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Full Length Article

Adult hippocampal neurogenesis is impaired by transient and moderate developmental thyroid hormone disruption☆

M.E. Gilbert[a,*], J.H. Goodman[b,c], J. Gomez[b], A.F.M. Johnstone[a], R.L. Ramos[d]

[a] Toxicity Assessment Division, National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC, USA
[b] Department of Developmental Neurobiology, NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA
[c] Departments of Physiology and Pharmacology and Neurology, SUNY Downstate Medical Center Brooklyn, NY 11203, USA
[d] Department of Biomedical Sciences, New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY 11568, USA

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ABSTRACT

The hippocampus maintains a capacity for neurogenesis throughout life, a capacity that is reduced in models of adult onset hypothyroidism. The effects of developmental thyroid hormone (TH) insufficiency on neurogenesis in the adult hippocampus, however, has not been examined. Graded degrees of TH insufficiency were induced in pregnant rat dams by administration of 0, 3 or 10 ppm of 6-propylthiouracil (PTU) in drinking water from gestational day (GD) 6 until weaning. Body, brain, and hippocampal weight were reduced on postnatal day (PN) 14, 21, 78 and hippocampal volume was smaller at the 10 but not 3 ppm dose level. A second experiment examined adult hippocampal neurogenesis following developmental or adult onset hypothyroidism. Two male offspring from 0 and 3 ppm exposed dams were either maintained on control water or exposed to 3 ppm PTU to create 4 distinct treatment conditions (Control-Control; Control-PTU, PTU-Control, PTU-PTU) based on developmental and adult exposures. Beginning on the 28th day of adult exposure to 0 or 3 ppm PTU, bromodeoxyuridine (BrdU, 50 mg/kg, ip) was administered twice daily for 5 days, and one male from each treatment was sacrificed 24 h and 28 days after the last BrdU dose and brains processed for immunohistochemistry. Although no volume changes were seen in the hippocampus of the neonate at 3 ppm, thinning of the granule cell layer emerged in adulthood. Developmental TH insufficiency produced a reduction in newly born cells, reducing BrdU + ve cells at 1 with no further reduction at 28-days post-BrdU. Similar findings were obtained using the proliferative cell marker Ki67. Neuronal differentiations was also altered with fewer doublecortin (Dcx) expressing cells and a higher proportion of immature Dcx phenotypes seen after developmental but not adult TH insufficiency. An impaired capacity for neurogenesis may contribute to impairments in synaptic plasticity and cognitive deficits previously reported by our laboratory and others following moderate degrees of developmental TH insufficiency induced by this PTU model.

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1. Introduction

Thyroid hormone (TH) is critical for brain organization during early development and for normal brain function throughout life (Bernal, 2002; Williams, 2008). In the developing brain, TH is required for optimal neurogenesis, synaptogenesis, neuronal migration, plasticity, and myelination (Auso et al., 2004; Morreale de Escobar et al., 2004; Berbel et al., 2010; Mohan et al., 2012). Severe restrictions of TH undermine these developmental processes producing smaller animals with smaller brains, with particular sensitivity in the hippocampus (Rami et al., 1986a,b; Madeira et al., 1988, 1991, 1992; Rami and Rabie, 1990; Hasegawa et al., 2010; Powell et al., 2012). However, the dose-response relationship of more modest degrees of TH deprivation have not been widely reported. The dentate gyrus (DG) of the hippocampus also represents a brain region where neurogenesis, a process typically relegated to the immature brain, continues throughout

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* Corresponding author at: Toxicity Assessment Division (MD-B105-05) National Health and Environmental Effects Research Laboratory U.S. Environmental Protection Agency Research Triangle Park, NC 27711, USA.
E-mail address: gilbert.mary@epa.gov (M.E. Gilbert).

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life. A significant fraction of these newly born granule cells integrate into the existing DG circuitry to support learning and memory, modulate affect, and respond to injury (Jacobs et al., 2000; Kempermann et al., 2000; Shors et al., 2001; Santarelli et al., 2003; Snyder et al., 2005; Laplagne et al., 2006; Saxe et al., 2006; Samuels and Hen, 2011; Christie and Turnley, 2012; Turnley et al., 2014).

Data from several laboratories have implicated TH in the proliferation of neural stem/progenitor cells (NPC) in the adult rodent brain (Montero-Pedrazuela et al., 2006; Lopez-Juarez et al., 2012; Shiraki et al., 2012b), while others have reported that adult onset hypothyroidism reduces the survival of newly born neurons (Ambrogini et al., 2005; Desouza et al., 2005; Zhang et al., 2009; Kapoor et al., 2011, 2012). These observations derive from studying neurogenesis in adult animals with adult onset hypothyroidism, and under conditions of very severe reductions in TH. It is unclear if milder degrees of TH insult, closer to those that would accompany exposure to environmental contaminants, sub-clinical hypothyroidism, or hypothyroxinemia, are sufficient to impair adult neurogenesis. Neither is it known if transient TH deficiencies initiated *in utero* but from which animals recover could lead to a persistent impairment adult neurogenesis.

