change in the distribution of estrogen receptors between the cytoplasm and nucleus. Estrogen treatment is known to promote a depletion of cytoplasmic receptors that is accompanied by a parallel increase in nuclear RE (*11*). Furthermore, Lee and Jacobson (*12*) have shown that the concentration of free cytoplasmic receptors is at a minimum in proestrus, and at a maximum in estrus. Thus, the ratio of nuclear RE (our results) to free cytoplasmic binding sites (*12*) is high when the rate of estrogen secretion is at a maximum in proestrus (*10*). Conversely, the ratio of nuclear RE (our results) to free cytoplasmic sites (*12*) is low when the rate of estrogen secretion is at a minimum, as in estrus (*10*).

The linearity of the double reciprocal plots, and the similarity in the K^R_d (*13*) (1.2×10^{-9} to $2.6 \times 10^{-9} M$, 37°C) throughout the estrous cycle (Table 1), and after estradiol treatment, suggest that the receptors are homogenous in their affinity for estradiol. These K^R_d values are in agreement with those obtained by others for both cytoplasmic and nuclear estrogen binding sites (*3, 14*).

A causal relation between the elevated uterine weight, the protein content or the protein to DNA ratio, and the maximum concentration of nuclear RE during proestrus remains to be established. Estrogen is known to augment uterine protein and RNA synthesis, and these effects are blocked by puromycin and actinomycin (*15*). Moreover, the RE complex has been implicated in the estrogen stimulation of RNA polymerase activity (*16*). While the possibility that the estrogen receptor exerts its effects at the cytoplasmic level cannot be excluded, the results of our study suggest that nuclear RE complexes may be of physiological importance, and are not simply a pharmacologic phenomenon.

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Synaptogenesis in the Rat Cerebellum: Effects of Early Hypo- and Hyperthyroidism

Abstract. *The number of synapses in the molecular layer of the rat cerebellum is reduced by early hypo- and hyperthyroidism within 30 days. Hypothyroidism retards synaptogenesis after 10 days, while hyperthyroidism accelerates synaptogenesis initially, but by 21 days the number of synapses is reduced. The sensitivity of developing synapses to thyroid hormone may permit analysis of the events triggering synaptogenesis.*

Neonatal hypo- and hyperthyroidism produce various deficits in postnatal neural development, including a decrease in cerebral and cerebellar weights (*1, 2*), and changes in the number, size, and packing density of cells (*1–3*). Changes in the amount and composition of the neuropil have been demonstrated in the sensorimotor cortex (*2, 4*), the visual cortex (*5*), and the cerebellum (*6*). Changes have also been found in development of metabolic compartmentation, which is thought to reflect maturation of dendritic processes and nerve terminals (*7*). Together these results indicate a retardation of neuropil development in hypothyroidism, and an acceleration in hyperthyroidism. Also, there is behavioral and electrophysiological evidence for neurological changes in these conditions in the form of retarded or accelerated maturation of innate behavioral patterns (*8, 9*), and abnormalities in the electrical patterns of the brain (*8, 10*).

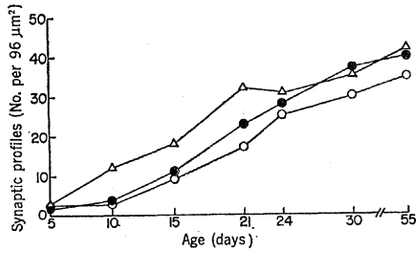

Fig. 1. Density of synaptic profiles in cerebellar molecular layer. △, Hyperthyroid; ○, hypothyroid; ●, control. Statistical significance: control and hypothyroid, at 21 and 30 days, $P < .01$; at 55 days, $P < .05$; control and hyperthyroid, at 10, 15, and 21 days, $P < .01$.

We examined the effects of early hypo- and hyperthyroidism on synaptogenesis in the cerebellar molecular layer with quantitative light and electron microscope methods. Our results provide evidence that both hypo- and hyperthyroidism cause a reduction in the total number of synapses formed in the cerebellar molecular layer, but by different processes.

Groups of animals were injected from birth with either physiological saline (controls), propylthiouracil (producing a hypothyroidism), or L-thy-

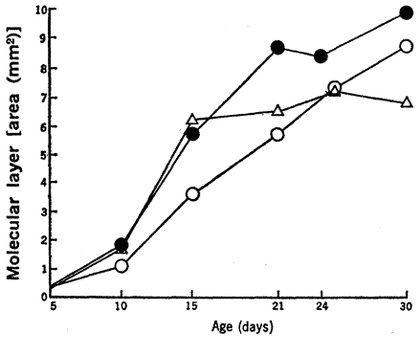

Fig. 2. Area of cerebellar molecular layer. △, Hyperthyroid; ○, hypothyroid; ●, control. Statistical significance: control and hypothyroid, 15 to 30 days, $P < .01$; control and hyperthyroid, 21 to 30 days, $P < .01$.

