JARO 02: 312–329 (2001)
DOI: 10.1007/s101620010076

JARO
Journal of the Association for Research in Otolaryngology

Consequences of Hypothyroidism on Auditory System Function in *Tshr* Mutant (*hyt*) Mice

PAMELA M. SPRENKLE,[1,2,4] JOANN MCGEE,[1–3] JOHN M. BERTONI,[2,4] AND EDWARD J. WALSH[1–3]

[1]*Developmental Auditory Physiology Laboratory, Boys Town National Research Hospital, Omaha, NE 68131, USA*
[2]*Department of Biomedical Sciences, Creighton University, Omaha, NE 68178, USA*
[3]*Department of Otolaryngology, Creighton University, Omaha, NE 68178, USA*
[4]*Department of Neurology, Creighton University, Omaha, NE 68178, USA*

Received: 11 September 2000; Accepted: 11 April 2001; Online publication: 1 August 2001

ABSTRACT

The otological consequences of hypothyroidism and the outcome of thyroxin (T_4) administration during the developmental period preceding the onset of hearing were examined in mice that express a point mutation in the gene encoding the thyrotropin receptor (*Tshr*), the so-called *hyt* mouse. Progeny of sires homozygous for the trait and heterozygous dams were injected with T_4 or saline placebo from birth through the tenth postnatal day and auditory-evoked brainstem responses (ABRs) to acoustic clicks and tone bursts were recorded from young adults. Mutant (*hyt/hyt*) mice exhibited a distinctive pattern of sensory pathology that was characterized by their insensitivity to sound, prolonged response latencies, reduced peak amplitudes, and steep latency–intensity curves relative to the phenotypically normal, euthyroid, *+/hyt* littermates. Following thyroxin treatment, *hyt/hyt* mice responded to acoustic stimuli more frequently, were more sensitive to tone bursts throughout their audiometric range, and exhibited decreased latencies and increased amplitudes when compared with placebo-treated homozygous mutants. Although thresholds to acoustic stimuli were improved relative to the untreated group, T_4-treated homozygotes were less sensitive than normal, euthyroid individuals. In addition, energy consumption by auditory brainstem nuclei, measured by 2-deoxyglucose (2-DG) uptake, was significantly lower in *hyt/hyt* mice compared with heterozygotes, and T_4 treatment increased the level of 2-DG utilization. Moreover, mean ages for eye-opening and pinna-raising were delayed in animals that were homozygous for the *hyt* allele. When T_4 was administered to *hyt/hyt* animals, pinna-raising occurred earlier than in untreated animals. A subset of homozygotes exhibited circling behavior, indicative of vestibular and/or motor dysfunction, even though all individuals assumed a normal righting reflex. These findings, including recruitment-like behavior and the restoration of response magnitude at high levels but not low, suggest that the cochlear amplifier is the primary locus of an enduring otological defect associated with hypothyroidism in the *Tshr* mouse.

Keywords: hypothyroidism, hearing, auditory, development, thyroid hormone, thyroid stimulating hormone

INTRODUCTION

It is well known that thyroid hormones regulate the development and function of the mammalian nervous system (Bernal and Nunez 1995, for review) and that thyroid system disorders can lead to severe motor, cognitive, and sensory abnormalities (Comer and Norton 1982; Rovet et al. 1987; Vermiglio et al. 1990). Hypothyroidism is one such disorder and the disease is expressed in both late onset and congenital forms. In this study we concentrate on congenital hypothyroidism, the incidence of which is approximately 1:4000 individuals in North America, Europe, and Australia.

Correspondence to: Edward J. Walsh, Ph.D. • Boys Town National Research Hospital • 555 North 30th Street • Omaha, NE 68131. Telephone: (402) 498-6701; fax: (402) 498-6351; email: walsh@boystown.org

Although hormone replacement therapy usually corrects the disorder if detected early, it remains a common cause of mental retardation, particularly in developing countries (Delange 1998). It is also well known that numerous genetic and nongenetic factors are responsible for thyroid system pathology and that, in addition to impaired cognitive function, the untreated condition can produce unambiguous and extreme otological abnormalities. Moreover, it is generally accepted that the auditory system is particularly sensitive to thyroid system disorders when compared with other systems, especially during development (Deol 1973; Hébert et al. 1985a,b; Sohmer and Freeman 1996, for review).

One of the most common forms of syndromic deafness resulting from thyroid system pathology occurs in association with Pendred's syndrome (PS). PS is inherited as an autosomal recessive trait that has been linked to numerous mutations in the *PDS* gene that encodes pendrin, a transmembrane anion transport protein that is expressed in the thyroid gland, kidney, and inner ear. While it is generally held that pendrin transports iodide in the thyroid gland, the protein is thought to transport chloride/formate as well (Everett et al. 1997; Van Hauwe et al. 1998; Scott et al. 1999; Scott and Karniski 2000), implicating it as a homeostatic factor in the kidney and the stria vascularis of the inner ear. In the inner ear of mice, the *Pds* gene is expressed in the endolymphatic duct and sac and in the external spiral sulcus (Everett et al. 1999), suggesting that sequence abnormalities associated with this gene may lead directly to inner-ear pathophysiology.

In addition to otopathology resulting from the interruption of any one of the steps in the cascade of biochemical events that lead to the synthesis, release, or circulation of thyroid hormone, the expression of abnormal thyroid hormone receptors can also compromise normal audition. For example, the disease known as "thyroid hormone resistance" is an autosomally inherited disorder that is caused by mutation of β-subunit of the thyroid hormone receptor (TRβ) that produces a variable degree of hearing loss in a subset of affected individuals (Refetoff 1997; Brucker–Davis et al. 1996). In addition to these genetic causes, environmental factors, such as dietary insufficiency of iodine and exposure to polychlorinated biphenyls (PCBs), can also produce profound congenital hearing loss (Rajatanavin et al. 1997; Goldey et al. 1995a).

While progress is being made toward a more comprehensive understanding of the pathological basis of hearing loss associated with thyroid-related disorders, a full accounting remains incomplete. Abnormalities appear widespread, presumably extending from the periphery (Bargman and Gardner 1967; Kohonen et al. 1971; Deol 1973; Meyerhoff 1979; Uziel et al. 1981, 1983a; Anniko and Rosenkvist 1982; Gabrion et al. 1984; Legrand et al. 1988) to the highest central processing centers (Ruiz–Marcos et al. 1983; Dow-Edwards et al. 1986; Berbel et al. 1993). However, inconsistent findings across studies have complicated efforts to comprehensively understand the otherwise clear otopathology. This problem is compounded by the fact that hearing deficits arising from thyroid-related disorders range from mild to profound, as determined by assessing behavioral reflexes (Deol 1973; Comer and Norton 1982; Goldey et al. 1995b), as well as the outcome of tympanometric (Crifò et al. 1980), audiometric (de Luca et al. 1985), and evoked potential (Van Middlesworth and Norris 1980; Uziel et al. 1983a; Himelfarb et al. 1981; Hébert et al. 1985a,b; Bichsel et al. 1988; Goldey et al. 1995b) indices of the disease. Such wide-ranging variation in otology may be related to the observation that the degree of otopathology and its expression correlate with the onset, duration, and severity of the primary disease (Deol 1973; Hébert et al. 1985a, b; Uziel et al. 1985) and, in genetic forms of the disease, it may reflect the locus of mutation.

Hypothyroidism has been studied most frequently in experimental animals rendered hypothyroid using goitrogens (Deol 1973; Uziel et al. 1981; Albee et al. 1989), although ablative surgery (Withers et al. 1972) and radiation (Meyerhoff 1979) have also been used to produce the disease. In recent years, an increasingly popular approach is to take advantage of naturally occurring forms of the disease and, in that context, numerous strains of mice bearing heritable thyroid defects are currently available to the scientific community (Lyon et al. 1996). The *Tshr*^{hyt} or *hyt* mouse is a well-established animal model of sporadic congenital primary hypothyroidism (Beamer et al. 1981; Beamer and Cresswell 1982) that occurs in the late fetal period with the onset of autonomous thyroid hormone secretion (Beamer and Cresswell 1982; Stein et al. 1989). The condition is inherited as an autosomal recessive trait that results from a single base mutation in the gene encoding the thyrotropin receptor (*Tshr*) that is located on chromosome 12. The amino acid substitution resulting from this mutation occurs in a highly conserved region of the receptor, transforming the protein to a form that is unable to bind thyrotropin (TSH) (Stein et al. 1994; Gu et al. 1995). The phenotype of mice that are homozygous for the *hyt* allele is profound thyroid hypoplasia, resulting in persistent congenital hypothyroidism (Beamer et al. 1981; Adams et al. 1989) that mimics the clinical condition observed in patients with mutations in the *TSHR* gene and who are unresponsive to TSH (Refetoff et al. 1996; de Roux et al. 1996; Clifton–Bligh et al. 1997; Abramowicz et al. 1997; Biebermann et al. 1997). Based on work in our laboratory (Sprenkle 1997; Sprenkle et al. 1997; Walsh et al. 2000; Farrell et al. 2000) and the laboratory

of O'Malley (O'Malley et al. 1995; Li et al. 1999), it is clear that auditory function in the *Tshr* mutant mouse is abnormal, making individuals from this strain ideal subjects for studies of hypothyroid-induced congenital deafness.

The purpose of this investigation was to determine comprehensively the degree of physiological impairment associated with hypothyroidism in *Tshr* mutant mice. To achieve this, auditory brainstem responses (ABRs) and 2-deoxyglucose uptake profiles were studied in both phenotypically normal individuals (i.e., heterozygotes, *+/hyt*) and affected individuals (i.e., animals that were homozygous, *hyt/hyt,* for the trait). The consequences of replacing thyroxin (T_4) during a thyroxin-dependent critical period of development (Deol 1973) were also assessed.

METHOD

Study design

Adult male mice were divided into four groups: saline-treated (placebo) heterozygotes ($+/hyt_{(S)}$), thyroxin-treated heterozygotes ($+/hyt_{(T4)}$), saline-treated homozygous mutants ($hyt/hyt_{(S)}$), and thyroxin-treated homozygous mutants ($hyt/hyt_{(T4)}$). ABRs of 10–12 mice were evaluated in each group. Subjects were drawn from 15 litters. From each of those groups, 4–6 mice/group were evaluated for energy consumption by various central auditory nuclei using 2-deoxyglucose uptake determined 2 days after ABR recordings. The care and use of animals were approved by the Creighton University Animal Care and Use Committee.

