

AOP ID and Title:

SNAPSHOT

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AOP 42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals

Short Title: TPO Inhibition and Altered Neurodevelopment

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Status

Author status	OECD status	OECD project	SAAOP status
Open for citation & comment	EAGMST Under Review	1.10	Included in OECD Work Plan

Abstract

This AOP describes one adverse outcome that may result from the inhibition of thyroperoxidase (TPO) during mammalian development. Chemical inhibition of TPO, the molecular-initiating event (MIE), results in decreased thyroid hormone (TH) synthesis, and subsequent reduction in circulating concentrations of THs. THs are essential for normal human brain development, both prenatally and postnatally, modulating genes critical for a normal neuroanatomical development, with subsequent effects on neurophysiology, and finally neurological function. Therefore, chemicals that interfere with TH synthesis have the potential to cause TH insufficiency that may result in adverse neurodevelopmental effects in offspring. Herein, we discuss the implications of developmental TPO inhibition for hippocampal anatomy, function, and ultimately neural function controlled by the hippocampus. The biochemistry of TPO and its essentiality for TH synthesis is well known across species. The hippocampus is known to be critically involved in cognitive, emotional, and memory function. The adverse consequences of TH insufficiency depend both on severity and developmental timing, indicating that exposure to TPO inhibitors may produce different effects at different developmental windows of exposure. It is important to note that thyroid stimulating hormone (TSH) is not a KE in this AOP. While TSH may play a role in feedback-driven compensatory processes, it is not directly involved in brain development. The overall weight of evidence for this AOP is strong. Gaps in our understanding include the relationship of TH-dependent gene expression and complexities of brain development. Although quantitative information at all levels of KERs is limited a number of applications of this AOP have been identified.

Background

This AOP was originally started on the Chemical Mode of Action WIKI sponsored by WHO/IPCS. The MOA was originally described and published by Zoeller and Crofton (Crit Rev Toxicol 2005). Thanks to the following contributors whose work on the MOA-WIKI fostered further development on the AOP wiki: Michelle Embry, Richard Judson, Vicki Dellarco, Chihae Yang, Kevin Crofton.

Zoeller RT, Crofton KM. *Mode of action: developmental thyroid hormone insufficiency--neurological abnormalities resulting from exposure to propylthiouracil. Crit Rev Toxicol. 2005 Oct-Nov;35(8-9):771-81*

Summary of the AOP

Stressors

Name	Evidence
Methimazole	Strong
Propylthiouracil	Strong

Molecular Initiating Event

Title	Short name
Thyroperoxidase, Inhibition (https://aopwiki.org/events/279)	Thyroperoxidase, Inhibition

279: Thyroperoxidase, Inhibition (<https://aopwiki.org/events/279>)

Short Name: Thyroperoxidase, Inhibition

Key Event Component

Process	Object	Action
iodide peroxidase activity	thyroid peroxidase	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	MolecularInitiatingEvent
119: Inhibition of thyroid peroxidase leading to follicular cell adenomas and carcinomas (in rat and mouse) (https://aopwiki.org/aops/119)	MolecularInitiatingEvent
159: Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	MolecularInitiatingEvent
175: Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	MolecularInitiatingEvent

Stressors

Name
2(3H)-Benzothiazolethione
2-mercaptobenzothiazole
Ethylene thiourea
Mercaptobenzothiazole
Methimazole
Propylthiouracil
Resorcinol
Thiouracil
Ethylenethiourea
Amitrole
131-55-5
2,2',4,4'-Tetrahydroxybenzophenone

Name
Daidzein
Genistein
4-Nonylphenol
4-propoxyphenol
Sulfamethazine

Biological Organization

Level of Biological Organization
Molecular

Organ term

Organ term
thyroid follicle

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

There is a wealth of information on the inhibition of TPO by drugs such as MMI and PTU, as well as environmental xenobiotics. In the landmark paper on thyroid disruption by environmental chemicals, Brucker-Davis (1998) identified environmental chemicals that depressed TH synthesis by inhibiting TPO. Hurley (1998) listed TPO as a major target for thyroid tumor inducing pesticides. More recent work has tested over 1000 chemicals using a high-throughput screening assay (Paul-Friedman et al., 2016).

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
humans	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
pigs	Sus scrofa	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9823)
Xenopus laevis	Xenopus laevis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
chicken	Gallus gallus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9031)
zebrafish	Danio rerio	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)
fathead minnow	Pimephales promelas	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Female	Strong
Male	Strong

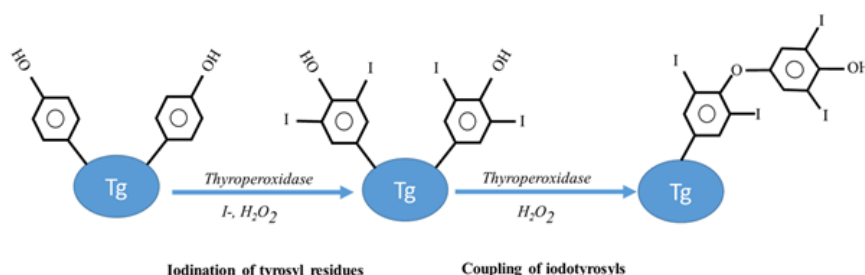
TPO inhibition is a MIE conserved across taxa, with supporting data from experimental models and human clinical testing. This conservation is likely a function of the high degree of protein sequence similarity in the catalytic domain of mammalian peroxidases (Taurog, 1999). Ample data available for human, rat, and porcine TPO inhibition demonstrate qualitative concordance across these species (Schmoltzer et al., 2007; Paul et al., 2013; Hornung et al., 2010). A comparison of rat TPO and pig TPO, bovine lactoperoxidase, and human TPO inhibition by genistein demonstrated good qualitative and quantitative (40–66%) inhibition across species, as indicated by quantification of MIT and DIT production (Doerge and Chang, 2002). Ealey et al. (1984) demonstrated peroxidase activity in guinea pig thyroid tissue using 3,3'-diaminobenzidine tetrahydrochloride (DAB) as a substrate that is oxidized by the peroxidase to form a brown insoluble reaction product. Formation of this reaction product was inhibited by 3-amino-1,2,4-triazole and the TPO inhibitor, methimazole (MMI). A comparative analysis of this action of MMI between rat- and human-derived TPO indicates concordance of qualitative response. Data also suggest an increased quantitative sensitivity to MMI in rat compared to human (Vickers et al., 2012). Paul et al. (2013) tested 12 chemicals using the guaiacol assay using both porcine and rat thyroid microsomes. The authors concluded that there was an excellent qualitative concordance between rat and porcine TPO inhibition, as all chemicals that inhibited TPO in porcine thyroid microsomes also inhibited TPO in rat thyroid microsomes when tested within the same concentration range.

How this Key Event Works

Thyroperoxidase (TPO) is a heme-containing apical membrane protein within the follicular lumen of thyrocytes that acts as the enzymatic catalyst for thyroid hormone (TH) synthesis. TPO catalyzes several reactions in the thyroid gland, including: the oxidation of iodide; nonspecific iodination of tyrosyl residues of thyroglobulin (Tg); and, the coupling of iodotyrosyls to produce Tg-bound monoiodotyrosine (MIT) and diiodotyrosine (DIT) (Divi et al., 1997; Kessler et al., 2008; Ruf et al., 2006; Taurog et al., 1996). The outcome of TPO inhibition is decreased synthesis of thyroxine (T4) and triiodothyronine (T3), a decrease in release of these hormones from the gland into circulation, and unless compensated, a consequent decrease in systemic concentrations of T4, and possibly T3. The primary product of TPO-catalyzed TH synthesis is T4 (Taurog et al., 1996; Zoeller et al., 2007) that would be peripherally or centrally deiodinated to T3.

The figure below illustrates the enzymatic and nonenzymatic reactions mediated by TPO.

Figure 1. Thyroperoxidase and thyroid hormone synthesis



Inhibition of TPO can be reversible, with transient interaction between the enzyme and the chemical, or irreversible, whereby suicide substrates permanently inactivate the enzyme. Reversible and irreversible TPO inhibition may be determined by the chemical structure, may be concentration dependent, or may be influenced by other conditions, including the availability of iodine (Doerge and Chang, 2002).

The ontogeny of TPO has been determined using both direct and indirect evidence. Available evidence suggests the 11th to 12th fetal week as the beginning of functional TPO in humans. In rodents, TPO function begins late in the second fetal week, with the first evidence of T4 secretion on gestational day 17 (Remy et al., 1980). Thyroid-specific genes appear in the thyroid gland according to a specific temporal pattern; thyroglobulin (Tg), TPO (Tpo), and TSH receptor (Tshr) genes are expressed by gestational day 14, and the sodium iodide symporter, NIS (Nis), is expressed by gestational day 16. Maturation to adult function is thought to occur within a few weeks after parturition in rats and mice, and within the first few months in neonatal humans (Santisteban and Bernal, 2005). Tg is first detected in human fetuses starting at 5th week of gestation and rises throughout gestation (Thorpe-Beeston et al., 1992), but iodine trapping and T4 production does not occur until around 10–12 weeks. Also, the dimerization of Tg, a characteristic of adult TH storage, is not found until much later in gestation (Pintar, 2000). In rats, Tg immunoreactivity does not appear until day 15 of gestation (Fukiishi et al., 1982; Brown et al., 2000). The vast majority of research and knowledge on Tg is from mammals, although genomic orthologs are known for a variety of other species (Holzer et al., 2016).

How it is Measured or Detected

There are no approved OECD or EPA guideline study protocols for measurement of TPO inhibition. From the early 1960's, microsomal fractions

prepared from porcine thyroid glands and isolated porcine follicles were used as a source of TPO for inhibition experiments (Taurog, 2005). Limited information has been published using microsomes from human goiter samples (Vickers et al., 2012) and rat thyroid glands (Paul et al., 2013; 2014; Paul-Friedman et al., 2016).

TPO activity has been measured for decades via indirect assessment by kinetic measurement of the oxidation of guaiacol (Chang & Doerge 2000; Hornung et al., 2010; Schmutzler et al., 2007). This method is a low-throughput assay due to the very rapid kinetics of the guaiacol oxidation reaction. More recently, higher-throughput methods using commercial fluorescent and luminescent substrates with rodent, porcine, and human microsomal TPO have been developed (Vickers et al., 2012; Paul et al., 2013; 2014; Kaczur et al., 1997). This assay substitutes a pre-fluorescent substrate (Amplex UltraRed) for guaiacol, that when incubated with a source of peroxidase and excess hydrogen peroxidase, results in a stable fluorescent product proportional to TPO activity (Vickers et al., 2012). The stability of the fluorescent reaction product allows this assay to be used in a higher throughput format (Paul-Friedman et al., 2016). This approach is appropriate for high-throughput screening, but does not elucidate the specific mechanism by which a chemical may inhibit TPO (Paul-Friedman et al., 2016), and as with most in vitro assays, is subject to various sources of assay interference (Thorne et al., 2010).

HPLC has been used to measure of the activity of TPO via formation of the precursors monoiodotyrosine (MIT), diiodotyrosine (DIT), and both T3 and T4, in a reaction mixture containing TPO, or a surrogate enzyme such as lactoperoxidase (Divi & Doerge 1994). The tools and reagents for this method are all available. However, HPLC or other analytical chemistry techniques make this a low throughput assay, depending on the level of automation. A primary advantage of this in vitro method is that it directly informs hypotheses regarding the specific mechanism by which a chemical may impact thyroid hormone synthesis in vitro.

References

- Brown RS, Shalhoub V, Coulter S, Alex S, Joris I, De Vito W, Lian J, Stein GS. Developmental regulation of thyrotropin receptor gene expression in the fetal and neonatal rat thyroid: relation to thyroid morphology and to thyroid-specific gene expression. *Endocrinology*. 2000 Jan;141(1):340-5.
- Brucker-Davis F. 1998. Effects of environmental synthetic chemicals on thyroid function. *Thyroid* 8:827-856.
- Chang, H. C. and D. R. Doerge (2000) Dietary genistein inactivates rat thyroid peroxidase in vivo without an apparent hypothyroid effect. *Toxicol Appl Pharmacol*. 168:244-252.
- Divi, R. L., & Doerge, D. R. (1994). Mechanism-based inactivation of lactoperoxidase and thyroid peroxidase by resorcinol derivatives. *Biochemistry* 33(32), 9668–9674.
- Divi, R. L., Chang, H. C., & Doerge, D. R. (1997). Anti-Thyroid Isoflavones from Soybean. *Biochem. Pharmacol.* 54(10), 1087–1096.
- Doerge DR, Chang HC. Inactivation of thyroid peroxidase by soy isoflavones, in vitro and in vivo. *J Chromatogr B Anal Technol Biomed Life Sci*. 2002 Sep 25;777(1-2):269-79.
- Ealey PA, Henderson B, Loveridge N.A quantitative study of peroxidase activity in unfixed tissue sections of the guinea-pig thyroid gland. *Histochem J*. 1984 Feb;16(2):111-22.
- Fukiishi Y, Harauchi T, Yoshizaki T, Hasegawa Y, Eguchi Y. Ontogeny of thyroid peroxidase activity in perinatal rats. *Acta Endocrinol (Copenh)*. 1982 101(3):397-402.
- Holzer G, Morishita Y, Fini JB, Lorin T, Gillet B, Hughes S, Tohmé M, Deléage G, Demeneix B, Arvan P, Laudet V. Thyroglobulin Represents a Novel Molecular Architecture of Vertebrates. *J Biol Chem*. 2016 Jun 16.
- Hornung, M. W., Degitz, S. J., Korte, L. M., Olson, J. M., Kosian, P. a, Linnun, A. L., & Tietge, J. E. (2010). Inhibition of thyroid hormone release from cultured amphibian thyroid glands by methimazole, 6-propylthiouracil, and perchlorate. *Toxicol Sci* 118(1), 42–51.
- Hurley PM. 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environ Health Perspect* 106:437-445.
- Kaczur, V., Vereb, G., Molnár, I., Krajczár, G., Kiss, E., Farid, N. R., & Balázs, C. (1997). Effect of anti-thyroid peroxidase (TPO) antibodies on TPO activity measured by chemiluminescence assay. *Clin. Chem* 43(8 Pt 1), 1392–6.
- Kessler, J., Obinger, C., Eales, G., 2008. Factors influencing the study of peroxidase- generated iodine species and implications for thyroglobulin synthesis. *Thyroid* 18, 769–774.
- Paul KB, Hedge JM, Macherla C, Filer DL, Burgess E, Simmons SO, Crofton KM, Hornung MW. Cross-species analysis of thyroperoxidase inhibition by xenobiotics demonstrates conservation of response between pig and rat. *Toxicology*. 2013. 312:97-107
- Paul, K.B., Hedge, J.M., Rotroff, D.M., Hornung, M.W., Crofton, K.M., Simmons, S.O. 2014. Development of a thyroperoxidase inhibition assay for high-throughput screening. *Chem. Res. Toxicol.* 27(3), 387-399.
- Paul-Friedman K, Watt ED, Hornung MW, Hedge JM, Judson RS, Crofton KM, Houck KA, Simmons SO. 2016. Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries. *Toxicol Sci*. 151:160-80.
- Pintar, J.E. (2000) Normal development of the hypothalamic-pituitary-thyroid axis. In: Werner & Ingbar's *The Thyroid*. (8th ed), Braverman. L.E. and Utiger, R.D. (eds) Lippincott Williams and Wilkins, Philadelphia.
- Remy L, Michel-Bechet M, Athouel-Haon AM, Magre S. Critical study of endogenous peroxidase activity: its role in the morphofunctional setting of the thyroid follicle in the rat fetus. *Acta Histochem*. 1980;67(2):159-72.
- Ruf, J., & Carayon, P. (2006). Structural and functional aspects of thyroid peroxidase. *Archives of Biochemistry and Biophysics*, 445(2), 269–77.
- Santisteban P, Bernal J. Thyroid development and effect on the nervous system. *Rev Endocr Metab Disord*. 2005 Aug;6(3):217-28.

Schmutzler, C., Bacinski, A., Gotthardt, I., Huhne, K., Ambrugger, P., Klammer, H., Schlecht, C., Hoang-Vu, C., Gruters, A., Wuttke, W., Jarry, H., Kohrle, J., 2007a. The ultraviolet filter benzophenone 2 interferes with the thyroid hormone axis in rats and is a potent in vitro inhibitor of human recombinant thyroid peroxidase. *Endocrinology* 148, 2835–2844.

Taurog A. 2005. Hormone synthesis. In: Werner and Ingbar's *The Thyroid: A Fundamental and Clinical Text* (Braverman LE, Utiger RD, eds). Philadelphia:Lippincott, Williams and Wilkins, 47–81

Taurog, a, Dorris, M. L., & Doerge, D. R. (1996). Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. *Archives of Biochemistry and Biophysics*, Taurog A. Molecular evolution of thyroid peroxidase. *Biochimie*. 1999 May;81(5):557-62

Thorne N, Auld DS, Inglese J. Apparent activity in high-throughput screening: origins of compound-dependent assay interference. *Curr Opin Chem Biol*. 2010 Jun;14(3):315-24.

Thorpe-Beeston JG, Nicolaides KH, McGregor AM. Fetal thyroid function. *Thyroid*. 1992 Fall;2(3):207-17. Review.

Vickers AE, Heale J, Sinclair JR, Morris S, Rowe JM, Fisher RL. Thyroid organotypic rat and human cultures used to investigate drug effects on thyroid function, hormone synthesis and release pathways. *Toxicol Appl Pharmacol*. 2012 Apr 1;260(1):81-8.

Zoeller, R. T., Tan, S. W., & Tyl, R. W. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Critical Reviews in Toxicology*, 37(1-2), 11–53.

Key Events

Title	Short name
Thyroid hormone synthesis, Decreased (https://aopwiki.org/events/277)	TH synthesis, Decreased
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/events/281)	T4 in serum, Decreased
Thyroxine (T4) in neuronal tissue, Decreased (https://aopwiki.org/events/280)	T4 in neuronal tissue, Decreased
Hippocampal gene expression, Altered (https://aopwiki.org/events/756)	Hippocampal gene expression, Altered
Hippocampal anatomy, Altered (https://aopwiki.org/events/757)	Hippocampal anatomy, Altered
Hippocampal Physiology, Altered (https://aopwiki.org/events/758)	Hippocampal Physiology, Altered

277: Thyroid hormone synthesis, Decreased (<https://aopwiki.org/events/277>)

Short Name: TH synthesis, Decreased

Key Event Component

Process	Object	Action
thyroid hormone generation	thyroid hormone	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
65: XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
128: Kidney dysfunction by decreased thyroid hormone (https://aopwiki.org/aops/128)	MolecularInitiatingEvent
134: Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
54: Inhibition of Na ⁺ /I ⁻ symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
159: Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent

AOP42

AOP ID and Name	Event Type
175: Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	KeyEvent
176: Sodium Iodide Symporter (NIS) Inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/176)	KeyEvent
188: Iodotyrosine deiodinase (IYD) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/188)	KeyEvent
192: Pendrin inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/192)	KeyEvent
193: Dual oxidase (DUOX) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/193)	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Organization

Level of Biological Organization
Cellular

Cell term

Cell term
thyroid follicular cell

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

not applicable as this KE is not an MIE

Propylthiouracil

6-n-propylthiouracil is a common positive control

Methimazole

Methimazole is a very common positive control

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
Pig	Pig	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)
Xenopus laevis	Xenopus laevis	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Decreased TH synthesis resulting from TPO or NIS inhibition is conserved across taxa, with *in vivo* evidence from humans, rats, amphibians, some fish species, and birds, and *in vitro* evidence from rat and porcine microsomes. Indeed, TPO and NIS mutations result in congenital hypothyroidism in humans (Bakker et al., 2000; Spitzweg and Morris, 2010), demonstrating the essentiality of TPO and NIS function toward maintaining euthyroid status. Though decreased serum T4 is used as a surrogate measure to indicate chemical-mediated decreases in TH synthesis, clinical and veterinary management of hyperthyroidism and Grave's disease using propylthiouracil and methimazole, known to decrease TH synthesis, indicates strong medical evidence for chemical inhibition of TPO (Zoeller and Crofton, 2005).

How this Key Event Works

The thyroid hormones (TH), triiodothyronine (T3) and thyroxine (T4) are tyrosine based hormones. Synthesis of TH is regulated by thyroid-stimulating hormone (TSH) binding to its receptor and thyroidal availability of iodine via the sodium iodide symporter (NIS). Other proteins contributing to TH production in the thyroid gland, including thyroperoxidase (TPO), dual oxidase enzymes (DUOX), and pendrin are also necessary for iodothyronine production (Zoeller et al., 2007).

The production of THs in the thyroid gland and resulting serum concentrations are controlled by a negatively regulated feedback mechanism. Decreased T4 and T3 serum concentrations activates the hypothalamus-pituitary-thyroid (HPT) axis which upregulates thyroid-stimulating hormone (TSH) that acts to increase production of additional THs (Zoeller and Tan, 2007). This regulatory system includes: 1) the hypothalamic secretion of the thyrotropin-releasing hormone (TRH); 2) the thyroid-stimulating hormone (TSH) secretion from the anterior pituitary; 3) hormonal transport by the plasma binding proteins; 4) cellular uptake mechanisms at the tissue level; 5) intracellular control of TH concentration by deiodinating mechanisms; 6) transcriptional function of the nuclear TH receptor; and 7) in the fetus, the transplacental passage of T4 and T3 (Zoeller et al., 2007).

TRH and the TSH primarily regulate the production of T4, often considered a "pro-hormone," and to a lesser extent of T3, the transcriptionally active TH. Most of the hormone released from the thyroid gland into circulation is in the form of T4, while peripheral deiodination of T4 is responsible for the majority of circulating T3. Outer ring deiodination of T4 to T3 is catalyzed by the deiodinases 1 and 2 (DIO1 and DIO2), with DIO1 expressed mainly in liver and kidney, and DIO2 expressed in several tissues including the brain (Bianco et al., 2006). Conversion of T4 to T3 takes place mainly in liver and kidney, but also in other target organs such as in the brain, the anterior pituitary, brown adipose tissue, thyroid and skeletal muscle (Gereben et al., 2008; Larsen, 2009).

Most evidence for the ontogeny of TH synthesis comes from measurements of serum hormone concentrations. And, importantly, the impact of xenobiotics on fetal hormones must include the influence of the maternal compartment since a majority of fetal THs are derived from maternal blood early in fetal life, with a transition during mid-late gestation to fetal production of THs that is still supplemented by maternal THs. In humans, THs can be found in the fetus as early as gestational weeks 10-12, and concentrations rise continuously until birth. At term, fetal T4 is similar to maternal levels, but T3 remains 2-3 fold lower than maternal levels. In rats, THs can be detected in the fetus as early as the second gestational week, but fetal synthesis does not start until gestational day 17 with birth at gestational day 22-23. Maternal THs continue to supplement fetal production until parturition. (see Howdeshell, 2002; Santisteban and Bernal, 2005 for review).

Decreased TH synthesis in the thyroid gland may result from several possible molecular-initiating events (MIEs) including: 1) Disruption of key catalytic enzymes or cofactors needed for TH synthesis, including TPO, NIS, or dietary iodine insufficiency. Theoretically, decreased synthesis of Tg could also affect TH production (Kessler et al., 2008; Yi et al., 1997). Mutations in genes that encode requisite proteins in the thyroid may also lead to impaired TH synthesis, including mutations in pendrin associated with Pendred Syndrome (Dossena et al., 2011), mutations in TPO and Tg (Huang and Jap 2015), and mutations in NIS (Spitzweg and Morris, 2010). 2) Decreased TH synthesis in cases of clinical hypothyroidism may

be due to Hashimoto's thyroiditis or other forms of thyroiditis, or physical destruction of the thyroid gland as in radioablation or surgical treatment of thyroid lymphoma. 3) It is possible that TH synthesis may also be reduced subsequent to disruption of the negative feedback mechanism governing TH homeostasis, e.g. pituitary gland dysfunction may result in a decreased TSH signal with concomitant T3 and T4 decreases. 4) More rarely, hypothalamic dysfunction can result in decreased TH synthesis.

It should be noted that different species and different lifestages store different amounts of TH precursor and iodine within the thyroid gland. Thus, decreased TH synthesis via transient iodine insufficiency or inhibition of TPO may not affect TH release from the thyroid gland until depletion of stored iodinated Tg. Adult humans may store sufficient Tg-DIT residues to serve for several months to a year of TH demand (Greer et al., 2002). Neonates and infants have a much more limited supply of less than a week.

How it is Measured or Detected

Decreased TH synthesis is often implied by measurement of TPO and NIS inhibition measured clinically and in laboratory models as these enzymes are essential for TH synthesis. Rarely is decreased TH synthesis measured directly, but rather the impact of chemicals on the quantity of T4 produced in the thyroid gland, or the amount of T4 present in serum is used as a marker of decreased T4 release from the thyroid gland (e.g., Romaldini et al., 1988). Methods used to assess TH synthesis include, incorporation of radiolabel tracer compounds, radioimmunoassay, ELISA, and analytical detection.

Recently, amphibian thyroid explant cultures have been used to demonstrate direct effects of chemicals on TH synthesis, as this model contains all necessary synthesis enzymes including TPO and NIS (Hornung et al., 2010). For this work THs was measured by HPLC/ICP-mass spectrometry. Decreased TH synthesis and release, using T4 release as the endpoint, has been shown for thiouracil antihyperthyroidism drugs including MMI, PTU, and the NIS inhibitor perchlorate (Hornung et al., 2010).

References

- Bakker B, Bikker H, Vulsma T, de Randamie JS, Wiedijk BM, De Vijlder JJ. 2000. Two decades of screening for congenital hypothyroidism in The Netherlands: TPO gene mutations in total iodide organification defects (an update). *The Journal of clinical endocrinology and metabolism*. 85:3708-3712.
- Bianco AC, Kim BW. (2006). Deiodinases: implications of the local control of thyroid hormone action. *J Clin Invest*. 116: 2571–2579.
- Dossena S, Nofziger C, Brownstein Z, Kanaan M, Avraham KB, Paulmichl M. (2011). Functional characterization of pendrin mutations found in the Israeli and Palestinian populations. *Cell Physiol Biochem*. 28: 477-484.
- Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeöld A, Bianco AC. (2008). Cellular and molecular basis of deiodinase-regulated thyroid hormone signalling. *Endocr Rev*. 29:898–938.
- Gereben B, Zeöld A, Dentice M, Salvatore D, Bianco AC. Activation and inactivation of thyroid hormone by deiodinases: local action with general consequences. *Cell Mol Life Sci*. 2008 Feb;65(4):570-90
- Greer MA, Goodman G, Pleus RC, Greer SE. Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. *Environ Health Perspect*. 2002. 110:927-937.
- Howdeshell KL. 2002. A model of the development of the brain as a construct of the thyroid system. *Environ Health Perspect*. 110 Suppl 3:337-48.
- Hornung MW, Degitz SJ, Korte LM, Olson JM, Kosian PA, Linnum AL, Tietge JE. 2010. Inhibition of thyroid hormone release from cultured amphibian thyroid glands by methimazole, 6-propylthiouracil, and perchlorate. *Toxicol Sci* 118:42-51.
- Huang CJ and Jap TS. 2015. A systematic review of genetic studies of thyroid disorders in Taiwan. *J Chin Med Assoc*. 78: 145-153.
- Kessler J, Obinger C, Eales G. Factors influencing the study of peroxidase-generated iodine species and implications for thyroglobulin synthesis. *Thyroid*. 2008 Jul;18(7):769-74. doi: 10.1089/thy.2007.0310
- Larsen PR. (2009). Type 2 iodothyronine deiodinase in human skeletal muscle: new insights into its physiological role and regulation. *J Clin Endocrinol Metab*. 94:1893-1895.
- Romaldini JH, Farah CS, Werner RS, Dall'Antonia Júnior RP, Camargo RS. 1988. "In vitro" study on release of cyclic AMP and thyroid hormone in autonomously functioning thyroid nodules. *Horm Metab Res*.20:510-2.
- Santisteban P, Bernal J. Thyroid development and effect on the nervous system. *Rev Endocr Metab Disord*. 2005 Aug;6(3):217-28.
- Spitzweg C, Morris JC. 2010. Genetics and phenomics of hypothyroidism and goiter due to NIS mutations. *Molecular and cellular endocrinology*. 322:56-63.
- Thienpont B, Tingaud-Sequeira A, Prats E, Barata C, Babin PJ, Raldúa D. Zebrafish eleutheroembryos provide a suitable vertebrate model for screening chemicals that impair thyroid hormone synthesis. *Environ Sci Technol*. 2011. 45(17):7525-32.
- Yi X, Yamamoto K, Shu L, Katoh R, Kawaoi A. Effects of Propylthiouracil (PTU) Administration on the Synthesis and Secretion of Thyroglobulin in the Rat Thyroid Gland: A Quantitative Immuno-electron Microscopic Study Using Immunogold Technique. *Endocr Pathol*. 1997 Winter;8(4):315-325.
- Zoeller RT, Crofton KM. 2005. Mode of action: developmental thyroid hormone insufficiency--neurological abnormalities resulting from exposure to propylthiouracil. *Crit Rev Toxicol*. 35:771-81
- Zoeller RT, Tan SW, Tyl RW. 2007. General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Critical reviews in toxicology*. 37:11-53.

281: Throxine (T4) in serum. Decreased (<https://aopwiki.org/events/281>)

AOP42

Short Name: T4 in serum, Decreased

Key Event Component

Process	Object	Action
	thyroxine	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
54: Inhibition of Na ⁺ /I ⁻ symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
8: Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/8)	KeyEvent
65: XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
134: Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
152: Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent
159: Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent
175: Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	KeyEvent
176: Sodium Iodide Symporter (NIS) Inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/176)	KeyEvent
194: Hepatic nuclear receptor activation leading to altered amphibian metamorphosis (https://aopwiki.org/aops/194)	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Organization

Level of Biological Organization
Tissue

Organ term

Organ term
serum

Evidence for Perturbation by Stressor

Propylthiouracil

6-n-propylthiouracil is a classic positive control for inhibition of TPO

Methimazole

Classic positive control

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
chicken	Gallus gallus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9031)
Xenopus laevis	Xenopus laevis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
Pig	Pig	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=0)
zebra fish	Danio rerio	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=7955)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Female	Strong
Male	Strong

The overall evidence supporting taxonomic applicability is strong. THs are evolutionarily conserved molecules present in all vertebrate species (Hulbert, 2000; Yen, 2001). Moreover, their crucial role in zebra fish (Thienpont et al., 2011), amphibian and lamprey metamorphoses is well established (Manzon and Youson, 1997; Yaoita and Brown, 1990; Furlow and Neff, 2006). Their existence and importance has also been described in many different animal and plant kingdoms (Eales, 1997; Heyland and Moroz, 2005), while their role as environmental messenger via exogenous routes in echinoderms confirms the hypothesis that these molecules are widely distributed among the living organisms (Heyland and Hodin, 2004). However, the role of TH in the different species depends on the expression and function of specific proteins (e.g receptors or enzymes) under TH control and may vary across species and tissues. As such extrapolation regarding TH action across species should be done with caution.

With few exceptions, vertebrate species have circulating T4 (and T3) that are bound to transport proteins in blood. Clear species differences exist in serum transport proteins (Dohler et al., 1979; Yamauchi and Ishihara, 2009). There are three major transport proteins in mammals; thyroid binding globulin (TBG), transthyretin (TTR), and albumin. In adult humans, the percent bound to these proteins is about 75, 15 and 10 percent, respectively (Schussler 2000). In contrast, in adult rats the majority of THs are bound to TTR. Thyroid binding proteins are developmentally regulated in rats. TBG is expressed in rats until approximately postnatal day (PND) 60, with peak expression occurring during weaning (Savu et al., 1989). However, low levels of TBG persist into adult ages in rats and can be experimentally induced by hypothyroidism, malnutrition, or caloric restriction (Rouaze-Romet et al., 1992). While these species differences impact TH half-life (Capen, 1997) and possibly regulatory feedback

mechanisms, there is little information on quantitative dose-response relationships of binding proteins and serum hormones during development across different species. Serum THs are still regarded as the most robust measurable key event causally linked to downstream adverse outcomes.

How this Key Event Works

All iodothyronines are derived from the modification of tyrosine molecules (Taurog, 2000). There are two biologically active thyroid hormones (THs) in serum, triiodothyronine (T3) and T4, and a few inactive iodothyronines (rT3, 3,5-T2). T4 is the predominant TH in circulation, comprising approximately 80% of the TH excreted from the thyroid gland and is the pool from which the majority of T3 in serum is generated (Zoeller et al., 2007). As such, serum T4 changes usually precede changes in other serum THs. Decreased thyroxine (T4) in serum results result from one or more MIEs upstream and is considered a key biomarker of altered TH homeostasis (DeVito et al., 1999).

Serum T4 is used as a biomarker of TH status because the circulatory system serves as the major transport and delivery system for TH delivery to tissues. The majority of THs in the blood are bound to transport proteins (Bartalena and Robbins, 1993). In serum, it is the unbound, or 'free' form of the hormone that thought to be available for transport into tissues. Free hormones are approximately 0.03 and 0.3 percent for T4 and T3, respectively. There are major species differences in the predominant binding proteins and their affinities for THs (see below). However, there is broad agreement that changes in serum concentrations of THs is diagnostic of thyroid disease or chemical-induced disruption of thyroid homeostasis (DeVito et al., 1999; Miller et al., 2009; Zoeller et al., 2007).

Normal serum T4 reference ranges can be species and lifestage specific. In rodents, serum THs are low in the fetal circulation, increasing as the fetal thyroid gland becomes functional on gestational day 17, just a few days prior to birth. After birth serum hormones increase steadily, peaking at two weeks, and falling slightly to adult levels by postnatal day 21 (Walker et al., 1980; Harris et al., 1978; Goldely et al., 1995; Lau et al., 2003). Similarly, in humans, adult reference ranges for THs do not reflect the normal ranges for children at different developmental stages, with TH concentrations highest in infants, still increased in childhood, prior to a decline to adult levels coincident with pubertal development (Corcoran et al. 1977; Kapelari et al., 2008). In some frog species, there is an analogous peak in thyroid hormones in tadpoles that starts around NF stage 56, peaks at Stage 62 and the declines to lower levels by Stage 56 (Sternberg et al., 2011; Leloup and Buscaglia, 1977).

How it is Measured or Detected

Serum T3 and T4 can be measured as free (unbound) or total (bound + unbound). Free hormone concentrations are clinically considered more direct indicators of T4 and T3 activities in the body, but in animal studies, total T3 and T4 are typically measured. Historically, the most widely used method in toxicology is radioimmunoassay (RIA). The method is routinely used in rodent endocrine and toxicity studies. The ELISA method is a commonly used as a human clinical test method. Least common is analytical determination of iodothyronines (T3, T4, rT3, T2) and their conjugates, though methods employing HPLC and mass spectrometry exist (Hornung et al., 2015; DeVito et al., 1999; Spencer, 2013).

Any of these measurements should be evaluated for the relationship to the actual endpoint of interest, repeatability, reproducibility, and lower limits of quantification using a fit-for-purpose approach (i.e., different regulatory needs will require different levels of confidence in the AOP). All three of the methods summarized above would be fit-for-purpose, depending on the number of samples to be evaluated and the associated costs of each method. Both RIA and ELISA measure THs by an indirect methodology, whereas analytical determination is the most direct measurement available. All these methods, particularly RIA, are repeatable and reproducible.

