Thyroxine: Effects of Neonatal Administration on Maturation, Development, and Behavior

Abstract. *Thyroxine was administered to infant rats within the first 3 days of postnatal life; controls receiving 0.01*N *NaOH were from the same litter. Thyroxine accelerated the maturation of the pituitary-adrenal response to electric shock. The "startle response" appeared earlier in the experimental animals, as did the development and response of the electroencephalogram to novel stimuli. The thyroxine-treated rats, when 16 to 18 days old, acquired a conditioned-avoidance response faster than did controls.*

Thyroxine deficiency during the early postnatal period has profound consequences for the subsequent life of an organism. This deficiency impairs learning ability, and the development of the central nervous system, and delays skeletal and somatic maturation (*1*). In the adult, one of the major functions of thyroxine is the regulation of temperature and of cellular metabolism. Thyroxine does not increase the metabolic rate (oxygen consumption) of the infant rat less than 10 days old. However, during this time thyroxine accelerates the deposition of brain cholesterol (which presumably represents myelinization), advances the age of eye-opening, and increases spontaneous locomotor activity (*2*). Thyroxine added to an in vitro cerebellum from a newborn rat or mouse has also been shown to accelerate myelinogenesis (*3*). Etkin (*4*) established the fundamental role of thyroxine in the maturation of the median-eminence area of the tadpole hypothalamus, a region of the brain which, in higher animals, controls many neuroendocrine regulatory mechanisms. One of these mechanisms, the secretion of adrenocorticotropin in response to stress, does not function during the early postnatal period (*5*).

We here demonstrate that administration of thyroxine to the newborn rat will: (i) accelerate the maturation of the pituitary-adrenal response to stress, (ii) accelerate the development of the electroencephalogram and advance the age at which the rat will respond behaviorally and neurophysiologically to acute environmental stimuli, and (iii) increase the ability of the infant rat to learn a conditioned-avoidance response.

Sprague-Dawley rats bred in this laboratory were used in all experiments. Thyroxine (1 μg per gram of body weight) was administered by intraperitoneal injection on postnatal days 1, 2, and 3 to half of each litter. The other half served as controls and received injections of the vehicle (0.01*N* NaOH).

For the test of pituitary-adrenal response to stress, the hormone was administered as described. When they were 5 days old, half of the thyroxine-treated animals from each litter and their corresponding controls were decapitated; the adrenals were removed, weighed, and frozen for determination of corticosterone (*6*). The animals from the remaining half of the litter were each placed on a grid and received electric shock for 3 minutes (3 ma; 15 seconds on, 15 seconds off). Fifteen minutes after the shock treatment they were decapitated and their adrenals were removed, weighed, and frozen.

A second experiment assessed the maturation of the "startle response." Beginning at 9 days of age, control and thyroxine-treated rats were placed individually in a round (14 cm in diameter) plastic container on shavings from the home cage; the container was inside a sound-proof box which had a transparent glass door. The containers rested on water-filled tubing connected to strain gauges whose output was recorded on an Offner dynograph. After 3 minutes of quiet, a loud auditory stimulus was delivered at 10-second intervals through a loudspeaker placed inside the box directly above the containers. Activity of the animal within its container was transmitted to the recorder.

Finally, the development and response of the electroencephalogram to novel stimuli was determined. Flattened silver wire electrodes were placed on the cortex of thyroxine-treated and control rats while the animals were anesthetized with sodium methohexital (brevital). These electrodes were implanted approximately 0.5 to 1.0 mm anterior and lateral of bregma when the animals were from 3 to 14 days old. The electrodes were cemented in place and the rats were returned to their mothers (*7*). The next day an experimental and a control animal were placed in adjacent, identical, plastic containers within the sound-proof box and the external lead wires were connected. The animals were observed for several minutes, during which time they became quiet and inactive. Continuous electroenceph-

Table 1. Effect of thyroxine administration (postnatal days 1, 2, and 3) upon adrenal corticosterone (ACS) response of 5-day-old rats to electric shock. Results are means plus or minus standard error. Not significant, n.s.

