation of 3, 9, 15 and 24 cps in waking state. Legend same as for Figure 15.

Plasma TSH and GH levels

Effect of Photic stimulation on plasma TSH levels is shown in Figure 17. The control level of TSH was 0.56 ± 0.13 mU/ml. TSH release seemed to be decreased by 3 and 9 cps photic stimulation whereas it was enhanced when 15 or 24 cps flicker was delivered. However, the difference between the levels of any groups was not statistically significant.

Figure 18 shows changes in GH level after photic stimulation. The control level of GH was 76.91 ± 26.01 ng/ml. Photic stimulation seemed to cause reduction of GH release from the anterior pituitary. Decreased GH levels, however, were not significantly different from the control level.

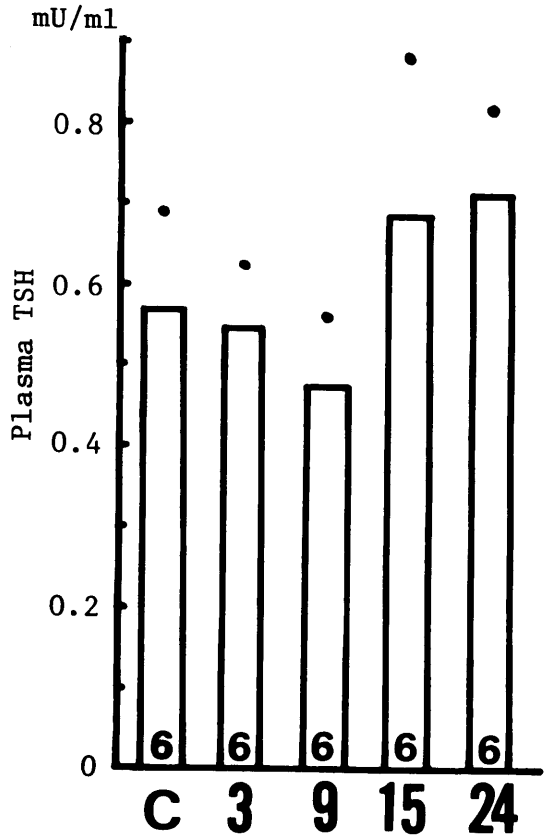

Figure 17. Plasma TSH levels in response to repetitive photic stimulation of 3, 9, 15 and 24 cps in waking state. Legend same as for Figure 15.

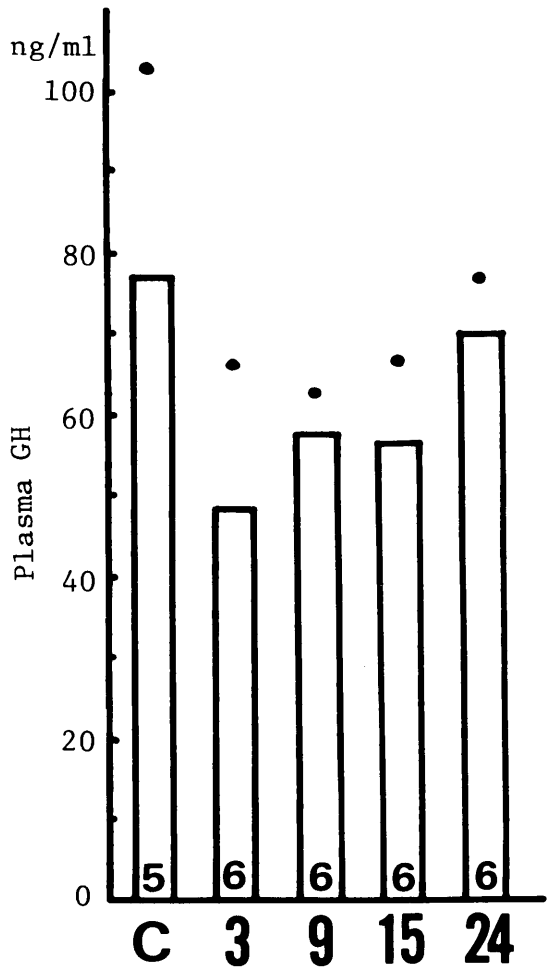

Figure 18. Plasma GH levels in response to repetitive photic stimulation of 3, 9, 15 and 24 cps in waking state. Legend same as for Figure 15.

