AOP ID and Title:

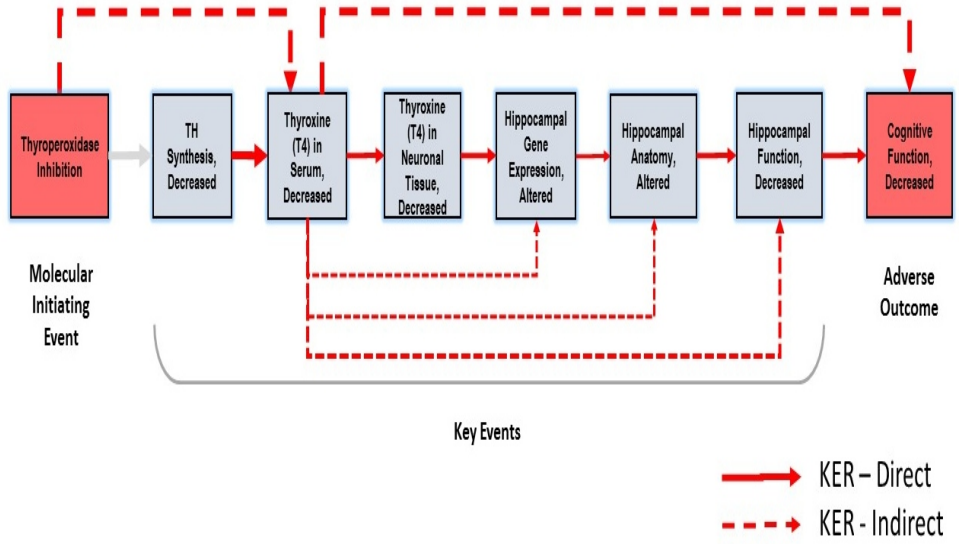
SNAPSHOT

Created at: 2018-08-10 16:14

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

Short Title: TPO Inhibition and Altered Neurodevelopment

Graphical Representation



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Status

Author status	OECD status	OECD project	SAAOP status
Open for citation & comment	EAGMST Approved	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

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	279	Thyroperoxidase, Inhibition (https://aopwiki.org/events/279)	Thyroperoxidase, Inhibition
2	KE	277	Thyroid hormone synthesis, Decreased (https://aopwiki.org/events/277)	TH synthesis, Decreased
3	KE	281	Thyroxine (T4) in serum, Decreased (https://aopwiki.org/events/281)	T4 in serum, Decreased
4	KE	280	Thyroxine (T4) in neuronal tissue, Decreased (https://aopwiki.org/events/280)	T4 in neuronal tissue, Decreased
5	KE	756	Hippocampal gene expression, Altered (https://aopwiki.org/events/756)	Hippocampal gene expression, Altered
6	KE	757	Hippocampal anatomy, Altered (https://aopwiki.org/events/757)	Hippocampal anatomy, Altered
7	KE	758	Hippocampal Physiology, Altered (https://aopwiki.org/events/758)	Hippocampal Physiology, Altered
8	AO	402	Cognitive Function, Decreased (https://aopwiki.org/events/402)	Cognitive Function, Decreased

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Thyroperoxidase, Inhibition (https://aopwiki.org/relationships/309)	adjacent	Thyroid hormone synthesis, Decreased	High	Low
Thyroid hormone synthesis, Decreased (https://aopwiki.org/relationships/305)	adjacent	Thyroxine (T4) in serum, Decreased	High	Moderate