Animal husbandry

Experimental subjects were bred in-house and were maintained on a 12:12 light/dark schedule, housed in 18 × 13 × 28-cm polycarbonate cages with pine-shaving bedding (Devil's Tower Co, Hulett, WY, USA), and were fed Formulab Chow 5008 (Purina Mills, Inc, Richmond, IN, USA); tap water was available *ad libitum.*

The breeding pairs from which the experimental subjects were derived were first- and second-generation offspring of mice obtained from the *hyt* colony at The Jackson Laboratory (Bar Harbor, ME); the *Tshr* colony is maintained on a BALB/c inbred line. Breeding pairs always consisted of a *hyt/hyt* male and a *+/hyt* female. This approach facilitated breeding efforts given the impaired reproductive status of hypothyroid females (i.e., their relative infertility and reduced capacity to maintain pregnancy). Males were housed separately and received thyroid powder supplements [desiccated porcine thyroid powder from Sigma Chemical Co. (St. Louis, MO); 25 mg/kg chow] prior to and between matings to enhance fertility. Litter size was culled to 6 within 24 hours of birth (postnatal day 0) and weanlings were housed separately on the basis of gender.

T_4 replacement

Either thyroxin or saline was administered to pups on a daily basis from birth (day 0) through postnatal day 10, a stage that has been identified as a critical, thyroxin-dependent developmental period in mice (Deol 1973). Serum thyroxin levels in *hyt/hyt* mice were restored to normal, age-matched values via subcutaneous injections of L-thyroxin (Sigma) on days 0–5 (4 ng/g), 6–8 (5.8 ng/g), and 9–10 (9.1 ng/g) according to the regimen used by Hébert et al. (1985b) and validated by Sprenkle et al. (2001a,b) for the mouse. Stock solutions of thyroxin (1.33 mg/100 mL in NaOH, pH 10) were frozen until use. Fresh working solutions (pH 7.7) were prepared for each litter on the day of birth by dilution with sterile saline. Concentrations were confirmed by radioimmunoassay (see below). Injections were performed between 0800 and 1000 hours using a 0.5-mL syringe with a 30-gauge needle.

Serum T_4 determinations

Serum T_4 levels were used to distinguish heterozygotes from homozygous mutants according to the procedure of Beamer et al. (1981). Animals with thyroxin levels $\leq$1.0 μg/dL were classified as *hyt/hyt* and those with thyroxin levels $\geq$4.0 μg/dL were placed in the *+/hyt* category. Thyroxin levels fell completely within these boundaries and the assignment of individuals to appropriate groups was, therefore, completely unambiguous. Blood samples were collected from the tail vein between postnatal days 40 and 44 and radioimmunoassays were performed, in duplicate, on 5-μL aliquots of serum (Serum T_4 Coated-Tube RIA, ICN Biochemicals, Costa Mesa, CA, USA). The assay detection limit for T_4 was 0.01 μg/dL.

Behavioral and physical assessments

Body weights and age associated with eye and pinna openings were monitored on a daily basis. Behavioral attributes were evaluated weekly, beginning on postnatal day 21. The tail-hanging test, as described by Llorens et al. (1993), was used to identify gross, overt vestibular impairment. Individuals were scored positively, indicating impairment, if they either ventroflexed their body or twirled while suspended by their tail; normal animals typically assume the posture for landing upright (i.e., extension of forelimbs in a righting reflex). To evaluate circling tendency, also an

indicator of vestibular pathology, mice were videotaped for one hour in a large enclosed space and the tapes were reviewed during three discrete and random 5-min periods. A circle was defined as a continuous 360° rotation in either the clockwise or counterclockwise direction. The difference between mice displaying "circling" behavior and those that did not was unambiguous and group affiliation was readily apparent upon even casual inspection.

ABR determinations

ABRs were recorded from adults (postnatal days 75–90) anesthetized with chloral hydrate (480 mg/kg IP) and supplemental doses (120 mg/kg) were administered as needed. Body temperature was thermostatically regulated and maintained at approximately 37.5°C throughout recording sessions. ABRs were recorded using subdermal electrodes positioned at the vertex (active, noninverting), mastoid region (reference, inverting), and neck musculature (ground). Differentially recorded scalp potentials were amplified 100,000× (Grass Model P511J), band-pass filtered between 0.03 and 10 kHz, and digitized (Cambridge Electronics Design 1401 *plus,* Cambridge, England) using a 20-kHz sampling rate over a 20-ms epoch, which included a 5-ms baseline recorded prior to stimulus delivery. A total of 500 trials were averaged.

Symmetrically shaped tone bursts were 3 ms long (1-ms linear on/off ramps and 1-ms plateau). Click stimuli were 60 μs in duration and both clicks and tone bursts were generated digitally. All acoustic stimuli were delivered free-field via a high-impedance piezoelectric tweeter (Realistic 40-1397) positioned 10 cm from the vertex. Stimulus levels were calibrated using a 0.5-in. microphone (Model 4134 Brüel and Kjær, Norcross, GA) positioned appropriately within the recording chamber (Industrial Acoustic Corp, New York, NY, USA) and are reported in decibels sound pressure level (dB SPL referenced to 20 μPa). Stimuli of alternating polarity were delivered at a rate of 5/s. ABR thresholds were determined for clicks and for tone bursts at 20, 16, 8, 4, and 2 kHz. Response intensity series were acquired using both clicks and tone bursts (20 and 8 kHz) incremented in 5-dB steps, starting below threshold and extending to 90 dB SPL. Three averages were acquired at each level.

Brain 2-deoxyglucose uptake

Energy consumption in specified auditory nuclei was mapped by measuring the uptake of 2-deoxyglucose (2-DG) (Sokoloff et al. 1977). Glucose utilization estimates were made two days following ABR recordings, a period of time that assured full recovery from residual influences of anesthesia. Mice received an intraperitoneal injection of ^{14}C-2-deoxyglucose (1 μCi/g, American Radiolabled Chemical Co, St. Louis, MO, USA) and were maintained in a quiet environment during the period immediately before injection until the time of sacrifice, which was achieved by sodium pentobarbital overdose. This occurred 45 min after injection of 2-DG. Brains were rapidly removed and frozen in isopentane at −40°C. Serial 20-μm sections were dried onto glass slides and apposed to Kodak SB5 X-ray film for 5 days. Autoradiograms were analyzed on a DUMAS computerized densitometer with BRAIN software (Drexel University, Philadelphia, PA, USA). The relative metabolic activity of each discrete structure was determined by calculating the ratio of the optical density of that structure to the optical density of the appropriate local commissural or peduncular white matter.

Data analysis

ABR threshold was defined as the lowest stimulus level capable of producing an unambiguous, reproducible response. For the purpose of statistical comparison only, when an animal was unresponsive to stimuli presented at the maximum level used in the study (90 dB SPL), "threshold" was assigned a value of 95 dB SPL. While useful and necessary in the determination of significant differences among groups studied here, this approach may underestimate actual thresholds in unresponsive individuals and may, therefore, lead to the false conclusion that significant differences do not exist where they actually do.

Peak latencies were determined relative to the onset of the stimulus, and peak amplitudes were computed using a triangulation procedure as in Walsh et al. (1986a,b). Custom software was used for data acquisition, measurements of peak amplitudes, latencies and interpeak intervals, and subsequent analyses, including curve-fitting procedures.

Slopes of latency–intensity curves were computed by first normalizing each latency–level curve to the latency value observed at 90 dB SPL (taking the difference and adding a value of 1) and, second, converting the normalized latencies to logarithmic values and performing a least-squares linear regression of logarithmic latency-vs.-stimulus level (in dB) curves; results are presented in log μs/dB units. Slopes of amplitude–level curves were computed according to a least-squares linear regression approach in which measurements used in the computation were restricted to responses produced by near-threshold level stimuli that fell in the linear response range.

One-way analysis of variance (ANOVA) was used to evaluate birth weights and dates of eye-opening and pinna-raising and 2-deoxyglucose data. Changes in body weights during development were analyzed by

TABLE 1

Somatic and behavioral characteristics

	$+/hyt_{(S)}$	$+/hyt_{(T4)}$	$hyt/hyt_{(S)}$	$hyt/hyt_{(T4)}$
Serum T_4[1] (μg/dL)	5.72 ± 0.36	6.38 ± 0.51	0.15 ± 0.09	0.11 ± 0.08
Birth weight[2] (g)	1.46 ± 0.27	1.39 ± 0.24	1.54 ± 0.25	1.46 ± 0.17
Eye-opening age[3]	14.0 ± 0.9	14.3 ± 0.6	16.9 ± 1.4[a,b]	16.4 ± 2.0[a,b]
Pinna-raising age[3]	15.2 ± 1.1	14.6 ± 0.9	21.0 ± 1.5[a,b]	18.2 ± 1.6[a,b]
Tail-hanging test	0/12	0/11	0/10	0/10
Circling behavior	0/12	0/11	6/10	5/10

Sample size for each measurement is indicated as the total number of animals tested for circling behavior under the appropriate column.
[1]Mean ± SEM
[2]Mean ± SD
[3]Mean ± SD in postnatal days
[a]$p < 0.001$ vs. $+/hyt_{(S)}$
[b]$p < 0.001$ vs. $+/hyt_{(T4)}$

two-way ANOVA. Tukey tests were used for multiple comparisons. Intergroup comparisons of electrophysiological data were performed nonparametrically using the Kruskal–Wallis test. Spearman's rank correlation coefficient was used to assess the relationships between the electrophysiological data and serum T_4 level. Differences were considered significant at $p < 0.01$ unless otherwise specified.

