presented in this report shows that the role of echolocation in the oilbird is the detection of relatively large objects. The oilbird's sonar system is crude in comparison with those of many insectivorous bats. Other animals known to use low frequency sonar systems include cave swiftlets *Collocalia* and the fruit bat *Rousettus*. Like the oilbird these animals echolocate only when the light is too dim to permit navigation by vision, and their signals are band-limited noises lacking systematic frequency modulation. The only aspect of echolocation in which these species differ radically from the oilbird is their reported ability to detect very small objects. *Collacalia vanikonensis granti,* using frequencies between 4.5 and 7.5 kHz, was said to have detected wires as thin as 6 mm in diameter. Similarly, one *Rousettus aegyptius* using perhaps 10 to 12 kHz, avoided wires as fine as 0.46 mm in diameter (*16*). It is difficult to reconcile these results with our observations of the oilbird (*17*).

MASAKAZU KONISHI
ERIC I. KNUDSEN
*Division of Biology, California Institute of Technology, Pasadena 91125*

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4. The aviary was a wire net enclosure 8 m wide by 10 m long and located at the former William Beebe Tropical Research Station, Simla, Trinidad.
5. Film rated as ASA 400 exposed for 1 second at F 1.4 did not show any sign of exposure. The luminence computed from the above data is less than $0.318 \times 10^{-2}$ cd/ft$^2$.
6. When 1.6 ml of 20 percent urethan (20 g of ethylcarbamate in 100 ml of water) was injected in a single dose into a bird weighing about 450 g, good anesthesia was induced, but no evoked potentials could be recorded from the forebrain nucleus. Cochlear potentials were still present, however. The same amount of urethan injected in many steps over a period of 1 hour did not abolish forebrain evoked potentials.
7. In order to reduce frequency biases due to the tonotopic organization in the forebrain nucleus, evoked potentials were recorded with a large electrode (1 mm of tip exposed) at several different loci in the nucleus.
8. A special battery-operated instrument containing oscillator, pulse generator, and electronic switch circuits was used. Cochlear potentials were obtained with continuous tones; forebrain potentials were evoked with tone bursts. For frequencies higher than 1 kHz, the duration of tone bursts was 4 msec with rise and decay times set at 1 msec. Longer durations were used for lower frequencies to include more cycles per burst. Sound frequencies were measured with a digital counter. Evoked as well as cochlear potentials were amplified with an amplifier (Grass P15) and displayed on an oscilloscope (Tektronix) for voltage measurement. Sound pressure level was controlled with a decade attenuator and was monitored with a calibrated 12 mm condenser microphone (Bruel and Kjaer), the output of which was displayed on the oscilloscope for voltage measurement. Since the sensitivity and stability of the condenser microphone was susceptible to high humidity, we also calibrated the loudspeaker with our own ears (E.K.), which were later calibrated with an audiometer.
9. The dynamic range is that range over which evoked or cochlear potentials vary as a function of stimulus sound level.
10. Sonar clicks were recorded with a tape recorder (Nagra 111) and a microphone (Sennheiser MKH 105) at a tape speed of 39 cm/sec. The frequency response of both instruments was essentially "flat" from 50 Hz to 20 kHz. Since the aviary was on top of a hill and was made of fine wire netting, it was largely free of echoes in the frequency range involved.
11. The spectral analysis of sonar clicks was made by W. Heiligenberg using a Fast Fourier Transform program on a computer (PDP 11/40). The recordings were played back on the same tape recorder at one-half the recording speed. The analog data were sampled at 50 kHz.
12. This part of the cave was totally dark at night.
13. The disks smaller than 20 cm were made of plastic sheet 6 mm thick. The larger disks, made of 6-mm Masonite sheet, were covered with plastic tape to reduce sound absorption and water damage.
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15. A rough test of aural directionality was made by comparing the amplitude of cochlear potentials as a function of speaker location. We also brought back a dead specimen to our laboratories in Pasadena for this purpose. The original shape of the ear canal and drum was carefully restored before these measurements. A curved probe tube 1 mm in diameter and 3 cm long was inserted through the external meatus so as to place its open end in front of the eardrum. The tube was connected through an adapter to a calibrated 12-mm condenser microphone (B & K). Sound level was measured with a wave analyzer as a function of speaker location. A special device was used to move a 5-cm speaker around the bird's head as described by E. I. Knudsen, M. Konishi, and J. D. Pettigrew [*Science* **198**, 1278 (1977)]. The results show little directionality for frequencies lower than 4 kHz and some location-dependent irregularities in sound level at 6 kHz.
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17. The sonar signal of *Rousettus* contains frequencies from about 10 kHz to about 50 to 60 kHz [G. Sales and D. Pye, *Ultrasonic Communication by Animals* (Wiley, New York, 1974)]. There is some disagreement as to its hearing range; in *R. aegyptius*, A. M. Brown [*J. Comp. Physiol.* **83**, 407 (1973)] found that auditory sensitivity is maximum at 11 to 12 kHz, and declines sharply on both sides of that range, while A. D. Grinnell and S. Hagiwara [*Z. Vergl. Physiol.* **76**, 82 (1972)] found a broad response range from 10 to 100 kHz in *R. amplexicaudatus*.
18. We thank D. R. Griffin, W. Heiligenberg, G. A. Manley, I. Lambie, J. Price, J. Simmons, and N. Suga. Supported by a grant from the National Geographic Society.