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/relationships/312)	adjacent	Thyroxine (T4) in neuronal tissue, Decreased	Moderate	Low
Thyroxine (T4) in neuronal tissue, Decreased (https://aopwiki.org/relationships/746)	adjacent	Hippocampal gene expression, Altered	Moderate	Low
Hippocampal gene expression, Altered (https://aopwiki.org/relationships/747)	adjacent	Hippocampal anatomy, Altered	Moderate	Low
Hippocampal anatomy, Altered (https://aopwiki.org/relationships/749)	adjacent	Hippocampal Physiology, Altered	Moderate	Low
Hippocampal Physiology, Altered (https://aopwiki.org/relationships/748)	adjacent	Cognitive Function, Decreased	High	Moderate
Thyroperoxidase, Inhibition (https://aopwiki.org/relationships/366)	non-adjacent	Thyroxine (T4) in serum, Decreased	High	Moderate
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/relationships/1387)	non-adjacent	Hippocampal gene expression, Altered	High	Low
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/relationships/1388)	non-adjacent	Hippocampal anatomy, Altered	High	Low
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/relationships/1389)	non-adjacent	Hippocampal Physiology, Altered	Moderate	Low
Thyroxine (T4) in serum, Decreased (https://aopwiki.org/relationships/403)	non-adjacent	Cognitive Function, Decreased	High	Moderate

Stressors

Name	Evidence
Methimazole	High
Propylthiouracil	High

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/2017/09/17/7kg720qgr1_TPO_AOP_Summary_Tables_20170916.pdf (https://aopwiki.org/system/dragonfly/production/2017/09/17/7kg720qgr1_TPO_AOP_Summary_Tables_20170916.pdf)

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Perinatal	High
Foetal	High

Life Stage	Evidence
During brain development	High

Taxonomic Applicability

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

- **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., 2016).
- **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. The influence of maternal thyroid status prior to onset of fetal thyroid function is an important consideration. 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). TPO is classically defined as a complex enzyme with multiple catalytic cycles capable of iodinating multiple species (Divi et al., 1997). However, in the context of this AOP we are using TPO inhibiton not in the classical sense, but instead to refer to the results derived from the assays commonly used assays to investigate environmental chemicals (e.g., guiacol oxidation). 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), with similar findings in adult rats (Cooper et al.,1984). Studies in adult animals show a similar recovery after cessation of dosing (e.g., Hill et al., 1998).
- Thyroxine (T4) in neuronal tissue, decreased: Mulitple 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/Nsfm 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 is also widely accepted that 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 quantitiative 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). A more recent model predicts neuroanatomical anomalies based on serum and brain T4 concentrations (Hassan et al., 2017). 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 susceptibity 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 (e.g., iodine deficiency, thyroidectomy, gene knockouts) 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; Genistein is an example of where a very large degree of inhibition may be required to have an impact on serum TH;
    - 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. Neither has this AOP considered the nongenomic actions of TH on cell signaling in brain.
- There is limited available data that inform a quantitative relationship between in vitro and in vivo inhibition of TPO (but see Vickers et al., 2012).
- 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, exposure to 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 inhibiton to serum hormone concentrations, but none for later KERs. And only one of these models the KERs during early development (Fisher et al., 2013). A recent study by Hassan et al. (2017) quantitatively linked PTU-induced TH synthesis declines in the dam and the fetus to decrements in serum and brain TH concentrations to a structural malformation in the postnatal brain. In this study, estimates of TPO inhibition were derived from glandular and serum PTU and TH concentrations. 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.

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Appendix 1

List of MIEs in this AOP

Event: 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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	MolecularInitiatingEvent
Aop:119 - Inhibition of thyroid peroxidase leading to follicular cell adenomas and carcinomas (in rat and mouse) (https://aopwiki.org/aops/119)	MolecularInitiatingEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	MolecularInitiatingEvent
Aop: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
Daidzein
Genistein
4-Nonylphenol
4-propoxyphenol
Sulfamethazine

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
thyroid follicular cell

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).

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rat	Rattus norvegicus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
humans	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)
pigs	Sus scrofa	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9823)
Xenopus laevis	Xenopus laevis	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
chicken	Gallus gallus	High	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	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=90988)

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

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 (Schmultzer 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. In addition, these authors noted a qualitative concordance that ranged from 1.5 to 50-fold differences estimated by relative potency. Similary, Takayama et al. (1986) found a very large species difference in potency for sulfamonomethoxine between cynomologus monkeys and rats.

