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Moderate perinatal thyroid hormone insufficiency alters visual system function in adult rats☆

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ABSTRACT

Thyroid hormone (TH) is critical for many aspects of neurodevelopment and can be disrupted by a variety of environmental contaminants. Sensory systems, including audition and vision are vulnerable to TH insufficiencies, but little data are available on visual system development at less than severe levels of TH deprivation. The goal of the current experiments was to explore dose-response relations between graded levels of TH insufficiency during development and the visual function of adult offspring. Pregnant Long Evans rats received 0 or 3 ppm (Experiment 1), or 0, 1, 2, or 3 ppm (Experiment 2) of propylthiouracil (PTU), an inhibitor of thyroid hormone synthesis, in drinking water from gestation day (GD) 6 to postnatal day (PN) 21. Treatment with PTU caused dose-related reductions of serum T4, with recovery on termination of exposure, and euthyroidism by the time of visual function testing. Tests of retinal (electroretinograms; ERGs) and visual cortex (visual evoked potentials; VEPs) function were assessed in adult offspring. Dark-adapted ERG a-waves, reflecting rod photoreceptors, were increased in amplitude by PTU. Light-adapted green flicker ERGs, reflecting M-cone photoreceptors, were reduced by PTU exposure. UV-flicker ERGs, reflecting S-cones, were not altered. Pattern-elicited VEPs were significantly reduced by 2 and 3 ppm PTU across a range of stimulus contrast values. The slope of VEP amplitude-log contrast functions was reduced by PTU, suggesting impaired visual contrast gain. Visual contrast gain primarily reflects function of visual cortex, and is responsible for adjusting sensitivity of perceptual mechanisms in response to changing visual scenes. The results indicate that moderate levels of pre-and post-natal TH insufficiency led to alterations in visual function of adult rats, including both retinal and visual cortex sites of dysfunction.

1. Introduction

Adequate supplies of thyroid hormone (TH) are critical for normal brain development. Lack of TH during gestation and the early postnatal period results in severe neurological deficits. Concentrations of TH measured in the serum are disrupted by a variety of environmental contaminants, including polyhalogenated aromatic hydrocarbons (PHAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), perchlorate, and triclosan (Brucker-Davis, 1998; USEPA, 2013). The integrity of sensory organs, including the cochlea and retina, require TH for normal development and function (Jones et al., 2003; Ng et al., 2013, 2015). Hypothyroidism in humans and in laboratory animals leads to cochlear hair cell loss, altered neocortical cytoarchitecture, and hearing impairments (Meyerhoff, 1979; Uziel et al., 1983; Berbel et al., 1985; Goldey et al., 1995; Knipper et al., 2000; Lavado-Autric et al., 2003; Auso et al., 2004).

In the retina, developmental hypothyroidism causes a reduction of retinal progenitor cells, reduced retinal thickness, altered photoreceptor patterning, improperly formed photoreceptor outer segments, and reduced cell density in the retinal ganglion cell layer (Navegantes et al., 1996; Ng et al., 2001; Sevilla-Romero et al., 2002; Harpavat and Cepko, 2003; Roberts et al., 2006; Ng et al., 2011; Pinazo-Duran et al., 2011; Ma and Ding et al., 2016). Post-mitotic retinal precursor cells differentiate into all types of

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photoreceptors including rods, middle-wavelength sensitive (M, "green") cones, and short-wavelength sensitive (S, "blue") cones. M-cones are formed from S-cones in response to TH signaling through the TRβ2 receptor (Ng et al., 2011). Knockout of thyroid hormone receptor TRβ2 results in a selective loss of M-cones and remaining cones all express S-cone opsin of (Ng et al., 2001).

Perturbation of the thyroid systems also negatively impacts the post-retinal components of the visual system, including the thalamus, visual and parietal cortices (Martinez-Galan et al., 2004). TH is necessary in the mature brain to maintain the neuronal spine density in the visual cortex and hippocampus (Ruiz-Marcos et al., 1979; Gould et al., 1990), whereas in the developing brain, thyroid signaling guides both the formation and refinement of CNS neural connections. Hypothyroidism beginning in early development alters the expression of trophic factors, deiodination enzymes, TH receptors, and neuronal and glial migratory patterns in the developing rodent neocortex (Berbel et al., 1985; Guadano-Ferraz et al., 1999; Martinez-Galan et al., 2004; Royland et al., 2008; Gilbert et al., 2016). Severe hypothyroidism beginning in late gestation alters axonal projections from the visual cortex to the spinal cord pyramidal tract (Li et al., 1995). Iodine deficiency resulting in reductions in maternal and offspring TH levels, reduces neuronal cell counts and synaptic number in the visual cortex (Mano et al., 1987). Developmental hypo- or hyper- thyroid conditions in mice disrupt the stability of axonal branching and synaptic boutons in layers 1 and 2 of visual cortex in grown offspring (Strobl et al., 2017). Overall, these experimental findings in rodents and nonhuman primates demonstrate the essential role of TH signaling in the structural integrity of the visual system.