Thyroxine-immunofluorescence quantitative disruption test (TIQDT) was designed to provide a simple, rapid, alternative bioassay for assessing the potential of chemical pollutants and drugs to disrupt thyroid gland function. Thyroid gland functionality was evaluated by measuring IT4C in 4768 120 hpf zebrafish eleutheroembryos, following the TIQDT protocol (Thienpont et al., 2011). This study demonstrated that zebrafish eleutheroembryos provided a suitable vertebrate model, not only for screening the potential thyroid disrupting effect of molecules, but also for estimating the potential hazards associated with exposure to chemicals directly impairing thyroxine (T4) synthesis. Amitrole, potassium perchlorate, potassium thiocyanate, methimazole (MMI), phloroglucinol, 6-propyl-2-thiouracil, ethylenethiourea, benzophenone-2, resorcinol, pyrazole, sulfamethoxazole, sodium bromide, mancozeb, and genistein were classified as thyroid gland function disruptors. Concordance between TIQDT on zebrafish and mammalian published data was very high. TIQDT performed on zebrafish eleutheroembryos is an alternative whole-organism screening assay that provides relevant information for environmental and human risk assessments.

References

- Axelrad DA, Baetcke K, Dockins C, Griffiths CW, Hill RN, Murphy PA, Owens N, Simon NB, Teuschler LK. Risk assessment for benefits analysis: framework for analysis of a thyroid-disrupting chemical. *J Toxicol Environ Health A*. 2005 68(11-12):837-55.
- Bartalena L, Robbins J. Thyroid hormone transport proteins. *Clin Lab Med*. 1993 Sep;13(3):583-98. Bassett JH, Harvey CB, Williams GR. (2003). Mechanisms of thyroid hormone receptor-specific nuclear and extra nuclear actions. *Mol Cell Endocrinol*. 213:1-11.
- Capen CC. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicol Pathol*. 1997 25(1):39-48.
- Cope RB, Kacew S, Dourson M. A reproductive, developmental and neurobehavioral study following oral exposure of tetrabromobisphenol A on Sprague-Dawley rats. *Toxicology*. 2015 329:49-59.
- Corcoran JM, Eastman CJ, Carter JN, Lazarus L. (1977). Circulating thyroid hormone levels in children. *Arch Dis Child*. 52: 716-720.
- Crofton KM. Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. *Risk Anal*. 2004 Dec;24(6):1665-71.
- DeVito M, Biegel L, Brouwer A, Brown S, Brucker-Davis F, Cheek AO, Christensen R, Colborn T, Cooke P, Crissman J, Crofton K, Doerge D, Gray E, Hauser P, Hurley P, Kohn M, Lazar J, McMaster S, McClain M, McConnell E, Meier C, Miller R, Tietge J, Tyl R. (1999). Screening methods for thyroid hormone disruptors. *Environ Health Perspect*. 107:407-415.

- Döhler KD, Wong CC, von zur Mühlen A (1979). The rat as model for the study of drug effects on thyroid function: consideration of methodological problems. *Pharmacol Ther B*. 5:305-18.
- Eales JG. (1997). Iodine metabolism and thyroid related functions in organisms lacking thyroid follicles: Are thyroid hormones also vitamins? *Proc Soc Exp Biol Med*. 214:302-317.
- Furlow JD, Neff ES. (2006). A developmental switch induced by thyroid hormone: *Xenopus laevis* metamorphosis. *Trends Endocrinol Metab*. 17:40–47.
- Goldley ES, Crofton KM. Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. *Toxicol Sci*. 1998 45(1):94-10
- Goldley ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. *Tox Appl Pharmacol*. 1995 135(1):77-88.
- Harris AR, Fang SL, Prosky J, Braverman LE, Vagenakis AG. Decreased outer ring monodeiodination of thyroxine and reverse triiodothyronine in the fetal and neonatal rat. *Endocrinology*. 1978 Dec;103(6):2216-22
- Heyland A, Hodin J. (2004). Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of non-feeding development. *Evolution*. 58: 524-538.
- Heyland A, Moroz LL. (2005). Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. *J Exp Biol*. 208:4355-4361.
- Hill RN, Crisp TM, Hurley PM, Rosenthal SL, Singh DV. Risk assessment of thyroid follicular cell tumors. *Environ Health Perspect*. 1998 Aug;106(8):447-57.
- Hornung MW, Kosian P, Haselman J, Korte J, Challis K, Macherla C, Nevalainen E, Degitz S (2015) In vitro, ex vivo and in vivo determination of thyroid hormone modulating activity of benzothiazoles. *Toxicol Sci* 146:254-264.
- Hulbert AJ. Thyroid hormones and their effects: a new perspective. *Biol Rev Camb Philos Soc*. 2000 Nov;75(4):519-631. Review.
- Kapelari K, Kirchlechner C, Högl W, Schweitzer K, Virgolini I, Moncayo R. 2008. Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study. *BMC Endocr Disord*. 8: 15.
- Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicol Sci*. 2003 Aug;74(2):382-92.
- Leloup, J., and M. Buscaglia. La triiodothyronine: hormone de la métamorphose des amphibiens. *CR Acad Sci* 284 (1977): 2261-2263.
- Liu J, Liu Y, Barter RA, Klaassen CD.: Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: a dose-response study. *J Pharmacol Exp Ther* 273, 977-85, 1994
- Manzon RG, Youson JH. (1997). The effects of exogenous thyroxine (T4) or triiodothyronine (T3), in the presence and absence of potassium perchlorate, on the incidence of metamorphosis and on serum T4 and T3 concentrations in larval sea lampreys (*Petromyzon marinus* L.). *Gen Comp Endocrinol*. 106:211-220.
- McClain RM. Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutat Res*. 1995 Dec;333(1-2):131-42
- Miller MD, Crofton KM, Rice DC, Zoeller RT. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. *Environ Health Perspect*. 2009 117(7):1033-41
- Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol*. 1996 Feb;136(2):269-79.
- NTP National Toxicology Program.: NTP toxicology and carcinogenesis studies of 3,3'-dimethylbenzidine dihydrochloride (CAS no. 612-82-8) in F344/N rats (drinking water studies). *Natl Toxicol Program Tech Rep Ser* 390, 1-238, 1991.
- O'Connor, J. C., J. C. Cook, et al. (1998). "An ongoing validation of a Tier I screening battery for detecting endocrine-active compounds (EACs)." *Toxicol Sci* 46(1): 45-60.
- O'Connor, J. C., L. G. Davis, et al. (2000). "Detection of dopaminergic modulators in a tier I screening battery for identifying endocrine-active compounds (EACs)." *Reprod Toxicol* 14(3): 193-205.
- Rouaze-Romet M, Savu L, Vranckx R, Bleiberg-Daniel F, Le Moullac B, Gouache P, Nunez EA. 1992. Reexpression of thyroxine-binding globulin in postweaning rats during protein or energy malnutrition. *Acta Endocrinol (Copenh)*.127:441-448.
- Savu L, Vranckx R, Maya M, Gripois D, Blouquit MF, Nunez EA. 1989. Thyroxine-binding globulin and thyroxinebinding prealbumin in hypothyroid and hyperthyroid developing rats. *BiochimBiophys Acta*. 992:379-384.
- Schneider S, Kaufmann W, Strauss V, van Ravenzwaay B. Vinclozolin: a feasibility and sensitivity study of the ILSI-HESI F1-extended one-generation rat reproduction protocol. *Regul Toxicol Pharmacol*. 2011 Feb;59(1):91-100.
- Schussler, G.C. (2000). The thyroxine-binding proteins. *Thyroid* 10:141–149.
- Spencer, CA. (2013). Assay of thyroid hormone and related substances. In De Groot, LJ et al. (Eds). *Endotext*. South Dartmouth, MA
- Sternberg RM, Thoemke KR, Korte JJ, Moen SM, Olson JM, Korte L, Tietge JE, Degitz SJ Jr. Control of pituitary thyroid-stimulating hormone synthesis and secretion by thyroid hormones during *Xenopus* metamorphosis. *Gen Comp Endocrinol*. 2011. 173(3):428-37

Taurog A. 2005. Hormone synthesis. In: Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text (Braverman LE, Utiger RD, eds). Philadelphia:Lippincott, Williams and Wilkins, 47–81 Walker P, Dubois JD, Dussault JH. Free thyroid hormone concentrations during postnatal development in the rat. *Pediatr Res*. 1980 Mar;14(3):247-9.

Thienpont B (https://www.ncbi.nlm.nih.gov/pubmed/?term=Thienpont%20B%5BAuthor%5D&cauthor=true&cauthor_uid=21800831), Tingaud-Sequeira A (https://www.ncbi.nlm.nih.gov/pubmed/?term=Tingaud-Sequeira%20A%5BAuthor%5D&cauthor=true&cauthor_uid=21800831), Prats E (https://www.ncbi.nlm.nih.gov/pubmed/?term=Prats%20E%5BAuthor%5D&cauthor=true&cauthor_uid=21800831), Barata C (https://www.ncbi.nlm.nih.gov/pubmed/?term=Barata%20C%5BAuthor%5D&cauthor=true&cauthor_uid=21800831), Babin PJ (https://www.ncbi.nlm.nih.gov/pubmed/?term=Babin%20PJ%5BAuthor%5D&cauthor=true&cauthor_uid=21800831), Raldúa D (https://www.ncbi.nlm.nih.gov/pubmed/?term=Rald%C3%BAa%20D%5BAuthor%5D&cauthor=true&cauthor_uid=21800831). Zebrafish eleutheroembryos provide a suitable vertebrate model for screening chemicals that impair thyroid hormone synthesis. *Environ Sci Technol*. (<https://www.ncbi.nlm.nih.gov/pubmed/21800831>) 2011 Sep 1;45(17):7525-32.

Yamauchi K1, Ishihara A. Evolutionary changes to transthyretin: developmentally regulated and tissue-specific gene expression. *FEBS J*. 2009. 276(19):5357-66.

Yaoita Y, Brown DD. (1990). A correlation of thyroid hormone receptor gene expression with amphibian metamorphosis. *Genes Dev*. 4:1917-1924.

Yen PM. (2001). Physiological and molecular basis of thyroid hormone action. *Physiol Rev*. 81:1097-1142.

Zoeller, R. T., R. Bansal, et al. (2005). "Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain." *Endocrinology* 146(2): 607-612.

Zoeller RT, Tan SW, Tyl RW. General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Crit Rev Toxicol*. 2007 Jan-Feb;37(1-2):11-53

280: Thyroxine (T4) in neuronal tissue, Decreased (<https://aopwiki.org/events/280>)

Short Name: T4 in neuronal tissue, Decreased

Key Event Component

Process	Object	Action
	thyroxine	decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
54: Inhibition of Na ⁺ /I ⁻ symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
8: Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/8)	KeyEvent
65: XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
134: Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
152: Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent

Stressors

Name
Methimazole
Propylthiouracil

Biological Organization

Level of Biological Organization
Organ

Organ term

Organ term
brain

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
chicken	Gallus gallus	Weak	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9031)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Female	Strong
Male	Strong

THs are critical for normal brain development in most vertebrates, primarily documented empirically in mammalian species (Bernal, 2013). However, there is compelling data that demonstrates the need for TH in brain development for many other taxa, including: birds, fish and frogs (Van Herck et al., 2013; Denver, 1998; Power et al., 2001). The most well known non-mammalian action of TH is to induce metamorphosis in amphibians and some fish species. However, there is a fundamental difference in the mechanisms by which T3 affects amphibian metamorphosis vs its role in mammalian brain development (Galton, 1983). In the rat, brain development proceeds, even if defective, despite the absence of TH. By contrast, TH administration to tadpoles induces early metamorphosis, whereas in its absence, tadpoles grow to extremely large size, but the metamorphosis program is never activated (Galton, 1983).

How this Key Event Works

Thyroid hormones (TH) are present in brain tissue of most vertebrate species, and thyroxine (T4) is converted to triiodothyronine locally in this tissue. The amount of THs in brain is known to vary during development and to differ among brain regions (Calvo et al., 1990; Kester et al., 2004; Tu et al., 1999). In human cerebral cortex, T3 increases steadily from 13-weeks, reaching adult levels by 20 weeks post conception. This occurs despite very low and unchanging levels in fetal serum T3, when fetal serum T4 increases 3-fold over the same period. This indicates that T3 in fetal brain is locally generated from serum-derived T4 via the activity of deiodinases, primarily DIO2. DIO2 serves to convert T4 to T3. During this time in fetal development DIO3 activity, which converts T3 to the inactive reverse T3 (rT3), remains very low in cortex. In contrast, in other brain regions including hippocampus and cerebellum, T3 remains low throughout early and mid-gestation and corresponds with high activity of DIO3 in these brain regions. In late gestation and after birth, DIO3 levels drop in hippocampus and cerebellum with a corresponding increase in T3 concentrations (Kester et al., 2004).

A similar spatial and temporal profile of deiodinase activity and corresponding brain hormone concentrations has been observed in rodent brain (Calvo et al., 1990; Tu et al., 1999). In the rat, either whole brain or cortex have been preferentially assessed as due to the low levels of hormones present and the small tissue volumes make quantification difficult. Brain T3 and T4 rise in parallel from gestational day 10 to gestational day 20 in rat. They are typically both quite low until gestational 17 with steep increases between GD18 and GD20 corresponding to the onset of fetal thyroid function (Calvo et al., 1990; Ruiz de Ono et al., 1988; Oberon et al., 1981). Just before birth, brain T3 and T4 concentrations are about one-third to one-half that of adult brain. Brain development in the early postnatal period in rat is roughly equivalent to the 3rd trimester in humans such that adult levels of T3 and T4 in brain are not reached in rodents until the 2nd-3rd postnatal week.

For THs to gain access to brain tissue they need to cross the blood brain barrier (BBB) which regulates the active transport of TH into neurons. Many transporter proteins have been identified, and the monocarboxylate transporters (Mct8, Mct10) and anion-transporting polypeptide

(OATP1c1) show the highest degree of affinity towards TH and are prevalent in brain (Jansen et al., 2007; Mayer et al., 2014). Transporters express a distinct distribution pattern that varies by tissue and age (Friesema et al., 2005; Henneman et al., 2001; Visser et al., 2007; Heuer et al., 2005; Muller and Heuer, 2007). Although several transporters have been identified, current knowledge of cell specific profile of transporters is limited.

Most of the hormone transported across the blood brain barrier is in the form of T4, primarily through the cellular membrane transporters (e.g., OATP1c1 transporter) into the astrocyte (Visser and Visser, 2012; Sugiyama et al., 2003; Tohyama et al., 2004). Within the astrocyte, T4 is converted into T3 via the local activity of deiodinase 2 (DIO2) (Guadano-Ferraz et al., 1997). A small amount of T3 may cross the blood brain barrier directly via the T3-specific transporter, MCT8 (Heuer et al., 2005). Although in mature brain T3 derives partially from the circulation and from the deiodination of T4, in the fetal brain T3 is exclusively a product of T4 deiodination (Calvo et al., 1990; Grijota-Martinez et al., 2011). In both cases, only the required amount of T3 is utilized in neurons and the excess is degraded by the neuron-specific deiodinase DIO3 (Tu et al., 1999; St. Germain et al., 2009; Hernandez et al., 2010).

Both deiodinase and transporter expression in brain peak in different brain regions at different times in fetal and neonatal life (Kester et al., 2004; Bates et al., 1999; Muller and Heuer, 2014; Heuer, 2007). Collectively, these spatial and temporal patterns of transporter expression and deiodinase activity provide exquisite control of brain T3 available for nuclear receptor activation and regulated gene expression.

How it is Measured or Detected

Radioimmunoassays (RIAs) are commonly used to detect TH in the brain (e.g., Obregon et al., 1982; Calvo et al., 1990; Morse et al., 1996; Bansal et al., 2005; Gilbert et al., 2013). The method (and minor variants) is well established in the published literature. However, it is not available in a simple 'kit' and requires technical knowledge of RIAs, thus has not been used in most routine toxicology studies. Evaluations in neuronal tissue are complicated by the difficulty of the fatty matrix, heterogeneity of regions within the brain, and low tissue concentrations and small tissue amounts especially in immature brain. Two analytical techniques, LC- and HPLC-inductively coupled plasma-mass spectrometry have recently been used to measure brain concentrations of TH. These techniques have proven capable of measuring very low levels in whole-body homogenates of frog tadpoles at different developmental stages (e.g., Simon et al., 2002; Tietge et al., 2010). The assay detects I-, MIT, DIT, T4, T3, and rT3. More recently, Wang and Stapleton (2010) and Donzelli et al. (2016) used liquid chromatography-tandem mass spectrometry for the simultaneous analysis of five THs including thyroxine (T4), 3,3',5-triiodothyronine (T3), 3,3',5'-triiodothyronine (rT3; reverse T3), 3,3'-diiodothyronine (3,3'-T2), and 3,5-diiodothyronine (3,5-T2) in serum and a variety of tissues including brain. These analytical methods require expensive equipment and technical expertise and as such are not routinely used.

References

- Bansal R, You SH, Herzig CT, Zoeller RT (2005). Maternal thyroid hormone increases HES expression in the fetal rat brain: an effect mimicked by exposure to a mixture of polychlorinated biphenyls (PCBs). *Brain Res Dev Brain Res* 156:13-22.
- Bates JM, St Germain DL, Galton VA. Expression profiles of the three iodothyronine deiodinases, D1, D2, and D3, in the developing rat. *Endocrinology*. 1999 Feb;140(2):844-51.
- Bernal J. (2013). Thyroid Hormones in Brain Development and Function. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, editors. *Endotext* [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-2015 www.thyroidmanager.org.
- Calvo R, Obregón MJ, Ruiz de Oña C, Escobar del Rey F, Morreale de Escobar G. (1990). Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. *J. Clin. Invest.* 86:889-899.
- Denver, RJ 1998 The molecular basis of thyroid hormone-dependent central nervous system remodeling during amphibian metamorphosis. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 119:219-228.
- Donzelli R, Colligiani D, Kusmic C, Sabatini M, Lorenzini L, Accorroni A, Nannipieri M, Saba A, Iervasi G, Zucchi R. Effect of Hypothyroidism and Hyperthyroidism on Tissue Thyroid Hormone Concentrations in Rat. *Eur Thyroid J*. 2016 Mar;5(1):27-34.
- Friesema EC, Jansen J, Milici C, Visser TJ (2005) Thyroid hormone transporters. *Vitam Horm* 70:137-167.
- Galton VH 1983 Thyroid hormone action in amphibian metamorphosis. In: Oppenheimer JH, Samuels HH (eds) *Molecular Basis of Thyroid Hormone Action*. Academic Press, New York, pp 445-483.
- Gilbert ME, Hedge JM, Valentin-Blasini L, Blount BC, Kannan K, Tietge J, Zoeller RT, Crofton KM, Jarrett JM, Fisher JW (2013) An animal model of marginal iodine deficiency during development: the thyroid axis and neurodevelopmental outcome. *Toxicol Sci* 132:177-195.
- Grijota-Martinez C, Diez D, Morreale de Escobar G, Bernal J, Morte B. (2011). Lack of action of exogenously administered T3 on the fetal rat brain despite expression of the monocarboxylate transporter 8. *Endocrinology*. 152:1713-1721.
- Guadano-Ferraz A, Obregon MJ, St Germain DL, Bernal J. (1997). The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc Natl Acad Sci USA*. 94: 10391-10396.
- Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ. (2001). Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. *Endocr Rev*. 22:451-476.
- Hernandez A, Quignodon L, Martinez ME, Flamant F, St Germain DL. Type 3 deiodinase deficiency causes spatial and temporal alterations in brain T3 signaling that are dissociated from serum thyroid hormone levels. *Endocrinology*. 2010 Nov;151(11):5550-8.
- Heuer H. (2007). The importance of thyroid hormone transporters for brain development and function. *Best Pract Res Clin Endocrinol Metab*. 21:265-276.

- Heuer H, Maier MK, Iden S, Mittag J, Friesema EC, Visser TJ, Bauer K. (2005). The monocarboxylate transporter 8 linked to human psychomotor retardation is highly expressed in thyroid hormone-sensitive neuron populations. *Endocrinology* 146:1701–1706.
- Jansen J, Friesema EC, Kester MH, Milici C, Reeser M, Gruters A, Barrett TG, Mancilla EE, Svensson J, Wemeau JL, Busi da Silva Canalli MH, Lundgren J, McEntagart ME, Hopper N, Arts WF, Visser TJ (2007) Functional analysis of monocarboxylate transporter 8 mutations identified in patients with X-linked psychomotor retardation and elevated serum triiodothyronine. *J Clin Endocrinol Metab* 92:2378-2381.
- Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, Hume R, Morreale de Escobar G. (2004). Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *J Clin Endocrinol Metab* 89:3117–3128.
- Mayer S, Müller J, Bauer R, Richert S, Kassmann CM, Darras VM, Buder K, Boelen A, Visser TJ, Heuer H. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. *J Clin Invest.* 2014 May 1;124(5):1987-99.
- Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol.* 1996 Feb;136(2):269-79
- Müller J, Heuer H. Expression pattern of thyroid hormone transporters in the postnatal mouse brain. *Front Endocrinol (Lausanne).* 2014 Jun 18;5:92.
- Obregon MJ, Mallol J, Escobar del Rey F, Morreale de Escobar G. (1981). Presence of l-thyroxine and 3,5,3-triiodo-l-thyronine in tissues from thyroidectomized rats. *Endocrinology* 109:908-913.
- Power DM, Llewellyn L, Faustino M, Nowell MA, Björnsson BT, Einarsdottir IE, Canario AV, Sweeney GE. Thyroid hormones in growth and development of fish. *Comp Biochem Physiol C Toxicol Pharmacol.* 2001 Dec;130(4):447-59.
- Ruiz de Oña C, Obregón MJ, Escobar del Rey F, Morreale de Escobar G. Developmental changes in rat brain 5'-deiodinase and thyroid hormones during the fetal period: the effects of fetal hypothyroidism and maternal thyroid hormones. *Pediatr Res.* 1988 Nov;24(5):588-94.
- Simon R, Tietge JE, Michalke B, Degitz S, Schramm KW. Iodine species and the endocrine system: thyroid hormone levels in adult *Danio rerio* and developing *Xenopus laevis*. *Anal Bioanal Chem.* 2002 Feb;372(3):481-5.
- St Germain DL, Galton VA, Hernandez A. (2009). Minireview: Defining the roles of the iodothyronine deiodinases: current concepts and challenges. *Endocrinology.* 150:1097-107.
- Sugiyama D, Kusuhara H, Taniguchi H, Ishikawa S, Nozaki Y, Aburatani H, Sugiyama Y. (2003). Functional characterization of rat brain-specific organic anion transporter (Oatp14) at the blood–brain barrier: high affinity transporter for thyroxine. *J Biol Chem.* 278:43489–43495.
- Tietge JE, Butterworth BC, Haselman JT, Holcombe GW, Hornung MW, Korte JJ, Kosian PA, Wolfe M, Degitz SJ. Early temporal effects of three thyroid hormone synthesis inhibitors in *Xenopus laevis*. *Aquat Toxicol.* 2010 Jun 1;98(1):44-50.
- Tohyama K, Kusuhara H, Sugiyama Y. (2004). Involvement of multispecific organic anion transporter, Oatp14 (Slc21a14), in the transport of thyroxine across the blood-brain barrier. *Endocrinology.* 145: 4384–4391.
- Tu HM, Legradi G, Bartha T, Salvatore D, Lechan RM, Larsen PR. (1999). Regional expression of the type 3 iodothyronine deiodinase messenger ribonucleic acid in the rat central nervous system and its regulation by thyroid hormone. *Endocrinology.* 140: 784–790.
- Van Herck SL, Geysens S, Delbaere J, Darras VM. Regulators of thyroid hormone availability and action in embryonic chicken brain development. *Gen Comp Endocrinol.* 2013.190:96-104.
- Visser EW, Visser TJ. (2012). Finding the way into the brain without MCT8. *J Clin Endocrinol Metab.* 97:4362-4365.
- Visser WE, Friesema EC, Jansen J, Visser TJ. (2007). Thyroid hormone transport by monocarboxylate transporters. *Best Pract Res Clin Endocrinol Metab.* 21:223–236.
- Wang, D. and Stapleton, HM. (2010) Analysis of thyroid hormones in serum by liquid chromatography -tandem mass spectrometry. *Anal Bioanal Chem.* 2010 Jul; 397(5): 1831–1839

756: Hippocampal gene expression, Altered (<https://aopwiki.org/events/756>)

Short Name: Hippocampal gene expression, Altered

Key Event Component

Process	Object	Action
regulation of gene expression	hippocampal formation	abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent

AOP ID and Name	Event Type
8: Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/8)	KeyEvent
134: Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
152: Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent

Stressors

Name
Methimazole
Propylthiouracil

Biological Organization

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
rats	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
human	Homo sapiens	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

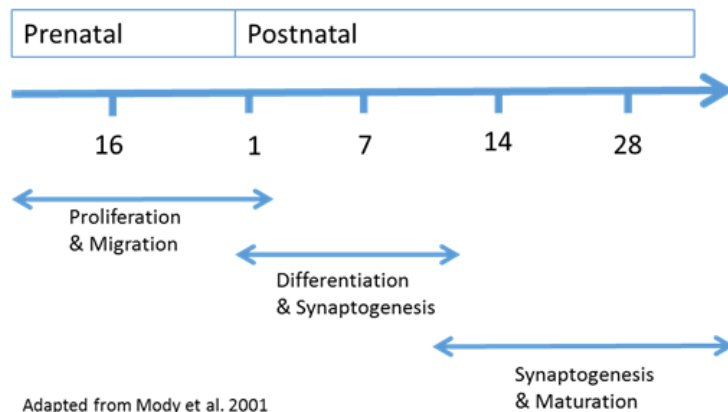
Sex	Evidence
Female	Strong
Male	Strong

Gene expression in the developing brain in general is analogous across most mammalian species (Kempermann, 2012). Most of the empirical data on gene expression in hippocampus is from rat, mouse and human studies.

How this Key Event Works

Gene expression profiles have been published for the developing human and rodent hippocampus (Zhang et al., 2002; Mody et al., 2001). In both humans and rodents, the hippocampus undergoes typical stages of neurodevelopment found in most brain regions, including: cell proliferation, migration, differentiation, synapse formation, and the maturation of synaptic function. In the rodent, peak windows during pre- and post-natal periods have been identified during which major cellular and physiological events occur (see Figure 1). Each window expresses distinct patterns of gene transcription and clusters of genes increase their expression corresponding to the progression of events of hippocampal ontogeny (see Mody et al., 2001). Tables of gene clusters associated with these phases can be found in Supplementary Tables of Mody et al. (2001).

Figure 1. Mouse Hippocampal Developmental Stages Controlled by Gene Expression



During the very early prenatal period, genes corresponding to general cellular function are prominent (Mody et al., 2001). These are followed in time by genes regulating neuronal differentiation and migration in the mid to late gestational period. From late gestation (gestational day 15) until birth almost all the cells in the CA fields switch from a highly active proliferation state to a postmitotic state, and undergo differentiation and migration. Expression of proliferative genes involved in cell cycle progression are highly expressed at gestational day 16, then subsequently are silent immediately after birth when genes directing neuronal growth switch on. The pyramidal neurons of the CA fields in the hippocampus proper develop in advance of the granule cells that comprise the principal cells of the dentate gyrus. As such, the genes controlling the distinct phases of neurodevelopment are expressed at different times in these two hippocampal subregions (Altman and Bayer, 1990a; b). In both subregions, however, many phenotypic changes within the hippocampal neuron occur in the period immediately after birth (postnatal day 1 to 7). Almost all neurons show extensive growth and differentiation during the first postnatal week. These cellular changes are marked by rapid cytoskeletal changes, production of cell adhesion molecules, and extracellular matrix formation. The gene families involved in these processes include actins, tubulins, and chaperonin proteins essential for promoting correct protein folding of cytoskeletal components. Cell adhesion and extracellular matrix proteins are also upregulated during this period as these genes are critical for differentiation and synaptogenesis.

During late postnatal hippocampal development (postnatal day 16-30), hippocampal circuits become more active and exhibit increased synaptic plasticity. Many genes upregulated during this phase of development are involved in synaptic function and include genes regulating vesicle associated proteins and calcium-mediated transmitter release, neurotrophins, and neurotransmitter receptors. Efficient energy utilization is essential during this period of increased synaptic activity, events mirrored by an upregulation of enzymes involved in glucose and oxidative metabolism.

How it is Measured or Detected

Measurement of genomic profiles in developing brain use methods that are well established and accepted in the published literature. Microarray studies with expression profile analyses have been conducted in cortex and hippocampus of humans (Zhang et al., 2002), non-human primates, and rodent brains of various ages (Mody et al., 2001; Royland et al., 2008; Dong et al., 2015). More commonly, quantitative rtPCR or in situ hybridization have been used to probe individual gene transcripts (Dowling et al., 2000; Morte et al., 2010) or their protein products (Alvarez-Dolado et al., 1994; Gilbert et al., 2007). Recently RNA-Seq technology was applied to T3-treated primary mouse cortical cells and gene targets enriched in astrocytes and neurons to identify TH-responsive genes (Gil-Ibanez et al, 2015).

References

- Altman J, Bayer SA. Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J Comp Neurol.* 1990a Nov 15;301(3):365-81.
- Altman J, Bayer SA. Prolonged sojourn of developing pyramidal cells in the intermediate zone of the hippocampus and their settling in the stratum pyramidale. *J Comp Neurol.* 1990b Nov 15;301(3):343-64.
- Alvarez-Dolado M, Ruiz M, Del Rio JA, Alcantara S, Burgaya F, Sheldon M, Nakajima K, Bernal J, Howell BW, Curran T, Soriano E, Munoz A (1999) Thyroid hormone regulates reelin and dab1 expression during brain development. *J Neurosci* 19:6979-6993.

AOP42

Dong H, You SH, Williams A, Wade MG, Yauk CL, Thomas Zoeller R (2015) Transient Maternal Hypothyroxinemia Potentiates the Transcriptional Response to Exogenous Thyroid Hormone in the Fetal Cerebral Cortex Before the Onset of Fetal Thyroid Function: A Messenger and MicroRNA Profiling Study. *Cereb Cortex* 25:1735-1745.

Dowling AL, Zoeller RT. 2000. Thyroid hormone of maternal origin regulates the expression of RC3/neurogranin mRNA in the fetal rat brain. *Brain Res: Molec Brain Res.* 82:126-132.

Gilbert ME, Sui L, Walker MJ, Anderson W, Thomas S, Smoller SN, Schon JP, Phani S, Goodman JH (2007) Thyroid hormone insufficiency during brain development reduces parvalbumin immunoreactivity and inhibitory function in the hippocampus. *Endocrinology* 148:92-102.

Gil-Ibanez P, Garcia-Garcia F, Dopazo J, Bernal J, Morte B. 2015. Global Transcriptome Analysis of Primary Cerebrocortical Cells: Identification of Genes Regulated by Triiodothyronine in Specific Cell Types. *Cerebral cortex*. Nov 2.

Kempermann G. New neurons for 'survival of the fittest'. *Nat Rev Neurosci.* 2012 Oct;13(10):727-36.

Mody M, Cao Y, Cui Z, Tay KY, Shyong A, Shimizu E, Pham K, Schultz P, Welsh D, Tsien JZ. Genome-wide gene expression profiles of the developing mouse hippocampus. *Proc Natl Acad Sci U S A.* 2001 Jul 17;98(15):8862-7.

Morte B, Ceballos A, Diez D, Grijota-Martinez C, Dumitrescu AM, Di Cosmo C, Galton VA, Refetoff S, Bernal J. Thyroid hormone-regulated mouse cerebral cortex genes are differentially dependent on the source of the hormone: a study in monocarboxylate transporter-8- and deiodinase-2-deficient mice. *Endocrinology.* 2010. 151:2381-2387.

Royland JE, Parker JS, Gilbert ME. A genomic analysis of subclinical hypothyroidism in hippocampus and neocortex of the developing rat brain. *J Neuroendocrinol.* 2008 Dec;20(12):1319-38.

Zhang Y, Mei P, Lou R, Zhang MQ, Wu G, Qiang B, Zhang Z, Shen Y. Gene expression profiling in developing human hippocampus. *J Neurosci Res.* 2002 Oct 15;70(2):200-8.

757: Hippocampal anatomy, Altered (<https://aopwiki.org/events/757>)

Short Name: Hippocampal anatomy, Altered

Key Event Component

Process	Object	Action
	hippocampal formation	morphological change

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
8: Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/8)	KeyEvent
134: Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
152: Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Organization

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The hippocampus is generally similar in structure function across most mammalian species (West, 1990). The vast majority of information on the structure of the hippocampus is from mice, rats and primates including humans.

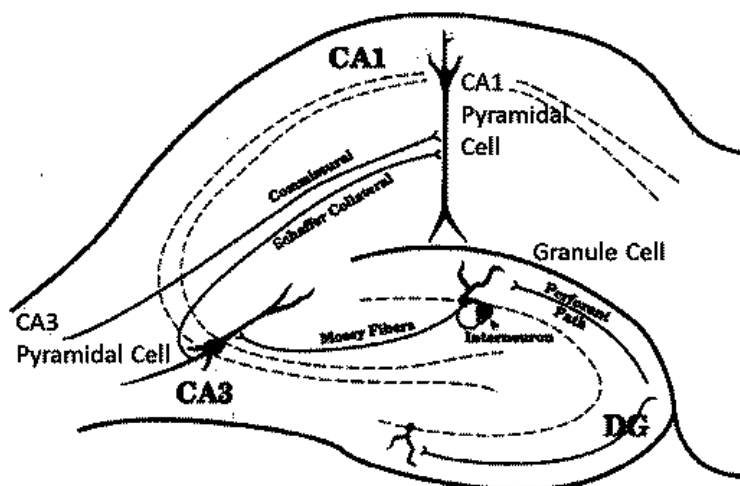
How this Key Event Works

The hippocampus is a major brain region located in the medial temporal lobe in humans and other mammals (West, 1990). Developmentally it is derived from neuronal and glial cells in the neural tube and differentiates in the proencephalon and telencephalon. The hippocampus is a cortical structure, but only contains 3-layers, distinct from the 6-layered neocortical structures. For this reason, it is known as archicortex or paleocortex meaning old cortex. Within humans, the structure is identified as early as fetal week 13 and matures rapidly until 2 to 3 years of age (Kier et al 1997), with continuing slow growth thereafter until adult ages (Utsunomiya et al., 1999). In rodents, the hippocampus begins to form in midgestation, with the CA fields forming in advance of the dentate gyrus. Dentate gyrus forms in late gestation with most of its development occurring in the first 2-3 postnatal weeks (Altman and Bayer, 1990a; 1990b).

The structure of the hippocampus has been divided into regions that include CA1 through CA4 and the dentate gyrus. The principal cell bodies of the CA field are pyramidal neurons, those of the dentate gyrus are granule cells. The dentate gyrus forms later in development than the CA fields of the hippocampus. These regions are generally found in all mammalian hippocampi.