Key Event Description

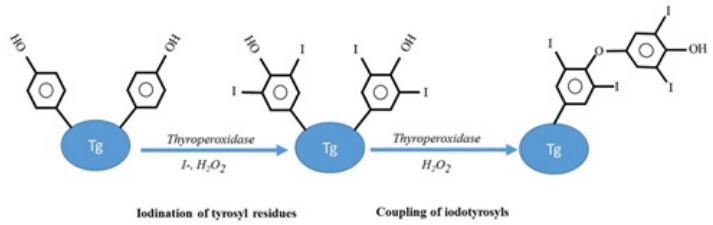
Thyroperoxidase (TPO) is a heme-containing apical membrane protein within the follicular lumen of thyrocytes that acts as the enzymatic catalyst for thyroid hormone (TH) synthesis. TPO catalyzes several reactions in the thyroid gland, including: the oxidation of iodide; nonspecific iodination of tyrosyl 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.

It is important to note that TPO is a complex enzyme and that has two catalytic cycles and is capable of iodinating multiple species (Divi et al., 1997). Alterations in all of these events are not covered by some of the commonly used assays that measure “TPO inhibition” (e.g., guicacol and AmplexUltraRed, see below). Therefore, in the context of this AOP we are using TPO inhibiton not in the classical sense, but instead to refer to the empirical data derived from the assays commonly used assays to investigate environmental chemicals.

Figure 1 below illustrates the enzymatic and nonenzymatic reactions mediated by TPO that result in the synthesis of thyroxine (T4) .

Figure 1. Synthesis of thyroxine (T4) by thryoperoxidase showing the iodination of tyrosyl residues and subsequent coupling of iodotyrosyls to form T4.



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 in rats, and the sodium iodide symporter, NIS (*Nis*), is expressed by gestational day 16 in rats. 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 human 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). It is important to note that prior to the onset of fetal thyroid function, TH are still required by the developing fetus which until that time relies solely on maternal sources. Chemical-induced TPO inhibition can affect synthesis in the maternal gland and in the fetal gland.

How it is Measured or Detected

There are no approved OECD or EPA guideline study protocols for measurement of TPO inhibition.. However, there is an OECD scoping document on identification of chemicals that modulate TH signaling that provides details on a TPO assay (OECD, 2017).

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.

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List of Key Events in the AOP

Event: 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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop:65 - XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
Aop:128 - Kidney dysfunction by decreased thyroid hormone (https://aopwiki.org/aops/128)	MolecularInitiatingEvent
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
Aop:54 - Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent
Aop:175 - Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	KeyEvent
Aop:176 - Sodium Iodide Symporter (NIS) Inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/176)	KeyEvent
Aop:188 - Iodotyrosine deiodinase (IYD) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/188)	KeyEvent
Aop:192 - Pendrin inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/192)	KeyEvent
Aop:193 - Dual oxidase (DUOX) inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/193)	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
thyroid follicular cell

Organ term

Organ term
thyroid gland

Evidence for Perturbation by Stressor

Overview for Molecular Initiating Event

not applicable as this KE is not an MIE

Propylthiouracil

6-n-proylthiouracil is a common positive control

Methimazole

Methimazole is a very common positve control

Domain of Applicability

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence
Male	High
Female	High

Decreased TH synthesis resulting from TPO or NIS inhibition is conserved across taxa, with *in vivo* evidence from humans, rats, amphibians, some fish specis, 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).

Key Event Description

The thyroid hormones (TH), triiodothyronine (T3) and thyroxine (T4) are thyrosine 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 concentations 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). *The ontogeny of TPO inhibition during development by environmental chemicals is a data gap.*

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.

Increased fetal thyroid levels are also possible. Maternal Graves disease results in fetal thyrotoxicosis (hyperthyroidism and increased serum T4 levels), has been successfully treated by maternal administration of TPO inhibitors (c.f., Sato et al., 2014).

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; Zoeller, 2004). 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 spectometry. 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).

TIQDT (Thyroxine-immunofluorescence quantitative disruption test) is a method that provides an immunofluorescent based estimate of thyroxine in the gland of zebrafish (Thienpont et al 2011). This method has been used for ~25 xenobiotics (e.g., amitrole, perchlorate, methimazole, PTU, DDT, PCBs). The method detected changes for all chemicals known to directly impact TH synthesis in the thyroid gland (e.g., NIS and TPO inbibitors), but not those that upregulate hepatic catabolism of T4.