DISCUSSION

Thyroid Responses to Photic Stimulation in the Chicken

In recent years considerable attention has been focussed upon the neural control of pituitary-thyroid function. It has been reviewed at regular intervals by several investigators, namely, D'Angelo (1963), Reichlin (1966), Brown-Grant (1966) and Knigge (1971). It was first demonstrated by Greer (1951) that hypothalamic lesions prevented the goitrogenic response to thiouracil feeding, which induced thyroxine defficiency and, in turn, TSH hypersecretion. This role of the CNS has been supported by many subsequent studies (Bogdanove and D'Angelo, 1959; Reichlin, 1960). Some neural influence on thyroid function are reduced after anterior pituitary is grafted (von Euler and Holmgren, 1956; Khazin and Reichlin, 1961) except when the pituitary is transplanted so as to receive blood supply from the median eminence (Halasz et al., 1962).

Electrical stimulation of the hypothalamus and other sites of the brain has also provided valuable evidence that the pituitary-thyroid function is influenced by neural activities in the brain (Harris and

Wood, 1958; Shizume et al., 1962a, 1962b; Martin and Reichlin, 1972). The specific points of the hypothalamus which control TSH secretion are inconsistent.

Apart from these studies, electrophysiological experiments have a possibility to represent actual phenomena taking place in the brain, which are involved with regulation of the endocrine function. The present study demonstrated defined evoked potentials at various parts of the chicken hypothalamus. In contrast, evoked potential at the brain surface was not observable in the EEG recordings in the chicken. Distinct evoked potentials at the brain surface have been well documented in mammals including man. In the course of studying the influence of repetitive photic stimulation on the EEG of the chicken, evoked potentials were observed not only in the area along the visual path but also in the areas distant from visual path (personal communication with J. Yano).

It is well known that the hypothalamus is linked with the visual path in birds (Benoit, 1964; Sharrer, 1964) as well as mammals (Feldman et al., 1959; Feldman, 1964, 1966; Dafny et al., 1965; Dafny and Feldman, 1967; Bogacz and Wilson, 1969; Sarne and Feldman, 1971) and the role of light in the neuroendocrine system is well established (Critchlow, 1963). Some electrophysiolog-

ical studies have indicated the relationship between thyroid hormone and brain function. Exogenous thyroid hormone influences visual evoked potentials as shown by changes in the latency and amplitude in the cat (Short et al., 1964), goldfish (Hara et al., 1966) and man (Short et al., 1968). These studies did not attempt to delineate the neural control of the pituitary-thyroid axis but attempted to demonstrate the effect of hormone on the CNS.

Many previous studies demonstrated that electrical stimulation of the hypothalamus caused release of TSH in anesthetized animals (Harris and Woods, 1958; Shizume et al., 1962a, 1962b; Martin and Reichlin, 1972). However, these studies did not always elucidate the electrophysiological aspects concerned with stimulation and hypothalamic TRH release activity. TSH secretion is difficult to elicit by electrical stimulation in intact, unanesthetized animals because of the fact that avoidance and aversive reactions occur with ventro-hypothalamic stimulation of sufficient intensity (Martin and Reichlin, 1972). In these studies, little attention was paid to the EEG of the hypothalamus during or after electrical stimulation.

The present findings demonstrate that thyroid hormone release was increased by electrical activity in

the hypothalamus represented by a frequency of 3 cps EEG waves, the release was inhibited when the EEG was entrained to 12 cps waves. This frequency-specific effect and the failure of a 24 cps flicker to affect thyroid activity were to prove that the effects are not due to a non-specific, simple excitation caused by lighting. This view might also be supported by the additional result that no effect was exerted on heart rate by any frequencies of flicker in unrestrained chickens under the same experimental conditions (unpublished data, Oshima) using radio telemetric measurement (Shimada and Oshima, 1973).