In the present study, we examined the dose-dependency of developmental TH insufficiency induced alterations of common anatomical metrics including brain and hippocampal weight, and hippocampal volume. Despite a wealth of data demonstrating the devastating effects on neuronal development accompanying severe hypothyroidism, fewer reports of low level, dose-dependent, quantitative assessments are available at relatively modest degrees of developmental hypothyroidism useful for computational modeling. A second study assessed hippocampal neurogenesis in adult offspring of dams experiencing moderate degrees of TH disruption induced by propylthiouracil (PTU). One objective was to examine the potential lasting effects of transient developmental hypothyroidism on neurogenesis in the adult following return to euthyroid status. An additional question focused on the exposure to a similar dose in the adult. Finally, a third objective was to determine if developmental hypothyroidism increased the vulnerability to subsequent hypothyroid insult in adulthood. We report that moderate levels of TH insufficiency limited to adulthood did not affect adult neurogenesis, but developmental hypothyroidism resulted in a significant reduction in cell proliferation/early survival phase of the neurogenesis process. This impairment was accompanied by a reduction in volume of the DG granule cell layer (GCL), an effect that was not detected until adulthood. Additional thyroid insult imposed on the adult after perinatal TH compromise did not produce further decrements in neurogenesis. Deficiencies in adult neurogenesis that persist in response to developmental TH insufficiency may contribute to impairments in synaptic transmission, long-term potentiation, and hippocampally-based learning and memory previously reported by our laboratory with this low dose PTU model (Gilbert and Sui, 2006; Gilbert, 2011; Gilbert et al., 2016).

2. Experimental procedures

2.1. Animals and treatment

Pregnant Long-Evans (LE) rats were obtained from Charles River (Raleigh, NC) on gestational day (GD) 2 and housed individually in standard plastic hanging cages in an AAALAC-approved animal facility. All animal treatments were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animal rooms were maintained on a 12:12 light:dark schedule, and animals were permitted free access to food (Purina 5008 rat chow) and filtered tap water. In Experiment 1 (Fig. 1A), pregnant dams were exposed to 0, 3 (0.0003%) or 10 (0.001%) ppm of PTU (Sigma, St. Louis, MO) in the drinking water from GD6 to postnatal day (PN) 30 when all pups were weaned to control drinking water. The lower dose was designed to emulate maternal hypothyroxinemia (reductions in circulating levels of thyroxine, T4), the high dose to induce hypothyroidism (significant

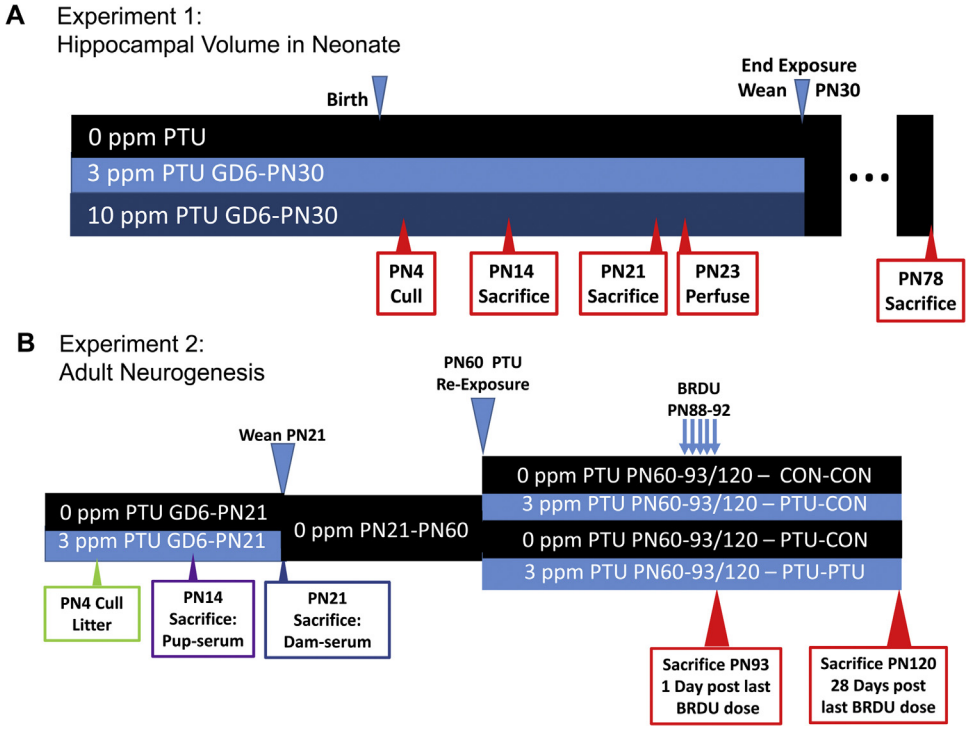

Fig. 1. Schematics for exposure and tissue collection for neonatal body and brain weights and hippocampal size of Experiment 1 (A) and adult neurogenesis of Experiment 2 (B).