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## Thyroxine Increases Nerve Growth Factor Concentration in Adult Mouse Brain

Abstract. *The effects of thyroxine and propylthiouracil on nerve growth factor concentrations in cerebral cortex, cerebellum, and brainstem of adult male mice were assessed by using a sensitive radioimmunoassay for the β-subunit of mouse nerve growth factor. Thyroxine administration significantly increased the concentration of nerve growth factor in all three brain areas compared to control values, whereas propylthiouracil was without effect. These results suggest that thyroid hormones stimulate nerve growth factor synthesis in the mature central nervous system, and raise the possibility that the influence of thyroid hormones on central nervous system development might be mediated or influenced by nerve growth factor.*

Nerve growth factor (NGF) is a protein that is essential for the maturation and maintenance of adrenergic neurons in sympathetic ganglia (*1*). The recent observations that brain tissue contains both NGF (*2*) and NGF membrane receptors (*3*) suggest that NGF may play an important role in central nervous system (CNS) function [reviewed in (*4*)]. We have found that administration of thyroid hormone to adult male mice significantly increases NGF content and concentration in submaxillary gland and liver (*5*). Because thyroid hormones affect brain growth and maturation in postnatal rodents (*6, 7*), we conducted a study in adult male mice to examine the possibility that thyroid hormones affect NGF in the mature CNS, using a recently developed specific and highly sensitive radioimmunoassay (RIA) for the biologically active β-subunit of NGF.

Table 1. Body and thyroid gland weights in control, PTU-treated, and $T_4$-treated adult male mice. Each value is the mean ± S.E. ($N$ = 10).

| Group | Body weight (g) | Thyroid weight (mg per 100 g body weight) |
|---|---|---|
| Control | 38.2 ± 1.4 | 7.6 ± 0.8 |
| PTU-treated | 36.8 ± 1.2 | 41.8 ± 2.7* |
| $T_4$-treated | 42.5 ± 1.9 | 7.2 ± 0.6 |

*Significantly different from controls at $P < .001$.

The β-NGF was purified by the method of Mobley *et al.* (*8*) and was used as both iodination and reference preparations. The antiserum to 2.5*S* NGF used in this study was provided by H. Herschman. The sensitivity of the RIA is 13 pg per assay tube with an intraassay coefficient of variation of 7 percent. Of the substances tested for immunological cross-reactivity (*9*), only mouse epidermal growth factor produced significant displacement (11 percent at a dose of 100 μg/ml). Parallel labeled ligand displacement was observed for all tissues studied, including brain.

Three groups of ten adult male Swiss-Webster mice were used. One group served as controls; the second was treated with 12 daily intraperitoneal injections of 25 μg of L-thyroxine ($T_4$); the third group received 0.05 percent propyl-


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Table 2. Nerve growth factor content in cerebral cortex, cerebellum, and brainstem of adult male mice. Each value is the mean ± S.E. ($N$ = 10). The $F$ ratios were highly significant for all three brain areas. Significance levels for the multiple comparisons by $t$-tests after logarithmic transformation of the data were corrected for the number of tests performed (*27*).

| Group | NGF content (ng) | | |
|---|---|---|---|
| | Cortex | Cerebellum | Brainstem |
| Control | 0.94 ± 0.08 | 0.40 ± 0.03 | 0.46 ± 0.13 |
| PTU-treated | 1.21 ± 0.14 | 0.44 ± 0.03 | 0.55 ± 0.07 |
| $T_4$-treated | 3.78 ± 0.77* | 0.78 ± 0.13† | 2.20 ± 0.58* |

*Significantly different from controls at $P < .001$. †Significantly different from controls at $P < .01$.

thiouracil (PTU) in the drinking water for 21 days. All animals had free access to mouse laboratory chow.