Visual system processing deficits have also been reported in children with compromised thyroid status (Zoeller and Rovet, 2004). Premature infants, who are deprived of a maternal source of TH in late gestation, exhibit deficiencies in visual attention, visual contrast sensitivity, color vision, and visuomotor skills (Rovet and Simic, 2008; Simic et al., 2013). Children born to hypothyroid mothers, or suffering from thyroid gland dysgenesis at birth, exhibit both impaired color vision and visual contrast sensitivity (Mirabella et al., 2005; Simic et al., 2013; Simic and Rovet, 2017).

In support of these clinical findings, animal studies linking TH deficits with impaired visual system development have been reported. These data, however, have been largely derived from experiments with severe hormone compromise (i.e., thyroid ablation, gene deletion, high doses of TH synthesis inhibitors) causing virtually complete elimination of measurable TH. In contrast, exposures to environmental contaminants typically occur at concentrations causing only moderate dysregulation of TH, much less extreme than the existing experimental data. Previously, we demonstrated that low-moderate doses of the TH synthesis inhibitor, propylthiouracil (PTU), inducing graded levels of TH insufficiency in pregnant rats, caused brain neurochemical and structural abnormalities, hippocampal electrophysiology impairment, and learning deficits in exposed offspring (Sharlin et al., 2008; Gilbert, 2011; Lasley and Gilbert, 2011; Gilbert et al., 2013, 2014; Gilbert et al., 2016). However, it is unclear what magnitude of TH disruption presents a concern for impaired visual system development. Therefore, using the same experimental model, the present study examined dose-response relationships between moderate TH reductions during development and visual function of grown adult offspring.

2. Methods

2.1. Subjects

Pregnant Long-Evans rats were obtained from Charles River (Raleigh, NC) on gestational day (GD) 2 and housed individually in standard plastic hanging cages. The housing rooms were maintained on a 12:12 light-dark cycle. Animals were permitted free access to standard laboratory chow and, outside of the dosing period, tap water. Beginning on GD6 and continuing until postnatal day (PN) 21, dams received 0 or 3 ppm (Experiment 1) or 0, 1, 2 or 3 ppm (Experiment 2) of PTU dissolved in deionized drinking water (0–0.0003% solutions). The day of birth was designated PN0 and all litters were culled to 10 pups on PN4. Exposure to PTU terminated when pups were weaned on PN21. At weaning, offspring were transferred to plastic hanging cages (two animals of same sex/cage) and were permitted free access to food and tap water. Body weights of dams were monitored throughout gestation and the postnatal period. Body weights of offspring were monitored from PN5-PN35 and are reported elsewhere (Lasley and Gilbert, 2011). Only male pups were selected for visual function testing because of the extensive time required for electrophysiological testing, the limited time available for testing, and the principle aim of the experiment being to assess dose-response relationships between PTU treatment and visual function changes. These considerations determined a need to restrict other factors, such as sex of the offspring, in order to limit measurement variance and maximize the statistical power to detect significant differences between treatment groups.

The animal facility followed the guidelines of the National Institutes for Health for animal care, and was fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the USEPA National Health and Environmental Effects Research Laboratory (NHEERL), which ensured conformance with the 2004 National Research Council "Guide for the Care and Use of Laboratory Animals, Eighth Edition", the Animal Welfare Act and Public Health Service Policy on the Humane Care and Use of Laboratory Animals.

2.2. Chemical sources

Phenylephrine hydrochloride (2.5%), Proparacaine hydrochloride (0.5%), Tropicamide (0.75%) and USP eyedrops were obtained from (Bausch and Lomb, Irvine CA). Xylazine (TranquiVed Injection, USP) was obtained from Vedco, Inc, St. Joseph MO). Ketamine (Ketaset, ketamine hydrochloride, USP) was obtained from Fort Dodge Animal Health (Fort Dodge, IA). Nembutal sodium solution (Pentobarbital sodium, USP) was obtained from Akorn, (Forest Hills IL). 6-*n*-Propylthiouracil was obtained from Sigma (St. Louis MO).

2.3. Experimental designs

Two experiments are reported. Adult male offspring of Experiment 1 (0 or 3 ppm PTU) were evaluated on a series of ERGs and pattern-elicited VEPs. Visual function tests were collected in animals PN 63–86 days of age. Each group was comprised of 6–7 rats derived from 3 to 4 litters/treatment group. Experiment 2 was designed to further explore dose-response relationships between developmental TH reductions and altered visual function in the adult. To accommodate the number of animals to be processed in dose-response assessment, testing was restricted to VEP recordings. Dose-response relationships for VEPs were examined in adult offspring exposed perinatally to 0, 1, 2, or 3 ppm PTU. A schematic of the timeline of the two experiments is presented in the figures to follow. Final sample sizes in Experiment 2 were: 0 ppm, n = 9 litters (16 pups); 1 ppm, n = 8 litters (15 pups); 2 ppm, n = 10 litters (19 pups); 3 ppm; n = 8 litters (12 pups) (Supplemental Table S1). No more than 3 pups were taken from any given litter. As discussed below, the statistical analysis considered litter to be the unit of analysis, and pups within a litter were treated as repeated within-subject samples.