	Control group		Thyroxine group		
	Litters (No.)	ACS (μg/g adrenal wt.)	Litters (No.)	ACS (μg/g adrenal wt.)	Significance
Prior to stress	12	20.2 ± 4.7	11	16.1 ± 1.6	n.s.
After stress	12	17.9 ± 1.7	8	23.9 ± 2.6	n.s.
Significance		n.s.		<.05	

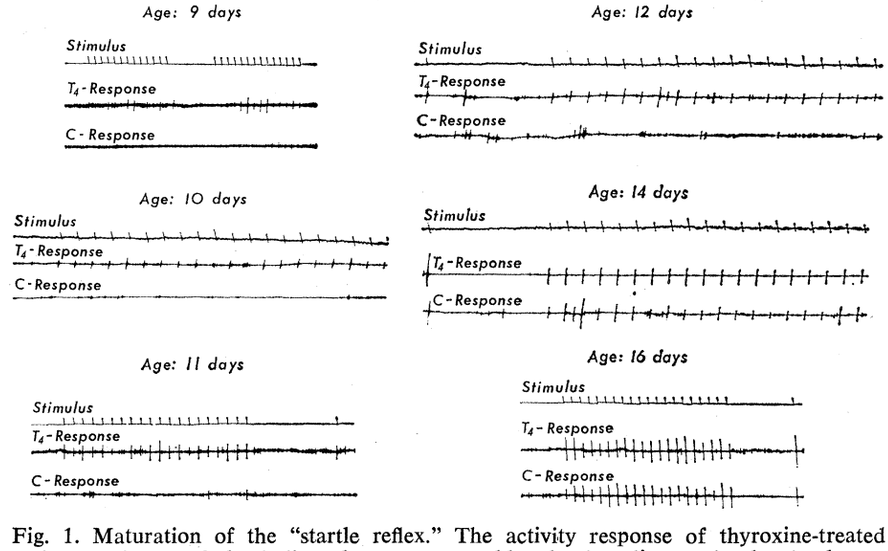

Fig. 1. Maturation of the "startle reflex." The activity response of thyroxine-treated and control rats of the indicated ages to a sudden loud auditory stimulus is shown. Stimulus was repeated at 10-second intervals. T_4, thyroxine-treated; *C*, control.

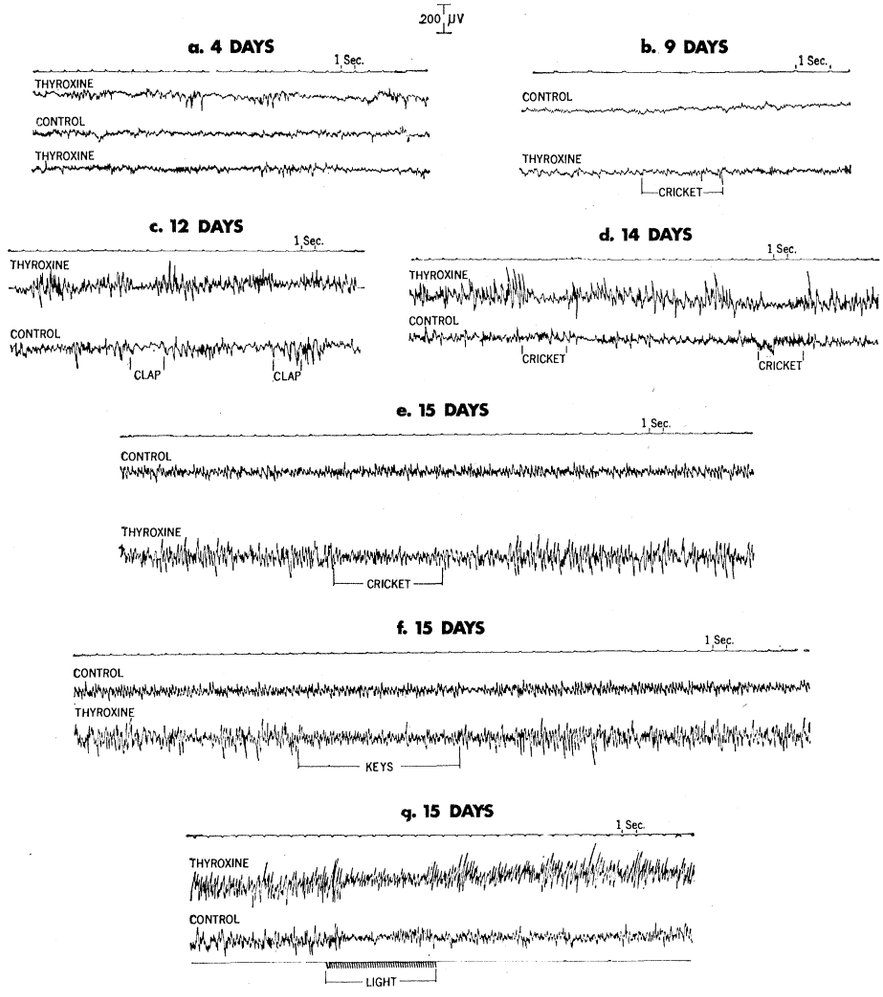

Fig. 3. Active avoidance behavior of thyroxine-treated and control rats 16, 17, and 18 rats at different ages. Effect of various stimuli on cortical activity is also shown.