roxine (producing hyperthyroidism) (*11*). Each litter was composed of ten rats (mostly males) originating from at least two different litters born on the same day. Animals were killed at 5, 10, 15, 21, 24, or 30 days of age. For light microscopy, the brains were fixed in Bouin's fluid followed by 10 percent neutral formalin, and were embedded in Paraplast. The brains were sectioned sagittally at 6 μm and parasagittal sections were stained with hematoxylin and eosin. The area of the molecular layer was determined with an Ott compensating polar planimeter applied to cerebellar tracings of these sections (magnified 65 times with a modified Leitz projection apparatus). For electron microscopy, the brains were fixed by perfusion with 6 percent glutaraldehyde buffered with phosphate (*p*H 7.2). Cerebellums were removed and were further fixed in cold 6 percent glutaraldehyde for at least 1 hour. This was followed by slicing at 235 μm, dehydration in ethanol, and staining with ethanolic phosphotungstic acid (*12*), after which tissue slices were embedded in Araldite-Epon. Samples for synaptic density counts were randomly selected from outer, middle, and inner zones of the molecular layers from coded animals. Pictures were taken at a magnification of × 6027 with a Philips 300 electron microscope. Synapse counts were made directly from coded negatives and expressed as the average number of synaptic profiles per 96 μm^2 of molecular layer. To obtain estimates of the total number of synapses in the sagittal area of the molecular layer, we applied the formula: total synaptic profiles equals synaptic profiles per 96 μm^2 of the molecular layer times the area of molecular layer in square micrometers. Analysis of variance and Duncan's multiple range test (*13*) were used as tests of significance ($P < .01$).

Early hypothyroidism caused retardation of synaptogenesis as shown by a reduced rate of increase in the density of synaptic profiles; this was significant at 21, 30, and 55 days (Fig. 1). A similar pattern was seen in the development of the molecular layer (Fig. 2). By 30 days the area of the molecular layer was still significantly reduced, although not as much as in the hyperthyroid group. The increase in the calculated total number of synapses was retarded from day 10 on, but by day 30, the reduction was not as great as in the hyperthyroid group.

Early hyperthyroidism caused a transient increase in density of synaptic profiles until 24 days of age followed by a decline to control values by 30 days (Fig. 1). This treatment also caused a pronounced decrease in area of the molecular layer after 15 days (Fig. 2). The calculated total number of synapses in the hyperthyroid animals was higher than in the controls until 21 days, followed by a significant reduction at 30 days as compared with controls.

Our results show that hypo- and hyperthyroidism lead to a pronounced reduction in the synaptic content of the cerebellar molecular layer. However, this effect at day 30 is probably the result of two different processes. In hyperthyroidism, the cells of the external granular layer (postnatal germinative matrix of the cerebellum) have been shown to cease proliferation early (*14*) producing fewer stem cells from which granule, basket, and stellate cells are formed. This premature termination is associated with early initiation of cell differentiation (*14*) leading to an initial acceleration of synaptogenesis but to an ultimate reduction in total number of synapses. In hypothyroidism, however, the main effect seems to be a general retardation of the differentiation of cerebellar neurons (*6, 14, 15*). The evidence of retarded synaptogenesis throughout development, shown in this study, supports this hypothesis.

In conclusion, it appears from these and other results (*14*) that both the acceleration of cell differentiation in the cerebellar cortex (produced by hyperthyroidism) and its retardation (produced by hypothyroidism) lead to reductions in the synaptic content of the neuropil. It remains to be determined whether or not acceleration or retardation produced by other means, and in other parts of the brain, also lead to synaptic deficits. These results not only suggest the possible role of thyroid hormone as a trigger in the process of synaptogenesis, but also indicate the usefulness of early thyroid treatments as a tool for studying synaptogenesis in the developing nervous system.

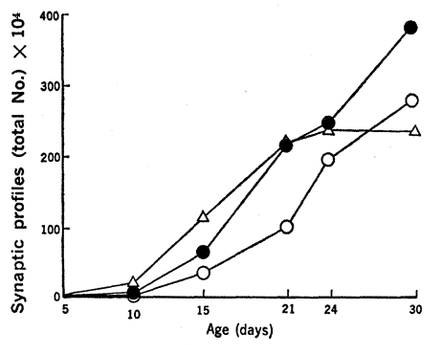

Fig. 3. Estimated total synaptic profiles in cerebellar molecular layer. △, Hyperthyroid; ○, hypothyroid; ●, control.

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Salicylate: Action on Normal Body Temperature in Rats

Abstract. *Rats receiving intraperitoneal injections of sodium salicylate (30 to 300 milligrams per kilogram of body weight) showed a decline in rectal temperature of up to 5.5°C when placed in a 5°C environment. High dosages of salicylate lowered the rectal temperatures of rats kept in a 23°C environment. The finding that salicylate can lower nonfebrile body temperature suggests that this class of antipyretic agents does affect normal temperature regulation.*

It is generally believed that the effectiveness of salicylates is restricted to the lowering of body temperature previously elevated by pyrogens and that salicylates have little or no effect on afebrile subjects (*1*). I present here evidence which demonstrates that salicylate does lower the normal body temperature of rats, especially if the animals are in a cold environment.