The rapid and considerable degree of alteration in the $PB^{131}I$ response to photic stimulation as compared to that seen with a relatively large dose of TRH (50 μg/kg body weight) would allow for the possible interpretation that the response is attributable to a change in endogenous TSH release. Newcomer and Huang (1974) also reported that 10-20 day-old chicks were responsive to TRH (50 to 400 μg/100 g body weight). Ochi et al., (1972) reported no effect of TRH on chick thyroid activity. We also have undertaken a similar experiment to study the effect of TRH on day-old chick thyroid activity by measuring the thyroidal ^{131}I uptake. No change in the thyroid activity was observed following TRH injection. These results indicate that the pituitary

gland of day-old chick is not responsive to TRH for releasing TSH and it takes some days for the pituitary gland to be responsive to its releasing hormone. The role of hypothalamus in regulation of TSH release in the chicken is as yet incompletely understood as compared with that of mammals. Kanematsu and Hikami (1970) have indicated a reduction of ^{131}I uptake by thyroid following destruction of the region of the anterior hypothalamus. EEG alteration was induced by flicker in the area coincident with the region described in that report. But at present, this finding does not contribute directly to the delineation of the anatomic location of the thyrotropin area in the hypothalamus of the chicken.

It is well known that thyroid function is dependent upon the environmental temperature and that exposure to a colder environment leads to increased activity of the thyroid gland (Brown-Grant et al., 1954; Reichlin, 1966). This alteration in thyroid activity, of course, is related to stimulation of TSH release from the anterior pituitary. Accordingly, the second experiment prompted to investigate how the hypothalamo-pituitary-thyroid activity in response to cold exposure is influenced by photic stimulation which entrains the EEG pattern.

When photic stimulation (9 cps) was provided to

the birds in addition to cold exposure, they reacted by significant reduction in thyroid activity as compared to birds exposed to cold only. Brown and Hedge (1972) have elegantly demonstrated that acute cold response showing an increase in thyroid activity was completely blocked by pretreatment of thyroxine. It has been reported excess amount of thyroxine causes its suppressive action on the pituitary (von Euler and Holmgren, 1956) or hypothalamus (Knigge and Joseph, 1971). Similar result was obtained in the present study but inhibition of thyroid activity was elicited by neural factor. It is unlikely that photic stimulation exerts its effect directly upon either pituitary or thyroid gland. The EEG waves which were entrained by the 9 cps flicker might be involved with alteration in responsiveness of the CNS to cold which normally results in TSH secretion.

Responses of the CNS and Anterior Pituitary to Photic Stimulation in the Rat

In order to elucidate regulatory roles of the hypothalamo-pituitary-adrenal axis, two major techniques such as lesion and stimulation have been employed previously. Divergent opinions, however, still exist with respect to the specific location of hypothalamic neurons which produce releasing factor (Mangili et al., 1966).

Some studies indicate that ACTH secretion is controlled primarily by the medial basal hypothalamus (Ganong and Hume, 1954; Halasz et al., 1967a, 1967b). On the other hand, Dunn and Critchlow (1973b) emphasize the hypothalamic elements directly involved in the ACTH secretion is not in the ventomedial hypothalamus but are localized to discrete hypothalamic sites generally close to the midline. In contrast, Brodish (1963; 1969) and D'Angelo (1964) suggest that the entire region of the ventral hypothalamus, extending from the optic chiasm to the mammillary bodies, is involved in the control of ACTH secretion. Recent study of Redgate and Fahringer (1973) shares the view that no circumscribed anatomic localization for ACTH secretion exists in rat hypothalamus.