The major input pathway to the hippocampus is from the layer 2 neurons of the entorhinal cortex to the dentate gyrus via the perforant path forming the first connection of the trisynaptic loop of the hippocampal circuit. Direct afferents from the dentate gyrus (mossy fibers) then synapse on CA3 pyramidal cells which in turn send their axons (Schaeffer Collaterals) to CA1 neurons to complete the trisynaptic circuit (Figure 1). From the CA fields information then passes through the subiculum entering the fiber pathways of the alveus, fimbria, and fornix and it routed to other areas of the brain (Amaral and Lavenex, 2006). Through the interconnectivity within the hippocampus and its connections to amygdala, septum and cortex, the hippocampus plays a pivotal role in several learning and memory processes, including spatial behaviors. The primary input pathway to the CA regions of the hippocampus is from the septum by way of the fornix and direct input from the amygdala. Reciprocal outputs from the hippocampus back to these regions and beyond also exist.

Trisynaptic Hippocampal Circuitry



How it is Measured or Detected

Data in support of this key event have been collected using a wide variety of standard biochemical, histological and anatomical methods (e.g., morphometrics, immunohistochemical staining, in situ hybridization and imaging procedures). Many of methods applied to reveal anatomical abnormalities are routine neurohistopathology procedures similar to those recommended in EPA and OECD developmental neurotoxicity guidelines (US EPA, 1998; OECD, 2007). Subtle cytoarchitectural features depend on more specialized birth dating procedures and staining techniques. It is essential to consider the timing of events during development for detection to occur, as well as the timing for detection (Hevner, 2007; Garman et al., 2001; Zraggen et al., 2012). Similar techniques used in rodent studies have been applied to postmortem tissue in humans.

In humans, structural neuroimaging techniques are used to assess hippocampal volume with an analysis technique known as voxel-based morphometry (VBM). Volume of brain regions is measured by drawing regions of interest (ROIs) on images from brain scans obtained from magnetic resonance imaging (MRI) or positron emission tomography (PET) scans and calculating the volume enclosed. (Mechelli et al., 2005). Similar imaging techniques can be applied in rodent models (Powell et al., 2009; Hasegawa et al., 2010; Pirko et al., 2005; Pirko and Johnson, 2008).

References

- Altman J, Bayer SA. Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J Comp Neurol*. 1990a Nov 15;301(3):365-81.
- Altman J, Bayer SA. Prolonged sojourn of developing pyramidal cells in the intermediate zone of the hippocampus and their settling in the stratum pyramidale. *J Comp Neurol*. 1990b Nov 15;301(3):343-64.
- Amaral D, Lavenex P (2006). "Ch 3. Hippocampal Neuroanatomy". In Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J. *The Hippocampus Book*. Oxford University Press. ISBN 978-0-19-510027-3.
- Garman RH, Fix AS, Jortner BS, Jensen KF, Hardisty JF, Claudio L, Ferenc S. Methods to identify and characterize developmental neurotoxicity for human health risk assessment. II: neuropathology. *Environ Health Perspect*. 2001 Mar;109 Suppl 1:93-100.
- Hasegawa M, Kida I, Wada H. A volumetric analysis of the brain and hippocampus of rats rendered perinatal hypothyroid. *Neurosci Lett*. 2010 Aug 2;479(3):240-4.
- Hevner RF. Layer-specific markers as probes for neuron type identity in human neocortex and malformations of cortical development. *J Neuropathol Exp Neurol*. 2007 66(2):101-9.
- Kier, EL, Kim, JH, Fulbright, K, Bronen, RA. Embryology of the human fetal hippocampus: MR imaging, anatomy, and histology. *AJNR Am J Neuroradiol*. 1997, 18(3):525-32.
- Mechelli A, Price C, Friston K, Ashburner J (2005) Voxel-Based Morphometry of the Human Brain: Methods and Applications. *Curr Med Imaging Rev* 1:105-113.
- OECD. 2007. OECD guidelines for the testing of chemicals/ section 4: Health effects. Test no. 426: Developmental neurotoxicity study. <http://www.oecd.org/dataoecd/20/52/37622194>.
- Powell MH, Nguyen HV, Gilbert M, Parekh M, Colon-Perez LM, Mareci TH, Montie E. Magnetic resonance imaging and volumetric analysis: novel tools to study the effects of thyroid hormone disruption on white matter development. *Neurotoxicology*. 2012 Oct;33(5):1322-9.
- Pirko I, Fricke ST, Johnson AJ, Rodriguez M, Macura SI. Magnetic resonance imaging, microscopy, and spectroscopy of the central nervous system in experimental animals. *NeuroRx*. 2005 Apr;2(2):250-64.
- Pirko I, Johnson AJ. Neuroimaging of demyelination and remyelination models.

Curr Top Microbiol Immunol. 2008; 318:241-66.

U.S.EPA. 1998. Health effects guidelines OPPTS 870.6300 developmental neurotoxicity study. EPA Document 712-C-98-239. Office of Prevention Pesticides and Toxic Substances.

Utsunomiya, H., K Takano, M Okazaki, A Mitsudome Development of the temporal lobe in infants and children: analysis by MR-based volumetry. AJNR Am J Neuroradiol: 1999, 20(4);717-23.

West MJ (1990). "Stereological studies of the hippocampus: a comparison of the hippocampal subdivisions of diverse species including hedgehogs, laboratory rodents, wild mice and men". Progress in Brain Research. Progress in Brain Research 83: 13–36.

Zraggen E, Boitard M, Roman I, Kanemitsu M, Potter G, Salmon P, Vutskits L, Dayer AG, Kiss JZ. Early postnatal migration and development of layer II pyramidal neurons in the rodent cingulate/retrosplenial cortex. Cereb Cortex. 2012 Jan;22(1):144-57.

758: Hippocampal Physiology, Altered (<https://aopwiki.org/events/758>)

Short Name: Hippocampal Physiology, Altered

Key Event Component

Process	Object	Action
chemical synaptic transmission		abnormal

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
8: Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/8)	KeyEvent
134: Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
152: Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent

Stressors

Name
Propylthiouracil
Iodine deficiency
Methimazole

Biological Organization

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Female	Strong
Male	Strong

The majority of evidence for this key event come from work in rodent species (i.e., rat, mouse). There is a moderate amount of evidence from other species, including humans (Clapp et al., 2012).

How this Key Event Works

The hippocampus functions as a highly integrated and organized communication and information processing network with millions of interconnections among its constitutive neurons. Neurons in the hippocampus and throughout the brain transmit and receive information largely through chemical transmission across the synaptic cleft, the space where the specialized ending of the presynaptic axon terminus of the transmitting neuron meets the specialized postsynaptic region of the neuron that is receiving that information (Kandell et al., 2012).

During development (see KE: Hippocampal anatomy, Altered), as neurons reach their final destination and extend axonal processes, early patterns of electrical synaptic activity emerge in the hippocampus. These are large fields of axonal innervation of broad synaptic target sites that are replaced by more elaborate but highly targeted and refined axonal projections brought about by activity-dependent synaptic pruning and synapse elimination. This is a classic case of the interaction between physiological and anatomical development, where anatomy develops first, and can be 'reshaped' by physiological function (Kutsarova et al., 2017).

In the rat, excitatory processes are fully mature in area CA1 of hippocampus within 2 weeks of birth with inhibitory processes lagging begin by several weeks (Muller et al., 1989; Michelson and Lothman, 1988; Harris and Teyler, 1984). In hippocampal slices, inhibitory function in area CA1s is first seen on postnatal day 5 and increases in strength at postnatal day 12 through 15. In vivo studies fail to detect inhibition until postnatal day 18 with steady increase thereafter to adult levels by postnatal day 28. Synaptic plasticity in the form of long-term potentiation (LTP) is absent in the very young animal, only emerging about postnatal day 14, appearing to require the stability of both excitatory and inhibitory function to be established (Muller et al., 1989; Bekenstein and Lothman, 1991). These features of the maturation of hippocampal physiology are paralleled in dentate gyrus, but as with anatomical indices in the rat, the development of these physiological parameters lag behind the CA1 by about 1 week.

How it is Measured or Detected

In animals, synaptic function in the hippocampus has been examined with imaging techniques, but more routinely, electrical field potentials recorded in two subregions of the hippocampus, area CA1 and dentate gyrus, have been assessed in vivo or in vitro from slices taken from naive or exposed animals. Field potentials reflect the summed synaptic response of a population of neurons following direct stimulation of input pathways across a monosynaptic connection. Changes in response amplitude due to chemical perturbations and other stressors (e.g., iodine deficiency, thyroidectomy, gene knockouts) is evidence of altered synaptic function. This can be measured in vitro, in vivo, or in hippocampal slices taken from treated animals (Gilbert and Burdette, 1995). The most common physiological measurements used to assess function of the hippocampus are excitatory synaptic transmission, inhibitory synaptic transmission, and synaptic plasticity in the form of long-term potentiation (LTP).

Excitatory Synaptic Transmission: Two measures, the excitatory postsynaptic potential (EPSP) and the population spike are derived from the compound field potential at increasing stimulus strengths. The function described by the relationship of current strength (input, I) and evoked response (output, O), the I-O curve is the measure of excitatory synaptic transmission (Gilbert and Burdette, 1995).

Inhibitory Synaptic Transmission: Pairs of stimulus pulses delivered in close temporal proximity is used to probe the integrity of inhibitory synaptic transmission. The response evoked by the second pulse of the pair at brief intervals (<30 msec) arrives during the activation of feedback inhibitory loops in the hippocampus. An alteration in the degree of suppression to the 2nd pulse of the pair reflects altered inhibitory synaptic

function (Gilbert and Burdette, 1995).

Long Term Potentiation (LTP): LTP is widely accepted to be a major component of the cellular processes that underlie learning and memory (Malenka and Bear, 2004; Bramham and Messaoudi, 2005). LTP represents, at the synapse and molecular level, the coincident firing of large numbers of neurons that are engaged during a learning event. The persistence of LTP emulates the duration of the memory. Synaptic plasticity in the form of LTP is assessed by delivering trains of high frequency stimulation to induce a prolonged augmentation of synaptic response. Probe stimuli at midrange stimulus strengths are delivered before and after application of LTP-inducing trains. The degree of increase in EPSP and PS amplitude to the probe stimulus after train application, and the duration of the induced synaptic enhancement are metrics of LTP. Additionally, contrasting I-O functions of excitatory synaptic transmission before and after (hours to days) LTP is induced is also a common measure of LTP maintenance (Bramham and Messaoudi, 2005; Kandell et al., 2012; Malenka and Bear, 2004).

Synaptic function in the human hippocampus has been assessed using electroencephalography (EEG) and functional neuroimaging techniques (Clapp et al., 2012). EEG is a measure of electrical activity over many brain regions but primarily from the cortex using small flat metal discs (electrodes) placed over the surface of the skull. It is a readily available test that provides evidence of how the brain functions over time. Functional magnetic resonance imaging or functional MRI (fMRI) uses MRI technology to measure brain activity by detecting associated changes in blood flow. This technique relies on the fact that cerebral blood flow and neuronal activation are coupled. Positron emission tomography (PET) is a functional imaging technique that detects pairs of gamma rays emitted indirectly by a radionuclide (tracer) injected into the body (Tietze, 2012; McCarthy, 1995). Like fMRI, PET scans indirectly measure blood flow to different parts of the brain – the higher the blood flow, the greater the activation (McCarthy, 1995). These techniques have been widely applied in clinical and research settings to assess learning and memory in humans and can provide information targeted to hippocampal functionality (McCarthy, 1995; Smith and Jonides, 1997; Willoughby et al., 2014; Wheeler et al., 2015; Gilbert et al., 1998).

Assays of this type are fit for purpose, have been well accepted in the literature, and are reproducible across laboratories. The assay directly measures the key event of altered neurophysiological function.

References

- Bekenstein JW, Lothman EW. An in vivo study of the ontogeny of long-term potentiation (LTP) in the CA1 region and in the dentate gyrus of the rat hippocampal formation. *Brain Res Dev Brain Res*. 1991 Nov 19;63(1-2):245-
- Bramham CR, Messaoudi E (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. *Prog Neurobiol* 76:99-125.
- Clapp WC, Hamm JP, Kirk IJ, Teyler TJ. Translating long-term potentiation from animals to humans: a novel method for noninvasive assessment of cortical plasticity. *Biol Psychiatry*. 2012 Mar 15;71(6):496-502.
- Gilbert, M.E. and Burdette, L.J. (1995). Hippocampal Field Potentials: A Model System to Characterize Neurotoxicity. In *Neurotoxicology: Approaches and Methods*. L.W Chang and W. Slikker (Eds). Academic Press:New York, 183-204.
- Gilbert ME, Mack CM. Chronic lead exposure accelerates decay of long-term potentiation in rat dentate gyrus in vivo. *Brain Res*. 1998 Apr 6;789(1):139-49.
- Harris KM, Teyler TJ. Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. *J Physiol*. 1984. 346:27-48.
- Kandell, E., Schwartz, J., Siegelbaum, A. and Hudspeth, A.J. (2012) *Principles of Neural Science*, 5th Edition. Elsevier, North Holland.
- Kutsarova E, Munz M, Ruthazer ES. Rules for Shaping Neural Connections in the Developing Brain. *Front Neural Circuits*. 2017 Jan 10;10:111. doi: 10.3389/fncir.2016.00111.
- Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. *Neuron* 44:5-21.
- McCarthy, G. (1995) Review: Functional Neuroimaging and Memory. *The Neuroscientist*, 1:155-163.
- Michelson HB, Lothman EW. An in vivo electrophysiological study of the ontogeny of excitatory and inhibitory processes in the rat hippocampus. *Brain Res Dev Brain Res*. 1989 May 1;47(1):113-22.
- Muller D, Oliver M, Lynch G. Developmental changes in synaptic properties in hippocampus of neonatal rats. *Brain Res Dev Brain Res*. 1989 Sep 1;49(1):105-14.
- Smith, E and Jonides, J. (1997). Working Memory: A View from Neuroimaging. *Cognitive Psychology*, 33:5-42.
- Tietze, KJ. (2012). Review of Laboratory and Diagnostic Tests- Positron Emission Tomography. In *Clinical Skills for Pharmacists*, 3rd Edition, pp 86-122.
- Wheeler SM, McLelland VC, Sheard E, McAndrews MP, Rovet JF (2015) Hippocampal Functioning and Verbal Associative Memory in Adolescents with Congenital Hypothyroidism. *Front Endocrinol (Lausanne)* 6:163.
- Willoughby KA, McAndrews MP, Rovet JF (2014) Effects of maternal hypothyroidism on offspring hippocampus and memory. *Thyroid* 24:576-584.

Adverse Outcomes

Title	Short name

AOP42

Title	Short name
Cognitive Function, Decreased (https://aopwiki.org/events/402)	Cognitive Function, Decreased

402: Cognitive Function, Decreased (<https://aopwiki.org/events/402>)

Short Name: Cognitive Function, Decreased

Key Event Component

Process	Object	Action
learning or memory		decreased
cognition		decreased

AOPs Including This Key Event

AOP ID and Name	Event Type
42: Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	AdverseOutcome
65: XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	AdverseOutcome
134: Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	AdverseOutcome
152: Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	AdverseOutcome

Stressors

Name
Methimazole
Propylthiouracil
Iodine deficiency

Biological Organization

Level of Biological Organization
Individual

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Basic forms of learning behavior such as habituation have been found in many taxa from worms to humans (Alexander, 1990). More complex cognitive processes such as executive function likely reside only in higher mammalian species such as non-human primates and humans.

How this Key Event Works

Learning and memory depend upon the coordinated action of different brain regions and neurotransmitter systems constituting functionally integrated neural networks (D'Hooge and DeDeyn, 2001). Among the many brain areas engaged in the acquisition of, or retrieval of, a learned event, the hippocampal-based memory systems have received the most study. The main learning areas and pathways are similar in rodents and primates, including man (Eichenbaum, 2000; Stanton and Spear, 1990; Squire, 2004).

In humans, the hippocampus is involved in recollection of an event's rich spatial-temporal contexts and distinguished from simple semantic memory which is memory of a list of facts (Burgess et al., 2000). Hemispheric specialization has occurred in humans, with the left hippocampus specializing in verbal and narrative memories (i.e., context-dependent episodic or autobiographical memory) and the right hippocampus, more prominently engaged in visuo-spatial memory (i.e., memory for locations within an environment). The hippocampus is particularly critical for the formation of episodic memory, and autobiographical memory tasks have been developed to specifically probe these functions (Eichenbaum, 2000; Willoughby et al., 2014). In rodents, there is obviously no verbal component in hippocampal memory, but reliance on the hippocampus for spatial, temporal and contextual memory function has been well documented. Spatial memory deficits and fear-based context learning paradigms engage the hippocampus, amygdala, and prefrontal cortex (Eichenbaum, 2000; Shors et al., 2001; Samuels et al., 2011; Vorhees and Williams, 2014; D'Hooge and DeDeyn, 2001; Lynch, 2004; O'Keefe and Nadal, 1978). These tasks are impaired in animals with hippocampal dysfunction (O'Keefe and Nadal, 1978; Morris and Frey, 1987; Gilbert et al., 2016).

How it is Measured or Detected

In rodents, a variety of tests of learning and memory have been used to probe the integrity of hippocampal function. These include tests of spatial learning like the radial arm maze (RAM), the Barnes maze, and most commonly, the Morris water maze (MWM). Test of novelty such as novel object recognition, and fear based context learning are also sensitive to hippocampal disruption. Finally, trace fear conditioning which incorporates a temporal component upon traditional amygdala-based fear learning engages the hippocampus. A brief description of these tasks follows.

- 1) RAM, Barnes, MWM are examples of spatial tasks in which animals are required to learn: the location of a food reward (RAM); an escape hole to enter a preferred dark tunnel from a brightly lit open field area (Barnes maze); or a hidden platform submerged below the surface of the water in a large tank of water (MWM) (Vorhees and Williams, 2014).
- 2) Novel Object recognition. This is a simpler task that can be used to probe recognition memory. Two objects are presented to animal in an open field on trial 1, and these are explored. On trial 2, one object is replaced with a novel object and time spent interacting with the novel object is taken evidence of memory retention (i.e., I have seen one of these objects before, but not this one. Cohen and Stackman, 2015).
- 3) Contextual Fear conditioning is a hippocampal based learning task in which animals are placed in a novel environment and allowed to explore for several minutes before delivery of an aversive stimulus, typically a mild foot shock. Upon reintroduction to this same environment in the future (typically 24-48 hours after original training), animals will limit their exploration, the context of this chamber being associated with an aversive event. The degree of suppression of activity after training is taken as evidence of retention, i.e., memory (Curzon et al., 2009).
- 4) Trace fear conditioning. Standard fear conditioning paradigms require animals to make an association between a neutral conditioning stimulus (CS, a light or a tone) and an aversive stimulus (US, a footshock). The unconditioned response (CR) that is elicited upon delivery of the footshock US is freezing behavior. With repetition of CS/US delivery, the previously neutral stimulus comes to elicit the freezing response. This type of learning is dependent on the amygdala, a brain region associated with, but distinct from the hippocampus. Introducing a brief delay between presentation of the neutral CS and the aversive US, a trace period, requires the engagement of the amygdala and the hippocampus (Shors et al., 2004).

Most methods are well established in the published literature and many have been engaged to evaluate the effects of developmental thyroid disruption. The US EPA and OECD Developmental Neurotoxicity (DNT) Guidelines (OCSP 870.6300 or OECD 426) both require testing of learning and memory (USEPA, 1998; OECD, 2007). These DNT Guidelines have been deemed valid to identify developmental neurotoxicity and adverse neurodevelopmental outcomes (Makris et al., 2009).

A variety of standardized learning and memory tests have been developed for human neuropsychological testing. These include episodic autobiographical memory, word pair recognition memory; object location recognition memory. Some components of these tests have been incorporated in general tests of adult intelligence (IQ) such as the WAIS and the Wechsler. Modifications have been made and norms developed

for incorporating of tests of learning and memory in children. Examples of some of these tests include:

- 1) Rey Osterieth Complex Figure (RCFT) which probes a variety of functions including as visuospatial abilities, memory, attention, planning, and working memory (Shin et al., 2006).
- 2) Children's Auditory Verbal Learning Test (CAVLT) is a free recall of presented word lists that yields measures of Immediate Memory Span, Level of Learning, Immediate Recall, Delayed Recall, Recognition Accuracy, and Total Intrusions. (Lezak 1995; Talley, 1986).
- 3) Continuous Visual Memory Test (CVMT) measures visual learning and memory. It is a free recall of presented pictures/objects rather than words but that yields similar measures of Immediate Memory Span, Level of Learning, Immediate Recall, Delayed Recall, Recognition Accuracy, and Total Intrusions. (Lezak, 1984; 1994).
- 4) Story Recall from Wechsler Memory Scale (WMS) Logical Memory Test Battery, a standardized neuropsychological test designed to measure memory functions (Lezak, 1994; Talley, 1986).
- 5) Autobiographical memory (AM) is the recollection of specific personal events in a multifaceted higher order cognitive process. It includes episodic memory- remembering of past events specific in time and place, in contrast to semantic autobiographical memory is the recollection of personal facts, traits, and general knowledge. Episodic AM is associated with greater activation of the hippocampus and a later and more gradual developmental trajectory. Absence of episodic memory in early life (infantile amnesia) is thought to reflect immature hippocampal function (Herold et al., 2015; Fivush, 2015).
- 6) Staged Autobiographical Memory Task. In this version of the AM test, children participate in a staged event involving a tour of the hospital, perform a series of tasks (counting footprints in the hall, identifying objects in wall display, buy lunch, watched a video). It is designed to contain unique event happenings, place, time, visual/sensory/perceptual details. Four to five months later, interviews are conducted using Children's Autobiographical Interview and scored according to standardized scheme (Willoughby et al., 2014).

Regulatory Examples Using This Adverse Outcome

A prime example of impairments in cognitive function as the adverse outcome for regulatory action is developmental lead exposure and IQ function in children (Bellinger, 2012). In addition, testing for the impact of chemical exposures on cognitive function, often including spatially-mediated behaviors, is an integral part of both EPA and OECD developmental neurotoxicity guidelines (USEPA, 1998; OECD, 2007).

References

- Alexander RD (1990) Epigenetic rules and Darwinian algorithms: The adaptive study of learning and development. *Ethology and Sociobiology* 11:241-303.
- Bellinger DC (2012) A strategy for comparing the contributions of environmental chemicals and other risk factors to neurodevelopment of children. *Environ Health Perspect* 120:501-507.
- Burgess N (2002) The hippocampus, space, and viewpoints in episodic memory. *Q J Exp Psychol A* 55:1057-1080.
- Cohen, SJ and Stackman, RW. (2015). Assessing rodent hippocampal involvement in the novel object recognition task. A review. *Behav. Brain Res.* 285: 105-1176.
- Curzon P, Rustay NR, Browman KE. Cued and Contextual Fear Conditioning for Rodents. In: Buccafusco JJ, editor. *Methods of Behavior Analysis in Neuroscience*. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2009
- D'Hooge R, De Deyn PP (2001) Applications of the Morris water maze in the study of learning and memory. *Brain Res Brain Res Rev* 36:60-90.
- Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. *Nat Rev Neurosci* 1:41-50.
- Fivush R. The development of autobiographical memory. *Annu Rev Psychol.* 2011. 62:559-82.
- Gilbert ME, Sanchez-Huerta K, Wood C (2016) Mild Thyroid Hormone Insufficiency During Development Compromises Activity-Dependent Neuroplasticity in the Hippocampus of Adult Male Rats. *Endocrinology* 157:774-787.
- Gilbert ME, Sui L (2006) Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. *Brain Res* 1069:10-22.
- Herold, C, Lässer, MM, Schmid, LA, Seidl, U, Kong, L, Fellhauer, I, Thomann, PA, Essig, M and Schröder, J. (2015). Neuropsychology, Autobiographical Memory, and Hippocampal Volume in "Younger" and "Older" Patients with Chronic Schizophrenia. *Front. Psychiatry*, 6: 53.
- Lezak MD (1984) Neuropsychological assessment in behavioral toxicology--developing techniques and interpretative issues. *Scand J Work Environ Health* 10 Suppl 1:25-29.
- Lezak MD (1994) Domains of behavior from a neuropsychological perspective: the whole story. *Nebr Symp Motiv* 41:23-55.
- Lynch, M.A. (2004). Long-Term Potentiation and Memory. *Physiological Reviews*. 84:87-136.
- Makris SL, Raffaele K, Allen S, Bowers WJ, Hass U, Alleva E, Calamandrei G, Sheets L, Amcoff P, Delrue N, Crofton KM. A retrospective performance assessment of the developmental neurotoxicity study in support of OECD test guideline 426. *Environ Health Perspect.* 2009 Jan;117(1):17-25.
- Morris RG, Frey U. Hippocampal synaptic plasticity: role in spatial learning or the automatic recording of attended experience? *Philos Trans R Soc Lond B Biol Sci.* 1997 Oct 29;352(1360):1489-503. Review
- O'Keefe, J. and Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. Oxford: Oxford University Press.

OECD. 2007. OECD guidelines for the testing of chemicals/ section 4: Health effects. Test no. 426: Developmental neurotoxicity study. www.Oecd.Org/dataoecd/20/52/37622194.Pdf [accessed may 21, 2012].

Samuels BA, Hen R (2011) Neurogenesis and affective disorders. *Eur J Neurosci* 33:1152-1159.

Shin, MS, Park, SY, Park, SR, Oeol, SH and Kwon, JS. (2006). Clinical and empirical applications to the Rey-Osterrieth complex figure test. *Nature Protocols*, 1: 892-899.

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 410:372-376. Squire LR (2004) Memory systems of the brain: a brief history and current perspective. *Neurobiol Learn Mem* 82:171-177.

Stanton ME, Spear LP (1990) Workshop on the qualitative and quantitative comparability of human and animal developmental neurotoxicity, Work Group I report: comparability of measures of developmental neurotoxicity in humans and laboratory animals. *Neurotoxicol Teratol* 12:261-267.

Talley, JL. (1986). Memory in learning disabled children: Digit span and eh Rey Auditory verbal learning test. *Archives of Clinical Neuropsychology*, Elsevier.

U.S.EPA. 1998. Health effects guidelines OPPTS 870.6300 developmental neurotoxicity study. EPA Document 712-C-98-239. Office of Prevention Pesticides and Toxic Substances.

Vorhees CV, Williams MT (2014) Assessing spatial learning and memory in rodents. *ILAR J* 55:310-332.

Willoughby KA, McAndrews MP, Rovet JF. Accuracy of episodic autobiographical memory in children with early thyroid hormone deficiency using a staged event. *Dev Cogn Neurosci*. 2014. 9:1-11.

Scientific evidence supporting the linkages in the AOP

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Thyropoxidase, Inhibition	directly leads to	Thyroid hormone synthesis, Decreased	Strong	Weak
Thyroid hormone synthesis, Decreased	directly leads to	Thyroxine (T4) in serum, Decreased	Strong	Moderate
Thyropoxidase, Inhibition	indirectly leads to	Thyroxine (T4) in serum, Decreased	Strong	Moderate
Thyroxine (T4) in serum, Decreased	directly leads to	Thyroxine (T4) in neuronal tissue, Decreased	Moderate	Weak
Thyroxine (T4) in neuronal tissue, Decreased	directly leads to	Hippocampal gene expression, Altered	Moderate	Weak
Hippocampal gene expression, Altered	directly leads to	Hippocampal anatomy, Altered	Moderate	Weak
Hippocampal anatomy, Altered	directly leads to	Hippocampal Physiology, Altered	Moderate	Weak
Hippocampal Physiology, Altered	directly leads to	Cognitive Function, Decreased	Strong	Moderate
Thyroxine (T4) in serum, Decreased	indirectly leads to	Hippocampal gene expression, Altered	Strong	Weak
Thyroxine (T4) in serum, Decreased	indirectly leads to	Hippocampal anatomy, Altered	Strong	Weak
Thyroxine (T4) in serum, Decreased	indirectly leads to	Hippocampal Physiology, Altered	Moderate	Weak
Thyroxine (T4) in serum, Decreased	indirectly leads to	Cognitive Function, Decreased	Strong	Moderate

Thyropoxidase, Inhibition leads to TH synthesis, Decreased (<https://aopwiki.org/relationships/309>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	directly leads to	Strong	Weak
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	directly leads to		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
Xenopus laevis	Xenopus laevis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Inhibition of TPO activity is widely accepted to directly impact TH synthesis. This is true for both rats and humans, as well as some fishes, frogs and birds. Most of the data supporting a causative relationship between TPO inhibition and altered TH synthesis is derived from animal studies, in vitro thyroid microsomes from rats or pigs, and a limited number of human ex vivo (Nagasaka and Hidaka, 1976; Vickers et al., 2012) and clinical studies. There are data to support that gene mutations in TPO result in congenital hypothyroidism, underscoring the essential role of TPO in human thyroid hormone synthesis.

How Does This Key Event Relationship Work

Thyroperoxidase (TPO) is a heme-containing apical membrane protein within the follicular lumen of thyrocytes that acts as the enzymatic catalyst for thyroid hormone (TH) synthesis (Taurog, 2005). Two commonly used reference chemicals, propylthiouracil (PTU) and methimazole (MMI), are thiourene drugs that inhibit the ability of TPO to: a) activate iodine and transfer it to thyroglobulin (Tg) (Davidson et al., 1978); and, b) couple thyroglobulin (Tg)-bound iodotyrosyls to produce Tg-bound thyroxine (T4) and triiodothyronine (T3) (Taurog, 2005).

Weight of Evidence

The weight of evidence supporting a direct linkage between the MIE, TPO inhibition, and the KE of decreased TH synthesis, is strong and supported by more than three decades of research in animals, including humans (Cooper et al., 1982; Cooper et al., 1983; Divi and Doerge, 1994).

Biological Plausibility

The biological plausibility for this KER is rated Strong. TPO is the only enzyme capable of de novo synthesis of TH. TPO catalyzes several reactions, including the oxidation of iodide, nonspecific iodination of tyrosyl residues of thyroglobulin (Tg) to form moniodotyrosyl (MIT) or diiodotyrosyl (DIT) residues, and the coupling of these Tg-bound iodotyrosyls to produce Tg-bound T3 and T4 (Divi and Doerge, 1994; Kessler et al., 2008; Ruf et al., 2006; Taurog et al., 1996, 2005). Therefore, inhibition of TPO activity is widely accepted to directly impact TH synthesis.

Empirical Support for Linkage

Empirical support for this KER is strong. There are several papers that have measured alterations in TPO and subsequent effects on TH synthesis. Taurog et al. (1996) showed decreased guaiacol activity, decreased bound I^{125} , and subsequent decreases in newly formed T3 and T4 per molecule of Tg, following exposure to PTU, MMI and some antibiotics. Following in vivo exposure to PTU in rats (Cooper et al., 1982; 1983), there are concentration and time-dependent decreases in thyroid protein bound iodine and serum T4 and T3 that recovered one month after cessation of PTU exposure. In addition, measures of thyroidal iodine content were highly correlated with intra-thyroidal PTU concentration. Vickers et al. (2012) demonstrated dose- and time- dependent inhibition of TPO activity in both human and rat thyroid homogenates exposed to

MMI. Tietge et al (2010) recently showed decreases in thyroidal T4 following MMI exposure in *Xenopus*. Doerge et al (1998) showed that a tryphenylmethane dye, malachite green, inhibited TPO and lowered thyroxine production. A recent paper used a series of benzothiazoles and showed TPO inhibition (guaiacol assay) and inhibition of TSH stimulated thyroxine release from *Xenopus* thyroid gland explant cultures (Hornung et al., 2015).

Temporal Evidence: The temporal nature of this KER is applicable to all life stages, including development (Seed et al., 2005). The impact of decreased TPO activity on thyroidal hormone synthesis is similar across all ages. Good evidence for the temporal relationship of the KER comes from thyroid system modeling (e.g., Degon et al., 2008; Fisher et al., 2013). In addition, there is ample evidence of the temporal impacts of TPO inhibition on TH synthesis, using ex vivo and in vitro measures that demonstrate the time course of inhibition following chemical exposures, including some data from human thyroid microsomes and ex vivo thyroid slices (Vickers et al., 2012).

Future work is needed that measures both TPO inhibition and TH production during development.

Dose-Response Evidence: Dose-response data is available from a number of studies that correlate TPO inhibition with decreased TH production measured using a variety of endpoints including iodine organification (e.g., Taurog et al., 1996), inhibition of guaiacol oxidation in thyroid microsomes (e.g., Doerge and Chang, 2002), and direct measure of thyroid gland T4 concentrations (e.g., Hornung et al., 2015). However, there is a lack of dose-response data from developmental studies showing direct linkages from TPO inhibition to thyroidal TH synthesis.

Uncertainties or Inconsistencies

While it is clear that TPO inhibition will lead to altered hormone synthesis, there is a need for data that will inform quantitative modeling of the relationship between TPO inhibition and the magnitude of effects on thyroid hormone synthesis.

Quantitative Understanding of the Linkage

There are only a limited number of studies where both TPO inhibition and iodine organification have been measured in vivo, and there are not enough data available to make any definitive quantitative correlations. One in vivo study in rats exposed to the TPO inhibitor genistein found no in vivo impact on serum thyroid hormone concentrations, even when TPO was inhibited up to 80% (Chang and Doerge, 2000).

References

- Chang HC, Doerge DR. Dietary genistein inactivates rat thyroid peroxidase in vivo without an apparent hypothyroid effect. *Toxicol Appl Pharmacol* 168:244–252 (2000).
- Cooper DS, Kieffer JD, Halpern R, Saxe V, Mover H, Maloof F, Ridgway EC (1983) Propylthiouracil (PTU) pharmacology in the rat. II. Effects of PTU on thyroid function. *Endocrinology* 113:921-928.
- Cooper DS, Saxe VC, Meskell M, Maloof F, Ridgway EC. Acute effects of propylthiouracil (PTU) on thyroidal iodide organification and peripheral iodothyronine deiodination: correlation with serum PTU levels measured by radioimmunoassay. *J Clin Endocrinol Metab*. 1982 54(1):101-7.
- Davidson, B., Soodak, M., Neary, J.T., Strout, H.V., and Kieffer, J.D. (1978). The irreversible inactivation of thyroid peroxidase by methylmercaptimidazole, thiouracil, and propylthiouracil in vitro and its relationship to in vivo findings. *Endocrinology* 103:871–882.
- Divi, R. L., and Doerge, D. R. (1994). Mechanism-based inactivation of lactoperoxidase and thyroid peroxidase by resorcinol derivatives. *Biochemistry* 33(32), 9668-74.
- Doerge DR, Chang HC, Divi RL, Churchwell Mechanism for inhibition of thyroid peroxidase by leucomalachite green. *Chem Res Toxicol*. 1998 11(9):1098-104.
- Doerge DR, Chang HC. Inactivation of thyroid peroxidase by soy isoflavones, in vitro and in vivo. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2002 Sep 25;777(1-2):269-79
- Hornung MW, Kosian PA, Haselman JT, Korte JJ, Challis K, Macherla C, Nevalainen E, Degitz SJ. In Vitro, Ex Vivo, and In Vivo Determination of Thyroid Hormone Modulating Activity of Benzothiazoles. *Toxicol Sci*. 2015 146(2):254-64.
- Kessler, J., Obinger, C., and Eales, G. (2008). Factors influencing the study of peroxidase-generated iodine species and implications for thyroglobulin synthesis. *Thyroid* 18(7), 769-74, 10.1089/thy.2007.0310.
- Nagasaka, A., and Hidaka, H. (1976). Effect of antithyroid agents 6-propyl-2-thiouracil and 1-methyl-2-mercaptimidazole on human thyroid iodine peroxidase. *J. Clin. Endocrinol. Metab*. 43:152–158.
- Ruf, J., and Carayon, P. (2006). Structural and functional aspects of thyroid peroxidase. *Archives of biochemistry and biophysics* 445(2), 269-77, 10.1016/j.abb.2005.06.023.
- Taurog, A., Dorris, M. L., and Doerge, D. R. (1996). Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. *Archives of biochemistry and biophysics* 330(1), 24-32,
- Taurog A. 2005. Hormone synthesis. In: Werner and Ingbar's *The Thyroid: A Fundamental and Clinical Text* (Braverman LE, Utiger RD, eds). Philadelphia:Lippincott, Williams and Wilkins, 47–81.
- Tietge JE, Butterworth BC, Haselman JT, Holcombe GW, Hornung MW, Korte JJ, Kosian PA, Wolfe M, Degitz SJ. Early temporal effects of three thyroid hormone synthesis inhibitors in *Xenopus laevis*. *Aquat Toxicol*. 2010 98(1):44-50
- Vickers AE, Heale J, Sinclair JR, Morris S, Rowe JM, Fisher RL. Thyroid organotypic rat and human cultures used to investigate drug effects on thyroid function, hormone synthesis and release pathways. *Toxicol Appl Pharmacol*. 2012 260(1):81-8.