RESULTS

Somatic and behavioral characteristics

Serum T_4 levels in adult *Tshr* mutant mice fell into two distinct, nonoverlapping populations with mean values of 0.15 μg/dL ($n = 10$) and 5.72 μg/dL ($n = 12$) that ranged from 0.01 to 0.82 μg/dL and from 4.29 to 7.56 μg/dL, respectively. This bimodal distribution is characteristic of *Tshr* mutant mice (Adams et al. 1989; Stein et al. 1989) and served as the basis for sorting experimental subjects into *hyt/hyt* and *+/hyt* groups. T_4 treatment during the perinatal period did not significantly alter adult serum T_4 levels (Table 1).

Although individual differences were not observed at birth (Table 1), growth in body weight was significantly ($p < 0.01$) retarded among *hyt/hyt* mice relative to heterozygotes by postnatal day 9, and adult homozygotes weighed 35% less than their *+/hyt* counterparts (Fig. 1). T_4 treatment did appear to produce a slight weight gain in *+/hyt* mice during the latter half of the dosing period, but the effect was short lasting (Fig. 1). Also, although the mean body weight of T_4-treated *hyt/hyt* animals was within normal limits through postnatal day 21, as with *+/hyt* mice, there were no long-term effects of T_4 treatment on weight gain.

Average ages for eye-opening and pinna-raising in the four groups of mice studied here are shown in (Table 1). For heterozygotes, eye-opening occurred at postnatal day 14, whereas pinnae unfolded about one day later. T_4 treatment of *+/hyt* mice had no effect on either measure. Compared with heterozygotes, both eye-opening and ear-raising were delayed in *hyt/hyt* mice by 3 and 6 days ($p < 0.001$), respectively. T_4 treatment of *hyt/hyt* mice reduced the delay in ear-raising by 3 days, but had no effect on the age of eye-opening.

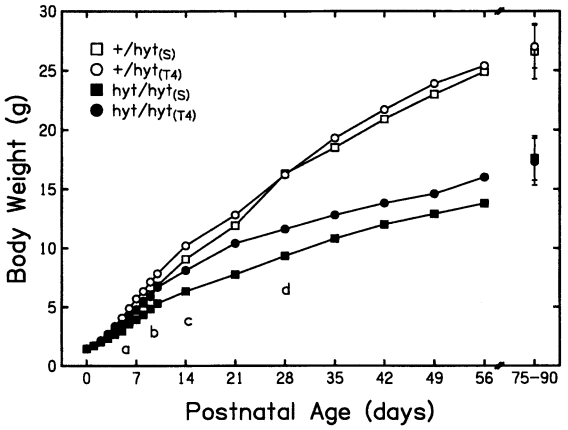

FIG. 1. Growth curves for hypothyroid and euthyroid mice. Mean body weights as a function of postnatal age are shown for each of the four experimental groups: hypothyroid (*hyt/hyt*) and euthyroid (*+/hyt*) mice given thyroxin (T_4) or saline placebo (S) injections (T_4 or S indicated in subscripts) from birth through the postnatal day 10. For the sake of clarity, error bars are not shown. Values at the far right are group weights (mean ± SD) at the time of ABR testing. Each group contained 10–12 mice. At the age indicated by "a," the weights of the $+/hyt_{(T4)}$ group began to surpass all other groups ($p < 0.05$); "b," $+/hyt_{(S)}$ began to surpass $hyt/hyt_{(S)}$ ($p < 0.01$); "c," $+/hyt_{(T4)}$ no longer surpassed $+/hyt_{(S)}$; "d," $+/hyt_{(S)}$ began to surpass $hyt/hyt_{(T4)}$ ($p < 0.01$).

Circling behavior was observed in 60% of saline-treated homozygotes ($hyt/hyt_{(S)}$) and in 50% of thyroxin-treated homozygotes ($hyt/hyt_{(T4)}$) but was not accompanied by other overt signs of vestibular impairment, such as head-tilting or jerking, or abnormal responses to the tail-hanging test (Table 1). None of the heterozygotes exhibited circling behavior or other

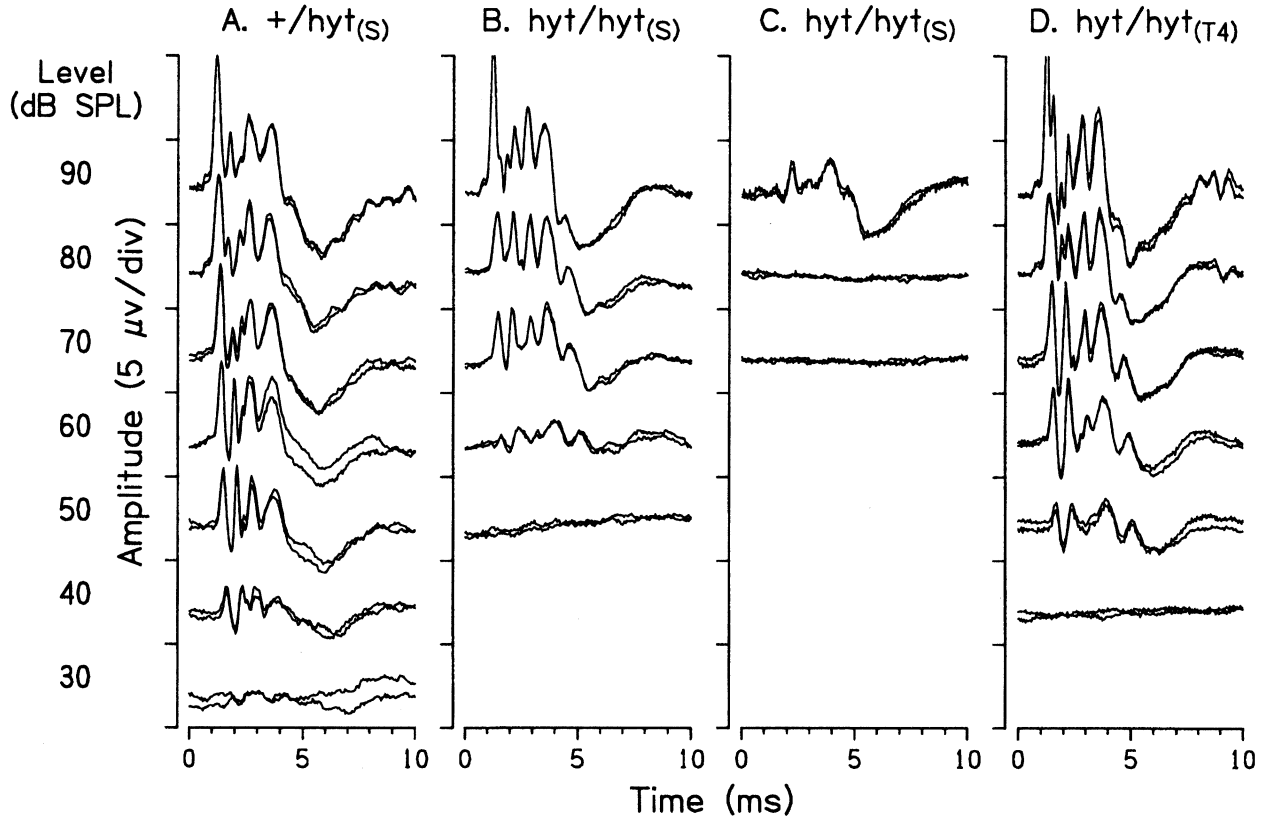

FIG. 2. Representative examples of ABR waveforms. Click-evoked waveforms were obtained from individual mice given thyroxin (T_4) or saline placebo (S) injections from birth through postnatal day 10 and are plotted across a range of stimulus levels. Two replicates are shown overlapping at each level. ABR level series from two $hyt/hyt_{(S)}$ individuals are shown (**B, C**) to represent the variability in responses within this group. Waveforms of $+/hyt_{(T4)}$ mice (data not shown) were very similar to those of $+/hyt_{(S)}$ mice.

signs of vestibular impairment. Mice exhibiting circling behavior were indistinguishable from other *hyt/hyt* mice with respect to T_4 levels, weights at birth and at the time of ABR testing, and ages of eye-opening and pinna-raising.

General ABR findings

The essential findings of this investigation are illustrated in Figure 2, in which ABRs from both heterozygotes (*+/hyt*) and homozygotes (*hyt/hyt*) are compared. ABRs to clicks generally consisted of four major waves, designated I, II, III, and IV in both mutant and normal animals. Response waveforms to tone bursts were similar, regardless of stimulus frequency or genetic category, although ABRs elicited by low-frequency tone bursts (<4 kHz) were less robust than those elicited by higher frequencies or click stimuli, most likely a reflection of higher low-frequency thresholds in *Tshr* heterozygotes. The capacity of thyroxin to partially reverse the pathophysiological consequence(s) of hypothyroidism in *Tshr* mutant mice is illustrated in Figure 2D. Although thyroxin-treated homozygotes were generally more sensitive to acoustic stimuli than those in the placebo-treated group, the recovery of function—here determined by considering threshold differences between treated homozygotes and untreated heterozygotes—was incomplete, i.e., treated homozygotes were less sensitive than untreated heterozygotes.

The pattern illustrated in Figure 2 concisely represents the salient findings of the larger study. Animals that were heterozygous for the *hyt* trait were indistinguishable from normal animals; those that expressed homozygosity for the trait were always impaired, and some function was recovered when thyroxin was replaced in mutants during the first 10 postnatal days.

Incidence of response

One of the clearest differences between the hypothyroid and euthyroid groups included in this study was the number of individuals from each category that responded to acoustic stimulation. We show this in the form of response incidence curves that were generated by plotting the percentage of mice in each experimental group that responded to specific stimuli as a function of stimulus level (Fig. 3). It is clear that curves depicting hypothyroid groups (filled symbols), regardless of their treatment category ($hyt/hyt_{(S)}$ or $hyt/hyt_{(T4)}$),

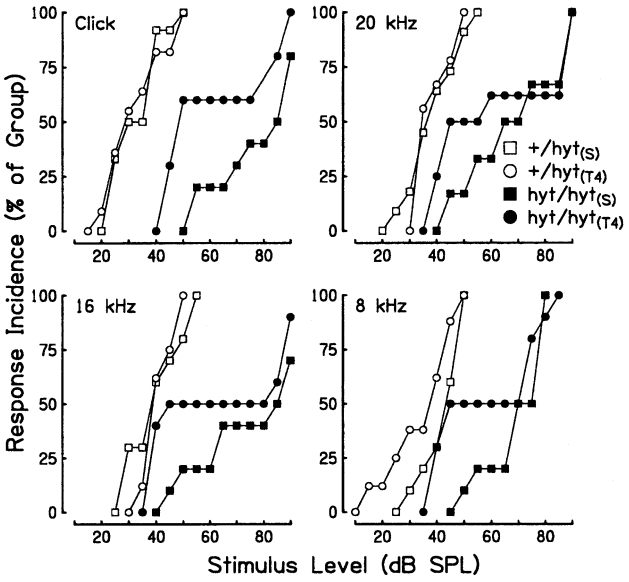

FIG. 3. Response incidence-vs.-stimulus level functions. The percentage of mice within each study group that responded to clicks and tone bursts is plotted as a function of stimulus level. The symbol key for all panels is located in the top right panel. For each group, 8–12 mice were tested.