When each animal was killed, the brain was removed immediately and the cerebral cortex, cerebellum, and brainstem were dissected on ice. Tissues were homogenized in ten volumes of 0.05*M* phosphate-buffered saline, *p*H 7.2, and were centrifuged at 25,000*g* for 40 minutes at 4°C. Supernatants from all tissue preparations were assayed for NGF concentration at two dilutions in the same RIA run. Supernatant protein concentrations were determined by the Folin phenol method of Lowry *et al.* (*10*), using bovine serum albumin as the standard. Statistical analyses were performed by one-way analysis of variance (*11*) and Student's two-tailed $t$-test.

No significant differences were noted in body weights among the three groups of animals (Table 1). Thyroid gland weights, however, were significantly increased in the PTU-treated animals compared to control mice (a mean increase of 550 percent). The lack of a significant difference in thyroid gland weights between the control and $T_4$-treated mice was presumably related to the relatively short period of exposure to $T_4$.

Baseline NGF concentrations were similar in all three brain areas of the control animals (Fig. 1). The $T_4$ treatment resulted in a consistent and significant increase in both NGF content (Table 2) and concentration (Fig. 1) in cerebral cortex, cerebellum, and brainstem tissues of adult male mice. Whether this increased NGF is due to increased production or decreased catabolism is not entirely clear. The observation that systemically administered $^{125}$I-labeled NGF does not accumulate in brain tissue (*12*) supports the view that the increased brain NGF after thyroxine treatment is the result of local factors, and suggests increased NGF synthesis. The possibility of decreased catabolism, however, cannot be excluded.

The failure of PTU to produce significant alterations in either NGF content or concentration may be due to the failure to produce complete biochemical hypothyroidism or the limited duration of the hypothyroidism. At the dosage and route of administration employed in this study, PTU has been shown to produce biochemical hypothyroidism in rats (*13*). Although our mice were definitely goitrous and clinically hypothyroid, it is possible that sufficient thyroid hormones were available for normal NGF metabolism. Ishii and Shooter (*14*) showed that in castrated adult male mice the submaxillary gland NGF concentration decreased with a half-life of 6 to 8 days and was readily restored by administration of testosterone. Thus, it is possible that the time required to induce severe hypothyroidism, in addition to the duration of the disappearance half-life of tissue NGF, was insufficient, in our study, to alter the NGF content of the tissues examined. Consonant with this possibility is the failure of the PTU-treated animals to lose a significant amount of weight (Table 1).

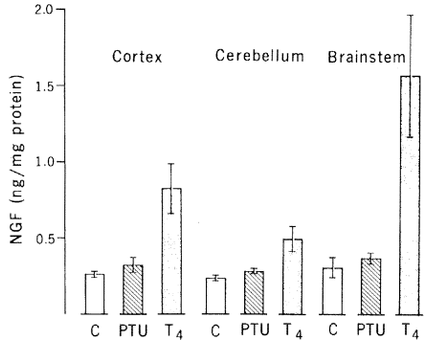


Fig. 1. Nerve growth factor concentration in cerebral cortex, cerebellum, and brainstem of adult male mice. Bars indicate mean ± standard error (S.E.). The $F$ ratios were highly significant for all three brain areas. Significance levels for the multiple comparisons by $t$-tests, after logarithmic transformation of the data, were corrected for the number of tests performed (*27*). The $P$ levels for the tissues when corrected in this manner were: cortex, $P < .002$; cerebellum, $P < .003$; and brainstem, $P < .001$. (All levels of significance are for $T_4$-treated compared to control mice.) No statistically significant differences were noted for PTU-treated compared to control mice.

The role of NGF in the CNS is of considerable interest. If adult rats bearing a transplanted iris (tissue that is normally supplied with sympathetic nerve fibers) in a lesion of the dorsal noradrenergic bundle are given intraventricular or intracerebral injections of NGF, axonal regeneration in the lesion is accelerated (*15*). Moreover, this accelerated regeneration can be inhibited by intracerebral injection of NGF antiserum at the time of iris implantation (*15*). A similar effect of NGF was observed when the severed optic nerve of the newt was used as a model for CNS neuronal regeneration (*16*). In addition, NGF accelerates recovery in rats following lesions in the lateral hypothalamus (*17*). Administration of triiodothyronine ($T_3$), the active metabolite of $T_4$ (*18*), has also been shown to accelerate axonal regeneration in the cerebrum of lesioned adult rats. Furthermore, $T_3$ appears to be selectively concentrated in adrenergic nerve terminals (*19*). The present demonstration of an interaction between thyroid hormones and NGF in the mature mouse brain suggests that the thyroid hormone acceleration of axonal regeneration in the brain of lesioned animals may be mediated through NGF.