TH synthesis, Decreased leads to T4 in serum, Decreased (<https://aopwiki.org/relationships/305>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	directly leads to	Strong	Moderate
XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	directly leads to	Strong	Moderate
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	directly leads to	Strong	Strong
Inhibition of Na ⁺ /I ⁻ symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	directly leads to	Strong	Strong
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	directly leads to		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
Xenopus laevis	Xenopus laevis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

While a majority of the empirical evidence comes from work with laboratory rodents, there is a large amount of supporting data from humans (with anti-hyperthyroidism drugs including propylthiouracil and methimazole), some amphibian species (e.g., frog), and some avian species (e.g., chicken). The following are samples from a large literature that supports this concept: Cooper et al. (1982; 1983); Hornung et al. (2010); Van Herck et al. (2013); Paul et al. (2013); Alexander et al. (2017).

How Does This Key Event Relationship Work

Thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3), are synthesized by TPO in the thyroid gland as iodinated thyroglobulin (Tg) and stored in the colloid of thyroid follicles. Secretion from the follicle into serum is a multi-step process. The first involves thyroid stimulating hormone (TSH) stimulation of the separation of the peptide linkage between Tg and TH. The next steps involve endocytosis of colloid, fusion of the endosome with the basolateral membrane of the thyrocyte, and finally release of TH into blood. More detailed descriptions of this process can be found in reviews by Braverman and Utiger (2012) and Zoeller et al. (2007).

Weight of Evidence

The weight of evidence linking these two KEs of decreased TH synthesis and decreased T4 in serum is strong. It is commonly accepted dogma that decreased synthesis in the thyroid gland will result in decreased circulating TH.

Biological Plausibility

The biological relationship between two KEs in this KER is well understood and documented fact within the scientific community.

Empirical Support for Linkage

It is widely accepted that TPO inhibition leads to declines in serum T4 levels in adult mammals. This is due to the fact that the sole source for circulating T4 derives from hormone synthesis in the thyroid gland. Indeed, it has been known for decades that insufficient dietary iodine will lead to decreased serum TH concentrations due to inadequate synthesis. Strong qualitative and quantitative relationships exist between reduced TH synthesis and reduced serum T4 (Ekerot et al., 2013; Degon et al., 2008; Cooper et al., 1982; 1983; Leonard et al., 2016; Zoeller and Tan, 2007). There is more limited evidence supporting the relationship between decreased TH synthesis and lowered circulating hormone levels during development. Lu and Anderson (1994) followed the time course of TH synthesis, measured as thyroxine secretion rate, in non-treated pregnant rats and correlated it with serum T4 levels. More recently, modeling of TH in the rat fetus demonstrates the quantitative relationship between TH synthesis and serum T4 concentrations (Hassan et al., 2017). Furthermore, a wide variety of drugs and chemicals that inhibit TPO are known to result in decreased release of TH from the thyroid gland, as well as decreased circulating TH concentrations. This is evidenced by a very large number of studies that employed a wide variety of techniques, including thyroid gland explant cultures, tracing organification of ¹³¹I and *in vivo* treatment of a variety of animal species with known TPO inhibitors (King and May, 1984; Atterwill et al., 1990; Brown et al., 1986; Brucker-Davis, 1998; Hornung et al., 2010; Hurley et al., 1998; Kohrle, 2008).

Temporal Evidence: The temporal nature of this KER is applicable to all life stages, including development (Seed et al., 2005). There are currently no studies that measured both TPO synthesis and TH production during development. However, the impact decreased TH synthesis on serum hormones is similar across all ages. Good evidence for the temporal relationship comes from thyroid system modeling (e.g., Degon et al., 2008; Fisher et al., 2013). In addition, recovery experiments have demonstrated that serum thyroid hormones recovered in athyroid mice following grafting of *in-vitro* derived follicles (Antonica et al., 2012).

Dose-response Evidence: Dose-response data is lacking from studies that include concurrent measures of both TH synthesis and serum TH concentrations. However, data is available demonstrating correlations between thyroidal TH and serum TH concentrations during gestation and lactation during development (Gilbert et al., 2013). This data was used to develop a rat quantitative biologically-based dose-response model for iodine deficiency (Fisher et al., 2013).

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. The first uncertainty stems from the paucity of data for quantitative modeling of the relationship between the degree of synthesis decrease and resulting changes in circulating T4 concentrations. In addition, most of the data supporting this KER comes from inhibition of TPO or NIS, and there are a number of other processes (e.g., endocytosis, lysosomal fusion, basolateral fusion and release) that are not as well studied.

Quantitative Understanding of the Linkage

Fisher et al. (2013) published a quantitative biologically-based dose-response model for iodine deficiency in the rat. This model provides quantitative relationships for thyroidal T4 synthesis (iodine organification) and predictions of serum T4 concentrations in developing rats. There are other computational models that include thyroid hormone synthesis. Ekerot et al. (2012) modeled TPO, T3, T4 and TSH in dogs and humans based on exposure to myeloperoxidase inhibitors that also inhibit TPO. This model was recently adapted for rat (Leonard et al., 2016) and Hassan et al (2017) have extended it to include the pregnant rat dam in response to TPO inhibition induced by PTU. While the original model predicted serum TH and TSH levels as a function of oral dose, it was not used to explicitly predict the relationship between serum hormones and TPO inhibition, or thyroidal hormone synthesis. Leonard et al. (2016) recently incorporated TPO inhibition into the model. Degon et al (2008) developed a human thyroid model that includes TPO, but does not make quantitative prediction of organification changes due to inhibition of the TPO enzyme.

References

- Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, Peeters RP, Sullivan S. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. *Thyroid*. 2017 Mar;27(3):315-389.
- Antonica F, Kasprzyk DF, Opitz R, Iacovino M, Liao XH, Dumitrescu AM, Refetoff S, Peremans K, Manto M, Kyba M, Costagliola S. Generation of functional thyroid from embryonic stem cells. *Nature*. 2012 491(7422):66-71.
- Atterwill CK, Fowler KF. A comparison of cultured rat FRTL-5 and porcine thyroid cells for predicting the thyroid toxicity of xenobiotics. *Toxicol In Vitro*. 1990. 4(4-5):369-74.
- Braverman, L.E. and Utiger, R.D. (2012). *Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text* (10 ed.). Philadelphia, PA: Lippincott Williams & Wilkins. pp. 775-786. ISBN 978-1451120639.
- Brown CG, Fowler KL, Nicholls PJ, Atterwill C. Assessment of thyrotoxicity using *in vitro* cell culture systems. *Food Chem Toxicol*. 1986 24(6-7):557-62.
- Brucker-Davis F. Effects of environmental synthetic chemicals on thyroid function. *Thyroid*. 1998 8(9):827-56.
- Calil-Silveira J, Serrano-Nascimento C, Laconca RC, Schmiedecke L, Salgueiro RB, Kondo AK, Nunes MT. (2016). Underlying Mechanisms of Pituitary-Thyroid Axis Function Disruption by Chronic Iodine Excess in Rats. *Thyroid*. Oct;26(10):1488-1498.
- Cooper DS, Kieffer JD, Halpern R, Saxe V, Mover H, Maloof F, Ridgway EC (1983) Propylthiouracil (PTU) pharmacology in the rat. II. Effects of PTU on thyroid function. *Endocrinology* 113:921-928.
- Cooper DS, Saxe VC, Meskeil M, Maloof F, Ridgway EC. Acute effects of propylthiouracil (PTU) on thyroidal iodide organification and peripheral iodothyronine deiodination: correlation with serum PTU levels measured by radioimmunoassay. *J Clin Endocrinol Metab*. 1982 54(1):101-7.

- Degon, M., Chipkin, S.R., Holot, C.V., Zoeller, R.T., and Chait, Y. (2008). A computational model of the human thyroid. *Mathematical Biosciences* 212, 22–53.
- Dong X, Dong J, Zhao Y, Guo J, Wang Z, Liu M, Zhang Y, Na X. (2017). Effects of Long-Term In Vivo Exposure to Di-2-Ethylhexylphthalate on Thyroid Hormones and the TSH/TSHR Signaling Pathways in Wistar Rats. *Int J Environ Res Public Health*. Jan 4;14(1). pii: E44.
- Ekerot P, Ferguson D, Glämsta EL, Nilsson LB, Andersson H, Rosqvist S, Visser SA. Systems pharmacology modeling of drug-induced modulation of thyroid hormones in dogs and translation to human. *Pharm Res*. 2013 30(6):1513-24.
- Fisher JW, Li S, Crofton K, Zoeller RT, McLanahan ED, Lumen A, Gilbert ME. Evaluation of iodide deficiency in the lactating rat and pup using a biologically based dose-response model. *Toxicol Sci*. 2013 132(1):75-86.
- Gilbert ME. (2004). Alterations in synaptic transmission and plasticity in area CA1 of adult hippocampus following developmental hypothyroidism. *Brain Res Dev Brain Res* 148:11-18.
- Gilbert ME, Hedge JM, Valentín-Blasini L, Blount BC, Kannan K, Tietge J, Zoeller RT, Crofton KM, Jarrett JM, Fisher JW. An animal model of marginal iodine deficiency during development: the thyroid axis and neurodevelopmental outcome. *Toxicol Sci*. 2013 132(1):177-95.
- Hassan, I, El-Masri, H., Kosian, PA, Ford, J, Degitz, SJ and Gilbert, ME. Quantitative adverse outcome pathway for neurodevelopmental effects of thyroid peroxidase-induced thyroid hormone synthesis inhibition. *Toxicological Sciences*, kfx163, <https://doi.org/10.1093/toxsci/kfx163> 2017, in press.
- Hornung MW, Degitz SJ, Korte LM, Olson JM, Kosian PA, Linnum AL, Tietge JE. Inhibition of thyroid hormone release from cultured amphibian thyroid glands by methimazole, 6-propylthiouracil, and perchlorate. *Toxicol Sci*. 2010 118(1):42-51.
- Hurley PM. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environ Health Perspect*. 1998 106(8):437-45.
- King DB, May JD. Thyroidal influence on body growth. *J Exp Zool*. 1984 Dec;232(3):453-60.
- Köhrle J. Environment and endocrinology: the case of thyroidology. *Ann Endocrinol (Paris)*. 2008 69(2):116-22.
- Leonard JA, Tan YM, Gilbert M, Isaacs K, El-Masri H. Estimating margin of exposure to thyroid peroxidase inhibitors using high-throughput in vitro data, high-throughput exposure modeling, and physiologically based pharmacokinetic/pharmacodynamic modeling. *Toxicol Sci*. 2016 151(1):57-70.
- Lewandowski TA1, Seeley MR, Beck BD. (2004). Interspecies differences in susceptibility to perturbation of thyroid homeostasis: a case study with perchlorate. *Regul Toxicol Pharmacol*. Jun;39(3):348-62.
- Liu C, Wang C, Yan M, Quan C, Zhou J, Yang K. (2012). PCB153 disrupts thyroid hormone homeostasis by affecting its biosynthesis, biotransformation, feedback regulation, and metabolism. *Horm Metab Res*. Sep;44(9):662-9.
- Lu, M-H, and Anderson, RR. Thyroxine secretion rats during pregnancy in the rat. *Endo Res*. 1994. 20(4):343-364.
- Paul KB, Hedge JM, Macherla C, Filer DL, Burgess E, Simmons SO, Crofton KM, Hornung MW. Cross-species analysis of thyroperoxidase inhibition by xenobiotics demonstrates conservation of response between pig and rat. *Toxicology*. 2013. 312:97-107.
- Pearce EN, Alexiou M, Koukkou E, Braverman LE, He X, Ilias I, Alevizaki M, Markou KB. (2012). Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women from Greece. *Clin Endocrinol (Oxf)*. Sep;77(3):471-4.
- Steinmaus CM. (2016a). Perchlorate in Water Supplies: Sources, Exposures, and Health Effects. *Curr Environ Health Rep*. Jun;3(2):136-43.
- Steinmaus C, Pearl M, Kharrazi M, Blount BC, Miller MD, Pearce EN, Valentin-Blasini L, DeLorenze G, Hoofnagle AN, Liaw J. (2016b). Thyroid Hormones and Moderate Exposure to Perchlorate during Pregnancy in Women in Southern California. *Environ Health Perspect*. Jun;124(6):861-7.
- Sui L, Gilbert ME. (2003). Pre- and postnatal propylthiouracil-induced hypothyroidism impairs synaptic transmission and plasticity in area CA1 of the neonatal rat hippocampus. *Endocrinology* 144:4195-4203.
- Tang JM, Li W, Xie YC, Guo HW, Cheng P, Chen HH, Zheng XQ, Jiang L, Cui D, Liu Y, Ding GX, Duan Y. (2013). Morphological and functional deterioration of the rat thyroid following chronic exposure to low-dose PCB118. *Exp Toxicol Pathol*. Nov;65(7-8):989-94.
- Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K, Gibbs J. (2004). Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid*, Dec;14(12):1012-9.
- Van Herck SL, Geysens S, Delbaere J, Darras VM. Regulators of thyroid hormone availability and action in embryonic chicken brain development. *Gen Comp Endocrinol*. 2013. 190:96-104.
- Wu F, Zhou X, Zhang R, Pan M, Peng KL. (2012). The effects of ammonium perchlorate on thyroid homeostasis and thyroid-specific gene expression in rat. *Environ Toxicol*. Aug;27(8):445-52.
- Wu Y, Beland FA1, Fang JL. (2016). Effect of triclosan, triclocarban, 2,2',4,4'-tetrabromodiphenyl ether, and bisphenol A on the iodide uptake, thyroid peroxidase activity, and expression of genes involved in thyroid hormone synthesis. *Toxicol In Vitro*. Apr;32:310-9.
- Zoeller, R. T., Tan, S. W., and Tyl, R. W. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Critical reviews in toxicology* 37(1-2), 11-53.

Thyroperoxidase, Inhibition leads to T4 in serum, Decreased (<https://aopwiki.org/relationships/366>)
AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	indirectly leads to	Strong	Moderate
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	indirectly leads to		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Xenopus laevis	Xenopus laevis	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
chicken	Gallus gallus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9031)
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
All life stages	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Use of TPO inhibitors as anti-hyperthyroidism drugs, in humans and pets (Emiliano et al., 2010; Trepanier, 2006), in amphibian and avian species (Coady et al., 2010; Grommen et al., 2011; Rosebrough et al., 2006; Tietge et al., 2012), demonstrate decreased serum TH concentrations in vivo in rats (US EPA, 2005) and strongly supports a causative linkage between inhibition of TPO and decreased serum T4 across species.

How Does This Key Event Relationship Work

TPO is the enzyme that catalyzes iodine organification of thyroglobulin to produce Tg-bound T3 and T4 in the lumen of thyroid follicles. Tg-bound THs are endocytosed across the apical lumen-follicular cell membrane, undergo thyroglobulin proteolysis, followed by hormone secretion into the blood stream (see Taurog, 2005 for review). This indirect KER describes the relationship of TPO inhibition to reduced circulating levels of thyroid hormone (TH) in the serum.

Weight of Evidence

The weight of evidence linking thyroperoxidase inhibition to reductions in circulating serum TH is strong. Many studies support this basic linkage. There is no inconsistent data.

Biological Plausibility

It is a well-accepted fact that inhibition of the only enzyme capable of synthesizing THs, TPO, results in subsequent decrease in serum TH concentrations. A large amount of evidence from clinical and animal studies clearly support the commonly accepted dogma that inhibition of TPO leads to decreased serum THs.

Empirical Support for Linkage

The majority of research in support of this KER involve exposure to known TPO inhibitors and measurement of serum hormones. There are many in vivo studies that link decreases in serum TH concentrations with exposure to xenobiotics that inhibit thyroperoxidase (TPO) (Brucker-Davis, 1998; Hurley, 1998; Boas et al., 2006; Crofton, 2008; Kohrle, 2008; Pearce and Braverman, 2009; Murk et al., 2013).

While these studies support the connection between exposure to a known TPO inhibitor and decreased TH, many of these studies do not empirically measure TPO inhibition or decreased TH synthesis. Thus, many studies support the indirect linkage between TPO inhibition (for chemicals identified as TPO inhibitors in in vivo or ex vivo studies) and decreased TH, with the well accepted theory that this proceeds via

decreased TH synthesis. That exposure to TPO inhibitors leads to decreased serum TH concentrations, via decreased TH synthesis is strongly supported by decades of mechanistic research in a variety of species.

This indirect relationship is also evidenced by the use of clinically-relevant anti-hyperthyroidism drugs, MMI and PTU (Laurberg & Anderson, 2014; Sundaresht et al., 2013). These drugs are both recognized TPO inhibitors and are part of a standard drug-based regimen of care for clinically hyperthyroid patients including those with Grave's disease. Serum THs are measured as the bioindicator of successful treatment with anti-hyperthyroidism drugs; the actual decrease in TH synthesis in the thyroid gland is implied in the efficacious use of these drugs (Trepanier, 2006).

In rats, MMI and PTU are often used as control chemicals to decrease serum THs to study biological phenomena related to disruption of TH homeostasis (many examples, including Zoeller and Crofton, 2005; Morreale de Escobar et al, 2004; Schwartz et al., 1997; Herwig et al., 2014; Wu et al., 2013; Pathak et al., 2011). Further, MMI is recommended as a positive control for use in the Amphibian Metamorphosis (Frog) Assay within Tier 1 of the U.S. EPA Endocrine Disruptor Screening Program (US EPA, 2009; Coady et al., 2010), an assay used to evaluate the potential for chemicals to disrupt TH homeostasis. PTU has been suggested a positive control chemical in the guidance for the Comparative Developmental Thyroid Assay (US EPA, 2005), a non-guideline assay used to evaluate the potential for chemicals to disrupt TH homeostasis during gestation and early neonatal development.

Thus, an indirect key event relationship between TPO inhibition and decreased serum THs is strongly supported by a large database of clinical medicine and investigative research with whole animals (with a great deal of supporting evidence in rats and frogs).

Temporal Evidence: The temporal nature of this KER is applicable to all life stages, including development (Seed et al., 2005). The qualitative impact of thyroperoxidase inhibition on serum hormones is similar across all ages. The temporal nature of the impact on serum THs by TPO inhibitors in developmental exposure studies is evidenced by the duration of exposure and developmental age (Goldey et al., 1995; Ahmed et al., 2010; Tietge et al., 2010), as well as recovery after cessation of exposure (Cooke et al., 1993; Goldey et al., 1995; Sawin et al., 1998; Axelstad et al., 2008; Shibutani et al., 2009; Lasley and Gilbert, 2011). The temporal relationship between TPO inhibitor exposure duration and serum hormone decreases in adult organisms has been widely demonstrated (e.g., Hood et al., 1999; Mannisto et al., 1979). In addition, MMI and PTU induced decreases in serum T4 are alleviated by thyroid hormone replacement in both fetal and postnatal age rats (Calvo et al., 1990; Sack et al., 1995; Goldey and Crofton, 1998). Computational modeling of the thyroid also provides evidence for the indirect temporal relationship between these two KEs (e.g., Degon et al., 2008; Fisher et al., 2013).

Dose-Response Evidence: Empirical data is available from enough studies in animals treated with TPO inhibitors during development to make it readily accepted dogma that a dose-response relationship exists between TPO inhibition and serum TH concentrations. Again, these studies do not empirically measure TPO inhibition or decreased TH synthesis, but rely on the strong support of decades of mechanistic research in a variety of species of the causative relationship between these KEs. Examples of dose-responsive changes in TH concentrations following developmental exposure to TPO inhibitors include studies a variety of species, including: rodents (Blake and Henning, 1985; Goldey et al., 1995; Sawin et al., 1998); frogs (Tietge et al., 2013); fish tissue levels (Elsalini and Rohr, 2003.); and, chickens (Wische et al., 1979). Computational modeling of the thyroid also provides evidence for the indirect dose-response relationship between these two KEs (e.g., Leonard et al., 2016; Fisher et al., 2013).

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. The predominant uncertainty regarding the indirect key event relationship between inhibition of TPO activity and decreased serum T4 is the quantitative nature of this relationship, i.e., to what degree must TPO be inhibited in order to decrease serum T4 by a certain magnitude. Many animal (rat) studies typically employ relatively high exposures of TPO-inhibiting chemicals that result in hypothyroidism (severe decrements in T4 and T3). Thus, a dose-response relationship between TPO inhibition and decreased serum T4 is not typically defined. However, there are numerous publications demonstrating clear dose- and duration-dependent relationships between TPO inhibitors dose and reduced serum T3 and T4 in rodent models (see for example: Cooper et al., 1983; Hood et al., 1999; Goldey et al., 2005; Gilbert, 2011).

Quantitative Understanding of the Linkage

The indirect linkage between exposure to known TPO inhibitors and decreased serum TH has not been defined quantitatively. The two key event relationships that mediate this relationship (TPO inhibition leading to decreased TH synthesis, and decreased TH synthesis leading to decreased serum TH) have been incorporated into some quantitative models. A quantitative biologically-based dose-response model for iodine deficiency in the rat includes relationships between thyroidal T4 synthesis and serum T4 concentrations in developing rats Fisher et al. (2013). Ekerot et al. (2012) modeled TPO, T3, T4 and TSH in dogs and humans based on exposure to myeloperoxidase inhibitors that also inhibit TPO and was has recently adapted for rat (Leonard et al., 2016). While the original model predicted serum TH and TSH levels as a function of oral dose, it was not used to explicitly predict the relationship between serum hormones and TPO inhibition, or thyroidal hormone synthesis. Leonard et al. (2016) recently incorporated TPO inhibition into the model. Degon et al (2008) developed a human thyroid model that includes TPO, but does not make quantitative prediction of organification changes due to inhibition of the TPO enzyme.

References

- Ahmed OM, Abd El-Tawab SM, Ahmed RG. Effects of experimentally induced maternal hypothyroidism and hyperthyroidism on the development of rat offspring: I. The development of the thyroid hormones-neurotransmitters and adenosinergic system interactions. *Int J Dev Neurosci.* 2010 28(6):437-54
- Axelstad M, Hansen PR, Boberg J, Bonnicksen M, Nellemann C, Lund SP, Hougaard KS, Hass U. Developmental neurotoxicity of propylthiouracil (PTU) in rats: relationship between transient hypothyroxinemia during development and long-lasting behavioural and functional changes. *Toxicol Appl Pharmacol.* 2008 232(1):1-13.
- Blake HH, Henning SJ. Effect of propylthiouracil dose on serum thyroxine, growth, and weaning in young rats. *Am J Physiol.* 1985 248(5 Pt 2):R524-30.
- Boas M, Feldt-Rasmussen U, Skakkebaek NE, Main KM. Environmental chemicals and thyroid function. *Eur J Endocrinol.* 2006 154:599-611.
- Brucker-Davis F. Effects of environmental synthetic chemicals on thyroid function. *Thyroid.* 1998 8:827-56.

- Calvo R, Obregón MJ, Ruiz de Oña C, Escobar del Rey F, Morreale de Escobar G. Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. *J Clin Invest.* 1990 Sep;86(3):889-99.
- Coady K, Marino T, Thomas J, Currie R, Hancock G, Crofoot J, McNalley L, McFadden L, Geter D, Klecka G. 2010. Evaluation of the amphibian metamorphosis assay: exposure to the goitrogen methimazole and the endogenous thyroid hormone L-thyroxine. *Environmental toxicology and chemistry / SETAC.* Apr;29:869-880.
- Cooke PS, Kirby JD, Porcelli J. Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: optimization of the propylthiouracil dose and effects of methimazole. *J Reprod Fertil.* 1993 97(2):493-9
- Cooper DS, Kieffer JD, Halpern R, Saxe V, Mover H, Maloof F, Ridgway EC (1983) Propylthiouracil (PTU) pharmacology in the rat. II. Effects of PTU on thyroid function. *Endocrinology* 113:921-928.
- Crofton KM Thyroid disrupting chemicals: mechanisms and mixtures. *Int J Androl.* 2008 31:209-23
- Degon, M., Chipkin, S.R., Hollot, C.V., Zoeller, R.T., and Chait, Y. (2008). A computational model of the human thyroid. *Mathematical Biosciences* 212: 22–53.
- Ekerot P, Ferguson D, Glämsta EL, Nilsson LB, Andersson H, Rosqvist S, Visser SA. Systems pharmacology modeling of drug-induced modulation of thyroid hormones in dogs and translation to human. *Pharm Res.* 2013 Jun;30(6):1513-24.
- Fisher JW, Li S, Crofton K, Zoeller RT, McLanahan ED, Lumen A, Gilbert ME. Evaluation of iodide deficiency in the lactating rat and pup using a biologically based dose-response model. *Toxicol Sci.* 2013 132(1):75-86.
- Elsalini OA, Rohr KB.: Phenylthiourea disrupts thyroid function in developing zebrafish. *Dev Genes Evol* 212, 593-8, 2003.
- Emiliano, A.B., Governale, L., Parks, M., Cooper, D.S., 2010. Shifts in propylthiouracil and methimazole prescribing practices: antithyroid drug use in the United States from 1991 to 2008. *J. Clin. Endocrinol. Metab.* 95, 2227–2233.
- Gilbert ME. 2011. Impact of low-level thyroid hormone disruption induced by propylthiouracil on brain development and function. *Toxicological Dec;*124:432-445.
- Goldes ES, Kehn LS, Rehnberg GL, Crofton KM. Effects of developmental hypothyroidism on auditory and motor function in the rat. *Toxicol Appl Pharmacol.* 1995 135(1):67-76.
- Goldes ES, Crofton KM. Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. *Toxicol Sci.* 1998 Sep;45(1):94-105.
- Grommen, S.V., Iwasawa, A., Beck, V., Darras, V.M., De Groef, B., 2011. Ontogenic expression profiles of thyroid-specific genes in embryonic and hatching chicks. *Domest. Anim. Endocrinol.* 40, 10–18.
- Herwig A, Campbell G, Mayer CD, Boelen A, Anderson RA, Ross AW, Mercer JG, Barrett P. 2014. A thyroid hormone challenge in hypothyroid rats identifies T3 regulated genes in the hypothalamus and in models with altered energy balance and glucose homeostasis. *Thyroid :* Nov;24:1575-1593.
- Hood A, Liu YP, Gattone VH, 2nd, Klaassen CD (1999) Sensitivity of thyroid gland growth to thyroid stimulating hormone (TSH) in rats treated with antithyroid drugs. *Toxicol Sci* 49:263-271.
- Hurley PM. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environ Health Perspect.* 1998 106:437-45.
- Köhrle J. Environment and endocrinology: the case of thyroidology. *Ann Endocrinol (Paris).* 2008 69:116-22.
- Lasley SM, Gilbert ME. Developmental thyroid hormone insufficiency reduces expression of brain-derived neurotrophic factor (BDNF) in adults but not in neonates. *Neurotoxicol Teratol.* 2011 33(4):464-72
- Laurberg P, Andersen SL. 2014. Therapy of endocrine disease: antithyroid drug use in early pregnancy and birth defects: time windows of relative safety and high risk? *Eur J Endocrinol.* 2014 Jul;171(1):R13-20.
- Leonard JA, Tan YM, Gilbert M, Isaacs K, El-Masri H. Estimating margin of exposure to thyroid peroxidase inhibitors using high-throughput in vitro data, high-throughput exposure modeling, and physiologically based pharmacokinetic/pharmacodynamic modeling. *Toxicol Sci.* 2016 151(1):57-70.
- Männistö PT, Ranta T, Leppäluoto J. Effects of methylmercaptoimidazole (MMI), propylthiouracil (PTU), potassium perchlorate (KClO₄) and potassium iodide (KI) on the serum concentrations of thyrotrophin (TSH) and thyroid hormones in the rat. *Acta Endocrinol (Copenh).* 1979 91(2):271-81.
- Morreale de Escobar G, Obregon MJ, Escobar del Rey F (2004) Role of thyroid hormone during early brain development. *Eur J Endocrinol* 151 Suppl 3:U25-37.
- Murk AJ, Rijntjes E, Blaauboer BJ, Clewell R, Crofton KM, Dingemans MM, Furlow JD, Kavlock R, Köhrle J, Opitz R, Traas T, Visser TJ, Xia M, Gutleb AC. Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals. *Toxicol In Vitro.* 2013 27:1320-46.
- Pathak A, Sinha RA, Mohan V, Mitra K, Godbole MM. 2011. Maternal thyroid hormone before the onset of fetal thyroid function regulates reelin and downstream signaling cascade affecting neocortical neuronal migration. *Cerebral Cortex.* 21:11-21.
- Pearce EN, Braverman LE. Environmental pollutants and the thyroid. *Best Pract Res Clin Endocrinol Metab.* 2009 23:801-1.
- Rosebrough, R.W., Russell, B.A., McMurtry, J.P., 2006. Studies on doses of methimazole (MMI) and its administration regimen on broiler metabolism. *Comp. Biochem. Physiol. A: Mol. Integr. Physiol.* 143, 35–41.
- Sack J, Weller A, Rigler O, Rozin A. A simple model for studying the correction of in utero hypothyroidism in the rat. *Pediatr Res.* 1995 37(4 Pt 1):497-501.

- Sawin S, Brodish P, Carter CS, Stanton ME, Lau C. Development of cholinergic neurons in rat brain regions: dose-dependent effects of propylthiouracil-induced hypothyroidism. *Neurotoxicol Teratol*. 1998 20(6):627-35
- Schwartz HL, Ross ME, Oppenheimer JH (1997) Lack of effect of thyroid hormone on late fetal rat brain development. *Endocrinology* 138:3119-3124.
- Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol*. 2005 35(8-9):664-72.
- Shibutani M, Woo GH, Fujimoto H, Saegusa Y, Takahashi M, Inoue K, Hirose M, Nishikawa A. Assessment of developmental effects of hypothyroidism in rats from in utero and lactation exposure to anti-thyroid agents. *Reprod Toxicol*. 2009 Nov;28(3):297-307
- Sundares V, Brito JP, Wang Z, Prokop LJ, Stan MN, Murad MH, Bahn RS. 2013. Comparative effectiveness of therapies for Graves' hyperthyroidism: a systematic review and network meta-analysis. *The Journal of clinical endocrinology and metabolism*. Sep;98:3671-3677.
- Taurog A. 2005. Hormone synthesis. In: Werner and Ingbar's *The Thyroid: A Fundamental and Clinical Text* (Braverman LE, Utiger RD, eds). Philadelphia:Lippincott, Williams and Wilkins, 47–81
- Taurog, a, Dorris, M. L., & Doerge, D. R. (1996). Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. *Archives of Biochemistry and Biophysics*, Taurog A. Molecular evolution of thyroid peroxidase. *Biochimie*. 1999 May;81(5):557-62
- Tietge JE, Butterworth BC, Haselman JT, Holcombe GW, Hornung MW, Korte JJ, Kosian PA, Wolfe M, Degitz SJ. Early temporal effects of three thyroid hormone synthesis inhibitors in *Xenopus laevis*. *Aquat Toxicol*. 2010 Jun 1;98(1):44-50
- Tietge, J.E., Degitz, S.J., Haselman, J.T., Butterworth, B.C., Korte, J.J., Kosian, P.A., Lindberg-Livingston, A.J., Burgess, E.M., Blackshear, P.E., Hornung, M.W., 2012. Inhibition of the thyroid hormone pathway in *Xenopus laevis* by 2- mercaptobenzothiazole. *Aquat. Toxicol*. 126C, 128–136.
- Tietge JE, Degitz SJ, Haselman JT, Butterworth BC, Korte JJ, Kosian PA, Lindberg-Livingston AJ, Burgess EM, Blackshear PE, Hornung MW. Inhibition of the thyroid hormone pathway in *Xenopus laevis* by 2-mercaptobenzothiazole. *Aquat Toxicol*. 2013 15;126:128-36
- Trepanier, L.A., 2006. Medical management of hyperthyroidism. *Clin. Tech. Small Anim. Pract*. 21, 22–28.
- U.S. Environmental Protection Agency. 2005. Guidance for Thyroid Assays in Pregnant Animals, Fetuses, and Postnatal Animals, and Adult Animals. Office of Pesticide Programs, Health Effects Division, Washington, DC
- U.S. Environmental Protection Agency. 2009. Endocrine Disruptor Screening Program Test Guidelines OPPTS 890.1100: Amphibian Metamorphosis (Frog). Washington, DC.
- Wise H I, Rolle-Getz G K, and Goldsmith E D.: The effects of aminotriazole (ATZ) on the thyroid gland and the development of the white leghorn chick. *Growth* 43, 238-251, 1979
- Wu S, Tan G, Dong X, Zhu Z, Li W, Lou Z, Chai Y. 2013. Metabolic profiling provides a system understanding of hypothyroidism in rats and its application. *PLoS one*.8:e55599.
- Zoeller RT, Crofton KM (2005) Mode of action: developmental thyroid hormone insufficiency--neurological abnormalities resulting from exposure to propylthiouracil. *Crit Rev Toxicol* 35:771-781.

T4 in serum, Decreased leads to T4 in neuronal tissue, Decreased (<https://aopwiki.org/relationships/312>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	directly leads to	Moderate	Weak
XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	directly leads to	Moderate	Weak
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	directly leads to	Strong	Weak
Inhibition of Na ⁺ /I ⁻ symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	directly leads to	Strong	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human	Homo sapiens	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong
All life stages	Moderate

Sex Applicability

Sex	Evidence
Male	Moderate
Female	Moderate

The majority of the information on this KER comes from in vivo studies with rodents (mainly MCT8 knock-out mice and thyroidectomized rats) and histopathological analyses of human brain tissues derived from patients affected by AHDS (Allan-Herndon-Dudley syndrome). The evolutionary conservation of the transport of TH from circulation to the developing brain suggests, with some uncertainty, that this KER is also applicable to other taxa, including birds, fish and frogs (Van Herck et al., 2013; Denver, 1998; Power et al., 2001).

How Does This Key Event Relationship Work

In mammals, thyroxine (T4) in brain tissue is derived almost entirely from the circulating pool of T4 in blood. Transfer of free T4 (and to a lesser extent, T3) from serum binding proteins (thyroid binding globulin (TBG), transthyretin (TTR) and albumin; see McLean et al., 2017, for a recent review) into the brain requires transport across the blood brain barrier (BBB) and/or indirect transport from the cerebral spinal fluid (CSF) into the brain through the blood-CSF-barrier. The blood vessels in rodents and humans express the main T4 transporter, MCT8, (Roberts et al. 2008), as does the choroid plexus which also expresses TTR and secretes the protein into the CSF (Alshehri et al. 2015).