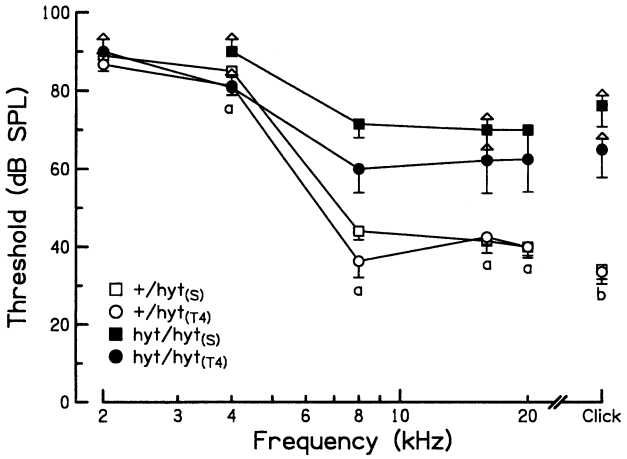

FIG. 4. ABR audiograms for euthyroid and hypothyroid mice. Average ABR thresholds in response to click and tone burst stimuli are plotted for each experimental group indicated in the symbol key. Upward-pointing arrows denote instances in which one or more mice exhibited no measurable response at the highest stimulus intensity used (90 dB SPL). Error bars represent the SEM of 8–12 mice tested per group. Letter designations next to symbols represent the following statistically significant differences ($p < 0.01$) compared with the +/$hyt_{(S)}$ group: "a," vs. *hyt*/$hyt_{(S)}$; "b," vs. *hyt*/$hyt_{(S)}$ and vs. *hyt*/$hyt_{(T4)}$.

are shifted toward higher levels (i.e., to the right) relative to the euthyroid (+/*hyt*) groups (open symbols), indicating that the percentage of responsive homozygotes was typically less than the percentage of responsive heterozygotes, regardless of level.

The percentage of euthyroid animals (+/$hyt_{(S)}$ and +/$hyt_{(T4)}$ groups) that responded to clicks and tone bursts between 20 and 8 kHz increased regularly as the stimulus level was raised from near threshold (15 to roughly 35 dB SPL) to approximately 50–60 dB SPL, with all euthyroid animals responding to stimuli in this frequency range when levels greater than 60 dB SPL were considered. The smooth, relatively rapid recruitment of responsive individuals that was observed as stimulus level increased is indicative of the relatively low intragroup variability observed in response thresholds among these animals.

In contrast to the relatively steep and smooth response incidence-vs.-level curves that characterized heterozygotes, growth curves denoting the percentage of homozygous mutant mice responding to clicks and tone bursts above 4 kHz were more complicated and irregular, a finding that is consistent with the high degree of intragroup variability in response thresholds observed for homozygous animals. Although the majority of homozygotes responded to clicks and tone bursts above 4 kHz at moderate to high stimulus levels, very few animals responded to 2 kHz, even at the highest level studied, and at 4 kHz only 30% of *hyt*/$hyt_{(S)}$ animals responded at 90 dB SPL. None responded below that level. This contrasts dramatically with the observation that 100% of +/*hyt* mice responded to 4-kHz tone bursts presented at 90 dB SPL.

The percentage of mutant homozygotes that responded to acoustic stimulation was higher in the thyroxin-treated group (filled circles) when compared with untreated animals (filled squares), regardless of stimulus conditions, but was less than that observed in the heterozygote category (Fig. 3). Interestingly, growth curves for clicks and tone bursts above 4 kHz were biphasic, exhibiting a low-level growth component that ranged between 40 and 50 dB SPL and a high-level growth phase in which the percentage of responsive animals grew again above 80 dB SPL, suggesting that distinct subgroups exist within the *hyt*/$hyt_{(T4)}$ category.

ABR threshold

Like their wild-type counterparts, heterozygotes responded to relatively low-level clicks and tone bursts ≥8 kHz, while thresholds for 4-kHz tone bursts and below exceeded 75 dB SPL (Fig. 4). In contrast, saline-treated homozygotes (*hyt*/$hyt_{(S)}$) were generally insensitive to all stimuli tested. The largest threshold difference between *hyt*/$hyt_{(S)}$ and +/$hyt_{(S)}$ mice was 42 dB in response to clicks ($p < 0.01$), but thresholds were significantly higher (~30 dB higher) for all tone bursts tested above 4 kHz in *hyt*/$hyt_{(S)}$ animals ($p < 0.01$). Thyroxin treatment improved the mean thresholds of the *hyt*/*hyt* group by approximately 10 dB, although the improvement was not statistically significant, with

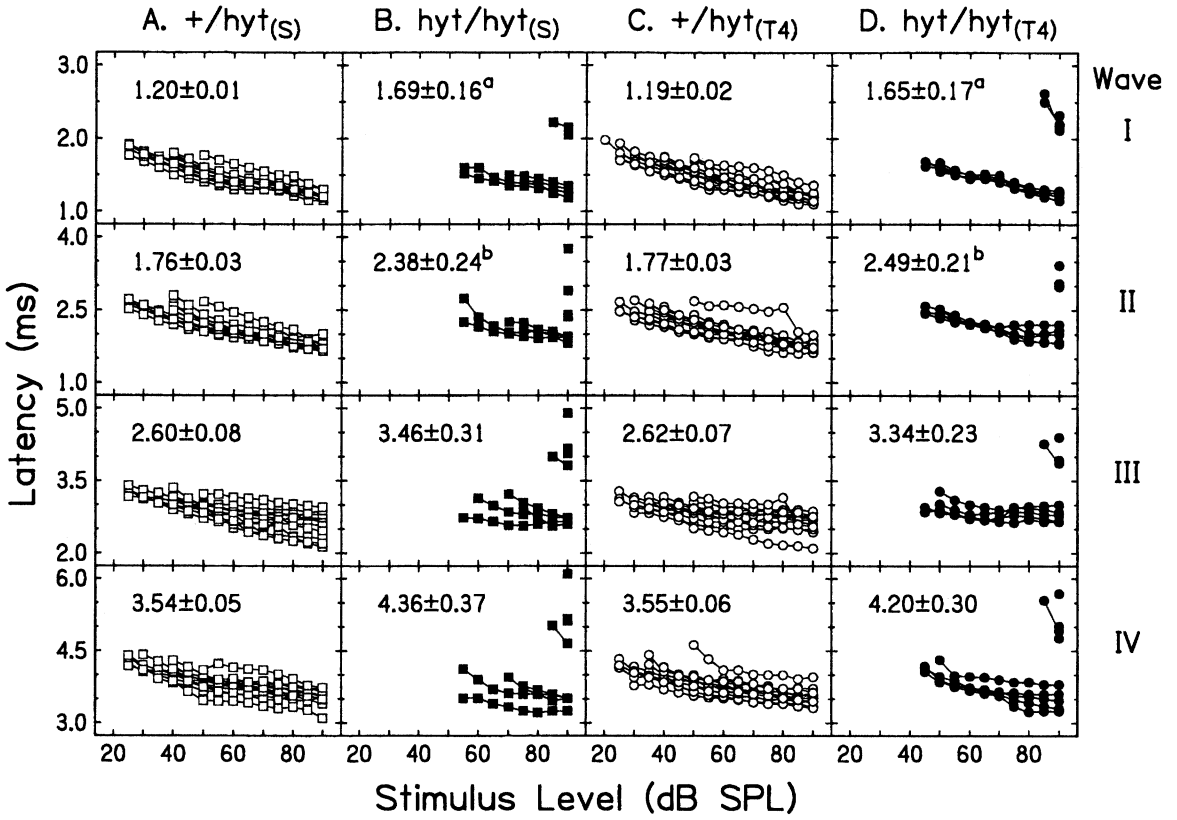

FIG. 5. Latency–level functions for click stimuli. Click-evoked latencies of each primary ABR wave (I–IV, top to bottom rows) are plotted as a function of stimulus level for each experimental group (columns). Each curve represents the responses from one individual. Mean latencies (in ms) at 90 dB SPL (±SEM) are indicated for each panel and associated letter designations represent statistically significant differences ($p < 0.001$) between the indicated group and the following groups: "a," vs. +/$hyt_{(T4)}$; "b," vs. +/$hyt_{(S)}$ and vs. +/$hyt_{(T4)}$

thresholds for this group remaining considerably higher than those observed in heterozygotes. T_4 treatment had no effect on thresholds of the +/*hyt* group across the frequency range or in response to click stimuli.

ABR wave latencies

Regardless of group affiliation, response latencies generally followed the well-established rule relating latency and stimulus level in that latencies of all ABR peaks decreased as level increased. However, latency–intensity curves from placebo-treated homozygotes (*hyt*/$hyt_{(S)}$) were unambiguously abnormal, as shown in Figures 5 and 6 which depict response latencies to clicks and 8-kHz tone bursts, respectively. The most evident abnormality associated with response latency was intragroup variability and the relatively steep slopes of latency–intensity curves associated with cases in which response times were significantly prolonged. At 90 dB SPL, the highest sound level used in this study, the difference between the tightly clustered distribution of latencies for +/$hyt_{(S)}$ animals and the broad distribution of component latencies for saline-treated homozygotes (*hyt*/$hyt_{(S)}$) is relatively clear (compare Figs. 5A with 5B and Figs. 6A with 6B). While latency differences were significant in the case of responses to 90-dB-SPL clicks and 8-kHz tone bursts for certain waves of the ABR (see legends for Figs. 5 and 6), response latencies of *hyt*/*hyt* mice to 20 kHz were not significantly different from their heterozygous cohorts. However, it is important to point out that average differences of the same basic magnitude were observed for all ABR waves and the failure to detect significance under those conditions most likely reflects the relatively small number of responsive homozygotes. Latency–level curves representing T_4-treated +/*hyt* mice were essentially like their untreated counterparts, although overall variability appeared to decrease some (Fig. 6).