Many investigations also have been made on the negative feedback action of ACTH or corticoid on the CNS which inhibits adrenocortical activity. Possible sites of blocking action in the brain are the anterior pituitary (De Wied., 1964; Kloet et al., 1974), the median eminance (ME) (Corbin et al., 1965; Davidson et al., 1968) or other area in the brain (Davidson and Feldman, 1967). These results are obtained in rats with lesions of the ME or corticoid intracerebral implantation. Changes in MUA in the ventromedial nuclei and arcuate nucleus (Sawyer et al., 1968) and in the hippocampus

(michal, 1974) following ACTH or corticoid administration were demonstrated. Feldman and Dafny (1970) showed that alteration in evoked potential to sensory stimuli could be brought about by exogenous corticoid, indicating that steroid feedback action might occur in different limbic structures but main effect was on the hypothalamus. Other studies provide similar indications that different areas in the brain showing hormone-induced changes in unit activity might be sites of blocking action on ACTH release (Slusher et al., 1966; Pfaff et al., 1971; Ondo and Kitay, 1972).

Most of the previous studies have attempted to reveal relationship between the CNS and endocrine system by monitoring electrical activity in the brain following administration of exogenous hormone. An approach of the present study, however, is quite different. A particular electrophysiological state in the brain was artificially induced by photic stimulation and levels of hormones were assessed.

Photic stimulation provoked well defined potentials in the rat (Bogacz and Wilson, 1969; Sarne and Feldman. 1971; Dafny, 1974) but the amplitude of the potentials was much smaller than that of the chicken. The patterns of evoked responses were altered by an increase in frequency of the stimulation and the specific

rhythmic patterns were produced depending on the frequency of stimulation. An increase in plasma corticosterone was resulted from any frequency in the wakeful rat. However, this result indicates that the degree of increase in plasma corticosterone was dependent on the frequencies of photic stimulation. Less increment in plasma corticosterone was produced in waking rats which received lower frequency flicker (3 and 9 cps), while more elevated levels were resulted from higher frequency flicker (15 and 24 cps). Feldman et al., (1969) reported that plasma corticosterone levels were significantly elevated following emitting flashes at the rate of 5 cps for 30 min in waking rats.

For a number of years it has been suggested that the hypothalamo-pituitary-adrenal axis and the hypothalamo-pituitary-thyroid axis are in someway inversely related (Bogoroch and Timiras, 1954; Brown-Grant et al., 1954; Ducommun et al., 1966; Sakiz and Guillemin, 1965). Jobin and Fortier (1965) reported that the inhibition of TSH secretion was observed with enhanced release of ACTH as a result of non-specific stress, whereas exposure to cold concurrently stimulates the secretion of both tropic hormones. This mechanism of the pituitary response to stresses is unknown. These studies have taken no consideration on electrophysio-

logical aspect of the brain activity in response to a non-specific stress or cold exposure. The present study shows similar results to these findings. Photic stimulation of 9 cps increased ACTH secretion whereas it tended to decrease TSH release. On the other hand, 15 and 24 cps photic stimulation elicited activation of adrenocortical function as well as thyroid activity. It is rather difficult to relate the present results to previous findings immediately, but it should be noted that the same modality of stimulus with different frequencies could induce alterations in the state of the CNS and corresponding changes in the anterior pituitary function. Namely, the dissociation of TSH and ACTH release was observed following the stimulation of lower frequency.

Neural control of GH secretion has been well documented (Müller, 1973). Most stimuli such as ether, cold or hypoglycemia have been shown to cause inhibition of GH secretion (Schalch and Reichlin, 1966; Takahashi et al., 1971; Collu et al., 1973) whereas it is reported that pentobarbital causes a rise in GH release (Martin, 1973/74) by affecting neural elements of the brain in the rat. The occurrence of a pattern of intermittent bursts of GH secretion during the time of deeper sleep was known in man (Quabbe et al., 1966; Takahashi et al.,

1968).