Sixty-four female albino rats, weighing between 260 and 360 g each, were placed, in groups of four or five at a time, in individual metal cages in a cold chamber maintained at 5° ± 1°C. The fur of half of the rats was shaved. After 15 minutes in the cold, the rats received an intraperitoneal injection of either sodium salicylate (30, 60, 120, 180, 240, or 300 mg per kilogram of body weight) dissolved in 2 ml of isotonic saline or 2 ml of saline alone. Each rat was tested only once at a single dosage of sodium salicylate or saline. They remained in the cold chamber for 1 hour more and were then removed to an environment with a temperature of 23° ± 1°C. A thermistor probe which recorded rectal temperatures was connected to a telethermometer (Yellow Springs Instrument Company). At the sampling intervals, the thermistor probe was inserted 5 cm into the anus of each rat. Temperatures were taken immediately before the rats were placed in the cold, every 15 minutes during the 1-hour cold test, and for up to 4 hours after they had been returned to the 23°C environment. Four rats each in the shaved and unshaved groups were tested at each dosage of salicylate, and eight rats in each group were tested with saline. A group of 20 unshaved rats received intraperitoneal injections of either sodium salicylate dissolved in saline (30, 60, 120, 180, or 300 mg/kg, three rats per dosage) or saline alone (five rats) and were kept at an ambient temperature of 23°C for 4 hours, during which time their rectal temperatures were recorded periodically.

Figure 1 shows the effect of the low, medium, and high dosages of sodium salicylate on the body temperature of

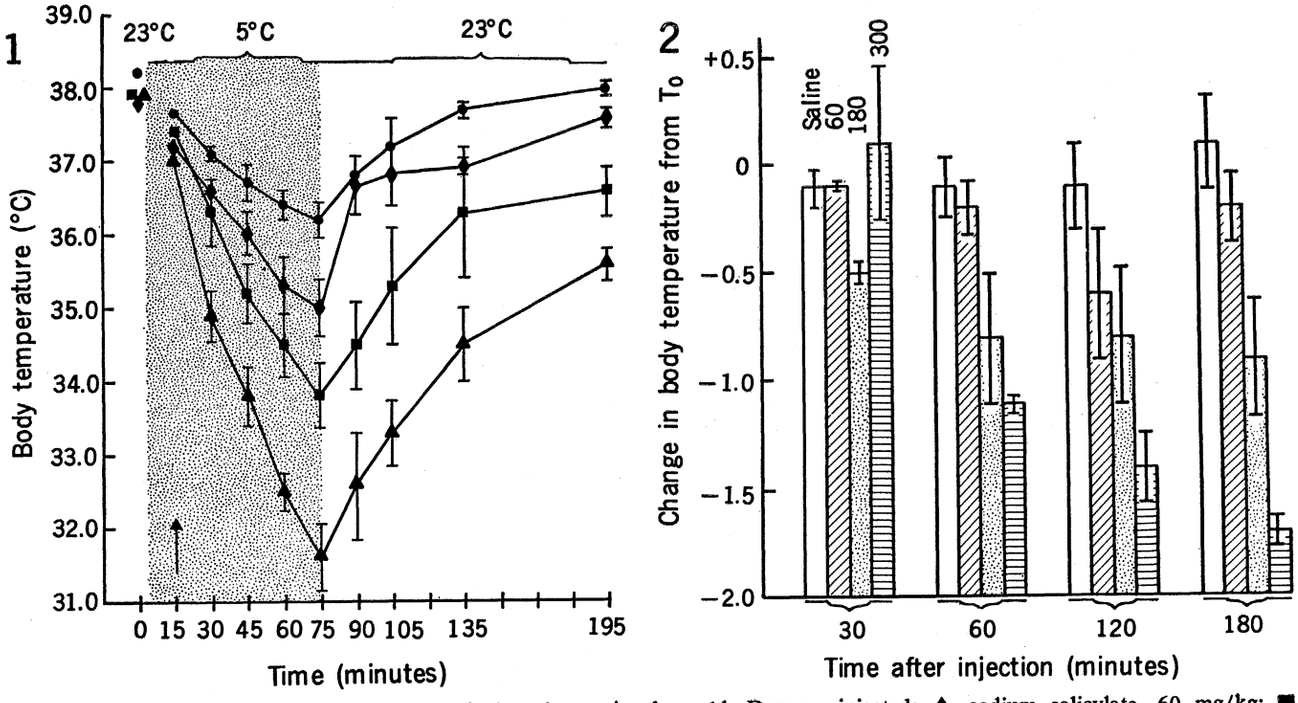

Fig. 1 (left). Change in body temperature of shaved rats in the cold. Dosages injected: ♦, sodium salicylate, 60 mg/kg; ■, sodium salicylate, 180 mg/kg; ▲, sodium salicylate, 300 mg/kg; ●, saline. The time of injection of sodium salicylate or saline is indicated by the arrow. Vertical lines indicate the standard error of the mean. Fig. 2 (right). Change in body temperature (body temperature before injection = T_0) of unshaved rats kept at 23°C for 30 to 180 minutes after the injection of sodium salicylate or saline. Numbers above bars indicate the dosages of sodium salicylate in milligrams per kilogram of body weight.