T4 entering the brain through the BBB is taken up into astrocytes via cell membrane iodothyronine transporters (e.g., organic anion-transporting polypeptides) (Visser et al., 2011). In astrocytes, T4 is then deiodinated by Type II deiodinase to triiodothyronine (T3) (St Germain and Galton, 1997), which is then transported via other iodothyronine transporters (e.g., monocarboxylate transporter) into neurons (Visser et al., 2011). While some circulating T3 may be taken up into brain tissue directly from blood (Dratman et al., 1991), the majority of neuronal T3 comes from deiodination of T4 in astrocytes. Decreases in circulating T4 will result in decreased brain T3 tissue concentrations. It is also known that Type II deiodinase can be up-regulated in response to decreased T4 concentrations to maintain tissue concentrations of T3 (Pedraza et al., 2007; Lavado-Autric et al., 2013; Morse et al., 1986), except in tanycytes of the paraventricular nucleus (Fekete and Lechan, 2014).

Weight of Evidence

The weight of evidence linking reductions in circulating serum TH and reduced brain concentrations of TH is moderate. Many studies support this basic linkage. However, there are compensatory mechanisms (e.g., upregulation of deiodinases, transporters) that may alter the relationship between hormones in the periphery and hormone concentrations in the brain. There is limited information available on the quantitative relationship between circulating levels of TH, these compensatory processes, and neuronal T4 concentrations, especially during development. Furthermore, in certain conditions, such as iodine deficiency, the decreases in circulating hormone might have greater impacts on tissue levels of TH (see for instance, Escobar del Rey, et al., 1989).

Biological Plausibility

The biological relationship between these two KEs is strong as it is well accepted dogma within the scientific community. There is no doubt that decreased circulating T4 leads to declines in tissue concentrations of T4 and T3 in a variety of tissues, including brain. However, compensatory mechanisms (e.g., increased expression of Type 2 deiodinase) may differ during different life stages and across different tissues, especially in different brain regions. Similarly, the degree to which serum TH must drop to overwhelm these compensatory responses has not been established.

Empirical Support for Linkage

Several studies have shown that tissue levels (including the brain) of TH are proportional to serum hormone level (Oppenheimer, 1983; Morreale de Escobar et al., 1987; 1990; Calvo et al., 1992; Porterfield and Hendrich, 1992, 1993; Broedel et al., 2003). In thyroidectomized rats, brain concentrations of T4 were decreased and Type II deiodinase (DII) activity was increased. Both brain T3 and T4 as well as DII activity returned to normal following infusion of T4 (Escobar-Morreale et al., 1995; 1997). Animals treated with PTU, MMI, or iodine deficiency during development demonstrate both lower serum and lower brain TH concentrations (Escobar-Morreale et al 1995; 1997; Taylor et al., 2008; Bastian et al., 2012; 2014; Gilbert et al., 2013).

Temporal Evidence: The temporal relationship between serum T4 and T4 in growing neuronal tissue described in this KER is developmental (Seed et al., 2005). While all brain regions will be impacted by changes in serum hormones, brain concentrations will be a function of development stage and brain region. Data is available from thyroid hormone replacement studies that demonstrate recovery of fetal brain T3 and T4 levels (following low iodine diets or MMI exposure) to control levels after maternal thyroid hormone replacement or iodine supplementation (e.g., Calvo et al., 1990; Obregon et al., 1991). For example, Calvo et al. (1990) carried out a detailed study of the effects of TPO inhibition on serum and tissues levels of TH in gestating rats. Clear dose-dependent effects of T4 replacement, but not T3 replacement were seen in all maternal tissues. However, for fetal tissues neither T4 nor T3, at any dose, could not completely restore tissue TH levels to control levels.

Dose-Response Evidence: There is good evidence, albeit from a limited number of studies of the correlative relationship between circulating thyroid hormone concentrations and brain tissue concentrations during fetal and early postnatal development following maternal iodine deficient diets or chemical treatments that depress serum THs (c.f., Calvo et al., 1990; Obregon et al., 1991; Morse et al., 1996).

Uncertainties or Inconsistencies

Limited data is available that demonstrates that the developing brain can compensate for chemical-induced alterations in TH concentrations (e.g., Calvo et al., 1990; Morse et al., 1996; Sharlin et al., 2010). There are likely different quantitative relationships between these two KEs depending on the compensatory ability based on both developmental stage and specific brain region (Sharlin et al., 2010). For these reasons, the empirical support for this linkage is rated as moderate. In addition, future work on cellular hormone transport mechanisms and deiodinase activity may inform the additional of KEs and KERs between serum and brain T4.

Quantitative Understanding of the Linkage

While it is well established that decreased in serum TH levels result in decreased brain TH concentrations, particularly fetal brain concentrations, a major gap is the lack of empirical data that allow direct quantification of this relationship.

References

- Ank A, Kersseboom S, Demir K, Catlı G, Yiş U, Böber E, van Mullem A, van Herebeek RE, Hız S, Abacı A, Visser TJ. (2014). Psychomotor retardation caused by a defective thyroid hormone transporter: report of two families with different MCT8 mutations. *Horm Res Paediatr.* 82(4):261-71.
- Alshehri B, D'Souza DG, Lee JY, Petratos S, Richardson SJ. The diversity of mechanisms influenced by transthyretin in neurobiology: development, disease and endocrine disruption. *J Neuroendocrinol.* 2015 May;27(5):303-23.
- Bastian TW, Anderson JA, Fretham SJ, Prohaska JR, Georgieff MK, Anderson GW (2012). Fetal and neonatal iron deficiency reduces thyroid hormone-responsive gene mRNA levels in the neonatal rat hippocampus and cerebral cortex. *Endocrinology* 153:5668-5680.
- Bastian TW, Prohaska JR, Georgieff MK, Anderson GW (2014) Fetal and neonatal iron deficiency exacerbates mild thyroid hormone insufficiency effects on male thyroid hormone levels and brain thyroid hormone-responsive gene expression. *Endocrinology* 55:1157-1167.
- Braverman LE. (2012). Various aspects of thyroid physiology and pathology contributed by international experts. *Curr Opin Endocrinol Diabetes Obes.* Oct;19(5):381.
- Broedel, O., Eravci, M., Fuxius, S., Smolarz, T., Jeitner, A., Grau, H., Stoltenburg-Diding, G., Plueckhan, H., Meinhold, H., and Baumgartner, A. (2003). Effects of hyper and hypothyroidism on thyroid hormone concentrations in regions of the rat brain. *Am. J. Physiol. Endocrinol. Metab.* 285:E470-480.
- Calvo, R., Obregon, M.J., Escobar del Rey, F., and Morreale de Escobar, G. (1992). The rat placenta and the transfer of thyroid hormones from the mother to the fetus. Effects of maternal thyroid status. *Endocrinology* 131:357-365.
- Calvo R, Obregon MJ, Ruiz de Ona C, Escobar del Rey F, Morreale de Escobar G 1990 Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. *J Clin Invest* 86:889-899.
- da Conceição RR, Laureano-Melo R, Oliveira KC, de Carvalho Melo MC, Kasamatsu TS, de Barros Maciel RM, de Souza JS, Giannocco G. (2016). Antidepressant behavior in thyroidectomized Wistar rats is induced by hippocampal hypothyroidism. *Physiol Behav.* Apr 1;157:158-64.
- Denver, RJ. (1998). The molecular basis of thyroid hormone-dependent central nervous system remodeling during amphibian metamorphosis. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 119:219-228.
- Dratman MB, Crutchfield FL, Schoenhoff MB. Transport of iodothyronines from bloodstream to brain: contributions by blood:brain and choroid plexus:cerebrospinal fluid barriers. *Brain Res.* 1991 Jul 19;554(1-2):229-36.
- Escobar del Rey F, Ruiz de Oña C, Bernal J, Obregón MJ, Morreale de Escobar G. Generalized deficiency of 3,5,3'-triiodo-L-thyronine (T3) in tissues from rats on a low iodine intake, despite normal circulating T3 levels. *Acta Endocrinol (Copenh).* 1989 Apr;120(4):490-8.
- Escobar-Morreale HF, Obregón MJ, Escobar del Rey F, Morreale de Escobar G. Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. *J Clin Invest.* 1995 Dec;96(6):2828-38.
- Escobar-Morreale HF1, Obregón MJ, Hernandez A, Escobar del Rey F, Morreale de Escobar G. Regulation of iodothyronine deiodinase activity as studied in thyroidectomized rats infused with thyroxine or triiodothyronine. *Endocrinology.* 1997 Jun;138(6):2559-68.
- Fekete C, Lechan RM. Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. *Endocr Rev.* 2014 Apr;35(2):159-94
- Fisher JW, Li S, Crofton K, Zoeller RT, McLanahan ED, Lumen A, Gilbert ME. (2013). Evaluation of iodide deficiency in the lactating rat and pup using a biologically based dose-response model. *Toxicol Sci.* 132(1):75-86.
- Gilbert ME, Hedge JM, Valentin-Blasini L, Blount BC, Kannan K, Tietge J, Zoeller RT, Crofton KM, Jarrett JM, Fisher JW (2013) An animal model of marginal iodine deficiency during development: the thyroid axis and neurodevelopmental outcome. *Toxicol Sci* 132:177-195.

- Lavado-Autric R, Calvo RM, de Mena RM, de Escobar GM, Obregon MJ. Deiodinase activities in thyroids and tissues of iodine-deficient female rats. *Endocrinology*. 2013 Jan;154(1):529-36.
- López-Espíndola D, Morales-Bastos C, Grijota-Martínez C, Liao XH, Lev D, Sugo E, Verge CF, Refetoff S, Bernal J, Guadaño-Ferraz A. (2014). Mutations of the thyroid hormone transporter MCT8 cause prenatal brain damage and persistent hypomyelination. *J Clin Endocrinol Metab*. Dec;99(12):E2799-804.
- McAninch EA, Bianco AC. (2014). Thyroid hormone signaling in energy homeostasis and energy metabolism. *Ann N Y Acad Sci*. Apr;1311:77-87.
- McLean TR, Rank MM, Smooker PM, Richardson SJ. Evolution of thyroid hormone distributor proteins. *Mol Cell Endocrinol*. 2017 Feb 27. pii: S0303-7207(17)30151-X. doi: 10.1016/j.mce.2017.02.038. [Epub ahead of print]
- Morreale de Escobar, G., Obregon, M.J., and Escobar del Ray, F. (1987). Fetal and maternal thyroid hormones. *Hormone Res*. 26:12–27.
- Morreale de Escobar, G., Calvo, R., Obregon, M.J., and Escobar del Rey, F. (1990). Contribution of maternal thyroxine to fetal thyroxine pools in normal rats near term. *Endocrinology* 126:2765–2767.
- Müller J, Mayerl S, Visser TJ, Darras VM, Boelen A, Frappart L, Mariotta L, Verrey F, Heuer H. (2014). Tissue-specific alterations in thyroid hormone homeostasis in combined Mct10 and Mct8 deficiency. *Endocrinology*. Jan;155(1):315-25.
- Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol*. 1996 Feb;136(2):269-79
- Obregon MJ, Ruiz de Ona C, Calvo R, Escobar del Rey F, Morreale de Escobar G 1991 Outer ring iodothyronine deiodinases and thyroid hormone economy: Responses to iodine deficiency in the rat fetus and neonate. *Endocrinology* 129:2663–2673.
- Oppenheimer, J.H. (1983). The nuclear Receptor-triiodothyronine complex: Relationship to thyroid hormone distribution, metabolism, and biological action. In: *Molecular Basis of Thyroid Hormone Action*, eds. J.H. Oppenheimer and H.H. Samuels, pp. 1–35. New York: Academic Press.
- Pedraza PE, Obregon MJ, Escobar-Morreale HF, del Rey FE, de Escobar GM (2006) Mechanisms of adaptation to iodine deficiency in rats: thyroid status is tissue specific. Its relevance for man. *Endocrinology* 147:2098-2108.
- Porterfield, S.P. and Hendrich, C.E. (1992). Tissue iodothyronine levels in fetuses of control and hypothyroid rats at 13 and 16 days gestation. *Endocrinology* 131:195–200.
- Porterfield, S.P. and Hendrich, C.E. (1993). The role of thyroid hormones in prenatal neonatal neurological development-current perspectives. *Endocrine Rev*. 14:94–106.
- Porterfield SP. (1994). Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. *Environ Health Perspect*. Jun;102 Suppl 2:125-30.
- Power DM, Llewellyn L, Faustino M, Nowell MA, Björnsson BT, Einarsdóttir IE, Canario AV, Sweeney GE. (2001). Thyroid hormones in growth and development of fish. *Comp Biochem Physiol C Toxicol Pharmacol*. Dec;130(4):447-59.
- Roberts LM, Woodford K, Zhou M, Black DS, Haggerty JE, Tate EH, Grindstaff KK, Mengesha W, Raman C, Zerangue N. Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. *Endocrinology*. 2008 Dec;149(12):6251-61.
- Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol*. 2005 35:664-72.
- Sharlin DS, Gilbert ME, Taylor MA, Ferguson DC, Zoeller RT. (2010). The nature of the compensatory response to low thyroid hormone in the developing brain. *J Neuroendocrinol*. Mar;22(3):153-65.
- St. Germain, D.L. and Galton, V.A. (1997). The deiodinase family of selenoproteins. *Thyroid* 7:655–668.
- Stohn JP, Martinez ME, Matoi K, Morte B, Bernal J, Galton VA, St Germain D, Hernandez A. (2016). MCT8 Deficiency in Male Mice Mitigates the Phenotypic Abnormalities Associated With the Absence of a Functional Type 3 Deiodinase. *Endocrinology*. Aug;157(8):3266-77.
- Taylor MA, Swant J, Wagner JJ, Fisher JW, Ferguson DC (2008) Lower thyroid compensatory reserve of rat pups after maternal hypothyroidism: correlation of thyroid, hepatic, and cerebrocortical biomarkers with hippocampal neurophysiology. *Endocrinology* 149:3521-3530.
- Utiger RD. (2006). Iodine nutrition--more is better. *N Engl J Med*. Jun 29;354(26):2819-21.
- Van Herck SL, Geysens S, Delbaere J, Darras VM. (2013). Regulators of thyroid hormone availability and action in embryonic chicken brain development. *Gen Comp Endocrinol*. 190:96-104.
- Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. *Mol Endocrinol*. 2011 Jan;25(1):1-14.
- Zoeller RT, Tan SW, Tyl RW. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Critical reviews in toxicology*. Jan-Feb;37:11-53.

T4 in neuronal tissue, Decreased leads to Hippocampal gene expression, Altered (<https://aopwiki.org/relationships/746>)
AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	directly leads to	Moderate	Weak
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	directly leads to	Moderate	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Most of the data available has come from rodent models. The evolutionary conservation of thyroid receptors (Holzer et al., 2017) coupled with their role in TR regulated gene transcription in neurodevelopment, suggests that this KER may also be applicable to other species.

How Does This Key Event Relationship Work

Many cellular and biochemical effects of thyroid hormones (TH) are mediated through regulation of gene expression (Oppenheimer, 1983; Bernal, 2007). Thyroxine (T4) is transferred from the serum to the brain (see KER: Thyroxine (T4) in Serum, Decreased leads to Thyroxine (T4) in Neuronal Tissue, Decreased), where it is converted to triiodothyronine (T3), the level of which is highly controlled by deiodinases. T3 binds to thyroid receptors (TR) in the nucleus of neuronal and glial cells to control gene expression. It is generally accepted that the modulation of TR gene expression in the hippocampus, or any other brain region, must therefore depend on the presence of hormone in these tissues.

Weight of Evidence

The weight of evidence is moderate for TH concentrations affecting gene expression in the developing brain is (Oppenheimer and Schwartz, 1997; Oppenheimer, 1983; Bernal, 2007; Morte et al., 2010a; 2010b; Williams, 2008). Direct measurement of TH in brain tissue, and in hippocampus in particular, has shown correlations with gene expression. Therefore, it is assumed that reductions in TH-responsive genes in the hippocampus stem from reduced availability of hormone in the brain from the serum. However, studies in which there are simultaneous assessments of hippocampal concentrations of thyroid hormone and hippocampal gene expression is limited.

Biological Plausibility

The biological relationship between these two KEs is strong. It is a generally accepted fact that TH produce their actions on brain development by binding to nuclear receptors to affect gene transcription. See KER (1387): "Thyroxine (T4) in serum, Decreased leads (*indirectly*) to Hippocampal gene expression, Altered" for more information on TR regulated genes. As the primary means whereby TH promotes its action is by binding to TR in brain, TH must be present in brain to affect this action. Circulating levels of T4 represent the primary source of T4 in the brain, which is then converted to the active hormone T3 by deiodinases within neuronal tissue.

Empirical Support for Linkage

The empirical support for this KER is moderate. Many in vitro studies have demonstrated a relationship between hormone concentrations TH and the induction of gene expression in brain cells, including hippocampal neurons in culture (Gil-Ibanez et al., 2015; Morte et al., 2010b). However, there are a limited number of studies investigating TH concentrations in the hippocampus and hippocampal gene expression. This is the case because thyroid hormone is difficult to measure in hippocampus and TH-induced gene expression changes can be subtle. We are aware of only four in vivo studies in which both thyroid hormones in the brain and gene expression in brain were simultaneously measured (Bastian et al., 2012; 2014; Hernandez et al., 2010; Sharlin et al., 2008). Only two of these reports, stemming from the same laboratory, specifically assessed thyroid

hormone and gene expression in hippocampus. In these studies, Bastian et al., (2012; 2014) measured decrements in hippocampal T3 using RIA and correlated these reductions with alterations in the expression of myelin associated genes (Mbp, Plp), the neurotrophin, Ngf, the calcium binding protein Parv, a TH-dependent transcription factor, Hr, and Agt.

Temporal Evidence: The temporal nature of this KER on TH dependent gene regulation is developmental (Seed et al., 2005). The impact of brain TH concentrations on regulation of TR regulated genes is age-dependent for a number of genes critical for normal hippocampal development. It is widely accepted that different genes are altered dependent upon the window of exposure in the fetal, neonatal or adult brain (c.f., Pathak et al, 2011; Mohan et al., 2012; Quignodon et al., 2004; Williams, 2008). Thyroid hormone supplementation has been shown to reverse some of the effects on gene expression (Mohan et al., 2012; Liu et al., 2010; Pathak et al., 2011).

Dose-Response Evidence: Dose-response data exists, but is limited to a small number of studies and a small number of genes (Bastian et al., 2012; 2014; Sharlin et al., 2008).

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are uncertainties. Uncertainties remain in the relationship of neuronal TH concentrations and gene expression in the brain because of the lack of studies simultaneously examining brain hormone and gene expression in the same study. This stems from the technological challenges associated with measuring brain hormone and the sometimes-subtle changes in brain gene expression induced by manipulations of the thyroid system. In addition, there are also some physiological actions of T4 that are mediated non-genomically at the cell membrane (Davis et al., 2016). However, the exact role for the non-genomic effects is not well accepted or understood (Galton, 2017).

Quantitative Understanding of the Linkage

There are no quantitative estimates of the degree of TH reduction in brain that is required to alter gene transcription because of limited data available where brain TH and gene expression are simultaneously assessed.

References

- Bastian TW, Anderson JA, Fretham SJ, Prohaska JR, Georgieff MK, Anderson GW (2012), Fetal and neonatal iron deficiency reduces thyroid hormone-responsive gene mRNA levels in the neonatal rat hippocampus and cerebral cortex. *Endocrinology* 153:5668-5680.
- Bastian TW, Prohaska JR, Georgieff MK, Anderson GW (2014) Fetal and neonatal iron deficiency exacerbates mild thyroid hormone insufficiency effects on male thyroid hormone levels and brain thyroid hormone-responsive gene expression. *Endocrinology* 155:1157-1167.
- Bernal J (2007) Thyroid hormone receptors in brain development and function. *Nat Clin Pract Endocrinol Metab* 3:249-259.
- Davis, P.J., Goglia, F., Leonard, J.L., 2016. Nongenomic actions of thyroid hormone. *Nat. Rev. Endocrinol.* 12, 111-121.
- Galton VA. The ups and downs of the thyroxine pro-hormone hypothesis. *Mol Cell Endocrinol.* 2017 Jan 24. pii: S0303-7207(17)30042-4. doi: 10.1016/j.mce.2017.01.029.
- Gil-Ibañez P, García-García F, Dopazo J, Bernal J, Morte B Global Transcriptome Analysis of Primary Cerebrocortical Cells: Identification of Genes Regulated by Triiodothyronine in Specific Cell Types. *Cereb Cortex.* 2015 Nov 2. pii: bhv273. [Epub ahead of print]
- Hernandez A, Quignodon L, Martinez ME, Flamant F, St Germain DL (2010), Type 3 deiodinase deficiency causes spatial and temporal alterations in brain T3 signaling that are dissociated from serum thyroid hormone levels. *Endocrinology* 151:5550-5558.
- Holzer G, Roux N, Laudet V. Evolution of ligands, receptors and metabolizing enzymes of thyroid signaling. *Mol Cell Endocrinol.* 2017 Mar 22. pii: S0303-7207(17)30191-0. doi: 10.1016/j.mce.2017.03.021.
- Liu D, Teng W, Shan Z, Yu X, Gao Y, Wang S, Fan C, Wang H, Zhang H. The effect of maternal subclinical hypothyroidism during pregnancy on brain development in rat offspring. *Thyroid.* 2010 Aug;20(8):909-15.
- Mohan V, Sinha RA, Pathak A, Rastogi L, Kumar P, Pal A, Godbole MM (2012) Maternal thyroid hormone deficiency affects the fetal neocorticalogenesis by reducing the proliferating pool, rate of neurogenesis and indirect neurogenesis. *Exp Neurol* 237:477-488.
- Morte B, Diez D, Auso E, Belinchon MM, Gil-Ibanez P, Grijota-Martinez C, Navarro D, de Escobar GM, Berbel P, Bernal J (2010a) Thyroid hormone regulation of gene expression in the developing rat fetal cerebral cortex: prominent role of the Ca²⁺/calmodulin-dependent protein kinase IV pathway. *Endocrinology* 151:810-820.
- Morte B, Ceballos A, Diez D, Grijota-Martínez C, Dumitrescu AM, Di Cosmo C, Galton VA, Refetoff S, Bernal J. (2010b) Thyroid hormone-regulated mouse cerebral cortex genes are differentially dependent on the source of the hormone: a study in monocarboxylate transporter-8- and deiodinase-2-deficient mice. *Endocrinology.* 2010 May;151(5):2381-7
- Oppenheimer, J. (1983). The nuclear-receptor-triiodothyronine complex: Relationship to thyroid hormone distribution, metabolism, and biological action. *Molecular Basis of Thyroid Hormone Action.* J. O. a. H. Samuels. New York, Academic Press: 1-34.
- Oppenheimer, J. H. and H. L. Schwartz (1997). "Molecular basis of thyroid hormone-dependent brain development." *Endocr Rev* 18(4): 462-75.
- Pathak A, Sinha RA, Mohan V, Mitra K, Godbole MM. 2011. Maternal thyroid hormone before the onset of fetal thyroid function regulates reelin and downstream signaling cascade affecting neocortical neuronal migration. *Cerebral cortex.* Jan;21:11-21.
- Quignodon L, Legrand C, Allioli N, Guadano-Ferraz A, Bernal J, Samarut J, Flamant F (2004) Thyroid hormone signaling is highly heterogeneous during pre- and postnatal brain development. *J Mol Endocrinol* 33:467-476.
- Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol.* 2005 35:664-72.
- Sharlin DS, Tighe D, Gilbert ME, Zoeller RT (2008) The balance between oligodendrocyte and astrocyte production in major white matter tracts is linearly related to serum total thyroxine. *Endocrinology* 149:2527-2536.

Hippocampal gene expression, Altered leads to Hippocampal anatomy, Altered (<https://aopwiki.org/relationships/747>)
AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	directly leads to	Moderate	Weak
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	directly leads to	Moderate	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)

Life Stage Applicability

Life Stage	Evidence
During brain development	Moderate

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The majority of data in support of this KER is from rodent models. The evolutionary conservation of thyroid receptors (Holzer et al., 2017) coupled with their role in TR regulated gene transcription in neurodevelopment, suggests that this KER may also be applicable to other species.

How Does This Key Event Relationship Work

The basic biological processes that link gene regulation in the structural formation and function of all organs of the body are similar throughout the developing organism. In the developing brain, genes encode proteins critical for developmental events intrinsic to structural development (e.g., neurogenesis, neuronal migration, synaptogenesis, myelination). The development of the hippocampus is no exception to this general rule of biology.

Weight of Evidence

The overall weight of evidence is moderate for a direct linkage between perturbation of the expression of genes in brain (and in hippocampus specifically) and neuroanatomical abnormalities. It is widely acknowledged that the development of the structure of the hippocampus is under the control of hippocampal gene expression. However, while an extensive body of literature exists linking some genes to hippocampal structure, there is no complete compendium on the total number of genes involved, nor direct causative links between the myriad of genes and the intricate development (both timing and location) of the majority of hippocampal structure.

Biological Plausibility

The biological plausibility of this KER is rated as strong. It is well established that gene regulation controls brain development. This also applies to the development of the hippocampus, where nuclear thyroid receptors that regulate gene transcription, directly or indirectly via transcription factor regulation, to control translation.

Empirical Support for Linkage

Empirical support for this KER is rated as strong. The number of publications in this area is extensive. A few examples are: Strange et al. (2014); Takei et al. (2016); and Shin et al. (2015). Work supporting the relationship includes use of a variety of animal models (i.e., nutritional deficiencies, chromosome abnormalities, gene deletions, knock out animals, toxicant exposures and developmental hormonal imbalance) (e.g., Frotscher,

2010; Castren and Castren, 2014; Spilker et al., 2016; Skucas et al., 2011; Lessman et al., 2011). Mutant mouse lines generated for genes involved in human cortical malformations such as doublecortin, reelin, Lis1 and Tuba1a also show gross disorganization within the hippocampus (Khalaf-Nazzal et al., 2013). Collectively, data from these studies clearly support the link between alterations in hippocampal gene expression and structural changes in hippocampal volume, cell number, and/or cytoarchitecture. A direct linkage between some specific gene targets and structural change in the hippocampus has been demonstrated using knock out and mutant mouse models (e.g., Grant et al., 1992; Lee et al., 2000; Frotscher, 2010; Castren and Castren, 2014; Spilker et al., 2016; Skucas et al., 2011; Lessman et al., 2011; Khalaf-Nazzal et al., 2013).

Temporal Evidence: The temporal nature of this KER is developmental (Seed et al., 2005). It is a well-recognized fact that there are critical developmental windows for disruption of TR-regulated genes and subsequent formation of the anatomy of the hippocampus. This has been demonstrated in multiple studies. Many of the gene-anatomy relationships critical to brain development only exist during development, or exist only to a very limited extent in the adult brain. For example, genes controlling neuronal proliferation and migration are critically essential in hippocampal development, and their disruption results in abnormal hippocampal anatomy. Whereas, in the adult brain the genes are largely without effect as these processes are completed in the early neonatal period. In support of this, a limited number of studies have defined critical periods for the interaction of some genes and resulting neuroanatomical organization of the hippocampus (Lee et al., 2015; Favaro et al., 2009; Lee et al., 2000). In addition, there are some 'rescue' experiments for a select number of genes (eg., Lee et al., 2015; Spilker et al., 2016). Several examples are described below:

In the Jacob/Nsrm knockout model, hippocampal dysplasia is seen in hippocampal areas CA1 and CA3, characterized by reduced complexity of the synapto-dendritic cytoarchitecture, shorter dendrites and fewer branches (Spilker et al., 2016). Simplified dendritic trees and reduced synaptogenesis were also observed in hippocampal primary neurons cultured from these knock out mice relative to cultures from wild type mice. The protein product of Jacob/Nsrm regulates activity-dependent brain-derived neurotrophic factor (*Bdnf*) transcription. Lower BDNF levels were seen in area CA1 of knock out mice on postnatal day 10. The dysplasia seen in hippocampal neuronal cultures from knock mice could be reversed by BDNF supplementation if administered in early (2-4 days in vitro) but not later (15 days in vitro) in development.

Neuregulin-2 (*Nrg2*) contributes to synaptogenesis of the granule cell layer of the hippocampus. In hippocampal slice cultures, inducible microRNA targeting strategies have demonstrated early suppression of *Nrg2* (4 days in vitro) but not late suppression (7 days in vitro) reduced synaptogenesis of inhibitory neurons. On the other hand, late treatment impaired the dendritic outgrowth of excitatory synaptic connections. These effects could be eliminated with overexpression of *Nrg2* (Lee et al., 2015).

Many of the gene-regulated processes involved in hippocampal development are also present in the developing cortex. In models of prenatal hypothyroidism, altered expression patterns of many genes involved in neuronal migration and apoptosis are associated with disruptions in hippocampal organization and cytoarchitecture of the cerebral cortex (Pathak et al., 2011; Mohan et al., 2012; Lui et al., 2010). Structural changes in hippocampus and cerebral cortex are dependent on time of exposure (Auso et al., 2003; Berbel et al., 2010; Pathak et al., 2011) and can be reversed with TH supplementation (Mohan et al., 2012; Pathak et al., 2011; Berbel et al., 2010).

Dose-Response Evidence: Dose-response data is lacking for this KER. Papers that utilize knock-out and mutant models do not provide 'dose-response' information for gene-anatomy relationships. Studies in which genes and anatomy were reported following developmental hypothyroidism were single high-dose studies that focused on varying the developmental window of exposure, but not necessarily the dose.

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. Few studies exist that report both gene expression changes and structural changes in the hippocampus in same study to provide direct causative evidence for this KER. Lacking also is the specific suite of genes that are altered in the hippocampus at particular developmental times that are causal to the structural defects reported. For future research, it is critical to generate data in which the upstream KE is modulated in a 'dose-response' manner to better support the causative relationship.

Quantitative Understanding of the Linkage

There are no data on the quantitative linkages between gene expression changes and altered hippocampal anatomy

References

- Auso E, Lavado-Autric R, Cuevas E, Del Rey FE, Morreale De Escobar G, Berbel P (2004) A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticalogenesis alters neuronal migration. *Endocrinology* 145:4037-4047.
- Berbel P, Navarro D, Ausó E, Varea E, Rodríguez AE, Ballesta JJ, Salinas M, Flores E, Faura CC, de Escobar GM. Role of late maternal thyroid hormones in cerebral cortex development: an experimental model for human prematurity. *Cereb Cortex*. 2010 20(6):1462-75.
- Castrén ML, Castrén E. BDNF in fragile X syndrome. *Neuropharmacology*. 2014 76:729-36.
- Favaro R, Valotta M, Ferri AL, Latorre E, Mariani J, Giachino C, Lancini C, Tosetti V, Ottolenghi S, Taylor V, Nicolis SK. Hippocampal development and neural stem cell maintenance require Sox2-dependent regulation of Shh. *Nat Neurosci*. 2009 12(10):1248-56.
- Frotscher M. Role for Reelin in stabilizing cortical architecture. *Trends Neurosci*. 2010 Sep;33(9):407-14.
- Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. *Science*. 1992 Dec 18;258(5090):1903-10.
- Holzer G, Roux N, Laudet V. Evolution of ligands, receptors and metabolizing enzymes of thyroid signaling. *Mol Cell Endocrinol*. 2017 Mar 22. pii: S0303-7207(17)30191-0. doi: 10.1016/j.mce.2017.03.021. [Epub ahead of print]
- Khalaf-Nazzal R, Bruel-Jungerman E, Rio JP, Bureau J, Irinopoulou T, Sumia I, Roumegous A, Martin E, Olaso R, Parras C, Cifuentes-Diaz C, Francis F. Organelle and cellular abnormalities associated with hippocampal heterotopia in neonatal doublecortin knockout mice. *PLoS One*. 2013 Sep 2;8(9):e72622.

Lee KH, Lee H, Yang CH, Ko JS, Park CH, Woo RS, Kim JY, Sun W, Kim JH, Ho WK, Lee SH. Bidirectional Signaling of Neuregulin-2 Mediates Formation of GABAergic Synapses and Maturation of Glutamatergic Synapses in Newborn Granule Cells of Postnatal Hippocampus. *J Neurosci*. 2015 Dec 16;35(50):16479-93.

Lee SM, Tole S, Grove E, McMahon AP. A local Wnt-3a signal is required for development of the mammalian hippocampus. *Development*. 2000 Feb;127(3):457-67.

Lessmann V, Stroh-Kaffei S, Steinbrecher V, Edelmann E, Brigadski T, Kilb W, Luhmann HJ. The expression mechanism of the residual LTP in the CA1 region of BDNF k.o. mice is insensitive to NO synthase inhibition. *Brain Res*. 2011 May 19;1391:14-23.

Liu D, Teng W, Shan Z, Yu X, Gao Y, Wang S, Fan C, Wang H, Zhang H. The effect of maternal subclinical hypothyroidism during pregnancy on brain development in rat offspring. *Thyroid*. 2010 Aug;20(8):909-15.

Mohan V, Sinha RA, Pathak A, Rastogi L, Kumar P, Pal A, Godbole MM (2012) Maternal thyroid hormone deficiency affects the fetal neocorticalogenesis by reducing the proliferating pool, rate of neurogenesis and indirect neurogenesis. *Exp Neurol* 237:477-488.

Pathak A, Sinha RA, Mohan V, Mitra K, Godbole MM. 2011. Maternal thyroid hormone before the onset of fetal thyroid function regulates reelin and downstream signaling cascade affecting neocortical neuronal migration. *Cerebral cortex*. Jan;21:11-21.

Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol*. 2005 35(8-9):664-72.

Shin JH, Kim YN, Kim IY, Choi DH, Yi SS, Seong JK. Increased Cell Proliferations and Neurogenesis in the Hippocampal Dentate Gyrus of Ahnak Deficient Mice. *Neurochem Res*. 2015 Jul;40(7):1457-62.

Skucas VA, Mathews IB, Yang J, Cheng Q, Treister A, Duffy AM, Verkman AS, Hempstead BL, Wood MA, Binder DK, Scharfman HE. Impairment of select forms of spatial memory and neurotrophin-dependent synaptic plasticity by deletion of glial aquaporin-4. *J Neurosci*. 2011 Apr 27;31(17):6392-7.

Spilker C, Nullmeier S, Grochowska KM, Schumacher A, Butnaru I, Macharadze T, Gomes GM, Yuanxiang P, Bayraktar G, Rodenstein C, Geiseler C, Kolodziej A, Lopez-Rojas J, Montag D, Angenstein F, Bär J, D'Hanis W, Roskoden T, Mikhaylova M, Budinger E, Ohi FW, Stork O, Zenclussen AC, Karpova A, Schwegler H, Kreutz MR. A Jacob/Nsmf Gene Knockout Results in Hippocampal Dysplasia and Impaired BDNF Signaling in Dendritogenesis. *PLoS Genet*. 2016 Mar 15;12(3):e1005907

Strange BA, Witter MP, Lein ES, Moser EI. Functional organization of the hippocampal longitudinal axis. *Nat Rev Neurosci*. 2014 Oct;15(10):655-69.