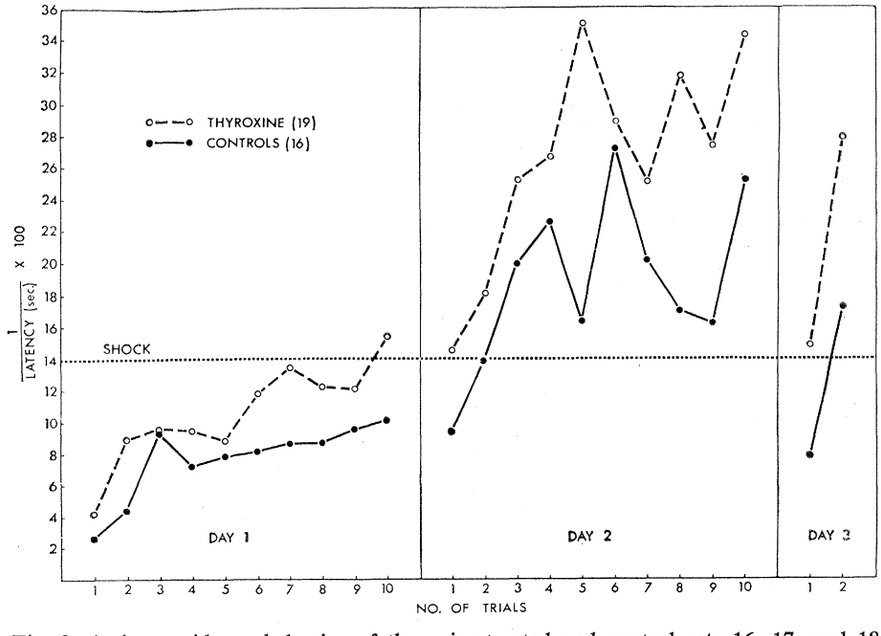

Fig. 3. Active avoidance behavior of thyroxine-treated and control rats 16, 17, and 18 days old. Dotted line indicates application of shock 7 seconds after the infants were placed on a charged grid. Latency refers to time (in seconds) before animals crawled to safe platform. Analysis of variance indicated that performance differences between groups on days 1 and 2 were significant, $P = .05$; on day 3, $P = .01$. Number of animals indicated in parentheses.

alograms were obtained. Stimuli to which the rats were subjected included flashing light, loud noise, mechanical cricket, and jangling keys.

A fourth experiment assessed conditioned-avoidance behavior. At 16, 17, and 18 days of age, experimental and control animals were placed individually on a grid (12.7 cm by 10.2 cm) with free access to a cardboard platform on the opposite side of which was a lighted, 100-watt bulb directed toward the grid. Shock was delivered through the grid 7 seconds later, and it was maintained until the rat reached the safe platform. The number of seconds between placing a rat on the grid and its climbing on to the platform was recorded. On days 16 and 17, ten trials were performed, and two trials were run on day 18.

Control, 5-day-old infant rats subjected to electric shock indicated no change in adrenal gland corticosterone 15 minutes after this stress. An increase, however, was exhibited in response to this same stress by rats that had received thyroxine (Table 1). Thus, thyroxine treatment appears to have advanced the age at which this stress provoked the secretion of adrenocorticotropin. The small decrease observed from the amount recorded before stress in the treated animals was not statistically significant.

In the test for the maturation of the "startle response" (Fig. 1), control animals exhibited no response (change in the amount of activity) to a sudden loud noise during the first 9 to 11 postnatal days. At day 12 this stimulus elicited a measurable "startle response," and by day 14 clear changes in activity occurred. In contrast, thyroxine-treated animals responded as early as 9 days of age. At 16 days responses of control and experimental animals appeared equivalent.

The infant rat brain, before 9 days of age, has very little electrical activity and recognizable frequency patterns are not yet present. Even when the rats are 9 days old, wave patterns are not yet regular or distinct. However, the thyroxine-treated animals have higher amplitudes of activity at all age groups studied (Fig. 2). At 12 days of age the experimental animals exhibited changes in both amplitude and frequency in response to a loud noise. Several days later (days 14 to 15) recognizable changes in the electroencephalogram occurred in the experimental animals in response to other external stimuli (cricket, light, and keys).

In the adult animal the secretion of adrenocorticotropin, as well as that of other tropic hormones, is moderated by the median eminence region of the hypothalamus. This area is undeveloped during the early postnatal period (*8*). Etkin (*4*) has shown that thyroxine accelerates the maturation of this region in the tadpole. We conclude that this hormone has accelerated the maturation of the central nervous system mechanism underlying this physiological adaptive response (Table 1).