It is especially interesting that peak latencies measured from responses to clicks for all four waves fell into two distinct subgroups among *hyt*/$hyt_{(S)}$ animals (Fig. 5B). One was clearly abnormal for all four waves, exhibiting greatly prolonged latencies at 90 dB SPL. The other subgroup was nearly normal, or "+/*hyt*-like," with respect to both latencies measured at 90 dB SPL and latency–level curve slopes under most conditions. The extent to which homozygotes exhibited distinct subgroups was stimulus-dependent.

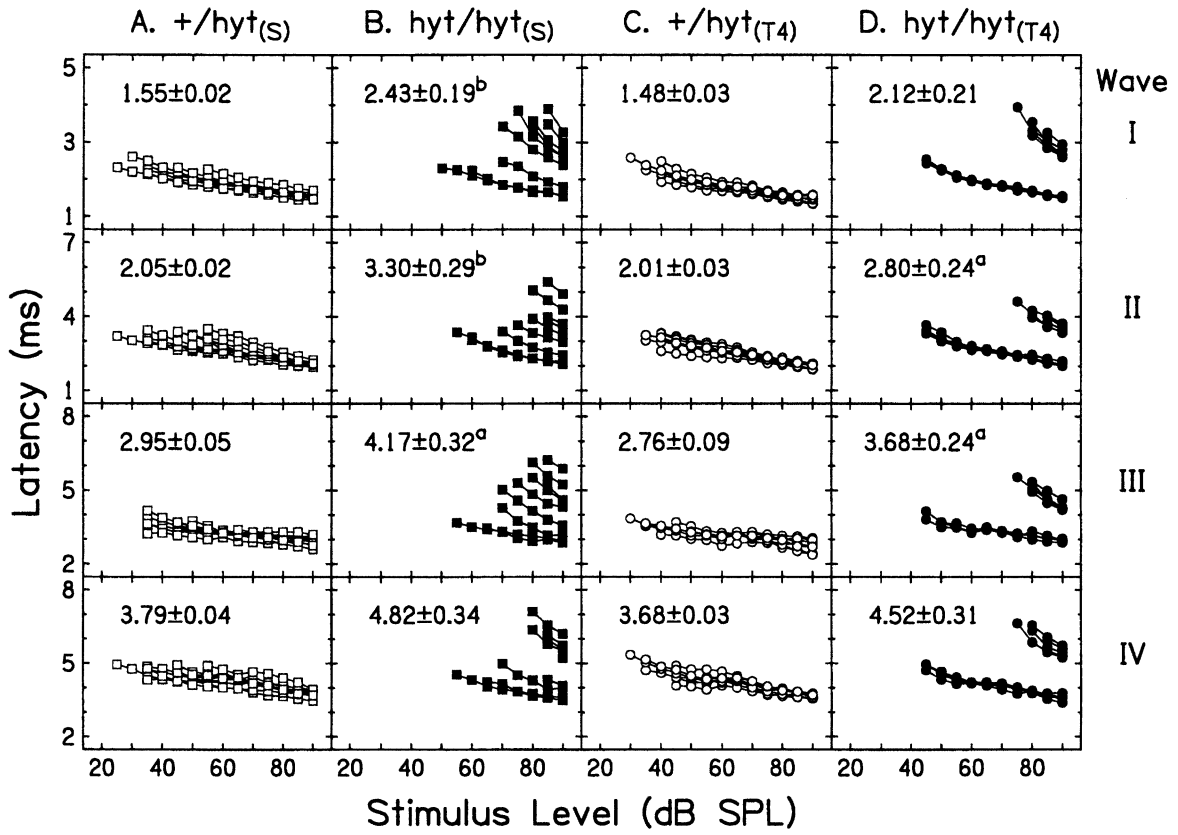

FIG. 6. Latency–level functions for 8 kHz. ABR wave latencies for each primary component (I–IV, top to bottom rows) are plotted as a function of stimulus level for each experimental group (columns). Each curve represents the responses from one individual. Mean latencies (in ms) at 90 dB SPL (±SEM) are indicated for each panel and associated letter designations represent statistically significant differences ($p < 0.001$) between the indicated group and the following groups: "a," vs. +/$hyt_{(T4)}$; "b," vs. +/$hyt_{(S)}$ and vs. +/$hyt_{(T4)}$.

Whereas two distinct subgroups were observed in response to clicks, latency–level curves derived from responses to tone bursts were more evenly distributed, although the range of latencies for a particular stimulus condition varied widely (Fig. 6B).

Slopes of latency–level curves were significantly steeper for all waves among homozygotes with extended latencies than in heterozygotes for 8 kHz, while at 20 kHz, statistically significant differences were apparent only for wave I and were not observed in the case of clicks (Fig. 7, left column). It is likely, however, that the relatively small number of homozygous animals that were responsive to clicks and 20 kHz contributed to the inability to assess statistical significance to differences observed between these groups.

The separation of latency–level curves into normal and abnormal groups emerged more evidently when homozygous mice were treated with T_4. Under these conditions, the grouping phenomenon was also observed for responses to 8 kHz (Fig. 6D). Also, more *hyt*/$hyt_{(T4)}$ mice (40%–60%) exhibited clear "+/*hyt*-like," or near-normal latencies to all stimuli studied. Latencies for the "+/*hyt*-like," group were tightly clustered and latency–level slopes were the same as those of +/*hyt* mice. On the other hand, latencies representing remaining members of the group were significantly prolonged ($p < 0.001$) and latency–level slopes were generally steep (Fig. 7, left column).

When latency–intensity plots were normalized relative to each individual's threshold (e.g., level converted from dB SPL to dB SL, or sensation level units), as shown in Figure 8 for wave I, overall variability associated with latencies from the *hyt*/*hyt* groups decreased slightly but did not change in the case of +/*hyt* animals. Latency estimates were still widely distributed in *hyt*/*hyt* mice, and mean latencies at threshold were generally elevated when compared with heterozygotes.

ABR interpeak intervals

Measurement of interpeak intervals I–II and I–IV (central conduction time, CCT) as a function of stimulus level for individual animals are shown in Figure 9. With increases in level, interpeak intervals, including the intervals between waves II–III and III–IV, tended to decrease. However, consistent differences between the interpeak intervals of +/*hyt* and *hyt*/*hyt* mice were not observed and T_4 treatment did not significantly

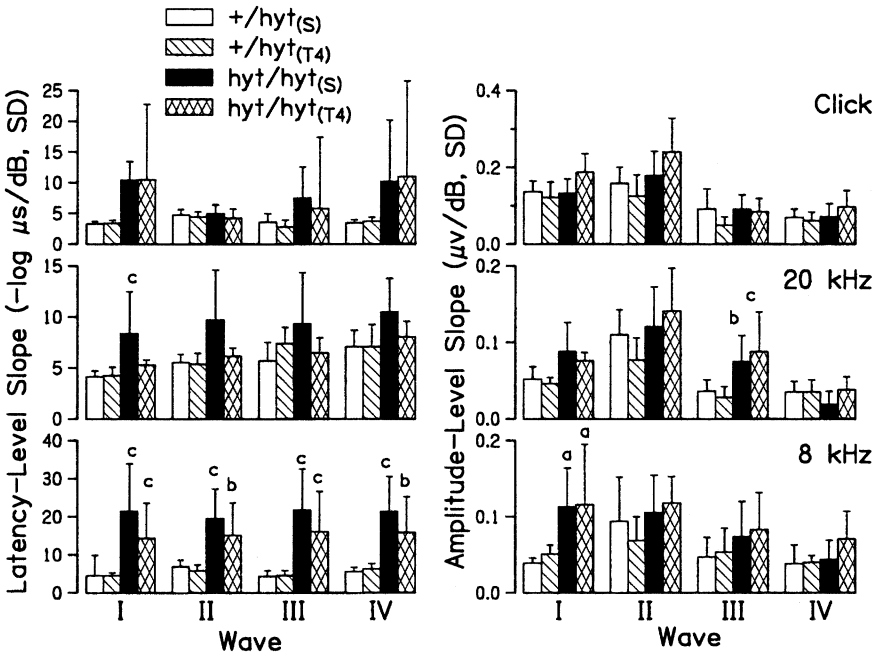

FIG. 7. Slopes of latency–level and amplitude–level curves. Mean slopes of latency-level curves (left column) and mean slopes of amplitude-level curves (right column) are compared across experimental groups (bar code key indicated in top left panel). Panels in the top, middle, and bottom rows are for responses to clicks, 20 kHz, and 8 kHz, respectively. Data are shown for waves I through IV. Latency–level slopes were computed by normalizing latency to the value observed at 90 dB SPL, converting to logarithmic units, and performing a least-squares linear regression fit to the data. Letter designations above bars represent statistically significant differences ($p < 0.001$) between the indicated group and the following groups: "a," vs. +/$hyt_{(S)}$; "b," vs. +/$hyt_{(T4)}$; "c," vs. +/$hyt_{(S)}$ and vs. +/$hyt_{(T4)}$.

