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# Dendritic spine density of adult hippocampal pyramidal cells is sensitive to thyroid hormone

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In order to determine whether pyramidal cells of the adult hippocampus are morphologically sensitive to thyroid hormone, we performed single-section Golgi impregnation analyses on brains from hyperthyroid and control rats. Quantitative analyses of Golgi-impregnated pyramidal cells from the CA1 region showed a significant decrease in the density of apical dendritic spines with hyperthyroidism. In contrast, no changes were observed in spine density of basal dendrites or in cross-sectional cell body area of CA1 pyramidal cells. No changes in any of these morphological variables were detected in pyramidal cells of the CA3 region with hyperthyroidism. These results suggest that spine density of the apical dendrites of CA1 pyramidal cells is specifically affected by thyroid hormone in adulthood. Since dendritic spines are thought to represent postsynaptic sites it is likely that this morphological change results in altered hippocampal function.

The effects of thyroid hormone on the development of the mammalian brain have been well documented. Thyroid hormone imbalances during a critical developmental period result in profound and permanent behavioral[1,8], chemical[11] and structural[3,4,7] abnormalities within specific neural regions. The hippocampus, a neural region which has been implicated in a number of important processes, such as learning and memory[10], is particularly sensitive to developmental thyroid hormone imbalances. Neonatal hypothyroidism and hyperthyroidism have been shown to dramatically alter the structural developmental of pyramidal cells in the hippocampus[4,12]. Although hypothyroidism in adulthood has been shown to affect the morphology of cells in the visual cortex[13], it is presently unknown whether or not thyroid hormone affects the morphology of hippocampal pyramidal cells in adulthood. In order to determine whether or not hippocampal pyramidal cells remain morphologically sensitive to thyroid hormone throughout adulthood, we performed single-section Golgi-impregnation analyses on brains from hyperthyroid and normal adult rats.

Fourteen adult female Sprague–Dawley rats (200–220 g; Charles River) were used for this study. The rats were injected with either 0.75 μg/g b. wt. 3,3′,5-triiodothyronine (T3) in sesame oil ($n$ = 7) or the vehicle alone ($n$ = 7) every day for 5 consecutive days. This treatment regimen was employed because it was sufficient to induce hyperthyroid behavioral characteristics (see below) without causing the premature death of any experimental animals. On the sixth day after the treatments began, these rats were perfused and the brains were processed using a modified version of the single-section Golgi impregnation method[2]. The rats were deeply anesthetized with Ketalar (Ketamine HCl) and transcardially perfused with 100–150 ml 4.0% paraformaldehyde in 0.1 M phosphate buffer with 1.5% (v/v) saturated picric acid. The brains were postfixed for 24 h in a solution of the same composition as the perfusate. Coronal sections, 100 μm thick, were cut by use of a Vibratome into a bath of 3.0% potassium dichromate in distilled water and incubated in this solution for 24 h. The sections were then rinsed in distilled water and mounted on ungelatinized glass slides. Coverslips were glued over the tissue sections at the 4 corners and the slide assemblies were placed in 1.5% silver nitrate in distilled water for 24 hours in the dark. The slide assemblies were then disassembled, the sections were rinsed in distilled water, dehydrated, cleared and coverslipped.

The slides containing Golgi-impregnated brain sections were coded prior to quantitative analysis and the code was not broken until after the analysis was finished. In order to qualify for analysis, Golgi-impregnated hippocampal pyramidal cells had to possess the following characteristics: (1) location within the dorsal aspect of the CA1 or CA3 hippocampal fields, (2) relative isolation from nearby impregnated cells in order to allow identification of dendrites which arose from specific cells, and (3) dark and even impregnation throughout the extent of the neuron. These selection criteria were used in an effort to analyze neurons from the same populations within and