No statistically significant change in mean GH concentration was observed in any of the treated groups. It may be attributable, in a part, to unfortunate choice of time when the blood sample was obtained. It has been reported that both plasma and pituitary GH levels varied markedly with the time of day in the rat as compared to those in other species (Schalch and Reichlin, 1966; Takahashi et al., 1971; Dunn et al., 1973/74).

It has been described that anesthetic agents are potent stimuli causing an increase in ACTH secretion (Gibbs, 1970; Brown and Hedge, 1972; Dunn et al., 1972; Cook et al., 1973). In accordance with these reports the present study demonstrates that HXB elicited a sharp increase in plasma corticosterone. A negative feedback action of glucocorticoids on ACTH secretion is well established (Sirett and Gibbs, 1969; Zimmerman et al., 1972; Dallman and Jones, 1973). The results of the present study showing that 200 μg/100 g body weight of dexamethasone significantly decreased corticosterone level of untreated and HXB treated rats are consistent with previous reports. It is particularly striking in the DHT rat that production of plasma corticosterone was reduced by photic stimulation of all the frequencies examined. This finding is contrast to that of wakeful

rats in which sharp increase in plasma corticosterone level was seen. No particular difference was found in EEG evoked responses between wakeful rats and anesthetized rats. But difference in EEG background activity existed.

Feldman et al (1969) have studied inhibitory effect of intrahypothalamic implantation of corticoids on adrenocortical responses to various stimuli. They observed that 30 min of auditory or photic stimulation was most prone to inhibition by steroid implants. They considered that this may be related in someway to the more neurogenic nature of the stimulus. This and our results imply that the ACTH release blocking effect by dexamethasone may not be solely exerted through humoral effect but neurogenic effect, at least in part. In other words dexamethasone may induce the neurological alterations resulting in transmission of the inhibitory signals for ACTH release. The alterations in the CNS activity induced by corticoids have been frequently reported. It seems that the neuronal activity which inhibits the activity of the effector is not exceptional as seen typically in IPSP. Thus, the present results that photic stimulation decreased the corticosterone levels in DHT rat may be explained as the consequence of potentiation of the signals for ACTH

release blocking elicited by dexamethasone. And the extent of this potentiation effect was marked in the rhythm of 3, 15 and 24 cps but not observed in 9 cps. In general, the direction of the effect of artificially induced rhythmic EEG activity on the adrenocortical function seems dependent on the background situation in the CNS, where the inputs of both stimulating and blocking signals mutually interrelated and there are frequency specificity in the extent of the effect irrespectively of the direction of the effect.

From the results obtained in the present study and the foregoing discussion, the situation we have arrived at may be depicted as follows:

a) The specific rhythmic EEG patterns are related with correspondent changes in neuroendocrine activity.

b) However, the mode in involvement of rhythmic EEG pattern in the neuroendocrine regulation is divergent; more precisely, the rhythmic EEG patterns themselves could not be always considered as the information carrier.

c) Thus, the rhythmic EEG patterns may probably affect the coding or decoding process in the neuroendocrine regulation.

At present, the problems concerning the coding in the

CNS are, of course, unsolved. However, the present study may indicate possibility of a new approach in attempt to elucidate the problems of coding and decoding of the CNS in the regulation of endocrine functions by artificially inducing the rhythmic EEG patterns.

SUMMARY AND CONCLUSIONS

1. The present study demonstrated the entrainment of hypothalamic EEG in the chicken by photic stimulation.
2. TRH elicited a marked increase in thyroid activity of the chicken.
3. The flicker frequency-specific effect on thyroid activity is mediated by changes in the hypothalamo-pituitary function.
4. Photic stimulation caused a modifying effect on responsiveness of the hypothalamo-pituitary function to cold exposure.
5. Photic stimulation elicited EEG evoked responses in midbrain reticular formation, amygdala, hippocampus and medial basal hypothalamus of the rat.
6. Photic stimulation elicited frequency-dependent increase in plasma corticosterone of the wakeful rat.
7. The present study, thus, indicates that the specific rhythmic EEG patterns may play an important role for neuroendocrine functions.

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