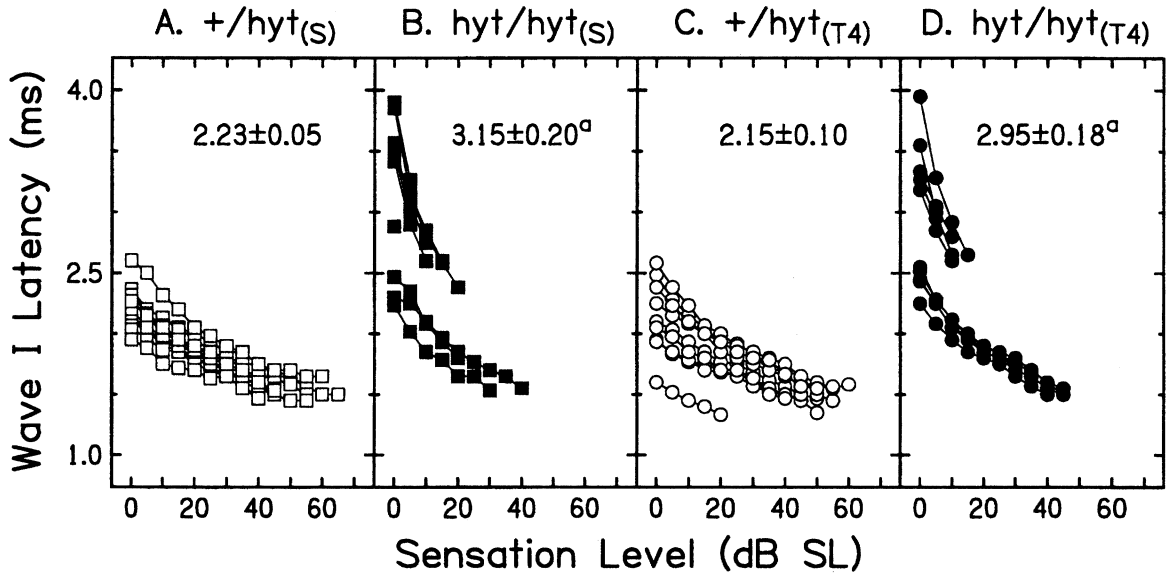

FIG. 8. Latency–sensation level functions. Wave I latencies at 8 kHz (from Fig. 6) are replotted as a function of sensation level for each experimental group (**A–D**). Each curve represents the responses of an individual animal. Sensation level was computed by subtracting the ABR threshold of each individual from the absolute pressure level. Mean latencies (in ms) at 0 dB SL (±SEM) are indicated for each panel and the associated letter designation ("a") represents statistically significant differences ($p < 0.001$) between the indicated group and both the +/$hyt_{(S)}$ and the +/$hyt_{(T4)}$ groups.

alter interpeak intervals or central conduction time in either group. At 90 dB SPL, the average I–II interval was shorter than were average II–III and III–IV intervals for the +/*hyt* group but not for the *hyt*/*hyt* groups in response to clicks and 8 kHz.

ABR wave amplitudes

Peak amplitudes were highly variable in response to all stimuli, as represented by responses to clicks in Figure 10. The amplitudes of waves I, III, and IV generally increased with stimulus level and saturated below 90 dB SPL, while wave II amplitudes were frequently nonmonotonic.

Figure 11 shows average amplitude-vs.-stimulus level curves for responses to clicks, 20 kHz, and 8 kHz; standard deviation bars were omitted to facilitate the comparison, but estimates of variability can be found in Figure 10. Peak amplitudes recorded from the majority of saline-treated homozygotes were smaller

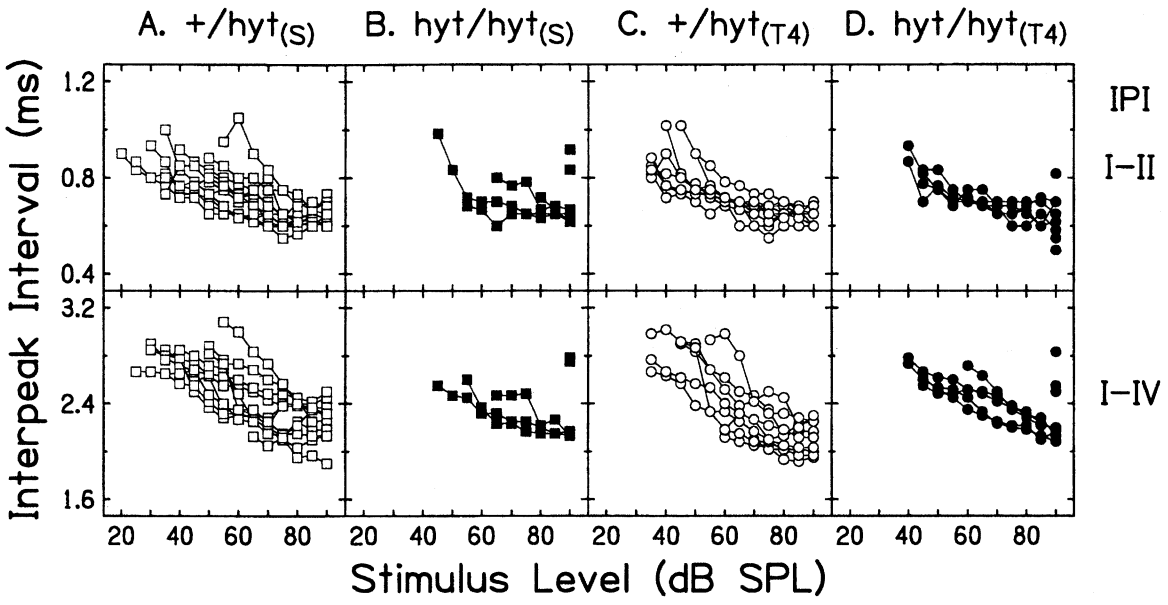

FIG. 9. Interpeak interval-vs.-stimulus level curves. Interpeak intervals between waves I and II (top row) and central conduction time (interval between wave I and IV, bottom row) are plotted as a function of stimulus level in response to 20 kHz for each experimental group (columns). Each curve represents the responses of an individual animal.

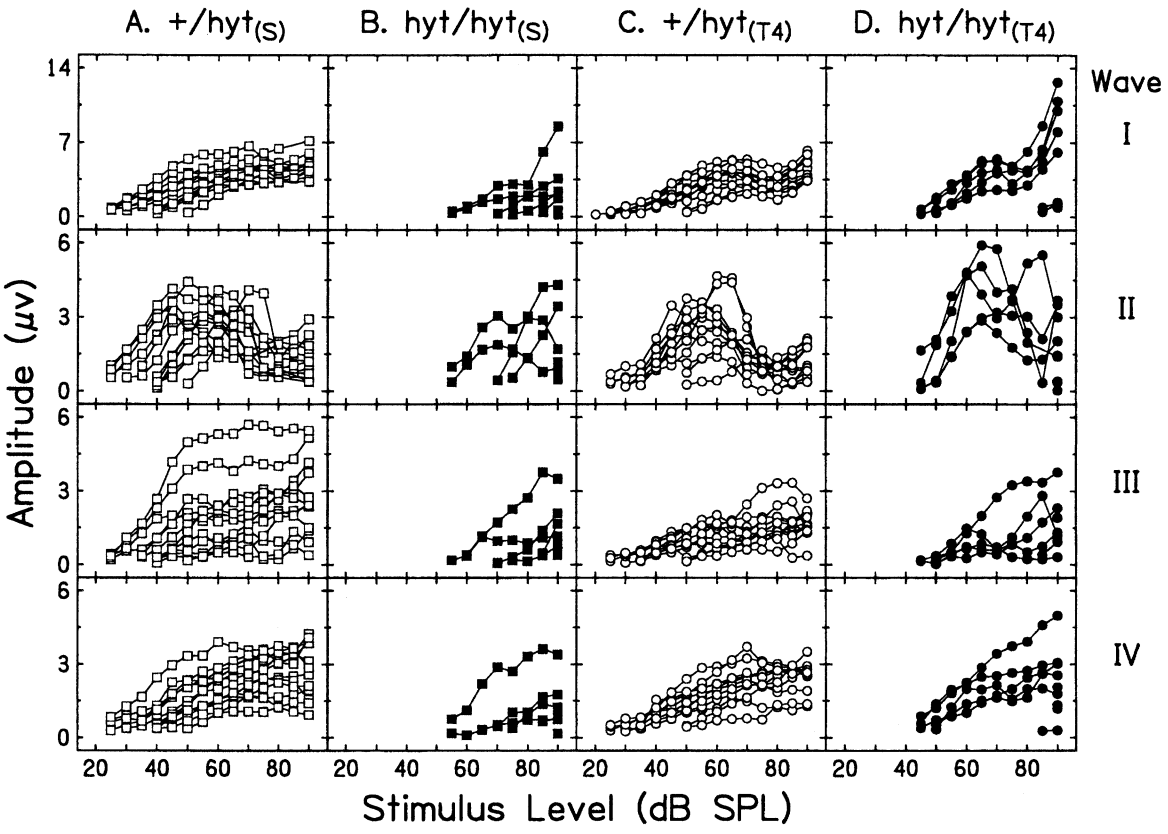

FIG. 10. Click-evoked amplitude–level functions. Click-evoked ABR amplitude–level functions for each primary ABR waves (I–IV, top to bottom rows) are plotted for each experimental group (columns). Each curve represents the responses from an individual animal.