The mechanism of the interaction between thyroid hormones and NGF is not known. Thyroid hormones act at the cellular level by binding to specific high-affinity, low-capacity nuclear binding sites, followed by induction of specific RNA polymerase activity and protein synthesis (*20*). By contrast, NGF interacts initially with membrane receptors (*3*), and this is followed by its internalization and retrograde axonal transport to the neuronal cell body (*21*). The fact that $^{125}$I-labeled NGF covalently bound to horseradish peroxidase is also internalized (*22*) suggests an interaction between NGF and membrane-associated microtubules (*23*). Microtubule formation may be important in axonal generation and neuronal spatial orientation (*7*), two major histological deficiencies in brain tissue of thyroid-deficient infant rats.

These observations and our data suggest that thyroid hormones may exert their control effect on CNS development by way of NGF. In support of this possibility, a role for thyroid hormones in the induction of an initiating factor for microtubule assembly in brain has been suggested (*24*), and we have shown (unpublished data) that NGF interacts with tubulin to promote microtubule polymer-

ization (*23*). The observations reported here, taken with earlier results, support the hypothesis that in immature animals, thyroid hormones and NGF interact at the cellular level to promote microtubule formation, axonogenesis, and possibly synaptogenesis (*7, 23, 25*). In the adult animal, such an interaction may be of importance for synaptic transmission and translocation of molecules across the cell membrane, possibly by the induction of a phosphatidylinositol effect (*26*) at the cell membrane.

PETER WALKER
MORTON E. WEICHSEL, JR.
DAVID A. FISHER
SHIRLEY M. GUO
DELBERT A. FISHER

*Fetal-Maternal Research Laboratories, Los Angeles County Harbor-University of California-Los Angeles Medical Center, Torrance, California 90509*

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## Temporal Pattern as a Cue for Species-Specific Calling Song Recognition in Crickets

Abstract. *Female crickets can recognize conspecific calling song from its temporal pattern alone. In* Teleogryllus oceanicus, *the song pattern consists of three classes of interpulse intervals arranged in a stereotyped sequence. Females recognize a model song in which the sequential order of intervals is random. This argues against the hypothesis that recognition results from matching auditory input to an internal template of the song.*

Many animals can recognize species-specific signals (*1*). A current problem in neuroethology concerns the mechanisms underlying such recognition. One class of possible mechanisms entails comparison of sensory input with an internal model of the signal. For example, birds have been suggested to judge auditory input against a species-specific song blueprint or template, both to select a conspecific song model to imitate (*2*) and to guide the development of song motor patterns (*3*). Comparison of auditory input with an internal template has also been suggested as underlying species-specific phonotaxis in crickets (*4, 5*). Male crickets produce a species-specific calling song, which attracts conspecific females (*6*). The song consists of a series of sound pulses delivered according to a stereotyped temporal pattern generated by the central nervous system (*7*). Females can recognize their conspecific song on the basis of its temporal pattern (*8, 9*).

It has been suggested that the neural machinery constituting the hypothesized template for song recognition in females has some elements in common with the male's song-pattern generator (*5, 10*). Such an overlap in the neural substrates for song production and song recognition could explain two important observations. (i) The song pattern produced by males and the pattern preferred by females are genetically coupled; interspecific hybrid males have songs that differ from either of the parental types, and hybrid females prefer these hybrid songs (*5*). (ii) Song production and song recognition vary in similar ways with temperature; females prefer the songs of males that are singing at the same temperature at which they are listening (*8*). An extreme version of the hypothesis of overlap between the neural substrates for song production and recognition is that the template in the female (who does not sing) consists of an internal copy of calling song, produced by the same neural machinery responsible for song pattern production in the male. We now present evidence that argues against this extreme view.

The calling song of *Teleogryllus oceanicus* consists of a series of sound pulses separated by three distinct classes of interpulse intervals, arranged in a repeating stereotyped sequence (Fig. 1, A, B, and E). The repeating unit (phrase) of the song (Fig. 1A) can be described as a string of four intrachirp intervals followed by nine pairs of alternating inter- and intratrill intervals, followed by an additional intertrill interval, all of the intervals being separated by sound pulses (*11*). We reasoned that if female *T. oceanicus* recognize their species calling


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