Eayrs and Lishman (*9*) have shown that thyroid hormone deficiency delays the appearance of certain behavioral responses, including the "startle response," in early postnatal life. The maturation of this defensive reflex (*10*) is shown in Fig. 1. Thyroxine-treated rats develop this response several days earlier than controls. Electroencephalograms were obtained from rats 4 to 15 days of age (Fig. 2). In agreement with the results of Bradley *et al.* (*11*), only sporadic electrical deflections of small amplitude appear before 10 days of age (*12*). However, in all age groups the preponderance of high-amplitude waves in the thyroxine-treated animals was in contrast to control animals which generally exhibited waves of lower amplitude. These high amplitude waves changed to low amplitude when arousal stimuli were presented. Thyroxine-treated animals are already responding at day 12, and by day 15 there are characteristic changes in the electroencephalogram. The apparent single response of a control animal on day 12 (Fig. 2c) was not reproducible. Light elicited changes in the electroencephalogram in control animals at an age (15 days) when their eyes were not yet fully opened. At this time the eyes of thyroxine-treated animals are already opened (*2*). The accelerated development of bio-environmental interaction, as evidenced by the earlier onset of the adrenalcortical response to stress, development and response of the electroencephalogram to novel stimuli, and the "startle response," complements the additional observations that the treated animals, when 16 to 18 days of age, acquire a conditioned-avoidance response more rapidly than untreated controls (Fig. 3). The biochemical and histological events underlying accelerated maturation of the central nervous system are not yet known (*13*).

The development of the central nervous system is severely retarded when there is a neonatal thyroid deficiency. The thyroid hormone may therefore play an important role for developing neurons to establish, during certain "critical" (or plastic) periods in early postnatal life, the web of interconnections that lay the foundation for the later behavioral repertoire of the organism.

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References and Notes

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12. L. B. Flexner [*Plzensky Lekarsky Sbornik* **3**, 77, (1961)] has shown that in the 10-day-old rat the cortical nerve cells acquire properties of the mature neuron; they sprout dendrites and axons, and shortly thereafter rhythmic electric activity is observed.
13. Thyroxine treatment in the infant rat, for example, does not induce formation of brain α-glycerophosphate dehydrogenase [S. Schapiro and C. J. Percin, *Endocrinology* **79**, 1075 (1966)]. In addition, it does not increase the activity of succinic dehydrogenase or acetycholinesterase in the whole brain (S. Schapiro, in preparation).
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Magnesium Pemoline: Effect on Avoidance Conditioning in Rats

Abstract. *Rats administered 20 milligrams of magnesium pemoline per kilogram of body weight learned to avoid shock in a jump-out apparatus in fewer trials than did controls. However, the results suggested that the principal effect of the drug was to facilitate the avoidance behavior of those animals that tended to "freeze" in response to electric grid shock. No differences in retention were observed between experimental and control animals that had achieved equal levels of learning.*

Plotnikoff (*1*) has reported that oral administration of magnesium pemoline in rats enhances acquisition and retention of a conditioned avoidance response in a jump-out apparatus. Bowman (*2*) subsequently questioned Plotnikoff's interpretation of the retention data because the drug and control groups had not achieved equal levels of learning prior to the retention test. We have assessed the generality of Plotnikoff's acquisition findings and tested the retention effect when all animals had achieved equal levels of learning.

Plotnikoff (*1*) tested rats which had been selected as "slow learners." Our data from preliminary experiments suggested that such "slow learners" are rats which adopt a freezing response in the jump-out apparatus. That is, rats which fail to exit from the chamber within the first few acquisition trials tend to adopt a motionless, rigid posture when placed in the box on subsequent trials; hence they learn the required jump-out avoidance response more slowly (*3*). We attempted to determine the extent to which the facilitative effects of magnesium pemoline upon avoidance learning is restricted to rats that freeze in response to shock.

Male Sprague-Dawley rats (230 to 250 g) were caged singly in a large colony room and had free access to food and water. Each rat was handled 1 minute each day for 3 days before selection and testing. The apparatus, essentially that described by Plotnikoff (*1*), consisted of a wooden chamber (20 by 30 by 50 cm) with a grid floor (3-mm diameter bars spaced 2 cm apart). An escape opening 20 cm square was cut into one wall 30 cm above the grid floor, and an escape platform extended outside the box. The escape platform and adjoining interior wall of the chamber were covered with wire mesh (13 mm squares) which enabled the rats to climb out of the box. The testing room was lighted by a 10-watt bulb located approximately 1 m directly above the test chamber. A white-noise