Takei Y, Kikkawa YS, Atapour N, Hensch TK, Hirokawa N. Defects in Synaptic Plasticity, Reduced NMDA-Receptor Transport, and Instability of Postsynaptic Density Proteins in Mice Lacking Microtubule-Associated Protein 1A. *J Neurosci*. 2015 Nov 25;35(47):15539-54

Hippocampal anatomy, Altered leads to Hippocampal Physiology, Altered (<https://aopwiki.org/relationships/749>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	directly leads to	Moderate	Weak
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	directly leads to	Moderate	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human	Homo sapiens	Not Specified	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The majority of data in support of this KER is from rodent models. The evolutionary conservation of hippocampal anatomy in mammals, birds, and reptiles (see Hevner, 2016; Streidter, 2015) suggests, with some uncertainty, that this KER is also applicable to multiple species.

How Does This Key Event Relationship Work

The hippocampus is a highly integrated and organized communication and information processing network with millions of interconnections among its constitutive neurons (see Andersen et al, 2006). The neuronal spine is the primary site of action for synaptic interface between neurons. Although difficult to measure due to their small size, large number and variable shapes, changes in the frequency and structure of dendritic spines of hippocampal neurons has dramatic effects on synaptic physiology and plasticity (Harris et al., 1992). Anatomical integrity at a more macro-level is also essential for physiological function. The connectivity of axons emanating from one set of cells that synapse on the dendrites of the receiving cells must be intact for effective communication between neurons to be possible. Synaptogenesis is a critical step for neurons to be integrated into neural networks during development. Changes in the placement of cells within the network due to delays or alterations in neuronal migration, the absence of a full proliferation of dendritic arbors and spine upon which synaptic contacts are made, and the lagging of transmission of electrical impulses due to insufficient myelination will independently and cumulatively impair synaptic function.

Weight of Evidence

The weight of evidence supporting the relationship between structural abnormalities in brain induced and altered synaptic function is moderate. There is no doubt that altered structure can lead to altered function. Many examples from knock out models, genetic mutations, prenatal alcohol, nutritional deficits demonstrate a correlative link between altered structure and impaired synaptic function within the hippocampus (Gil-Mohapel et al., 2010; Berman and Hannigan, 2000; Grant et al., 1992; Palop et al., 2010; Ieraci and Herrera, 2007). However, the scientific understanding of the causative and quantitative relationship between the two KEs is incomplete.

Biological Plausibility

The biological plausibility of alterations in hippocampal structure having an impact on synaptic function and plasticity in brain is strong. Because synaptic transmission in the hippocampus relies on the integrity of contacts and the reliability of electrical and chemical transmission between pre- and post-synaptic neurons, it is well accepted that interference on the anatomical levels will very much impact the functional output on the neurophysiological level (Knowles, 1992; Schultz and Engelhardt, 2014).

Empirical Support for Linkage

Empirical support for this KER is rated as moderate. Numerous examples of a direct linkage between hippocampal anatomy and hippocampal physiology are evident in knock out or transgenic mouse models (eg., Lessman et al., 2011). Other data is derived from nutritional deficiencies, alcohol exposure, and hippocampal slice culture models (Berman and Hannigan, 2000; Ieraci and Herrera, 2007; Gilbert et al., 2016). Although several examples are evident to demonstrate direct linkages between alterations in hippocampal anatomy and disruptions in hippocampal physiology, there is not a mechanism, anatomical insult, or signature pattern of synaptic impairment that accompanies each of these treatments.

Below are a few examples where direct linkages have been reported and they serve to bear witness to a direct relationship between altered hippocampal anatomy and altered hippocampal physiology.

Fyn is a tyrosine kinase gene involved in synaptic plasticity. Mutations of this gene lead to a lack of expression during development and result in an increase in the number of neurons in the dentate gyrus and CA subfields of the hippocampus. Fyn mutant mice also exhibited impairments in long term potentiation in hippocampal CA1 whereas two other forms of short-term plasticity remained intact (Grant et al., 1992).

Neuregulin-2 (NRG2) is a growth factor and is highly expressed in the hippocampal dentate where it contributes to synaptogenesis of newborn granule cells. In hippocampal slice cultures, inducible microRNA targeting strategies have demonstrated suppression of NRG2 reduced synaptogenesis of inhibitory neurons and impaired dendritic outgrowth and maturation of glutamatergic synapses. These anatomical alterations were accompanied by reductions in the amplitude of excitatory synaptic currents. The magnitude of the impairment was dependent on the timing of the infection and could be eliminated with overexpression of NRG2 in this in vitro model (Lee et al., 2015).

Brain-derived neurotrophic factor (BDNF) activation of CREB-activated gene expression plays a documented role in hippocampal synaptogenesis, dendrite formation, and synaptic plasticity in the developing and adult nervous system (Lessmann et al., 2011; Panja and Bramham, 2014). Jacob is a protein that translocates to the nucleus upon activation of BDNF-dependent pathways and is involved in both neuronal plasticity and neurodegeneration. Hippocampal neurons in culture derived from Jacob/Nsmf knockout mice exhibit shorter neurite length, reduced branching, and a few synaptic contacts. This effect was specific to hippocampal neurons, as cortical cells derived from the same animals did not display these abnormalities. In vivo, these animals exhibited a reduction of dendritic complexity of CA1 neurons, lower number of branches, decreased spine density. Deficits in synaptic plasticity in the form of LTP accompanied these structural impairments (Spilker et al., 2016).

In Alzheimer's Disease, amyloid- β protein accumulates in the hippocampus and leads to the formation of amyloid plaques, neuritic dystrophy and aberrant sprouting of axon terminals of the hippocampus. In a developmental germ-line knockout mouse model, high levels of amyloid- β induced aberrant neuronal network excitability and altered innervation of inhibitory interneurons. Deficits in hippocampal plasticity were seen in the dentate

gyrus without change in basal levels of synaptic transmission. In contrast, in area CA1, synaptic transmission was impaired while measures of synaptic plasticity remained intact (Palop et al., 2007).

Other evidence for a direct linkage between hippocampal anatomy and hippocampal physiology comes from the area of adult neurogenesis. The neurogenesis process refers to the acquisition of new neurons on the hippocampus of the adult brain and is associated with enhanced hippocampal synaptic function and learning ability (Deng et al., 2010). Manipulations such as caloric restriction, exercise and hormones can enhance neurogenesis and increase synaptic transmission and plasticity (Kapoor et al., 2015; Trivino-Paredes et al., 2016; Deng et al., 2010). A reciprocal relationship also exists whereby increases in hippocampal neural activity serves to increase neurogenesis (Bruehl-Jungerman et al., 2007; Bruehl-Jungerman et al., 2009; Kameda et al., 2012). Manipulations that decrease hippocampal neurogenesis including exposure to antidepressants, hormone disruption, stress, and alcohol are associated with impaired synaptic function (Herrera et al., 2003; Saxe et al., 2006; Gilbert et al., 2016; Montero-Pedrazuela et al., 2006; Gil-Mohapel et al., 2006; Sofroniew et al., 2006).

Temporal Evidence: The temporal nature of this KER is developmental (Seed et al., 2005). This has been demonstrated in multiple studies. A few examples detailed above defined critical periods for the manipulation that alters the structural development of the hippocampus that persists to adulthood to disrupt the synaptic physiology measured in the hippocampus in adulthood (Lee et al., 2015; Grant et al., 1992). A more limited number of 'rescue' experiments have been reported. Lee et al (2015), using an in vitro model, demonstrated impaired synaptogenesis that was dependent on the timing of the infection and could be eliminated with overexpression of NRG2. In Spliker et al (2016), BDNF application rescued the morphological deficits in hippocampal pyramidal neurons from Jacob/Nsmf mice.

Dose-Response Evidence: Dose-response data is lacking for this KER. For future research, it is critical to generate data in which the upstream KE is modulated in a 'dose-response' manner to better support the causative relationship.

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are uncertainties. Although several examples are evident to demonstrate direct linkages between alterations in hippocampal anatomy and disruptions in hippocampal physiology, there is not a common cellular mechanism, anatomical insult, or signature pattern of synaptic impairment that defines a common anatomically driven physiological phenotype. In addition, it is also known that there is an interaction between physiological and anatomical development, where anatomy develops first, and can be 'reshaped' by the ongoing maturation of physiological function (e.g., Kutsarova et al., 2017)

Quantitative Understanding of the Linkage

Little information exists to develop quantitative relationships between the KEs in this KER. Papers that utilize knock-out and mutant models have not provided 'dose-response' information for anatomy-physiology relationships.

References

- Andersen, P., Morris, R., Amaral, D., Bliss, T., O'Keefe, J. (Editors). The Hippocampus Book. Oxford University Press, 2006. ISBN: 9780195100273
- Berman RF, Hannigan JH. Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. *Hippocampus*. 2000;10(1):94-110.
- Bruehl-Jungerman E, Davis S, Laroche S (2007) Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 13:492-505.
- Bruehl-Jungerman E, Veyrac A, Dufour F, Horwood J, Laroche S, Davis S (2009) Inhibition of PI3K-Akt signaling blocks exercise-mediated enhancement of adult neurogenesis and synaptic plasticity in the dentate gyrus. *PLoS One* 4:e7901.
- Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci* 11:339-350.
- Gil-Mohapel J, Boehme F, Kainer L, Christie BR. Hippocampal cell loss and neurogenesis after fetal alcohol exposure: insights from different rodent models. *Brain Res Rev*. 2010 Sep 24;64(2):283-303.
- Gilbert ME, Goodman JH, Gomez J, Johnstone AF, Ramos RL. Adult hippocampal neurogenesis is impaired by transient and moderate developmental thyroid hormone disruption. *Neurotoxicology*. 2016 Dec 31;59:9-21.
- Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science*. 1992 Dec 18;258(5090):1903-10.
- Harris KM, Teyler TJ. Developmental onset of long-term potentiation in area CA1 of the rat hippocampus. *J Physiol*. 1984 Jan;346:27-48.
- Herrera DG, Yague AG, Johnsen-Soriano S, Bosch-Morell F, Collado-Morente L, Muriach M, Romero FJ, Garcia-Verdugo JM (2003) Selective impairment of hippocampal neurogenesis by chronic alcoholism: protective effects of an antioxidant. *Proc Natl Acad Sci U S A* 100:7919-7924.
- Hevner RF. Evolution of the mammalian dentate gyrus. *J Comp Neurol*. 2016 524(3):578-94.
- Ieraci A, Herrera DG. Single alcohol exposure in early life damages hippocampal stem/progenitor cells and reduces adult neurogenesis. *Neurobiol Dis*. 2007 Jun;26(3):597-605.
- Kameda M, Taylor CJ, Walker TL, Black DM, Abraham WC, Bartlett PF (2012) Activation of latent precursors in the hippocampus is dependent on long-term potentiation. *Transl Psychiatry* 2:e72.
- Kapoor R, Fanibunda SE, Desouza LA, Guha SK, Vaidya VA (2015) Perspectives on thyroid hormone action in adult neurogenesis. *J Neurochem* 133:599-616.
- Knowles WD. Normal anatomy and neurophysiology of the hippocampal formation. *J Clin Neurophysiol*. 1992 Apr;9(2):252-63.
- Kutsarova E, Munz M, Ruthazer ES. Rules for Shaping Neural Connections in the Developing Brain. *Front Neural Circuits*. 2017. 10:111. doi: 10.3389/fncir.2016.00111.

Lee KH, Lee H, Yang CH, Ko JS, Park CH, Woo RS, Kim JY, Sun W, Kim JH, Ho WK, Lee SH. Bidirectional Signaling of Neuregulin-2 Mediates Formation of GABAergic Synapses and Maturation of Glutamatergic Synapses in Newborn Granule Cells of Postnatal Hippocampus. *J Neurosci*. 2015 Dec 16;35(50):16479-93.

Lessmann V, Stroh-Kaffei S, Steinbrecher V, Edelmann E, Brigadski T, Kilb W, Luhmann HJ. The expression mechanism of the residual LTP in the CA1 region of BDNF k.o. mice is insensitive to NO synthase inhibition. *Brain Res*. 2011. 1391:14-23.

Montero-Pedrazuela A, Venero C, Lavado-Autric R, Fernandez-Lamo I, Garcia-Verdugo JM, Bernal J, Guadano-Ferraz A (2006) Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior. *Mol Psychiatry* 11:361-371.

Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L. Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron*. 2007 Sep 6;55(5):697-711.

Panja, D. and C. R. Bramham (2014). "BDNF mechanisms in late LTP formation: A synthesis and breakdown." *Neuropharmacology* 76 Pt C: 664-676. Schultz C, Engelhardt M. Anatomy of the hippocampal formation. *Front Neurol Neurosci*. 2014. 34:6-17

Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, Garcia AD,

Sofroniew MV, Kandel ER, Santarelli L, Hen R, Drew MR. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Natl Acad Sci U S A*. 2006 Nov 14;103(46):17501-6.

Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol*. 2005 35:664-72.

Schultz C, Engelhardt M. Anatomy of the hippocampal formation. *Front Neurol Neurosci*. 2014. 4:6-17.

Sofroniew et al., 2006

Spilker C, Nullmeier S, Grochowska KM, Schumacher A, Butnaru I, Macharadze T, Gomes GM, Yuanxiang P, Bayraktar G, Rodenstein C, Geiseler C, Kolodziej A, Lopez-Rojas J, Montag D, Angenstein F, Bär J, D'Hanis W, Roskoden T, Mikhaylova M, Budinger E, Ohl FW, Stork O, Zenclussen AC, Karpova A, Schwegler H, Kreutz MR. A Jacob/Nsmf Gene Knockout Results in Hippocampal Dysplasia and Impaired BDNF Signaling in Dendritogenesis. *PLoS Genet*. 2016 Mar 15;12(3):e1005907

Striedter GF. Evolution of the hippocampus in reptiles and birds. *J Comp Neurol*. 2016 Feb 15;524(3):496-517

Triviño-Paredes J, Patten AR, Gil-Mohapel J, Christie BR. The effects of hormones and physical exercise on hippocampal structural plasticity. *Front Neuroendocrinol*. 2016. 41:23-43.

Hippocampal Physiology, Altered leads to Cognitive Function, Decreased (<https://aopwiki.org/relationships/748>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	directly leads to	Strong	Moderate
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	directly leads to	Moderate	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
humans	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The majority of data in support of this KER is from rodent models. The evolutionary conservation of the role of the hippocampus in spatial cognitive functions suggests, with some uncertainty, that this KER is also applicable to other mammalian species.

How Does This Key Event Relationship Work

It is a well-accepted assertion that hippocampal synaptic integrity and plasticity are essential for spatial information processing in animals and spatial and episodic memory in humans (Burgess, 2002; Martin et al., 2000; Sweatt, 2016). A large number of studies with a variety of techniques and approaches have linked hippocampal functional deficits to decreased spatial ability, context learning, and fear learning. Study of human disease states and conditions where hippocampal function is impaired (i.e., brain trauma, Alzheimer's disease, temporal lobe epilepsy, Down's Syndrome), and imaging studies of hippocampal activation during memory challenge, makes it irrefutable that the hippocampus is essential for specific types of cognitive abilities. Decades of animal research has reinforced this assertion.

Weight of Evidence

The weight of evidence for proper hippocampal function and episodic memory in humans and the animal analogue, spatial and fear based context learning, is strong. Seminal studies over the past 60 years firmly established the cellular basis of behavior with synaptic plasticity (LTP and LTD). And recent work has provided details on the local hippocampal circuitry needed for memory formation and behavioral change (Sweatt, 2016). In humans, virtual reality experiments in large-scale spatial contexts demonstrate the convergence of spatial memory performance in normal patients with fMRI of the hippocampus clearly demonstrating the essentiality of hippocampal function to spatial learning (Burgess, 2002). This assertion is consistent with a wealth of animal data on hippocampal learning and memory. In rodent models, functional impairment of the hippocampus assessed using electrophysiological techniques is correlated with deficits in spatial memory typically assessed using mazes, and memory for context often assessed in fear-based learning paradigms (O'Keefe and Nadel, 1978; Clark et al., 2000; Squire, 2004; Eichenbaum, 2000; Panja and Bramham, 2014).

Biological Plausibility

The biological plausibility of the KER is rated as strong. It is well accepted that the normal hippocampal function is critical for the acquisition and memory of context and spatially mediated tasks in rodents and humans (Sweatt, 2016).

Empirical Support for Linkage

Empirical support for this KER is strong. The requisite of hippocampal integrity to optimal visuo-spatial context learning (i.e., episodic memory) in humans and spatial learning in rodents is well documented. In vivo recording in conscious behaving animals has demonstrated activity-dependent neural changes taking place in the hippocampus during spatial learning (Gruart and Delgado-Garcia, 2007). Impairments in hippocampal function induced by drugs, chemicals, lesions, mutant or knock out models that cause changes in synaptic transmission, plasticity, and hippocampal network activity, are coincident with deficits in spatial and context-based fear learning (O'Keefe and Nadel, 1978; Bannerman et al., 2014; Lynch, 2004; Verret et al., 2012). Similarly, treatments found to enhance or facilitate hippocampal synaptic transmission and plasticity are associated with improved learning and memory (Deng et al., 2010; Novkovic et al., 2015; Andrade et al., 2015; Trivino-Paredes et al., 2016). For example, n-methyl-D-aspartate (NMDA)-mediated glutamatergic synaptic transmission is essential for the induction of hippocampal synaptic plasticity in the form of LTP. Blockade of this form of plasticity by selective NMDA-receptors blockers impairs LTP and hippocampal tests of learning and memory (reviewed in Sweatt, 2016). Perturbation of hippocampal plasticity and impaired spatial learning have been reported in adult offspring following prenatal ethanol exposure (An and Zhang, 2015). The fyn mutant mouse (fyn is a tyrosine kinase pathway) displays impairments in hippocampal synaptic transmission and plasticity, as well as spatial learning deficits (Grant et al., 1992). Brain-derived neurotrophic factor (BDNF) knock out animals exhibit synaptic plasticity deficits and learning impairments (Aarse et al., 2016; Panja and Bramham, 2014). In the Jacob/Nfsm model which also exhibits pronounced alterations in BDNF-mediated signaling, hippocampal synaptic transmission and plasticity impairments were accompanied by deficits in contextual fear conditioning and novel location recognition tasks (Spilker et al., 2016). Finally, in rodent models of developmental TH insufficiency, impairments in hippocampal synaptic transmission and plasticity are coincident with deficits in learning tasks that require the hippocampus (Opazo et al., 2008; Gilbert and Sui, 2006; Gilbert, 2011; Gilbert et al., 2016).

In humans, hippocampal physiology assessed using neuroimaging reveals activation of hippocampus upon engagement in spatial learning and episodic memory providing a direct linkage of these two specific KEs (Burgess, 2002). In fMRI studies of congenitally hypothyroid children, or children born to women with altered thyroid function during pregnancy, changes in hippocampal activity patterns during memory encoding and retention were observed and associated with memory impairments (Wheeler et al., 2012; 2015; Willoughby et al., 2013; 2014).

Temporal Evidence: The temporal nature of this KER is developmental (Seed et al., 2005). This has been demonstrated in multiple studies. It is well-recognized that there are critical developmental windows for disruption of the functional development of the hippocampus and the integrity of this structure is essential for later development of spatial ability, context learning, and fear learning. A wealth of studies have shown correlation between hippocampal LTP and spatial learning performance, as well as the role of glutamatergic synaptic transmission and BDNF-mediated signaling pathways in these processes (Bramham, 2007; Andero et al., 2014; Morris et al., 1986; Sweatt, 2016; Migaud et al., 1998). Although studies on reversibility are rare, deficits in hippocampal synaptic transmission and plasticity in slices from BDNF knockout animals can be rescued with recombinant BDNF (Patterson et al., 1996).

Dose-Response Evidence: Limited dose-response information is available. Studies have investigated dose-dependency of both electrophysiological and behavioral impairments in animals suffering from developmental TH insufficiency (e.g., Gilbert and Sui, 2006; Gilbert, 2011; Gilbert et al., 2016).

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. It is a widely-held assertion that synaptic transmission and plasticity in the hippocampus underlie spatial learning (Martin et al., 2000; Gruart and Delgado-García, 2007; Bramham, 2007). However, the causative relationship of which specific alterations in synaptic function are associated with specific cognitive deficits is difficult to ascertain given the many forms of learning and memory, and the complexity of synaptic interactions in even the simplest brain circuit.

Quantitative Understanding of the Linkage

There are many forms of synaptic plasticity and numerous ways in which physiological function of neural circuits can be assessed. Similarly, there are many forms of learning and memory and multiple tasks and specifics associated with these tasks that vary from laboratory to laboratory. An emerging field of computational cognitive neuroscience lies at the intersection of computational neuroscience, machine learning and neural network theory. These computational and theoretical frameworks support the participation of the hippocampal synaptic transmission and plasticity in learning and memory in animals and humans (for review see: Ashby and Helie, 2012).

References

- Aarse J, Herlitze S, Manahan-Vaughan D. The requirement of BDNF for hippocampal synaptic plasticity is experience-dependent. *Hippocampus*. 2016 Jun;26(6):739-51.
- An L, Zhang T. Prenatal ethanol exposure impairs spatial cognition and synaptic plasticity in female rats. *Alcohol*. 2015 Sep;49(6):581-8.
- Andero R, Choi DC, Ressler KJ. BDNF-TrkB receptor regulation of distributed adult neural plasticity, memory formation, and psychiatric disorders. *Prog Mol Biol Transl Sci*. 2014. 122:169-92.
- Andrade-Talavera Y, Benito I, Casañas JJ, Rodríguez-Moreno A, Montesinos ML. Rapamycin restores BDNF-LTP and the persistence of long-term memory in a model of Down's syndrome. *Neurobiol Dis*. 2015. 82:516-25
- Ashby FG, Helie S. The Neurodynamics of Cognition: A Tutorial on Computational Cognitive Neuroscience. *J Math Psychol*. 2011 Aug 1;55(4):273-289.
- Bannerman DM, Sprengel R, Sanderson DJ, McHugh SB, Rawlins JNP, Monyer H, Seeburg PH (2014) Hippocampal synaptic plasticity, spatial memory and anxiety. *Nat Rev Neurosci* 15:181-192.
- Bramham CR. Control of synaptic consolidation in the dentate gyrus: mechanisms, functions, and therapeutic implications. *Prog Brain Res*. 2007. 163:453-71.
- Burgess N (2002) The hippocampus, space, and viewpoints in episodic memory. *Q J Exp Psychol A* 55:1057-1080. Clark RE, Zola SM, Squire LR. Impaired recognition memory in rats after damage to the hippocampus. *J Neurosci*. 2000 Dec 1;20(23):8853-60.
- Deng W, Aimone JB, Gage FH (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory *Nat Rev Neurosci* 11:339-350.
- Gilbert ME (2011) Impact of low-level thyroid hormone disruption induced by propylthiouracil on brain development and function. *Toxicol Sci* 124:432-445.
- Gilbert ME, Sanchez-Huerta K, Wood C (2016) Mild Thyroid Hormone Insufficiency During Development Compromises Activity-Dependent Neuroplasticity in the Hippocampus of Adult Male Rats. *Endocrinology* 157:774-787.
- Gilbert ME, Sui L (2006) Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. *Brain Res* 1069:10-22.
- Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science*. 1992 Dec 18;258(5090):1903-10.
- Gruart A, Delgado-García JM. Activity-dependent changes of the hippocampal CA3-CA1 synapse during the acquisition of associative learning in conscious mice. *Genes Brain Behav*. 2007 Jun;6 Suppl 1:24-31.
- Lynch, M.A. (2004). Long-Term Potentiation and Memory. *Physiological Reviews*. 84:87-136.
- Martin SJ, Grimwood PD, Morris RG. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci*. 2000. 23:649-711.
- Migaud M, Charlesworth P, Dempster M, Webster LC, Watabe AM, Makhinson M, He Y, Ramsay MF, Morris RG, Morrison JH, O'Dell TJ, Grant SG. Enhanced long-term potentiation and impaired learning in mice with mutant postsynaptic density-95 protein. *Nature*. 1998 Dec 3;396(6710):433-9.
- Morris RG, Frey U. Hippocampal synaptic plasticity: role in spatial learning or the automatic recording of attended experience? *Philos Trans R Soc Lond B Biol Sci*. 1997 Oct 29;352(1360):1489-503. Review
- Novkovic T, Mittmann T, Manahan-Vaughan D. BDNF contributes to the facilitation of hippocampal synaptic plasticity and learning enabled by environmental enrichment. *Hippocampus*. 2015 Jan;25(1):1-15.
- O'Keefe, J. and Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. Oxford: Oxford University Press.
- Opazo MC, Gianini A, Pancetti F, Azkcona G, Alarcón L, Lizana R, Noches V, Gonzalez PA, Marassi MP, Mora S, Rosenthal D, Eugenin E, Naranjo D, Bueno SM, Kalergis AM, Riedel CA (2008), Maternal hypothyroxinemia impairs spatial learning and synaptic nature and function in the offspring. *Endocrinology* 149:5097-5106

Panja, D. and C. R. Bramham (2014). "BDNF mechanisms in late LTP formation: A synthesis and breakdown." *Neuropharmacology* 76 Pt C: 664-676.

Schultz C, Engelhardt M, Anatomy of the hippocampal formation. *Front Neurol Neurosci*. 2014. 34:6-17

Patterson SL, Abel T, Deuel TA, Martin KC, Rose JC, Kandel ER. Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron*. 1996 Jun;16(6):1137-45.

Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol*. 2005 35:664-72.

Spilker C, Nullmeier S, Grochowska KM, Schumacher A, Butnaru I, Macharadze T, Gomes GM, Yuanxiang P, Bayraktar G, Rodenstein C, Geiseler C, Kolodziej A, Lopez-Rojas J, Montag D, Angenstein F, Bär J, D'Hanis W, Roskoden T, Mikhaylova M, Budinger E, Ohl FW, Stork O, Zenclussen AC, Karpova A, Schwegler H, Kreutz MR. A Jacob/Nsmf Gene Knockout Results in Hippocampal Dysplasia and Impaired BDNF Signaling in Dendritogenesis. *PLoS Genet*. 2016 Mar 15;12(3):e1005907 Squire LR 2004. Memory systems of the brain: A brief history and current perspective. *Neurobiology of Learning and Memory*, 82: 171-177

Sweatt JD. Neural plasticity and behavior - sixty years of conceptual advances. *J Neurochem*. 2016 Oct;139 Suppl 2:179-199. doi: 10.1111/jnc.13580. Review. PubMed PMID: 26875778.

Triviño-Paredes J, Patten AR, Gil-Mohapel J, Christie BR. The effects of hormones and physical exercise on hippocampal structural plasticity. *Front Neuroendocrinol*. 2016. 41:23-43.

Verret L, Mann EO, Hang GB, Barth AM, Cobos I, Ho K, Devidze N, Masliah E, Kreitzer AC, Mody I, Mucke L, Palop JJ. Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell*. 2012 Apr 27;149(3):708-21.

Wheeler SM, McAndrews MP, Sheard ED, Rovet J (2012) Visuospatial associative memory and hippocampal functioning in congenital hypothyroidism. *J Int Neuropsychol Soc* 18:49-56.

Wheeler SM, McLelland VC, Sheard E, McAndrews MP, Rovet JF (2015) Hippocampal Functioning and Verbal Associative Memory in Adolescents with Congenital Hypothyroidism. *Front Endocrinol (Lausanne)* 6:163.

Willoughby KA, McAndrews MP, Rovet JF (2014) Effects of maternal hypothyroidism on offspring hippocampus and memory. *Thyroid* 24:576-584.

Willoughby KA, McAndrews MP, Rovet J (2013) Effects of early thyroid hormone deficiency on children's autobiographical memory performance. *J Int Neuropsychol Soc* 19:419-429.

T4 in serum, Decreased leads to Hippocampal gene expression, Altered (<https://aopwiki.org/relationships/1387>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	indirectly leads to	Strong	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong

Sex	Evidence
Female	Strong

Most of the data available has come from rodent models.

How Does This Key Event Relationship Work

Many of the physiological effects of thyroid hormones (THs) are mediated through regulation of gene expression by zinc finger nuclear receptor proteins that are encoded by thyroid hormone genes alpha (Thra) and beta (Thrb). It is widely accepted that TH regulates gene transcription during brain development (Bernal, 2007; Anderson et al., 2003). The sole source of TH to the brain is from the circulating levels of the prohormone, thyroxine (T4). Once taken up from the serum to reach the brain, T4 is converted to triiodothyronine (T3) which binds to TH nuclear receptors (TR α and TR β). On binding, and in the presence of regulatory cofactors, transcription of certain genes is either up- or down-regulated (Oppenheimer, 1983). However, only a small number of genes have been shown to be directly influenced by TH receptor binding, and of these, most are transcription factors (Quignodon et al., 2008; Thompson and Potter, 2000; Horn and Heuer, 2010). In this manner, THs do influence a wide variety of genes.

Weight of Evidence

The weight of evidence for this indirect relationship is strong. It is well established that serum TH is the primary source of brain T4 from which neuronal T3, the active hormone, is locally generated and presented to the receptors in the nucleus of neurons to control gene transcription.

Biological Plausibility

The biological plausibility of this KER is rated as strong. This is consistent with the known biology of the relationship between serum TH concentrations and brain TH concentrations, and the known action of TH to mediate gene transcription in brain and many other tissues

Empirical Support for Linkage

The empirical support for this KER is strong. A global transcriptome analysis of primary cerebrocortical cells was recently published in which a number of genes regulated by T3 were identified (Gil-Ibanez et al., 2015). Although the bulk of literature in which serum TH reductions have been associated with gene expression changes in the brain have been focused on the cortex, several reports in hippocampus are available. Genes directly regulated by TH include the transcription factors Hr and Klf9 (Bteb) (Thompson and Potter, 2000; Cayrou et al., 2002; Denver and Williamson, 2009). The expression of a number of genes modulated by TH are expressed in the hippocampus. Many of genes that regulate processes involved in hippocampal development are also present in the developing cortex. Thus, Table 1 lists TH responsive genes whose expression in either area are altered by TH reduction. This list is not meant to be exhaustive, just exemplary.

Gene Name	Tissue	Model	Age	Reference
FETAL				
Klf9 (Bteb)	Rat- Cortex	MMI+CLO4	Fetus-GD17	Dong et al., 2015
Nurr1	Mouse-cortex	Thyroidectomy, MMI + CIO4	Fetal GD17; PN90	Navarro et al., 2014
Bdnf	Rat- Cortex	MMI	Fetus GD14-18	Pathak et al., 2011
Trkb	Rat- Cortex	MMI	Fetus GD14-18	Pathak et al., 2011
MCT8	Rat- Cortex	MMI	Fetus GD14-18	Mohan et al, 2012
Dio2	Rat -Cortex	MMI	Fetus GD14-18	Mohan et al, 2012
CyclinD1	Rat- Cortex	MMI	Fetus GD14-18	Mohan et al, 2012
Cyclin D2	Rat- Cortex	MMi	Fetus GD14-18	Mohan et al, 2012
Pax6	Rat- Cortex	MMI	Fetus GD14	Mohan et al, 2012
Hr	Mouse- cortex	MMI + CIO4	Fetal GD17	Morte et al., 2010
Sema7a	Mouse- Cortex	MMI + CIO4	Fetal GD17	Morte et al., 2010
RC3 (Neurogranin)	Rat- Hippocampus, Cortex	MMI	Fetus-GD16	Dowling and Zoeller, 2001

Camk4	Mouse-cortex	Thyroidectomy, MMI + CIO4	Fetal GD17; PN90	Morte et al., 2010; Navarro et al., 2014
NEONATAL				
Klf9 (Bteb)	Rat, Mouse-Cortex	PTU	Neonate-PN14	Royland et al., 2008; Bastian et al., 2012; Denver and Williamson, 2009; Denver et al., 1999
Hr	Rat- Cortex, Hippocampus, Cerebellum	PTU, MMI	Neonate-PN14	Royland et al., 2008; Bastian et al., 2012; Thompson and Potter, 2000; Morte et al., 2010
Parv	Rat- cortex	PTU	Neonate-PN14/21	Royland et al., 2008; Bastian et al., 2012; 2014, Shiraki et al., 2014
Ngf	Rat- Hippocampus, cortex	PTU	Neonate-PN14, PN90	Royland et al., 2008; Bastian et al., 2012; Gilbert et al., 2016
Agt	Rat- Cortex	PTU	Neonate, PN14	Royland et al., 2008; Bastian et al., 2012; 2014
Col11a2	Rat- Cortex	PTU	Neonate, PN14	Royland et al., 2008
Itih2	Rat- Cortex	PTU	Neonate, PN14	Royland et al., 2008
Sema7a	Rat- Cortex	PTU	Neonate-PN14	Royland et al., 2008
Reelin	Rat- Hippocampus, cortex, cerebellum	Thyroidectomy, PTU		Alvarez-Dolado et al. 1999; Shiraki et al., 2014
Mbp	Rat- Hippocampus, Cortex, Cerebellum	PTU, MMI	Neonate-PN14/21	Ibarrola et al., 1997; Royland et al., 2008; Bastian et al., 2012; 2014, Shiraki et al, 2014
Plp2	Hippocampus, Cortex	PTU, MMI		Royland et al., 2008; Bastian et al., 2012
Camk4	Rat/Mouse-cortex	PTU	Neonate-PN14	Royland et al., 2008
RC3 (Neurogranin)	Rat-hippocampus	Thyroidectomy + MMI	Neonate-PN5, PN21	Iniquez et al., 1993; Dong et al., 2010

Temporal Evidence: The temporal nature of this KER is developmental (Seed et al., 2005). It is a well-recognized fact that there are critical developmental windows for disruption of the serum THs that result in altered gene expression in the developing brain, including the hippocampus. Rescue experiments for this endpoint of gene expression in hypothyroid models are limited. In one, a combination of T3 and T4 treatment delivered on the last day of a 3-day gestational MMI hypothyroxinemia mouse model altered the pattern of gene expression observed in the cortex of offspring relative to euthyroid controls and MMI alone (Dong et al., 2015).

Dose-Response Evidence: There are a limited number of studies that have reported on the dose-dependent nature of the correlation between serum THs and hippocampal gene expression (Bastian et al., 2012; 2014; Royland et al., 2008).

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. It is widely accepted that changes in serum THs will result in alterations in hippocampal gene expression. Several different animal models have been used to manipulate serum TH concentrations that also measure gene expression changes. Varying windows of exposure to TH disruption and developmental sample time and region examined have also varied across studies. However, dose-response data is lacking. Most investigations of hippocampal gene expression have employed treatments that induce severe hormone reductions induced by PTU or MMI, or by thyroidectomy. In addition, few reports have studied the genes in the hippocampus, the cortex being more accessible in young animals. Finally, when the hippocampus is the target, different genes at different ages are reported, making it difficult to compare findings.