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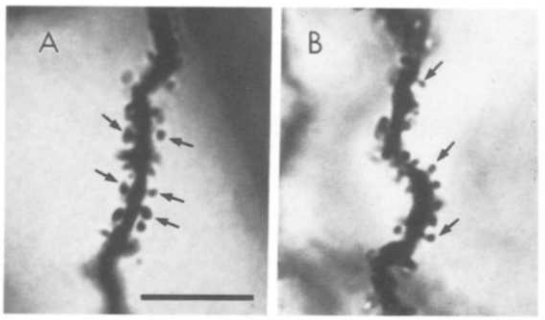


Fig. 1. Representative photomicrographs of Golgi-impregnated apical dendrites from CA1 pyramidal cells of control (A) and hyperthyroid (B) rats. Observe the decrease in dendritic spine density in B compared to A. Arrows indicate dendritic spines with pronounced heads. Bar in A = 10 $\mu$m and applies to both frames.

between groups. For each selected neuron, the cross sectional cell body area was determined by use of an image analysis morphometry program (Southern Micro Instrument Inc., Atlanta, GA). In addition, two measurements of spine density were obtained from specific regions of the dendritic tree for each neuron. For each pyramidal cell, spine density was determined from the most lateral tertiary dendrite on the apical tree and the most lateral secondary dendrite on the basal tree. These dendrites were always located within the stratum lacunosum moleculare and the stratum oriens of Ammon's horn, respectively. The criteria by which dendritic segments were selected for analysis were used to reduce the possibility that we were comparing spine density from different portions of the dendritic tree within and between animals. Camera lucida tracings (1250×) were obtained from selected dendritic segments that did not leave the plane of focus. All spines of the selected dendritic segment were counted, the length of the

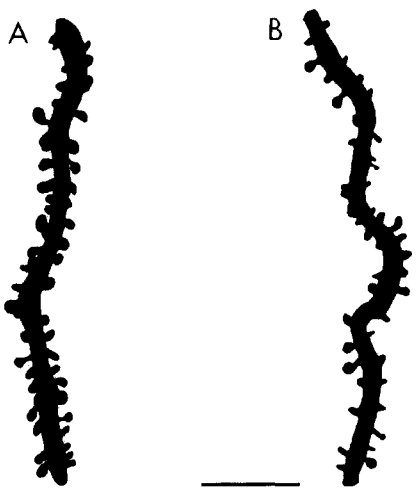


Fig. 2. Camera lucida tracings of the entire extent of the Golgi-impregnated apical dendrites partially depicted in Fig. 1. Observe the dramatic decrease in dendritic spines with thyroid hormone treatment (B) compared to the control (A). Bar in B = 10 $\mu$m and applies to both tracings.

TABLE I

*The effects of adult hyperthyroidism on the morphology of hippocampal pyramidal cells*

Values equal mean ± S.E.M.

| *Morphological variable* | *Control* | *Hyperthyroid* |
|---|---|---|
| Density of CA1 pyramidal cell apical dendritic spines (spines/10 $\mu$m) | 11.6 ± 0.5 | 8.6 ± 1.1* |
| Density of CA1 pyramidal cell basal dendritic spines (spines/10 $\mu$m) | 8.2 ± 0.5 | 6.7 ± 0.8 |
| Cell body area of CA1 pyramidal cells ($\mu m^2$) | 220.4 ± 5.5 | 219.4 ± 3.7 |
| Density of CA3 pyramidal cell apical dendritic spines (spines/10 $\mu$m) | 12.1 ± 1.0 | 9.6 ± 0.8 |
| Density of CA3 pyramidal cell basal dendritic spines (spines/10 $\mu$m) | 10.5 ± 1.2 | 9.6 ± 0.7 |
| Cell body area of CA3 pyramidal cells ($\mu m^2$) | 358.4 ± 8.5 | 372.1 ± 25.2 |

* $P < 0.025$; all other comparisons showed no significant differences ($P > 0.05$); *t*-test.

segment was determined with the image analysis system and spine density values were expressed as number of spines/10 $\mu$m of dendrite. For each animal, a total of 24 dendritic segments were analyzed (12 for each of the two cell types). The means of each variable were determined for each animal and these data were subjected to a two-tailed *t*-test.