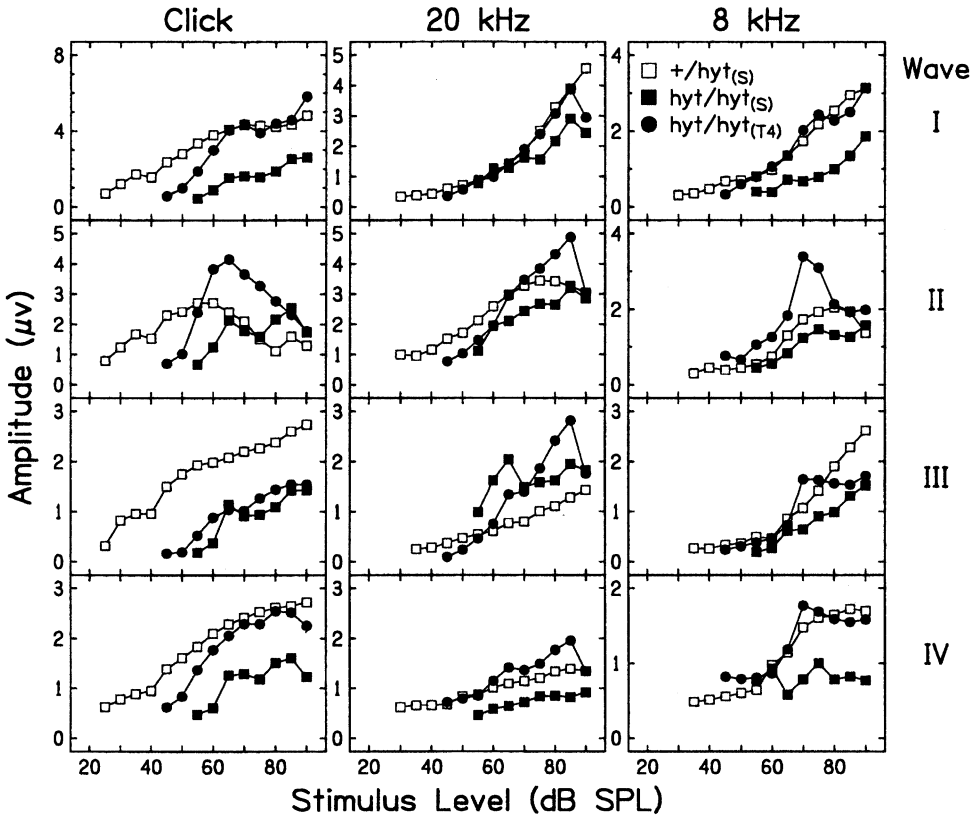

FIG. 11. Average amplitude–level functions. Amplitudes of the four primary ABR waves (I–IV, top to bottom rows) in response to clicks (left column), 20 kHz (middle column), and 8 kHz (right column) were averaged within each experimental group and are plotted as a function of stimulus level. The +/$hyt_{(T4)}$ group was omitted for greater clarity. Symbol key for all panels is indicated in the top right panel. Values are included only if two or more individuals from a group were responsive.

and amplitude–level curves were shifted to the right (i.e., higher stimulus levels were required to elicit equal amplitude responses) when compared with those associated with heterozygotes. The only exception to this rule was in the case of wave III for the 20-kHz condition; the fact that this peak was larger, on average, in hypothyroid animals than in euthyroid animals is paradoxical but may simply reflect the high degree of variability associated with responses from homozygous animals and the small number of responsive individuals in the mutant group.

It is especially interesting that treatment of homozygotes with thyroxin resulted in wave I amplitudes that were indistinguishable from those of normal, euthyroid animals at relatively high stimulus levels only (compare filled circles and open squares in Fig. 11). The observation that the amplitude of responses to "low"-level stimuli remained smaller than observed in euthyroid counterparts, combined with the fact that average thresholds of thyroxin-treated *hyt/hyt* animals were relatively high, is consistent with the view that the underlying pathology affects the gain of the cochlear amplifier and that thyroxin-dependent intracochlear developmental events blocked by hypothyroidism cannot be reversed by thyroxin therapy during the first 10 postnatal days. While T_4 treatment improved the amplitudes of ABR waves recorded from homozygotes, it had no effect on heterozygotes. Clear evidence of wave I recruitment—the phenomenon characterized by the abnormally high rate of response amplitude growth—was observed in responses to tone bursts (Fig. 7, right column).

Brain 2-deoxyglucose uptake

Consistent with the electrophysiological findings reported here, 2-deoxyglucose uptake was reduced throughout the central auditory pathway when homozygotes were compared with heterozygotes (Fig. 12). Depression of 2-DG uptake was evident in the cochlear nuclear complex, the superior olive, the lateral lemniscus, and the inferior colliculus. Among these structures the greatest reduction was seen in the inferior colliculus (37%). Thyroxin-treated homozygous mice exhibited intermediate values relative to those representing heterozygotes and saline-treated homozygotes, but differences were not significant.

DISCUSSION

In summary, consistent with observations made in the laboratory of O'Malley (O'Malley et al. 1995; Li et al. 1999), findings from this study confirm that *Tshr* mutant mice exhibit a constellation of physiological abnormalities that collectively reveal unambiguous

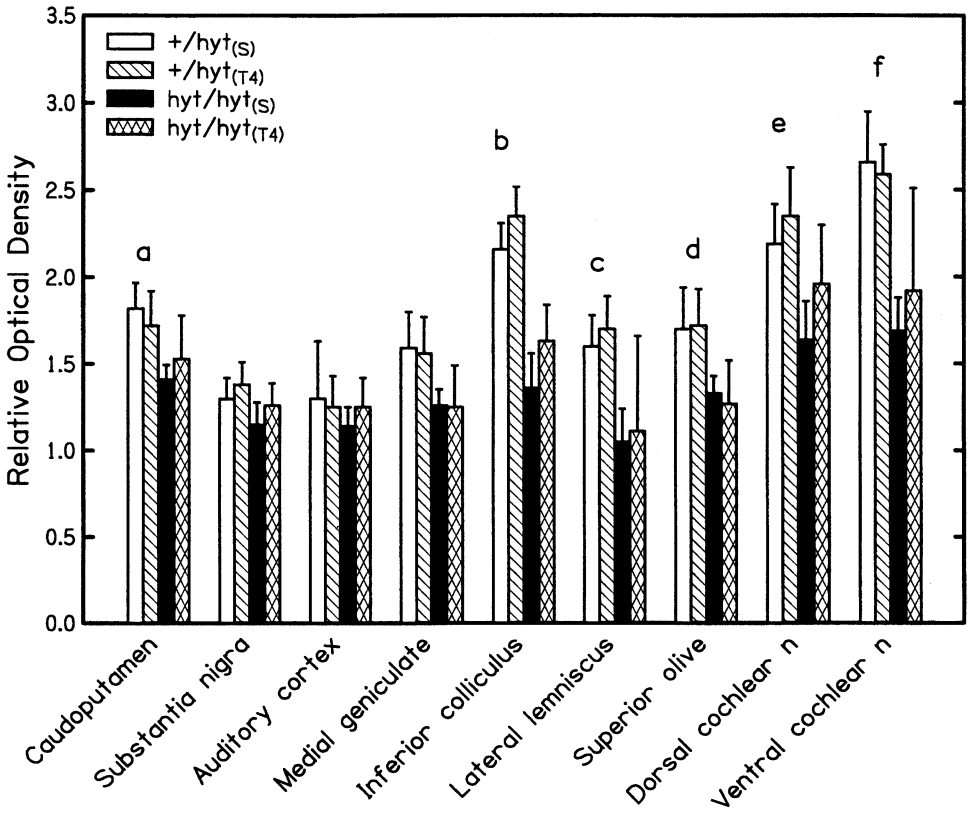

FIG. 12. Energy consumption by auditory brainstem nuclei. Measurements of 2-deoxyglucose uptake within the indicated brain regions are shown for each experimental group. Values were normalized to the value recorded for the appropriate local commissural or peduncular white matter and represent the mean ± SD of 4–6 mice per group. Letter designations above each group of bars represent the following statistically significant differences (no S or T_4 subscript designates either group): "a," $p <$ 0.01 +/$hyt_{(S)}$ vs. *hyt*/$hyt_{(S)}$; "b," $p <$ 0.001 +/*hyt* vs. *hyt*/*hyt;* "c," $p <$ 0.01 +/$hyt_{(T4)}$ vs. *hyt*/*hyt;* "d," $p <$ 0.005 +/*hyt* vs. *hyt*/$hyt_{(T4)}$; "e," $p <$ 0.005 +/*hyt* vs. *hyt*/$hyt_{(S)}$; "f," $p <$ 0.001 +/*hyt*/$hyt_{(S)}$.

peripheral auditory pathology. These findings are also consistent with reports of hypothyroid-associated changes in ABRs recorded from other species, including humans (Laureau et al. 1987; Anand et al. 1989; Norcross–Nechay et al. 1989; Bellman et al. 1996), dogs (Bichsel et al. 1988), and rats (Uziel et al. 1983a; Hébert et al. 1985a,b; Albee et al. 1989; Ben–Tovim et al. 1985). Specifically, relatively few *hyt/hyt* mice responded to acoustic stimulation except at high sound pressure levels, ABR latencies were prolonged, peak amplitudes were diminished, and response-vs.-level curves were steeper in hypothyroid animals compared with their euthyroid counterparts, consequently exhibiting recruitment-like behavior. In addition, glucose uptake under quiet conditions was reduced throughout brainstem auditory nuclei of *hyt/hyt* mice compared with +/*hyt* animals.

Thyroxin is required after the onset of function for normal auditory development

One goal of the present study was to determine the functional consequence(s) of thyroxin replacement in *Tshr* homozygotes during the critical period of development described by Deol (1973) for C57BL/Gr mice in an effort to further determine whether or not those findings generalize to other strains of mice. Deol's observation was based on the finding that auditory function was qualitatively normal in mice treated with the goitrogen propylthiouracil (PTU) from the time of birth through postnatal day 10. Because cochlear potentials are first elicited by intense airborne sounds around the middle of the second postnatal week, the outcome of Deol's study suggested that thyroxin's influence is directed toward morphological changes preceding the final stage of inner-ear differentiation during which the system acquires function. Based on Deol's qualitative observations in one strain of mice, we hypothesized that T_4-treated *hyt/hyt* mice would be as sensitive, or nearly as sensitive, to acoustic signals as their heterozygous littermates. Clearly, however, they are not. The observation that *hyt/hyt* mice born to euthyroid dams and subsequently treated with T_4 for the first 10 postnatal days fail to develop normal auditory function was unambiguous in this study. On the contrary, average thresholds were grossly elevated among the *hyt/*$hyt_{(T4)}$ animals. This is an important finding in that it dispels the notion that thyroxin's influence is limited to developmental events that precede the onset of cochlear function. This discovery is consistent with the finding that thyroid hormone receptors TRα, TRβ1, and TRβ2 are expressed during the period that cochlear function is achieved (Lautermann and Ten Cate 1997; Knipper et al. 1998, 1999).