Quantitative Understanding of the Linkage

There are no quantitative models that predict the degree of serum TH reduction that is required to alter hippocampal gene transcription. Most investigations for hippocampus have been conducted in the neonate after severe hormone reductions. Only two laboratories have reported dose-dependent effects at less than maximal hormone depletion (Bastian et al., 2012; 2014; Royland et al., 2008). In addition, there is very little known about whether compensatory processes are available in the developing hippocampus that may modulate the impact of serum levels on hippocampal gene transcription. These available data suggest that a 50% decrement in serum T4 in the pup, without a change in T3, is sufficient to observe changes in hippocampal gene expression. This is similar to finding for loss of hearing function in rats following postnatal chemical-induced hypothyroxinemia (Crofton, 2004)

References

- Alvarez-Dolado M, Ruiz M, Del Rio JA, Alcantara S, Burgaya F, Sheldon M, Nakajima K, Bernal J, Howell BW, Curran T, Soriano E, Munoz A (1999) Thyroid hormone regulates *reelin* and *dab1* expression during brain development. *J Neurosci* 19:6979-6993.
- Anderson GW, Schoonover CM, Jones SA (2003) Control of thyroid hormone action in the developing rat brain. *Thyroid* 13:1039-56.
- Bastian TW, Anderson JA, Fretham SJ, Prohaska JR, Georgieff MK, Anderson GW (2012). Fetal and neonatal iron deficiency reduces thyroid hormone-responsive gene mRNA levels in the neonatal rat hippocampus and cerebral cortex. *Endocrinology* 153:5668-5680.
- Bastian TW, Prohaska JR, Georgieff MK, Anderson GW. 2014. Fetal and neonatal iron deficiency exacerbates mild thyroid hormone insufficiency effects on male thyroid hormone levels and brain thyroid hormone-responsive gene expression. *Endocrinology*. 155:1157-1167.
- Bernal J. 2007. Thyroid hormone receptors in brain development and function. *Nature clinical practice Endocrinology & metabolism*. 3:249-259.
- Cayrou C, Denver RJ, Puymirat J. Suppression of the basic transcription element-binding protein in brain neuronal cultures inhibits thyroid hormone-induced neurite branching. *Endocrinology*. 2002 Jun;143(6):2242-9.
- Crofton KM. Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. *Risk Anal*. 2004 Dec;24(6):1665-71.
- Denver RJ, Ouellet L, Furling D, Kobayashi A, Fujii-Kuriyama Y, Puymirat J. Basic transcription element binding protein (BTEB) is a thyroid hormone-regulated gene in the developing central nervous system. Evidence for a role in neurite outgrowth. *J Biol Chem*. 1999 Aug 13;274(33):23128-34.
- Denver RJ, Williamson KE (2009) Identification of a thyroid hormone response element in the mouse Kruppel-like factor 9 gene to explain its postnatal expression in the brain. *Endocrinology* 150:3935-3943.
- Dong J, Liu W, Wang Y, Xi Q, Chen J. 2010. Hypothyroidism following developmental iodine deficiency reduces hippocampal neurogranin, CaMK II and calmodulin and elevates calcineurin in lactational rats. *International journal of developmental neuroscience* 28:589-596.
- Dong H, You SH, Williams A, Wade MG, Yauk CL, Thomas Zoeller R (2015) Transient Maternal Hypothyroxinemia Potentiates the Transcriptional Response to Exogenous Thyroid Hormone in the Fetal Cerebral Cortex Before the Onset of Fetal Thyroid Function: A Messenger and MicroRNA Profiling Study. *Cereb Cortex* 25:1735-1745.
- Dowling AL, Zoeller RT. Thyroid hormone of maternal origin regulates the expression of RC3/neurogranin mRNA in the fetal rat brain. *Brain research Molecular brain research*. 2000. 82:126-132.
- Gil-Ibanez P, Garcia-Garcia F, Dopazo J, Bernal J, Morte B. 2015. Global Transcriptome Analysis of Primary Cerebrocortical Cells: Identification of Genes Regulated by Triiodothyronine in Specific Cell Types. *Cerebral cortex*. Nov 2.
- Horn S. and Heuer H. Thyroid hormone action during brain development: more questions than answers. *Mol Cell Endocrinol*. 2010 Feb 5;315(1-2):19-26.
- Ibarrola N, Rodriguez-Pena A (1997) Hypothyroidism coordinately and transiently affects myelin protein gene expression in most rat brain regions during postnatal development. *Brain Res* 752:285-293.
- Iñiguez MA, Rodríguez-Peña A, Ibarrola N, Aguilera M, Muñoz A, Bernal J. Thyroid hormone regulation of RC3, a brain-specific gene encoding a protein kinase-C substrate. *Endocrinology*. 1993 Aug;133(2):467-73.
- Mohan V, Sinha RA, Pathak A, Rastogi L, Kumar P, Pal A, Godbole MM (2012) Maternal thyroid hormone deficiency affects the fetal neocorticalogenesis by reducing the proliferating pool, rate of neurogenesis and indirect neurogenesis. *Exp Neurol* 237:477-488.
- Morte B, Diez D, Auso E, Belinchon MM, Gil-Ibanez P, Grijota-Martinez C, Navarro D, de Escobar GM, Berbel P, Bernal J) Thyroid hormone regulation of gene expression in the developing rat fetal cerebral cortex: prominent role of the Ca²⁺/calmodulin-dependent protein kinase IV pathway. *Endocrinology* 2010a. 151:810-820.
- Morte B, Ceballos A, Diez D, Grijota-Martinez C, Dumitrescu AM, Di Cosmo C, Galton VA, Refetoff S, Bernal J. Thyroid hormone-regulated mouse cerebral cortex genes are differentially dependent on the source of the hormone: a study in monocarboxylate transporter-8- and deiodinase-2-deficient mice. *Endocrinology*. 2010b. 151:2381-2387.
- Navarro D, Alvarado M, Morte B, Berbel D, Sesma J, Pacheco P, Morreale de Escobar G, Bernal J, Berbel P. Late Maternal Hypothyroidism Alters the Expression of *Camk4* in Neocortical Subplate Neurons: A Comparison with *Nurr1* Labeling. *Cereb Cortex* 2014. 10:2694-2706.
- Oppenheimer J. The nuclear-receptor-triiodothyronine complex: Relationship to thyroid hormone distribution, metabolism, and biological action, In: Samuels HH, eds: *Molecular Basis of Thyroid Hormone Action*. Academic Press: New York. 1983: 1-34.
- Pathak A, Sinha RA, Mohan V, Mitra K, Godbole MM (2011). Maternal thyroid hormone before the onset of fetal thyroid function regulates *reelin* and downstream signaling cascade affecting neocortical neuronal migration. *Cereb Cortex* 21:11-21.
- Quignodon L, et al. Thyroid hormone signaling is highly heterogeneous during pre- and postnatal brain development. *J Mol Endocrinol* 2004, 33(2), 467-476.

Royland JE, Parker JS, Gilbert ME. A genomic analysis of subclinical hypothyroidism in hippocampus and neocortex of the developing rat brain. *J Neuroendocrinol.* 2008 Dec;20(12):1319-38.

Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol.* 2005 35:664-72.

Shiraki A, Saito F, Akane H, Takeyoshi M, Imatanaka N, Itahashi M, Yoshida T, Shibutani M (2014) Expression alterations of genes on both neuronal and glial development in rats after developmental exposure to 6-propyl-2-thiouracil. *Toxicol Lett* 228:225-234.

Thompson CC, Potter GB. Thyroid hormone action in neural development. *Cereb Cortex* 2000, 10(10), 939-945.

T4 in serum, Decreased leads to Hippocampal anatomy, Altered (<https://aopwiki.org/relationships/1388>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	indirectly leads to	Strong	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	<i>Rattus norvegicus</i>	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	<i>Mus musculus</i>	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Most of the available data has come from rodent models. Human clinical studies have documented changes in hippocampal volume in children with congenital hypothyroidism (Wheeler et al., 2011).

How Does This Key Event Relationship Work

The vast majority of brain thyroxine (T4) is from the serum. Once taken up from the serum, T4 is converted to triiodothyronine (T3) which binds to the nuclear receptors (TR α and TR β) to control thyroid-mediated gene expression (Oppenheimer, 1983). It is well established that TH regulates genes critical for brain development (Bernal, 2007; Anderson et al., 2003). As such, the structural development of the hippocampus is modulated by TR-mediated gene transcription, and alterations in serum TH can adversely impact hippocampal neuroanatomy.

Weight of Evidence

The weight of evidence for this indirect relationship is strong. There is a vast amount of literature that supports this KER in multiple species.

Biological Plausibility

The biological plausibility of this KER is rated as strong. The relationship is consistent with the known biology of the regulation of serum TH concentrations, brain TH concentrations, and the known action of TH to modulate genes critical for developmental processes that control structural development of the brain in general, including the hippocampus.

Empirical Support for Linkage

The empirical support for this KER is strong. In humans, untreated congenital hypothyroidism and severe iodine deficiency are accompanied by reductions in circulating levels of TH, and result in severe structural alterations in brain size, including hippocampus (Wheeler et al., 2011). The tie to serum TH has been amply demonstrated in clinical therapy of hypothyroidism during pregnancy and in congenitally hypothyroid children born to

euthyroid mothers. In addition, there is a vast amount of data from animal studies that support this relationship. Gross structural changes in the hippocampus following severe TH insufficiency are widely reported (Hasegawa et al., 2010; Powell et al., 2010; Madiera et al., 1991; 1992; Rami et al., 1986a 1986b; Madeira and Paula-Barbosa 1993; Rabie et al., 1980; Berbel et al., 1996). Other studies reveal more subtle changes in hippocampal structure such as reductions in a specific subregions of the hippocampus or of a cell type (eg. parvalbumin expressing inhibitory neurons) or synaptic component (ie synapsin, postsynaptic density proteins) or misplacement of cells within the hippocampal cell layers (Berbel et al., 1997; 2010; Gilbert et al., 2007; Auso et al., 2003; Gilbert et al., 2016; Cattani et al., 2013). These observations at the histological level are correlated with reductions in serum T4. The most profound structural impairments are typically seen with severe reductions in both hormones.

Additional evidence for a relationship between serum TH and hippocampal anatomy comes from the study of adult neurogenesis. The propensity of the hippocampus to generate new neurons throughout the lifetime of the organism occurs in only two brain regions, the olfactory bulb and the hippocampus. Severe reductions in circulating levels of TH in adulthood reduces both neuroprogenitor cell proliferation and survival of newly generated neurons in the neurogenic niche of the hippocampal dentate gyrus (Ambrogini et al., 2005, Montero-Pedrazuela et al., 2006, Kapoor et al., 2015). These same effects on neurogenesis also occur during development. However, the impact of developmental TH disruption on neurogenesis in adult offspring shows that the developing brain is more sensitive to these persistent effects. For example, a reduction in the capacity for neurogenesis was recently demonstrated in adult euthyroid offspring of developmentally TH compromised dams (Gilbert et al., 2016). These data indicate a permanent deficit in the capacity for neurogenesis, a process that controls dentate gyrus volume and cell number, following moderate reductions in serum TH in the fetus/neonate.

Finally, from in vitro studies, T3 stimulation accelerates the formation of GABAergic boutons and alters the distribution of GABAergic axons among growing neurons in culture. This growth is dependent on both activity within the network and the presence of T3. It can be blocked by the T3 nuclear receptor antagonist, 1-850, or pharmacological block of synaptic activity (Westerholz et al., 2010; 2013). T3 is believed to have this effect by its action on synaptic pruning. This example reveals the dynamic interplay between synaptic activity and neuroanatomy in the developing nervous system (Kozorovitskiy 2012).

Temporal Evidence: The temporal nature of this KER is developmental (Seed et al., 2005). It is a well-recognized fact that there are critical developmental windows for disruption of the serum THs that result in altered hippocampal anatomy. Reductions in serum TH in the neonate produced alterations in hippocampal parvalbumin-expressing neurons while the same treatment in adulthood is without effect (Gilbert et al., 2007). In a rodent model of prenatal TH deficiency, decreased length and number of radial glial cells which are critical for neuronal migration was reversed by hormone replacement treatment to the dam (Pathak et al., 2011). Reversibility of cortical layering defects with thyroxine treatment have also been reported in models of maternal hypothyroidism (Pathak et al., 2011; Berbel et al., 2010; Mohan et al., 2012). In in vitro studies, temporal specificity of the influence of T3 on GABAergic synapses and synaptic pruning has also been demonstrated (Westerholz et al., 2013). In addition, clinical therapy of hypothyroidism during pregnancy, and in congenitally hypothyroid children born to euthyroid mothers ameliorates most of the adverse impacts on the developing human brain.

Dose-Response Evidence: There are limited data available to inform the dose-dependent nature of the correlation between serum THs and changes in hippocampal anatomy. Gilbert et al (2007) demonstrated dose-dependent declines in the expression of protein marker inhibitory neurons in both hippocampus and neocortex with graded exposures to PTU and resultant serum T4. Shiraki et al. (2014; 2016) report dose-dependent alterations in the expression patterns of several neuronal and glial protein markers in the hippocampus after developmental exposure to different doses of PTU or MMI. Gilbert et al. (2016) report dose-dependent reductions in linear morphometry and volume of hippocampal subfields following developmental exposure to the PTU.

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some uncertainties. It is widely accepted that changes in serum THs during development will result in alterations in hippocampal structure. This has been repeatedly demonstrated. However, with some studies noted above, most investigations have been conducted in the neonate after severe hormone reductions induced by PTU, MMI or thyroidectomy. These severe changes alter a wide variety of general growth and developmental processes. More data is needed that link more limited decrements in serum TH to specific hippocampal anatomical changes.

Quantitative Understanding of the Linkage

Most investigations for hippocampal anatomy have been conducted in the neonate after severe hormone reductions. There is currently insufficient data for quantitative analysis of serum T4 and hippocampal gene expression.

References

- Ambrogini P, Cuppini R, Ferri P, Mancini C, Ciaroni S, Voci A, Gerdoni E, Gallo G (2005) Thyroid hormones affect neurogenesis in the dentate gyrus of adult rat. *Neuroendocrinology* 81:244-253.
- Anderson GW, Schoonover CM, Jones SA (2003) Control of thyroid hormone action in the developing rat brain. *Thyroid* 13:1039-56.
- Auso E, Lavado-Autric R, Cuevas E, Del Rey FE, Morreale De Escobar G, Berbel P (2004) A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticalogenesis alters neuronal migration. *Endocrinology* 145:4037-4047.
- Berbel P, Marco P, Cerezo JR, DeFelipe J (1996) Distribution of parvalbumin immunoreactivity in the neocortex of hypothyroid adult rats. *Neurosci Lett* 204:65-68.
- Berbel P, Navarro D, Ausó E, Varea E, Rodríguez AE, Ballesta JJ, Salinas M, Flores E, Faura CC, de Escobar GM. Role of late maternal thyroid hormones in cerebral cortex development: an experimental model for human prematurity. *Cereb Cortex*. 2010 Jun;20(6):1462-75.
- Bernal J. 2007. Thyroid hormone receptors in brain development and function. *Nature clinical practice Endocrinology & metabolism*. 3:249-259.
- Cattani D, Goulart PB, Cavalli VL, Winkelman-Duarte E, Dos Santos AQ,
- Pierozan P, de Souza DF, Woehl VM, Fernandes MC, Silva FR, Gonçalves CA, Pessoa-Pureur R, Zamoner A. Congenital hypothyroidism alters the oxidative status, enzyme activities and morphological parameters in the hippocampus of developing rats. *Mol Cell Endocrinol*. 2013 Aug 15;375(1-2):14-26.

- Gilbert ME, Goodman JH, Gomez J, Johnstone AF, Ramos RL. Adult hippocampal neurogenesis is impaired by transient and moderate developmental thyroid hormone disruption. *Neurotoxicology*. 2016. 59:9-21.
- Gilbert ME, Sui L, Walker MJ, Anderson W, Thomas S, Smoller SN, Schon JP, Phani S, Goodman JH (2007) Thyroid hormone insufficiency during brain development reduces parvalbumin immunoreactivity and inhibitory function in the hippocampus. *Endocrinology* 148:92-102.
- Hasegawa M, Kida I, Wada H (2010) A volumetric analysis of the brain and hippocampus of rats rendered perinatal hypothyroid. *Neurosci Lett* 479:240-244.
- Kapoor R, Fanibunda SE, Desouza LA, Guha SK, Vaidya VA (2015) Perspectives on thyroid hormone action in adult neurogenesis. *J Neurochem* 133:599-616.
- Kozorovitskiy Y, Saunders A, Johnson CA, Lowell BB, Sabatini BL. Recurrent network activity drives striatal synaptogenesis. *Nature*. 2012 May 13;485(7400):646-50.
- Madeira MD, Cadete-Leite A, Andrade JP, Paula-Barbosa MM (1991) Effects of hypothyroidism upon the granular layer of the dentate gyrus in male and female adult rats: a morphometric study. *J Comp Neurol* 314:171-186.
- Madeira MD, Paula-Barbosa MM (1993) Reorganization of mossy fiber synapses in male and female hypothyroid rats: a stereological study. *J Comp Neurol* 337:334-352.
- Madeira MD, Sousa N, Lima-Andrade MT, Calheiros F, Cadete-Leite A, Paula-Barbosa MM (1992) Selective vulnerability of the hippocampal pyramidal neurons to hypothyroidism in male and female rats. *J Comp Neurol* 322:501-518.
- Mohan V, Sinha RA, Pathak A, Rastogi L, Kumar P, Pal A, Godbole MM (2012) Maternal thyroid hormone deficiency affects the fetal neocortical neurogenesis by reducing the proliferating pool, rate of neurogenesis and indirect neurogenesis. *Exp Neurol* 237:477-488.
- Montero-Pedrazuela A, Venero C, Lavado-Autric R, Fernandez-Lamo I, Garcia-Verdugo JM, Bernal J, Guadano-Ferraz A (2006) Modulation of adult hippocampal neurogenesis by thyroid hormones: implications in depressive-like behavior. *Mol Psychiatry* 11:361-371.
- Oppenheimer J. The nuclear-receptor-triiodothyronine complex: Relationship to thyroid hormone distribution, metabolism, and biological action, In: Samuels HH, eds: *Molecular Basis of Thyroid Hormone Action*. Academic Press: New York. 1983: 1-34.
- Pathak A, Sinha RA, Mohan V, Mitra K, Godbole MM (2011) Maternal thyroid hormone before the onset of fetal thyroid function regulates reelin and downstream signaling cascade affecting neocortical neuronal migration. *Cereb Cortex* 21:11-21.
- Powell MH, Nguyen HV, Gilbert M, Parekh M, Colon-Perez LM, Mareci TH, Montie E (2012) Magnetic resonance imaging and volumetric analysis: novel tools to study the effects of thyroid hormone disruption on white matter development. *Neurotoxicology* 33:1322-1329.
- Rabie A, Clavel MC, Legrand J (1980) Analysis of the mechanisms underlying increased histogenetic cell death in developing cerebellum of the hypothyroid rat: determination of the time required for granule cell death. *Brain Res* 190:409-414.
- Rami A, Patel AJ, Rabie A (1986a) Thyroid hormone and development of the rat hippocampus: morphological alterations in granule and pyramidal cells. *Neuroscience* 19:1217-1226.
- Rami A, Rabie A, Patel AJ (1986b) Thyroid hormone and development of the rat hippocampus: cell acquisition in the dentate gyrus. *Neuroscience* 19:1207-1216.
- Seed J, Carney EW, Corley RA, Crofton KM, DeSesso JM, Foster PM, Kavlock R, Kimmel G, Klaunig J, Meek ME, Preston RJ, Slikker W Jr, Tabacova S, Williams GM, Wiltse J, Zoeller RT, Fenner-Crisp P, Patton DE. Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data. *Crit Rev Toxicol*. 2005 35:664-72.
- Shiraki A, Saito F, Akane H, Takeyoshi M, Imatanaka N, Itahashi M, Yoshida T, Shibutani M (2014) Expression alterations of genes on both neuronal and glial development in rats after developmental exposure to 6-propyl-2-thiouracil. *Toxicol Lett* 228:225-234.
- Shiraki A, Saito F, Akane H, Akahori Y, Imatanaka N, Itahashi M, Yoshida T, Shibutani M. Gene expression profiling of the hippocampal dentate gyrus in an adult toxicity study captures a variety of neurodevelopmental dysfunctions in rat models of hypothyroidism. *J Appl Toxicol*. 2016 Jan;36(1):24-34.
- Westerholz S, de Lima AD, Voigt T. Thyroid hormone-dependent development of early cortical networks: temporal specificity and the contribution of trkB and mTOR pathways. *Front Cell Neurosci*. 2013. 7:121.
- Westerholz S, de Lima AD, Voigt T. Regulation of early spontaneous network activity and GABAergic neurons development by thyroid hormone. *Neuroscience*. 2010 Jun 30;168(2):573-89.
- Wheeler SM, Willoughby KA, McAndrews MP, Rovet JF. Hippocampal size and memory functioning in children and adolescents with congenital hypothyroidism. *J Clin Endocrinol Metab*. 2011. 96(9):E1427-34

T4 in serum, Decreased leads to Hippocampal Physiology, Altered (<https://aopwiki.org/relationships/1389>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	indirectly leads to	Moderate	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human	Homo sapiens	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

There is a plethora of data supporting this KER in rats, mice, and humans. In addition, it is commonly accepted dogma that changes in serum TH concentration will alter brain development in most vertebrate species.

How Does This Key Event Relationship Work

Thyroid hormones are critical for normal development of the structure and function of the brain, including the hippocampus (Anderson et al., 2003; Bernal, 2007). Brain concentrations of T4 are dependent on transfer of T4 from serum, through the vascular endothelia, into astrocytes. In astrocytes, T4 is converted to T3 by deiodinase and subsequently transferred to neurons cellular membrane transporters. In the brain T3 controls transcription and translation of genes responsible for normal hippocampal structural and functional development. Normal hippocampal structure and physiology are critical for the development of cognitive function. Thus, there is an indisputable indirect link between serum T4 and cognitive function.

Weight of Evidence

The weight of evidence for this indirect relationship is strong. Alterations in serum TH concentrations are very well correlated with adverse impacts on cognitive behaviors such as learning and memory. This includes a large amount of literature, from more than four decades of research, that links hypothyroidism and/or hypothyroxinemia with alterations in spatial cognitive function, a hippocampal dependent behavior. A number of reviews are cited below that are primarily from humans and rodents, but this indirect relationship has also been shown for a number of other species.

In humans, severe serum TH reductions that accompany congenital hypothyroidism dramatically impair brain function and lead to severe mental retardation. Lower global IQ scores, language delays and weak verbal skills, motor weakness, attentional deficits and learning impairments accompany low serum TH in children (Derksen-Lubsen and Verkerk 1996). Standard tests of IQ function in children born to mothers with even marginal hypothyroidism during pregnancy or in children with a defective thyroid gland who are then treated remain approximately 6 points below expected values. Selective deficits on visual spatial, motor, language, memory and attention tests are observed, the exact phenotype largely dependent on the developmental window over which the insufficiency occurred and the severity of the hormone deficit (Mirabella et al. 2000; Rovet 2002; Zoeller and Rovet 2004; Willoughby et al 2014). Indeed, this link is recognized as being so clinically important that T4 and TSH are monitored in all newborns in the US.

In rodent models, reductions in serum TH induced by TPO inhibitors such as MMI and PTU, when induced during development, lead to a variety of neurobehavioral impairments. These impairments can occur in the sensory, motor, and cognitive domains. The specific phenotype is dependent on both the window of exposure, the duration of exposure, and the severity of the hormone reduction (Zoeller and Rovet, 2004). This includes more than four decades of work linking serum TH changes to alterations in hippocampal-dependent spatial behaviors (Akaike et al., 2004; Axelstad et al., 2008; Brosvic et al; Kawada et al, 1988; Friedhoff et al, 2000; Gilbert and Sui, 2006; Gilbert et al., 2016; Gilbert, 2011).

Biological Plausibility

The biological plausibility of this KER is rated as strong. The relationship is consistent with the known biology of how the relationship between serum TH concentrations, brain TH concentrations, and TH control of brain development.

Empirical Support for Linkage

Empirical support for this KER is rated as strong. Empirical data from studies that measure serum TH concentrations and then assess alterations in cognitive function, including hippocampal dependent behaviors, is vast. The qualitative relationship between reduced serum hormone levels and adverse cognitive outcomes is well accepted in endocrinology, as well as developmental neuroendocrinology. Indeed, the relationship between serum T4 and T3 levels and adverse neurodevelopmental outcomes (e.g., IQ loss in children) is beyond reproach.

Temporal Evidence: The temporal nature of this KER is developmental (Seed et al., 2005). It is a well-recognized fact that there are critical developmental windows for disruption of serum THs that result in alterations in cognitive function later in development and adulthood. In humans, hormone insufficiency that occurs in mid-pregnancy due to maternal drops in serum hormone, and that which occurs in late pregnancy due to disruptions in the fetal thyroid gland lead to different patterns of subsequent cognitive impairment (Zoeller and Rovet, 2004; Willoughby et al., 2014). In animal models, deficits in hippocampal-dependent cognitive tasks result from developmental, but not adult hormone deprivation (Gilbert and Sui, 2006; Gilbert et al., 2016; Axelstad et al., 2009; Gilbert, 2011; Opazo et al., 2008). Replacement studies have demonstrated that varying adverse neurobehavioral outcomes, including cognitive function, can be reduced or eliminated if T4 (and/or T3) treatment is given during the critical windows (e.g., Kawada et al., 1988; Goldey and Crofton, 1998; Reid et al., 2007).

Dose-Response Evidence: An increasing amount of literature is now available that provides clear evidence of the 'dose-response' nature of this KER. Most research over the last 40 years has employed high doses of chemicals, or chemicals plus thyroidectomies, that results in severe depletion of circulating thyroid hormones. More recently, researchers employed models in which graded degrees of TH insufficiency were induced in dams and pups by administering varying doses of chemicals and have correlated them to the dose-dependency of the observed effects. This work has provided increased confidence in the relationship between serum TH decrements and a variety of neurodevelopmental impairments. It also to the specificity of the observed effects on brain development that is directly mediated by TH insufficiency (Goldey et al., 1995; Crofton, 2004; Gilbert and Sui, 2006; Gilbert, 2011; Bastian et al., 2014; Royland et al., 2008; Sharlin et al., 2008).

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some remaining uncertainties. It is widely accepted that changes in serum THs during development will result in alterations in behavior controlled by the hippocampus. This has been repeatedly demonstrated in animal models and in humans. The major remaining uncertainty is the precise relationship between the degree, timing and duration of serum TH changes that leads to these behavioral deficits.

Quantitative Understanding of the Linkage

Except for a quantitative relationship between serum T4 and hearing loss in rodents (Crofton, 2004), there are no other reports of development of quantitative predictive models linking serum TH and adverse neurological outcomes. Insufficient data exist to develop a quantitative predictive model of adverse cognitive outcomes from serum TH concentrations. However, evidence from human studies suggests that decreases as low as 25% in serum T4 in pregnant women will yield small decrements in IQ in children (e.g., Haddow et al., 1995). Based on this, regulatory authorities have used 10 and/or 20% changes in serum T4 as a point of departure for hazard assessments in rodent studies (EPA, 2011).

References

- Anderson GW, Schoonover CM, Jones SA (2003) Control of thyroid hormone action in the developing rat brain. *Thyroid* 13:1039-56.
- Bernal J. 2007. Thyroid hormone receptors in brain development and function. *Nature clinical practice Endocrinology & metabolism*. 3:249-259.
- Akaike M, Kato N, Ohno H, Kobayashi T (1991) Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. *Neurotoxicol Teratol* 13:317-322.
- Axelstad M, Hansen PR, Boberg J, Bonnicksen M, Nellemann C, Lund SP, Hougaard KS, Hass U. Developmental neurotoxicity of propylthiouracil (PTU) in rats: relationship between transient hypothyroxinemia during development and long-lasting behavioural and functional changes. *Toxicol Appl Pharmacol*. 2008 Oct 1;232(1):1-13
- Bastian TW, Prohaska JR, Georgieff MK, Anderson GW (2014) Fetal and neonatal iron deficiency exacerbates mild thyroid hormone insufficiency effects on male thyroid hormone levels and brain thyroid hormone-responsive gene expression. *Endocrinology* 155:1157-1167.
- Brosvic GM, Taylor JN, Dihoff RE. (2002). Influences of early thyroid hormone manipulations: delays in pup motor and exploratory behavior are evident in adult operant performance. *Physiol Behav*. Apr 15;75(5):697-715.
- Crofton KM. Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. *Risk Anal*. 2004 Dec;24(6):1665-71.
- Derksen-Lubsen, G. and P. H. Verkerk (1996). "Neuropsychologic development in early treated congenital hypothyroidism: analysis of literature data." *Pediatr Res* 39(3): 561-6.
- EPA (2011) FIFRA Scientific Advisory Panel Consultation, Integrated Approaches to Testing and Assessment Strategy: Use of New Computational and Molecular Tools, US Environmental Protection Agency, Office of Pesticide Programs, Washington DC May 24-26, 2011.
- Friedhoff AJ, Miller JC, Armour M, Schweitzer JW, Mohan S. Role of maternal biochemistry in fetal brain development: effect of maternal thyroidectomy on behaviour and biogenic amine metabolism in rat progeny. *Int J Neuropsychopharmacol*. 2000 Jun;3(2):89-97.
- Gilbert ME. Impact of low-level thyroid hormone disruption induced by propylthiouracil on brain development and function. *Toxicol Sci*. 2011;124(2):432-445.
- Gilbert ME, Sui L. Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. *Brain Res*. 2006 Jan 19;1069(1):10-2
- Gilbert ME, Sanchez-Huerta K, Wood C (2016) Mild Thyroid Hormone Insufficiency During Development Compromises Activity-Dependent Neuroplasticity in the Hippocampus of Adult Male Rats. *Endocrinology* 157:774-787.
- Goldey ES, Kehn LS, Rehnberg GL, Crofton KM. Effects of developmental hypothyroidism on auditory and motor function in the rat. *Toxicol Appl Pharmacol*. 1995 Nov;135(1):67-76.
- Goldey ES, Crofton KM. (1998) Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. *Toxicol Sci*. 45:94-105.
- Haddow, J. E., G. E. Palomaki, et al. (1999). "Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child." *N Engl J Med* 341(8): 549-55.

Kawada J, Mino H, Nishida M, Yoshimura Y. (1988) An appropriate model for congenital hypothyroidism in the rat induced by neonatal treatment with propylthiouracil and surgical thyroidectomy: studies on learning ability and biochemical parameters. *Neuroendocrinology*. 47:424-30.

Reid RE, Kim EM, Page D, O'Mara SM, O'Hare E. Thyroxine replacement in an animal model of congenital hypothyroidism. *Physiol Behav*. 2007 91(2-3):299-303. Epub 2007 Mar 15.

Rovet, J. F. (2002). Congenital hypothyroidism: an analysis of persisting deficits and associated factors." *Child Neuropsychol* 8(3): 150-62.

Royland JE, Parker JS, Gilbert ME. 2008a. A genomic analysis of subclinical hypothyroidism in hippocampus and neocortex of the developing rat brain. *Journal of neuroendocrinology*. Dec;20:1319-1338.

Sharlin DS, Tighe D, Gilbert ME, Zoeller RT (2008) The balance between oligodendrocyte and astrocyte production in major white matter tracts is linearly related to serum total thyroxine. *Endocrinology* 149:2527-2536.

Willoughby KA, McAndrews MP, Rovet J. Effects of maternal hypothyroidism on offspring hippocampus and memory. *Thyroid*, 2014;24:576-584.

Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol*. 2004 Oct;16(10):809-18

T4 in serum, Decreased leads to Cognitive Function, Decreased (<https://aopwiki.org/relationships/403>)

AOPs Referencing Relationship

AOP Name	Directness	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	indirectly leads to	Strong	Moderate
XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	indirectly leads to	Strong	Moderate
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	indirectly leads to	Strong	Weak

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	<i>Rattus norvegicus</i>	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	<i>Mus musculus</i>	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)
human	<i>Homo sapiens</i>	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

Life Stage Applicability

Life Stage	Evidence
During brain development	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

There is a plethora of data supporting this KER in rats, mice, and humans.

How Does This Key Event Relationship Work

Thyroid hormones (TH) are critical for normal development of the structure and function of the brain, including hippocampal development and cognitive function (Anderson et al., 2003; Bernal, 2007; Willoughby et al., 2014). Brain concentrations of T4 are dependent on transfer of T4 from

serum, through the vascular endothelia, into astrocytes. In astrocytes, T4 is converted to T3 by deiodinase and subsequently transferred to neurons cellular membrane transporters. In the brain T3 controls transcription and translation of genes responsible for normal hippocampal structural and functional development. Normal hippocampal structure and physiology are critical for the development of cognitive function. Thus, there is an indisputable indirect link between serum T4 and cognitive function.

Weight of Evidence

The weight of evidence for this indirect relationship is strong. Alterations in serum TH concentrations are very well correlated with adverse impacts on cognitive behaviors such as learning and memory. This includes a large amount of literature, from more than four decades of research, that links hypothyroidism and/or hypothyroxinemia with alterations in spatial cognitive function, a hippocampal dependent behavior. A number of reviews are cited below that are primarily from humans and rodents, but this indirect relationship has also been shown for a number of other species.

In humans, severe serum TH reductions that accompany congenital hypothyroidism dramatically impair brain function and lead to severe mental retardation. Lower global IQ scores, language delays and weak verbal skills, motor weakness, attentional deficits and learning impairments accompany low serum TH in children (Derksen-Lubsen and Verkerk 1996). Standard tests of IQ function in children born to mothers with even marginal hypothyroidism during pregnancy or in children with a defective thyroid gland who are then treated remain approximately 6 points below expected values. Selective deficits on visual spatial, motor, language, memory and attention tests are observed, the exact phenotype largely dependent on the developmental window over which the insufficiency occurred and the severity of the hormone deficit (Mirabella et al. 2000; Rovet 2002; Zoeller and Rovet 2004; Willoughby et al 2014). Indeed, this link is recognized as being so clinically important that T4 and TSH are monitored in all newborns in the US.

In rodent models, reductions in serum TH induced by TPO inhibitors such as MMI and PTU, when induced during development, lead to a variety of neurobehavioral impairments. These impairments can occur in the sensory, motor, and cognitive domains. The specific phenotype is dependent on both the window of exposure, the duration of exposure, and the severity of the hormone reduction (Zoeller and Rovet, 2004). This includes more than four decades of work linking serum TH changes to alterations in hippocampal-dependent spatial behaviors (Akaike et al., 2004; Axelstad et al., 2008; Brosvic et al; Kawada et al, 1988; Friedhoff et al, 2000; Gilbert and Sui, 2006; Gilbert et al., 2016; Gilbert, 2011).

Biological Plausibility

The biological plausibility of this KER is rated as strong. The relationship is consistent with the known biology of how the relationship between serum TH concentrations, brain TH concentrations, and TH control of brain development.

Empirical Support for Linkage

Empirical support for this KER is rated as strong. Empirical data from studies that measure serum TH concentrations and then assess alterations in cognitive function, including hippocampal dependent behaviors, is vast. The qualitative relationship between reduced serum hormone levels and adverse cognitive outcomes is well accepted in endocrinology, as well as developmental neuroendocrinology. Indeed, the relationship between serum T4 and T3 levels and adverse neurodevelopmental outcomes (e.g., IQ loss in children) is beyond reproach.

Temporal Evidence: The temporal nature of this KER is developmental (Seed et al., 2005). It is a well-recognized fact that there are critical developmental windows for disruption of the serum THs that result in cognitive function. In humans, hormone insufficiency that occurs in mid-pregnancy due to maternal drops in serum hormone, and that which occurs in late pregnancy due to disruptions in the fetal thyroid gland lead to different patterns of cognitive impairment (Zoeller and Rovet, 2004). In animal models, deficits in hippocampal-dependent cognitive tasks result from developmental, but not adult hormone deprivation (Gilbert and Sui, 2006; Gilbert et al., 2016; Axelstad et al, 2009; Gilbert, 2011; Opazo et al., 2008). Replacement studies have demonstrated that varying adverse neurobehavioral outcomes, including cognitive function, can be reduced or eliminated if T4 (and/or T3) treatment is given during the critical windows (e.g., Kawada et al., 1988; Goldey and Crofton, 1998). Reid et al., 2007).