After 5 days of treatment, the rats which received thyroid hormone injections showed notable signs of hyperthyroidism, such as weight loss and a pronounced tremor.

Quantitative analysis of Golgi-impregnated apical dendrites of CA1 pyramidal cells revealed significantly lower dendritic spine density in the brains of thyroid hormone treated rats compared to controls ($P < 0.025$; see Table I, Figs. 1 and 2). Although no significant differences were detected in the basal dendrites of CA1 pyramidal cells following thyroid hormone treatment, a trend toward a decrease in dendritic spine density was also observed ($P < 0.1$; see Table I). No significant difference in the cross-sectional cell body areas of CA1 pyramidal cells was observed with thyroid hormone treatment ($P > 0.1$; see Table I). In contrast to the significant effect of thyroid hormone treatment on CA1 apical dendritic spine density, no significant changes in CA3 apical dendritic spine density were noted in response to thyroid hormone treatments ($P > 0.1$; see Table I). Likewise, no significant differences or notable trends toward differences in CA3 basal dendritic spine density or cross-sectional cell body area were detected with thyroid hormone treatment ($P > 0.1$ for both comparisons, see Table I).

The results of the present study demonstrate that CA1

pyramidal cells are morphologically sensitive to thyroid hormone in adulthood. Hyperthyroidism induced in adulthood resulted in a significant decrease in dendritic spine density of apical dendrites in CA1 pyramidal cells. In contrast, basal dendritic spine density and cell body area of CA1 pyramidal cells was unaffected by hyperthyroidism. Spine density of both apical and basal dendritic trees as well as the cell body area of CA3 pyramidal cells were unchanged by hyperthyroidism.

Neonatal thyroid hormone imbalances have been shown to exert dramatic influences over the morphologic characteristics of both CA1 and CA3 hippocampal pyramidal cells[4,12]. The present report suggests that CA1 pyramidal cells retain their sensitivity to thyroid hormone throughout adulthood. Thyroid hormone administration during development[4], and in adulthood (this study) results in altered dendritic spine density of CA1 pyramidal cells. Surprisingly, the changes in dendritic spine density are not in the same direction; developmental hyperthyroidism results in increased dendritic spine density[4], whereas adult hyperthyroidism results in decreased dendritic spine density despite the similarity in the dose of thyroid hormone given in both experiments. These results are particularly surprising because some studies suggest that thyroid hormone exerts a growth-promoting effect on neurons[6]. However, the observation that developmental hyperthyroidism can lead to decreases in the size and number of cholinergic basal forebrain neurons[3] suggests that excess thyroid hormone is capable of exerting a destructive influence over neurons.

In contrast to the thyroid hormone sensitivity of adult CA1 pyramidal cells, CA3 pyramidal cells do not appear to retain their sensitivity to this hormone throughout adulthood. Excess thyroid hormone administered during the early postnatal period results in CA3 pyramidal cells with increased cell body areas compared to controls[4]. The results of the present study suggest that the cell body area of adult CA3 pyramidal cells is no longer sensitive to thyroid hormone. One possible explanation for the difference in responsiveness between the two cell types is that adult CA1 pyramidal cells inherently possess a greater degree of structural plasticity than do adult CA3 pyramidal cells. In this regard, it is interesting that a previous study has shown that dendritic spine density of adult CA1 pyramidal cells, but not of adult CA3 pyramidal cells, responds to changes in the levels of ovarian steroids[5].

Since dendritic spines are thought to be postsynaptic sites[14], it is likely that changes in spine density reflect changes in synaptic density. Changes in synaptic density probably result in altered electrical activity and, ultimately, altered behavior. In this regard, it is interesting to note that clinical studies have shown that adult-onset hyperthyroidism can result in learning and memory deficits which, if treated, are reversible[9]. It is possible that such functional changes are the result of structural changes which occur in the hippocampus. The extent to which the structural changes reported here are reversible and affect processes, such as learning and memory and neuroendocrine function, which have been attributed to the hippocampus remains to be determined.

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