Defective expression of cochlear amplification in hypothyroid animals

It is important to point out that Deol's impression that animals treated with PTU during the postnatal period were nearly normal was based on his measurement of the Preyer reflex, a notoriously poor indicator of auditory function in general and acoustic sensitivity in particular. It is interesting, however, that his subjective impression matches, at least qualitatively, the amplitude-level finding of this study, i.e., moderate- to high-level stimuli produce normal or near-normal responses in T_4-treated homozygotes, while low-level signals that are suprathreshold in normal heterozygotes are ineffective in homozygotes (cf. Fig. 11). On the basis of that observation, and especially when combined with the finding that response amplitude-vs.-level functions for wave I responses to tone bursts studied here tend to grow at an abnormally high rate in *hyt/hyt* animals (i.e., they recruit), we suggest that the essential otological malfunction in *Tshr* mutants lies in the cochlear amplifier or its expression. The rationale for that argument is centered on the well-known fact that the cochlear amplifier operates most efficiently at low levels and that a prominent feature of outer hair cell (OHC) malfunction (e.g., amplifier malfunction) is loss of sensitivity and response recruitment. The conclusion that defective cochlear amplification lies at the pathological center of hypothyroidism is also supported by the observation that otoacoustic emissions are absent at low stimulus levels in *Tshr* mutant mice (Li et al. 1999; Walsh et al. 2000; Farrell et al. 2000). This is a more direct indicator of OHC malfunction, although more direct tests of this theory are required for its proof.

While we tentatively conclude that pathology in the *hyt/hyt* mouse reflects an enduring abnormality in the micromechanics of cochlear amplification or in the configuration of anatomical and/or molecular elements required for its expression, it is more difficult to identify the specific pathological locus, or loci, of the disease. A wide range of cochlear abnormalities has been reported in PTU-treated animals, and prominent among those most commonly reported is the tectorial membrane (TM) (Deol 1973; Uziel et al. 1981; Anniko and Rosenkvist 1982; Legrand et al. 1988). Because the influence of cochlear amplification would presumably be blocked, or at least diminished, if TM abnormalities led to its decoupling from OHCs, the question is important in our search for the source of pathophysiological findings reported here. However, the TM does not appear to be the locus of pathology, at least in *hyt/hyt* animals, and perhaps not in hypothyroidism generally. We base that argument on several observations, the first and most significant of which is that existing physiological evidence does not support the idea that the TM of *hyt/hyt* mice functions abnormally. This view is based on a comparison of threshold-frequency or tuning curve comparisons recorded from *hyt/hyt* and *+/hyt* animals (Walsh et al. 2000). While the thresholds at the tips of tuning curves from hypothyroid *hyt* animals are clearly elevated, tail thresholds are the same as in normal heterozygotes. This finding suggests that the cochlear amplifier malfunctions in diseased animals, while passive aspects of transduction, are normal.

Additional evidence supporting this view is anatomical. Specifically, TM abnormalities are evident in animals treated with PTU during the prenatal period. However, in mice treated with PTU at the time of birth, a condition that is similar to our $hyt/hyt_{(S)}$ group, the TM is nearly normal in appearance (Deol 1973), as is the overall organ of Corti, at least at the light level of microscopy. However, individuals from this group are clearly hearing-impaired. The most remarkable aspect of this discovery, however, is that the TM remains completely normal when PTU treatment is delayed until postnatal day 10 (Deol 1973), a condition that is similar to our $hyt/hyt_{(T4)}$ group. Notably, we continue to see significant otopathophysiology among individuals belonging to the $hyt/hyt_{(T4)}$ group. Collectively, these findings strongly suggest that the TM is probably not the source of pathophysiology observed in *Tshr* mutant mice or the source of hypothyroidism generally. Further indirect yet compelling evidence in support of this conclusion comes from the work of Forrest et al. (1996). These authors found normal cochlear anatomy in mice with null mutations in the gene encoding the β form of the thyroid receptor (TRβ), even though ABR thresholds are clearly elevated, effectively demonstrating that thyroid system abnormalities that produce otopathology are not necessarily linked to gross morphological abnormalities in the cochlea.

Those observations notwithstanding, Li et al. (1999) observed stereociliary abnormalities in some, but not all, OHCs of *hyt/hyt* mice and pointed out that it was necessary to view tissue under the scanning electron microscope to detect certain defects, including tufted or clumped stereociliary bundles. Although speculative, it is equitable to consider the possibility that the material binding stereocilia in *hyt/hyt* animals constitutes a residue of the TM left behind after being stripped from the surface of the organ of Corti. In that context, although the elemental and molecular composition of the material in question is not known, it is reasonable to hypothesize that the chemical composition of the TM in *hyt/hyt* animals is abnormal, as in PTU- treated rats (Gil–Loyzaga et al. 1990; Prieto et al. 1990; Remezal and Gil–Loyzaga 1993), and reacts to fixation differentially. The question of relevance in this discussion is whether or not this finding has functional implications. We conclude, based on the

physiological argument presented above, that it does not.

One aspect of congenital hypothyroidism that complicates the effort to identify the source of what appears to be an enduring locus of otopathology is that many poorly developed anatomical features associated with the disease eventually acquire adultlike status. For example, in PTU-treated animals, the tunnel of Corti eventually opens and associated pillar cells eventually differentiate. Likewise, given time, Kolliker's organ regresses and normal sensory cell morphology is observed, at least at the light level of microscopy, even though immature features are retained for an abnormally long time (Deol 1976; Uziel et al. 1981; 1983b; Gabrion et al. 1984; Prieto et al. 1990). It is noteworthy, but not surprising, that developmental delays are not exclusively morphological. Functional properties of the system are acquired slowly as well. The most notable feature thus far studied in this category is delayed appearance of a fast-activating potassium conductance in inner hair cells (IHC) in animals with thyroid pathology (Rüsch et al. 1998).

After considering the list of anomalies that accompany hypothyroidism, it appears that the only clear, persistent abnormality lies in the olivocochlear (OC) system (Uziel et al. 1983b; Prieto et al. 1990; Cantos et al. 1997). Evidence that OC neurite extension is developmentally delayed in PTU-treated animals is convincing, as is the observation that the magnitude of the OC projection is diminished, even in adulthood. This condition leads, necessarily, to what can be thought of as partial de-efferentation, i.e., a reduction in the number of synaptic contacts between OC fibers and OHCs. This suggests that this CNS defect may be an important etiological factor in the evolution of auditory pathophysiology observed in hypothyroid animals. This view is greatly strengthened by the experimental finding that the cochlear amplifier malfunctions in adult cats experimentally de-efferented as neonates, prior to the formation of OC–OHC synapses (Walsh et al. 1998a,b). The pathophysiological and histological similarities between *hyt/hyt* mice and neonatally de-efferented cats are truly striking. It is particularly interesting in this context that the postnatal expression of the neurotrophin receptor TrkB in OHCs is temporally locked to synaptogenic events associated with the efferent OC innervation of OHCs in normal animals but not in hypothyroid animals that do not even express OHC TrkB (Knipper et al. 1999). That observation provides strong circumstantial support for the view that OC neurite outgrowth delays may be responsible for the auditory pathophysiology observed in animals with congenital disorders of the thyroid system. Clearly, developmental delays extending beyond a critical period of opportunity may have profound developmental consequences, as unambiguously demonstrated in numerous studies of visual system development (Berardi et al. 2000).

Response latency abnormalities due to combined conduction velocity and synapse delays

Wave I latencies associated with homozygotes observed in this study were wide-ranging, extending from normal or near-normal to prolongations that were at least twice the value observed in heterozygotes. While it is difficult to identify the source of this remarkable variability, it is reasonable to assume that the defect is associated with the ascending pathway, and response prolongation is often attributed to diminished myelination, a condition that is known to occur in hypothyroid individuals (Balázs et al. 1968, 1969; Comer and Norton 1985), including *hyt/hyt* mice (Noguchi and Sugisaki 1984). While it is entirely reasonable to conclude that myelination abnormalities might account for a significant fraction of the prolonged response times observed here and in other studies, it is possible that synaptic delays also contribute to total response latencies in hypothyroid animals. This view is based primarily on the notion that conduction velocity differences alone could not account for the total response delay times reported here, given the short transmission lengths connecting brainstem generators of ABR waves and the fact that Uziel et al. (1985) observed synapse abnormalities in PTU-treated rat pups (see Walsh et al. 1986b for a more complete discussion).

It has been suggested that synaptic delays associated with IHC–primary afferent dendrites contribute significantly, perhaps even equally, to latency prolongations associated with immaturity (Walsh et al. 1986a; Walsh and McGee 1986); we suggest that the same may be true in hypothyroidism. Support for this position comes from the study of myelination directly. Although the level of mRNA expression of myelin basic protein in spiral ganglion cells of hypothyroid rats is low in immature animals, normal values are eventually acquired (Knipper et al. 1998). This finding suggests that myelination may not be a factor when studying 3-month-old *hyt/hyt* mice, the age of animals used in this study. While that question requires a direct test, more direct evidence comes from the observation that threshold compensation does not account for the prolonged response times (cf. Fig. 8), an observation that points conclusively to either auditory nerve fiber myelination (in the case of wave I, at least) and/or synaptic delay at the IHC–auditory nerve synapse as the source of the delay. Based on the conduction velocity argument waged above, we suggest that synaptic delays may be an important component of latency prolongation in diseased individuals.

Vestibular system abnormalities

Although a subset of *hyt/hyt* mice exhibited signs of vestibular pathology in their circling behavior, affected animals always assumed appropriate landing postures when prompted, and no other signs of overt vestibular system pathology were observed. Our findings are generally consistent with previous reports of vestibular system abnormalities in hypothyroid rats (Dememes et al. 1986; Acuna et al. 1990; Meza et al. 1996), dogs (Jaggy et al. 1994), and humans (Bhatia et al. 1977; Fuggle et al. 1991; Bellman et al. 1996) that collectively suggest that vestibular pathology occurs less frequently and is less severe than auditory consequences of the disease. It is possible that *Tshr* mutant mice display variable degrees of vestibular impairment and that extant abnormalities were undetectable using the tests employed in this study. Alternatively, compensatory mechanisms may act to produce normal behavior in a subset of the *hyt* population, or circling behavior may reflect abnormal function in another related system, such as the cerebellum.

ACKNOWLEDGMENTS

This project was supported by grants from the Deafness Research Foundation and the NIH (DC01007, DC00215, DC00982). The authors are grateful to Dr. Charles Murrin, University of Nebraska Medical Center, for the use of his imaging equipment.

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