Dose-Response Evidence: An increasing amount of literature is now available that provides clear evidence of the 'dose-response' nature of this KER. Most research over that last 40 years has employed high doses of chemicals, or chemicals plus thyroidectomies, that results in severe depletion of circulating thyroid hormones. More recently, researchers produced graded degrees of TH insufficiency in dams and pups by administering varying doses of chemicals and have correlated them to the dose-dependency of the observed effects. This work has provided increased confidence in the relationship between serum TH decrements and a variety of neurodevelopmental impairments, and also to the specificity of the observed effects on brain development that is directly mediated by TH insufficiency (Goldey et al., 1995; Crofton, 2004; Gilbert and Sui, 2006; Gilbert, 2011; Bastian et al., 2014; Royland et al., 2008; Sharlin et al., 2008).

Uncertainties or Inconsistencies

There are no inconsistencies in this KER, but there are some remaining uncertainties. It is widely accepted that changes in serum THs during development will result in alterations in behavior controlled by the hippocampus. This has been repeatedly demonstrated in animal models and in humans. The major remaining uncertainty is the precise relationship between the degree, timing and duration of serum TH changes that leads to these behavioral deficits.

Quantitative Understanding of the Linkage

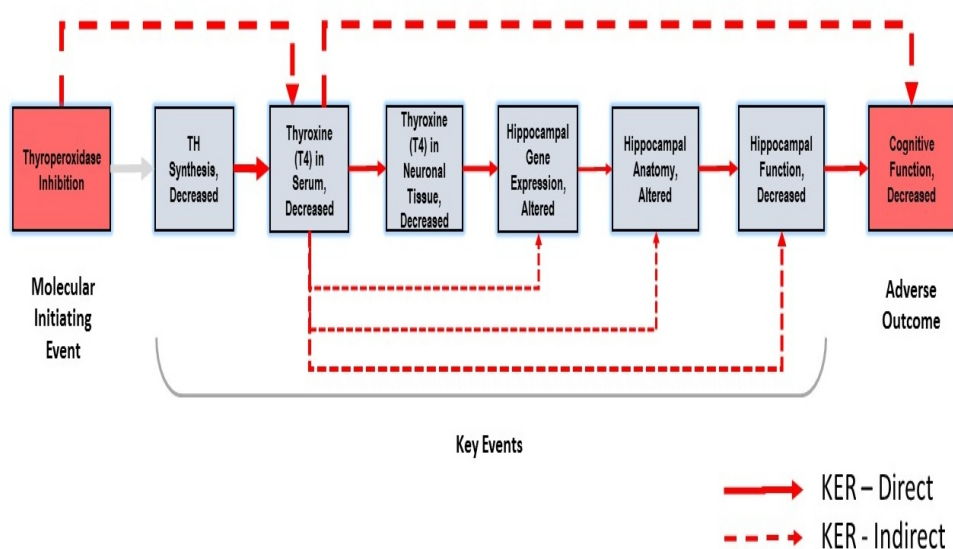
With the exception of a quantitative relationship between serum T4 and hearing loss in rodents (Crofton, 2004), there are no other reports of development of quantitative predictive models linking serum TH and adverse neurological outcomes. Insufficient data exist to develop a quantitative predictive model of adverse cognitive outcomes from serum TH concentrations. However, evidence from human studies suggests that decreases as low as 25% in serum T4 in pregnant women will yield small decrements in IQ in children (e.g., Haddow et al., 1995). Based on this, regulatory authorities have used 10 and/or 20% changes in serum T4 as a point of departure for hazard assessments in rodent studies (EPA, 2011).

References

Akaike M, Kato N, Ohno H, Kobayashi T (1991) Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. *Neurotoxicol Teratol* 13:317-322.

- Anderson GW, Schoonover CM, Jones SA (2003) Control of thyroid hormone action in the developing rat brain. *Thyroid* 13:1039-56.
- Axelstad M, Hansen PR, Boberg J, Bonnichsen M, Nellemann C, Lund SP, Hougaard KS, Hass U. Developmental neurotoxicity of propylthiouracil (PTU) in rats: relationship between transient hypothyroxinemia during development and long-lasting behavioural and functional changes. *Toxicol Appl Pharmacol.* 2008 Oct 1;232(1):1-13
- Bastian TW, Prohaska JR, Georgieff MK, Anderson GW (2014) Fetal and neonatal iron deficiency exacerbates mild thyroid hormone insufficiency effects on male thyroid hormone levels and brain thyroid hormone-responsive gene expression. *Endocrinology* 155:1157-1167.
- Bernal J. 2007. Thyroid hormone receptors in brain development and function. *Nature clinical practice Endocrinology & metabolism.* 3:249-259.
- Brosvic GM, Taylor JN, Dihoff RE. (2002). Influences of early thyroid hormone manipulations: delays in pup motor and exploratory behavior are evident in adult operant performance. *Physiol Behav.* Apr 15;75(5):697-715.
- Crofton KM. Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. *Risk Anal.* 2004 Dec;24(6):1665-71.
- Derksen-Lubsen, G. and P. H. Verkerk (1996). "Neuropsychologic development in early treated congenital hypothyroidism: analysis of literature data." *Pediatr Res* 39(3): 561-6.
- EPA (2011) FIFRA Scientific Advisory Panel Consultation, Integrated Approaches to Testing and Assessment Strategy: Use of New Computational and Molecular Tools, US Environmental Protection Agency, Office of Pesticide Programs, Washington DC May 24-26, 2011 Link to Document (<http://yosemite.epa.gov/sab/sabproduct.nsf/373C1DB0E0591296852579F2005BECB3/%24File/OPP+SAP+document-May2011.pdf>)
- Friedhoff AJ, Miller JC, Armour M, Schweitzer JW, Mohan S. Role of maternal biochemistry in fetal brain development: effect of maternal thyroidectomy on behaviour and biogenic amine metabolism in rat progeny. *Int J Neuropsychopharmacol.* 2000 Jun;3(2):89-97.
- Gilbert ME. Impact of low-level thyroid hormone disruption induced by propylthiouracil on brain development and function. *Toxicol Sci.* 2011;124(2):432-445.
- Gilbert ME, Sui L. Dose-dependent reductions in spatial learning and synaptic function in the dentate gyrus of adult rats following developmental thyroid hormone insufficiency. *Brain Res.* 2006 Jan 19;1069(1):10-2
- Gilbert ME, Sanchez-Huerta K, Wood C (2016) Mild Thyroid Hormone Insufficiency During Development Compromises Activity-Dependent Neuroplasticity in the Hippocampus of Adult Male Rats. *Endocrinology* 157:774-787.
- Goldey ES, Kehn LS, Rehnberg GL, Crofton KM. Effects of developmental hypothyroidism on auditory and motor function in the rat. *Toxicol Appl Pharmacol.* 1995 Nov;135(1):67-76.
- Goldey ES, Crofton KM. (1998) Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. *Toxicol Sci.* 45:94-105.
- Haddow, J. E., G. E. Palomaki, et al. (1999). "Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child." *N Engl J Med* 341(8): 549-55.
- Kawada J, Mino H, Nishida M, Yoshimura Y. (1988) An appropriate model for congenital hypothyroidism in the rat induced by neonatal treatment with propylthiouracil and surgical thyroidectomy: studies on learning ability and biochemical parameters. *Neuroendocrinology.* 47:424-30.
- Mirabella, G., D. Feig, et al. (2000). "The effect of abnormal intrauterine thyroid hormone economies on infant cognitive abilities." *J Pediatr Endocrinol Metab* 13(2): 191-4.
- Opazo MC, Gianini A, Pancetti F, Azkcona G, Alarcón L, Lizana R, Noches V, Gonzalez PA, Marassi MP, Mora S, Rosenthal D, Eugenin E, Naranjo D, Bueno SM, Kalergis AM, Riedel CA (2008), Maternal hypothyroxinemia impairs spatial learning and synaptic nature and function in the offspring. *Endocrinology* 149:5097-5106.
- Reid RE, Kim EM, Page D, O'Mara SM, O'Hare E. Thyroxine replacement in an animal model of congenital hypothyroidism. *Physiol Behav.* 2007 Jun 8;91(2-3):299-303. Epub 2007 Mar 15.
- Rovet, J. F. (2002). Congenital hypothyroidism: an analysis of persisting deficits and associated factors." *Child Neuropsychol* 8(3): 150-62.
- Royland JE, Parker JS, Gilbert ME. 2008a. A genomic analysis of subclinical hypothyroidism in hippocampus and neocortex of the developing rat brain. *Journal of neuroendocrinology.* Dec;20:1319-1338.
- Sharlin DS, Tighe D, Gilbert ME, Zoeller RT (2008) The balance between oligodendrocyte and astrocyte production in major white matter tracts is linearly related to serum total thyroxine. *Endocrinology* 149:2527-2536.
- Willoughby KA, McAndrews MP, Rovet J. Effects of maternal hypothyroidism on offspring hippocampus and memory. *Thyroid*, 2014;24:576-584.
- Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol.* 2004 Oct;16(10):809-18

Graphical Representation



Overall Assessment of the AOP

The following summary tables for:

1. Support for Biological Plausibility of KERS
2. Support for Essentiality of KEs
3. Empirical Support for KERs

Can be downloaded at: https://aopwiki.org/system/dragonfly/production/2017/03/24/6u60jhkjp8_TPO_AOP_Summary_Tables.pdf
 (https://aopwiki.org/system/dragonfly/production/2017/03/24/6u60jhkjp8_TPO_AOP_Summary_Tables.pdf)

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Perinatal	Strong
Foetal	Strong
During brain development	Strong

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
rat	Rattus norvegicus	Strong	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	Moderate	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Sex Applicability

Sex	Evidence
Male	Strong

Sex	Evidence
Female	Strong

- **Chemicals:** This AOP applies to a wide range of chemicals structures that inhibit TPO either in vivo or in vitro. Well recognized positive controls include propylthiouracil (PTU) and methimazole (MMI). There are 100s of other chemicals known to inhibit TPO in vitro (e.g., Paul-Friedman et al., 2015).
- **Sex:** This AOP applies to males and females. Disruption of thyroid hormone regulation during fetal and early postnatal develop, as well as the subsequent adverse impacts on nervous system development are similar in both sexes. There are no compelling data to suggest sex differences in susceptibility to TH disruption mediated by inhibition of TPO during development.
- **Life stages:** The relevant life stages for this AOP are fetal and early postnatal ages during critical windows of nervous system development where thyroid hormones guide normal development of the brain. There are clear windows of developmental susceptibility and different brain regions show distinct ontogenetic profiles for TH requirements. Distinct phenotypes have been described in both humans and animal models for different periods of TH insufficiency. This AOP does not apply to adult life states.
- **Taxonomic:** Based on the majority of the available evidence the taxonomic applicability domains of this AOP is mammals. Most evidence for this AOP has been gathered primarily from laboratory rodents and humans. However, there are supporting data from amphibians and birds for TPO inhibition leading to altered TH profiles. Due to the conserved nature of TH synthesis, transport, metabolism and transcriptional activity, this AOP is likely to be applicable to other classes of vertebrates where thyroid hormones drive development of the nervous system (e.g., birds, fish, reptiles). However, species-specific differences in development, ADME (adsorption, distribution, metabolism, and elimination), compensatory endocrine responses may influence the outcomes, particularly from a quantitative standpoint.

Essentiality of the Key Events

It is widely accepted that each of the key events is essential.

- **Molecular Initiating Event:** The molecular initiating event, i.e. inhibition of TPO, is the essential event to initiate this AOP, as supported by in vitro and in vivo evidence. TPO is the only enzyme capable of de novo TH synthesis (Taurog, 2005). A number of studies have demonstrated that cessation of exposure is known to result in a return to normal levels of TH synthesis and circulatory hormone levels (Cooper et al., 1983). Many in vivo and in vitro studies consistently demonstrate enzyme inhibition with similar chemicals for multiple species (Taurog, 1999; Paul et al., 2013; Vickers et al., 2012).
- **Thyroid hormone synthesis, Decreased.** A number of studies have demonstrated a correlation between TPO activity and decreased TH synthesis (e.g., Vickers et al., 2012). Thyroid gland T4 concentrations as well as serum TH are decreased in response to thyroidectomy, and recover when in-vitro derived follicles are grafted in athyroid mice (Antonica et al., 2012).
- **Thyroxine (T4) in serum, Decreased.** Inhibition of TPO is widely accepted as resulting in decreased TH synthesis in the thyroid gland, which results in decreased serum T4 concentration (Taurog, 2005). Stop/recovery experiments demonstrate recovery of serum thyroxine concentrations due to cessation of developmental exposure to TPO inhibitors (e.g., Crofton et al., 2000). Studies in adult animals show a similar recovery after cessation of dosing (e.g., Hill et al., 1998).
- **Thyroxine (T4) in neuronal tissue, decreased:** Multiple studies have demonstrated that fetal brain TH levels, previously decreased by maternal exposure to TPO inhibitors or thyroidectomy, recovered following maternal dosing with T4 (e.g., Calvo et al., 1990). In addition, upregulation of deiodinase has been shown compensate for some loss of neuronal T3 (Escobar-Morreale et al., 1997). Indirect evidence shows that T4 replacement that bring circulating T4 concentration back to normal, leads to recovery of brain TH and prevents downstream effects including alterations in gene expression in the developing brain.
- **Hippocampal Gene Expression, Altered:** It is well established specific genomic pathways underlie the progression of a number of neurodevelopmental processes in the hippocampus. There is some evidence from ex vivo studies that administration of growth factors will reverse the hippocampal dysplasia seen in Jacob/Nsfn knockout mice (Spilker et al., 2016). Less is known about the impact of hormone replacement on TH-responsive gene expression and the qualitative and quantitative relationships between altered TH-dependent gene expression in this brain region and altered hippocampal cytoarchitectural anatomy.
- **Hippocampal anatomy, altered:** It is well accepted that normal hippocampal anatomy is critical for hippocampal physiological function, and that alterations in anatomy lead to altered neuronal activity in the hippocampus (Lee et al., 2015; Grant et al., 1992; Spilker et al., 2016).
- **Hippocampal physiology, altered:** It is a well-accepted assertion that hippocampal synaptic integrity and neuronal plasticity are essential for spatial information processing in animals and spatial and episodic memory in humans. However, other brain regions also can influence these complex behaviors. Limited data from studies in BDNF knockout animals demonstrate that deficits in hippocampal synaptic transmission and plasticity, and downstream behaviors can be rescued with recombinant BDNF (Aarse et al., 2016; Andero et al., 2014).
- **Cognitive function, decreased:** It is a well-known fact that TH are critical for normal nervous system development (Williams et al., 2008). And this includes development of the hippocampus which plays a major role in spatial, temporal, and contextual memory. Indeed, most developed countries check for childhood hypothyroidism at birth to immediately begin replacement therapy. This has been shown to alleviate most adverse impacts of hypothyroidism in congenitally hypothyroid children (Derksen-Lubsen and Verkerk 1996; Zoeller and Rovet, 2004). The essentiality of the relationship between decreased TH levels and this adverse outcome is well accepted. Decreased cognitive function specific to the hippocampal region are particularly associated with decrements in memory and learning domains of cognition.

Weight of Evidence Summary

Biological plausibility: Biological plausibility refers to the structural or functional relationship between the key events based on our fundamental understanding of "normal biology". In general, the biological plausibility and coherence linking TPO inhibition through decreases in circulating concentrations of THs, to adverse impacts in the developing hippocampus and subsequent cognitive behaviors is very solid. That thyroidal TPO is the sole enzyme capable of de novo TH synthesis and the only source of circulating T4, is beyond doubt. It also widely accepted circulating T4 is the only source of nervous system T4 that is converted to the biological active T3. The direct link between reduced brain TH concentrations

and reduced expression of TR regulated genes is supported by a plethora of literature. However, the direct connection between exactly which genes are regulated and at which developmental periods is not as clear. Similarly, the precise relationships between gene expression and hippocampal anatomy is not completely known. A lot of the work in this area has been done for a limited number of genes and specific hippocampal anatomical anomalies that are known to alter both the physiological and function of the hippocampus, and subsequent cognitive function. That said, it is widely acknowledged that abnormal TH levels during fetal and early development lead to adverse hippocampally-driven cognitive function in humans and laboratory animals.

1. The biochemistry of TPO and its essentiality for TH synthesis is well known across species, with the evidence across vertebrate species, including amphibians, birds, rodents, pigs, and humans.
2. The relationship between TH synthesis and serum TH concentrations is well accepted scientific dogma. There are no other pathways in mammals that will maintain homeostatic serum TH concentrations.
3. Serum is the only source of thyroxine for the brain. In the brain, deiodinases convert T4 to T3, the more biologically active moiety. Some serum T3 may also contribute to total brain T3. These are well accepted scientific facts.
4. It is well established that T3 binding to thyroid receptors controls critical transcriptional and translational processes in the entire developing brain, including the hippocampus. Lack of TH results in abnormal development of the structure and physiological function in the hippocampus. What is not well known is exactly which genes, at what fetal and postnatal ages, are responsible for the development of the complexity of hippocampal anatomy and function.
5. Lastly, the biological plausibility that changes in brain structure and physiology, and specifically aberrations in the hippocampus, lead to abnormal cognitive function is well accepted.

Concordance of dose-response relationships:

There are a large number of studies that include correlative evidence between exposure to TPO inhibitors and downstream KEs, as well as the AO. In addition, there are also studies with dose-response relationships that indirectly link KEs, especially from serum TH concentrations to downstream KEs and the AO. There is a more limited set of studies in which two directly linked key events were considered in the same study following exposure to TPO inhibitors or other stressors (e.g., thyroidectomy, gene knockouts). These later studies, while providing critical data for causatively linking the key events, provide less information on the concordance of the dose-response relationship, especially for the latter KEs.

For earlier KEs, Zoeller and Crofton (2005) provide good dose response concordance for data derived from the TPO inhibitor 6-n-Propylthiuracil. While limited in number, in general these studies provide moderate confidence that downstream key events occurred at concentrations equal to or greater than those directly upstream. In addition, there are several quantitative models that, based on empirical data, can predict dose relationships between many of the early KEs up to and including serum hormone concentrations (e.g., Degon et al., 2008; Fisher et al 2013; Ekerot et al., 2012; Leonard et al., 2016). All this information taken together, provide strong concordance of the dose-relationships for all KEs.

Temporal concordance among the key events and adverse effect: There are two aspects of the temporal concordance of the key events in a developmental AOP. The first is the temporal concordance refers to the degree to which the data support the hypothesized sequence of the key events; i.e., the effect on KE1 is observed before the effect on KE2, which is observed before the effect on KE3, and so on. This translates to the temporal concordance of the AOP from TPO inhibition to decreased TH synthesis, reduced circulating TH concentrations, decreased nervous system TH, altered gene expression and anatomy in the hippocampus, and subsequent alterations in hippocampal physiology that result in decrements in cognition. The strength of the temporal concordance between these KEs varies from weak to strong (see Appendix Tables and individual KEs for detailed information). There is strong evidence for the early direct KEs from both empirical and modeling studies, and for many of the later KEs via the indirect KERs. The temporal concordance between TPO inhibition and TH synthesis is clearly evidenced by data from ex vivo and in vitro studies, as well as computational models (Leonard et al., 2016; Degon et al., 2008; Zoeller and Crofton, 2005; Cooper et al., 1983; Goldey et al. 1985; Christenson et al 1995). Data supporting the temporal concordance for the later KEs, i.e., from serum TH to changes in hippocampal physiology are limited or lacking.

The second aspect of temporal concordance for developmental AOPs is evidenced by demonstrations for critical windows of development where key events are perturbed, for which the effects are permanent and found during early development and throughout adulthood (Seed et al., 2005). It is a well-recognized fact that there are critical developmental windows for disruption of serum THs that result in subsequent alterations in all downstream KEs including the AO cognitive function later in development and adulthood. Indeed, the literature is replete with studies that demonstrate critical windows of susceptibility to thyroid disruption and adverse impacts on the developing brain. For reviews see: Morreale de Escobar (2001); Howdshell (2002). There are also many studies in which downstream direct and indirect consequences of TPO inhibition and other stressors have been ameliorated by administration of thyroxine. For example, based on the indirect link between serum TH hormone concentrations and decrements in hippocampally-mediated spatial behaviors, it commonly accepted dogma that there are critical windows of development in which exposure and hormone reduction lead to permanent effect on cognitive functions. Indeed, most developed countries have mandatory screening for congenital hypothyroidism, so that hormone replacement therapy can begin immediately, and thus prevent declines in IQ in childhood. (e.g., the temporal concordance between the MIE, KEs and AO. Overall, all available data are consistent with the temporal concordance of this AOP.

Consistency: There is no data that we are aware of that does not support the pattern of key events described in this AOP. A limited number of studies with measurements of directly linked KEs within the same study, the fact that the majority of the data was generated with single-stressor studies (e.g., one chemical dose, knockout, or thyroidectomy), coupled with likely differences in sensitivity of many of the measured endpoints (e.g., gene expression), make it difficult to determine quantitative consistency between studies. Nonetheless, the occurrence of the final AO, when upstream key events are observed is extremely consistent. It is also very important to note that the AO, alterations in cognitive function, is not likely to be specific solely to this AOP. Many of the key events included in this AOP overlap with AOPs linking other molecular initiating events to alterations in hippocampally-driven cognitive behaviors such as spatial learning in rats and IQ in humans.

Uncertainties, inconsistencies, and data gaps:

There are several areas of uncertainty and data gaps in the current AOP:

- There is a lack of quantitative information for several the KERs. These gaps hamper development of quantitative models that will allow linkages between the MIE and AO. Quantitative models are needed to facilitate efficient use of data on ~1000 chemicals from in vitro TPO assays (e.g., Paul-Friedman et al., 2016) to predict potential adverse outcomes. Computational models are needed to describe relationships

between serum and brain TH as a critical KER. With an additional metric of TH action in brain, this may be sufficient for application to computational prediction in the regulatory arena. These gaps include:

- Insufficient information exists to quantitatively link the degree of in vivo TPO inhibition required to elicit specific decrements in circulating T4 concentrations;
- There is a lack of data to quantitatively associate serum TH concentrations with TH concentrations in specific brain regions;
- Presently TH-responsive gene expression in hippocampus has not been quantitatively linked to changes in hippocampal anatomy, hippocampal function, and subsequent adverse cognitive effects.
- There is limited available data that inform a quantitative relationship between in vitro and in vivo inhibition of TPO (but see Leonard et al., 2016).
- Compensatory feedback systems are not included in this AOP. For example, it is well known that with chemicals that inhibit TPO (e.g., PTU) decrease circulating TH concentrations which activates the hypothalamic-pituitary feedback system (Capen, 1997). This leads to increased secretion of TSH, which upregulates TH synthesis in the thyroid gland (e.g., McCain, 1995; Capen, 1997; Hill et al., 1998). There is also compensation within the developing nervous system where low tissue T4 concentrations upregulates deiodinases in an attempt to maintain proper levels of T3 (e.g., Morse et al., 1996; Sharlin et al 2010). These and other compensatory systems are likely to be differentially active across different developmental ages and in different brain regions
- Lastly, there is some uncertainty in the literature about the role of thyroid stimulating hormone (TSH) in thyroid hormone based adverse outcome pathways and the relevance of rodent data for humans. It is clear that TSH is a key event in the AOP for rat thyroid follicular tumors (McCain, 1995; Hill et al., 1998) and this pathway is not deemed relevant to humans (Axelrad et al., 2005). However, it is critically important to note that the current AOP does not contain TSH as a KE. This is because, while TSH may play a role in feedback-driven compensatory processes to maintain peripheral hormone concentrations, it is not directly involved in brain development. In this AOP, TSH may be used as a supporting biomarker for alterations in circulating THs, however, it is not a perfect surrogate. There are also numerous examples of pharmaceutical and industrial chemicals that alter circulating THs in rats without any measurable change in TSH (NTP, 1990; O'Connor et al., 1998 2000; Liu et al., 1994; Zoeller et al., 2005; Morse et al., 1996; Goldey et al., 1995; Lau et al 2003; Schneider et al., 2011). In the absence of TSH changes, some of these chemicals do result in adverse neurological outcomes (e.g., Goldey and Crofton, 1998; Crofton, 2004; Zoeller et al., 2005; Cope et al., 2015). Therefore, stressor-induced changes in TH, not in TSH, are responsible for adverse neurological outcomes.

Quantitative Consideration

Assessment of quantitative understanding of the AOP: Currently, there are quantitative models for the early KERs from TPO inhibition to serum hormone concentrations, but none for later KERs. And only one of these models the KERs during early development (Fisher et al., 2013). For the rest of the KERs in this AOP, there is a varying amount of data from dose-response studies that demonstrate increasing impact with increasing chemical dose for all the KEs, and the direct and indirect KERs. At present, the overall quantitative understanding of the AOP is insufficient to directly link a measure of chemical potency as a TPO inhibitor to a quantitative prediction of effect on cognitive function (e.g., IQ in humans, learning deficits in rodents). Empirical information on dose-response relationships for the intermediate KEs, currently unavailable, would inform a computational, predictive model for thyroid disruption via TPO inhibition.

Considerations for Potential Applications of the AOP (optional)

1. This AOP may provide context for interpretation of data derived from Key Events corresponding to an Integrated Approaches and Testing Assessment (IATA) strategy (e.g., US EPA, 2011). It should be noted that it not necessary to quantify all the intermediate KEs defined in an AOP pathway to enable computational modeling to proceed to a quantitative model that would predict cognitive outcomes from in vitro TPO data.
2. This AOP stimulated recent efforts to develop a high-throughput screening assay to detect potential thyroperoxidase inhibition (Paul et al., 2014; Paul-Friedman et al., 2016) that could inform quantitative structure activity relationships, read-across models, and/or systems biology models to prioritize chemicals for further testing.

References

- Aarse J, Herlitze S, Manahan-Vaughan D. The requirement of BDNF for hippocampal synaptic plasticity is experience-dependent. *Hippocampus*. 2016 Jun;26(6):739-51.
- Andero R, Choi DC, Ressler KJ. BDNF-TrkB receptor regulation of distributed adult neural plasticity, memory formation, and psychiatric disorders. *Prog Mol Biol Transl Sci*. 2014;122:169-92.
- Antonica F, Kasprzyk DF, Opitz R, Iacovino M, Liao XH, Dumitrescu AM, Refetoff S, Peremans K, Manto M, Kyba M, Costagliola S. Generation of functional thyroid from embryonic stem cells. *Nature*. 2012 491(7422):66-71.
- Axelrad DA, Baetcke K, Dockins C, Griffiths CW, Hill RN, Murphy PA, Owens N, Simon NB, Teuschler LK. Risk assessment for benefits analysis: framework for analysis of a thyroid-disrupting chemical. *J Toxicol Environ Health A*. 2005 68(11-12):837-55.
- Calvo R, Obregón MJ, Ruiz de Oña C, Escobar del Rey F, Morreale de Escobar G. Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. *J Clin Invest*. 1990 Sep;86(3):889-99.
- Capen CC Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicol Pathol*. 1997 Jan-Feb;25(1):39-48. Review.

- Cooper, D.S., Kieffer, J.D., Halpern, R., Saxe, V., Mover, H., Maloof, F., and Ridgway, E.C. (1983). Propylthiouracil (PTU) pharmacology in the rat. II. Effects of PTU on thyroid function. *Endocrinology* 113:921–928.
- Cope RB, Kacew S, Dourson M. A reproductive, developmental and neurobehavioral study following oral exposure of tetrabromobisphenol A on Sprague-Dawley rats. *Toxicology*. 2015 329:49-59.
- Crofton KM, Kodavanti PR, Derr-Yellin EC, Casey AC, Kehn LS. PCBs, thyroid hormones, and ototoxicity in rats: cross-fostering experiments demonstrate the impact of postnatal lactation exposure. *Toxicol Sci*. 2000 57(1):131-40.
- Crofton KM. Developmental disruption of thyroid hormone: correlations with hearing dysfunction in rats. *Risk Anal*. 2004 Dec;24(6):1665-71.
- Degon, M., Chipkin, S.R., Hollo, C.V., Zoeller, R.T., and Chait, Y. (2008). A computational model of the human thyroid. *Mathematical Biosciences* 212, 22–53
- Derksen-Lubsen, G. and P. H. Verkerk (1996). "Neuropsychologic development in early treated congenital hypothyroidism: analysis of literature data." *Pediatr Res* 39(3): 561-6.
- Ekerot P, Ferguson D, Glämså EL, Nilsson LB, Andersson H, Rosqvist S, Visser SA. Systems pharmacology modeling of drug-induced modulation of thyroid hormones in dogs and translation to human. *Pharm Res*. 2013 30(6):1513-24.
- Escobar-Morreale HF, Obregón MJ, Hernandez A, Escobar del Rey F, Morreale de Escobar G. Regulation of iodothyronine deiodinase activity as studied in thyroidectomized rats infused with thyroxine or triiodothyronine. *Endocrinology*. 1997 Jun;138(6):2559-68.
- Fisher JW, Li S, Crofton K, Zoeller RT, McLanahan ED, Lumen A, Gilbert ME. Evaluation of iodide deficiency in the lactating rat and pup using a biologically based dose-response model. *Toxicol Sci*. 2013 132(1):75-86.
- Goldey ES, Crofton KM. Thyroxine replacement attenuates hypothyroxinemia, hearing loss, and motor deficits following developmental exposure to Aroclor 1254 in rats. *Toxicol Sci*. 1998 45(1):94-10
- Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats. *Toxicol Appl Pharmacol*. 1995; 135(1):77-88.
- Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science*. 1992 Dec 18;258(5090):1903-10.
- Howdeshell, K.L. A Model of the Development of the Brain as a Construct of the Thyroid System. *Env Hlth Perspect*. 2002. 100(suppl 3):337-348.
- Hill RN, Crisp TM, Hurley PM, Rosenthal SL, Singh DV. Risk assessment of thyroid follicular cell tumors. *Environ Health Perspect*. 1998 Aug;106(8):447-57.
- Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, Butenhoff JL, Stevenson LA. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. *Toxicol Sci*. 2003 Aug;74(2):382-92
- Lee KH, Lee H, Yang CH, Ko JS, Park CH, Woo RS, Kim JY, Sun W, Kim JH, Ho WK, Lee SH. Bidirectional Signaling of Neuregulin-2 Mediates Formation of GABAergic Synapses and Maturation of Glutamatergic Synapses in Newborn Granule Cells of Postnatal Hippocampus. *J Neurosci*. 2015 Dec 16;35(50):16479-93.
- Liu J, Liu Y, Barter RA, Klaassen CD.: Alteration of thyroid homeostasis by UDP-glucuronosyltransferase inducers in rats: a dose-response study. *J Pharmacol Exp Ther* 273, 977-85, 1994.
- McClain RM. Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. *Mutat Res*. 1995 Dec;333(1-2):131-42
- Morreale de Escobar, G. The role of thyroid hormone in fetal neurodevelopment. *J Pediatr Endocrinol Metabol*. 2001. 14; 1453-62.
- Morse DC, Wehler EK, Wesseling W, Koeman JH, Brouwer A. Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol Appl Pharmacol*. 1996 Feb;136(2):269-79.
- NTP National Toxicology Program.: NTP toxicology and carcinogenesis studies of 3,3'-dimethylbenzidine dihydrochloride (CAS no. 612-82-8) in F344/N rats (drinking water studies). *Natl Toxicol Program Tech Rep Ser* 390, 1-238, 1991.
- O'Connor, J. C., J. C. Cook, et al. (1998). "An ongoing validation of a Tier I screening battery for detecting endocrine-active compounds (EACs)." *Toxicol Sci* 46(1): 45-60.
- O'Connor, J. C., L. G. Davis, et al. (2000). "Detection of dopaminergic modulators in a tier I screening battery for identifying endocrine-active compounds (EACs)." *Reprod Toxicol* 14(3): 193-205.
- Paul KB, Hedge JM, Rotroff DM, Hornung MW, Crofton KM, Simmons SO. Development of a thyroperoxidase inhibition assay for high-throughput screening. *Chem Res Toxicol*. 2014 Mar 17;27(3):387-99
- Paul KB, Hedge JM, Macherla C, Filer DL, Burgess E, Simmons SO, Crofton KM, Hornung MW. Cross-species analysis of thyroperoxidase inhibition by xenobiotics demonstrates conservation of response between pig and rat. *Toxicology*. 2013. 312:97-107
- Paul-Friedman K, Watt ED, Hornung MW, Hedge JM, Judson RS, Crofton KM, Houck KA, Simmons SO. 2016. Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries. *Toxicol Sci*. 151:160-80.
- Schneider S, Kaufmann W, Strauss V, van Ravenzwaay B. Vinclozolin: a feasibility and sensitivity study of the ILSI-HESI F1-extended one-generation rat reproduction protocol. *Regul Toxicol Pharmacol*. 2011 Feb;59(1):91-100.
- Sharlin DS, Gilbert ME, Taylor MA, Ferguson DC, Zoeller RT. The nature of the compensatory response to low thyroid hormone in the developing brain. *J Neuroendocrinol*. 2010 Mar;22(3):153-65. d

Spilker C, Nullmeier S, Grochowska KM, Schumacher A, Butnaru I, Macharadze T, Gomes GM, Yuanxiang P, Bayraktar G, Rodenstein C, Geiseler C, Kolodziej A, Lopez-Rojas J, Montag D, Angenstein F, Bär J, D'Hanis W, Roskoden T, MikhaylovaM, Budinger E, Ohl FW, Stork O, Zenclussen AC, Karpova A, Schwegler H, Kreutz MR. A Jacob/Nsmf Gene Knockout Results in Hippocampal Dysplasia and Impaired BDNF Signaling in Dendritogenesis. *PLoS Genet.* 2016. 12(3):e1005907

Taurog A. Molecular evolution of thyroid peroxidase. *Biochimie.* 1999 May;81(5):557-62

Taurog A. 2005. Hormone synthesis. In: Werner and Ingbar's *The Thyroid: A Fundamental and Clinical Text* (Braverman LE, Utiger RD, eds). Philadelphia: Lippincott, Williams and Wilkins, 47–81

US EPA (2011) FIFRA Scientific Advisory Panel Consultation. Integrated Approaches to Testing and Assessment Strategy: Use of New Computational and Molecular Tools, US. May 24, 26, 2011, US Environmental Protection Agency, Office of Pesticide Programs, Washington DC

Vickers AE1, Heale J, Sinclair JR, Morris S, Rowe JM, Fisher RL. Thyroid organotypic rat and human cultures used to investigate drug effects on thyroid function, hormone synthesis and release pathways. *Toxicol Appl Pharmacol.* 2012 260(1):81-8.

Zoeller RT, Crofton KM. Mode of action: developmental thyroid hormone insufficiency--neurological abnormalities resulting from exposure to propylthiouracil. *Crit Rev Toxicol.* 2005 35(8-9):771-81. Review. PubMed PMID: 16417044.

Zoeller, R. T., R. Bansal, et al. (2005). "Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain." *Endocrinology* 146(2): 607-612.

Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. *J Neuroendocrinol.* 2004 Oct;16(10):809-18