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Event: 281: Thyroxine (T4) in serum, Decreased (https://aopwiki.org/events/281)

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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop:54 - Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
Aop: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
Aop:65 - XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent

AOP ID and Name	Event Type
Aop:152 - Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent
Aop:159 - Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	KeyEvent
Aop:175 - Thyroperoxidase inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/175)	KeyEvent
Aop:176 - Sodium Iodide Symporter (NIS) Inhibition leading to altered amphibian metamorphosis (https://aopwiki.org/aops/176)	KeyEvent
Aop:194 - Hepatic nuclear receptor activation leading to altered amphibian metamorphosis (https://aopwiki.org/aops/194)	KeyEvent

Stressors

Name
Propylthiouracil
Methimazole

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
serum

Evidence for Perturbation by Stressor

Propylthiouracil

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

Methimazole

Classic positive control

Domain of Applicability

Taxonomic Applicability

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

Term	Scientific Term	Evidence	Links
mouse	Mus musculus	High	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	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=8355)
Pig	Pig	High	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	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

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 Isihara, 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.

Key Event Description

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; Chang et al., 2007).

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 is 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; Goldey 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. Analytical determination of iodothyronines (T3, T4, rT3, T2) and their conjugates, though methods employing HLPC, liquid chromatography, immuno luminescence, and mass spectrometry are less common, but are becoming increasingly available (Hornung et al., 2015; DeVito et al., 1999; Spencer, 2013). It is important to note that thyroid hormones concentrations can be influenced by a number of intrinsic and extrinsic factors (e.g., circadian rhythms, stress, food intake, housing, noise) (see for example, Döhler et al., 1979).

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). This is of particular significance when assessing the very low levels of TH present in fetal serum. Detection limits of the assay must be compatible with the levels in the biological sample. 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.

T4 can also be measured using immunoluminescence assay (Baret (https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorStored=Baret%2C+A) and Fert (https://onlinelibrary.wiley.com/action/doSearch?ContribAuthorStored=Fert%2C+V), 1989). The specific amplification (the xanthine oxidase dependent luminescence of luminol enhanced in the presence of Fe–EDTA complex) has been applied to T4 immunoassays, with T4–XO (xanthine oxidase). The performances of these assay is at least equivalent to those obtained with iodinated tracers, using the same solid phases and the same calibrators. The major advantages of these immunoassays are: (1) the long-term signal which can be repeatedly recorded over several days, (2) the high detection sensitivity, (3) the long-term stability of the luminescence reagent, and (4) the stability of the conjugates.

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Event: 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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop:54 - Inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment (https://aopwiki.org/aops/54)	KeyEvent
Aop: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
Aop:65 - XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	KeyEvent
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
Aop: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 Context

Level of Biological Organization
Organ

Organ term

Organ term
brain

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

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).

Key Event Description

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 due to the low levels of hormones present and the small tissue volumes make quantitification 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; Obergon 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 though 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.

Distribution of TH in the brain differs between brain regions; for instance midbrain and telencephalon appear to be places of high signalling. This signalling pattern in adult mouse is well correlated with T3 distribution measured in various brain areas (Constantinou et al., 2005; Chatonnet et al., 2011; Moog et al., 2017).

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. Most often whole brain homogenates are assessed, obfuscating the known temporal and regional differences in brain hormone present. 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-triidothyronine (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.

The data published by Constantinou et al., (2005) determinedthe changes in the circulating thyroid hormone (TH) and brain synaptosomal TH content and the relative levels of mRNA encoding different thyroid hormone receptor (TR) isoforms in adult rat brain. Region-specific quantitative differences in the expression pattern of all thyroid hormone receptor isoforms in euthyroid animals and hypothyroid animals were recorded and the obtained results show that *in vivo* depletion of TH regulates TR gene expression in adult rat brain in a region-specific manner (Constantinou et al., 2005).

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Event: 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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop: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
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
Aop: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 Context

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Domain of Applicability

Taxonomic Applicability

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

Sex Applicability

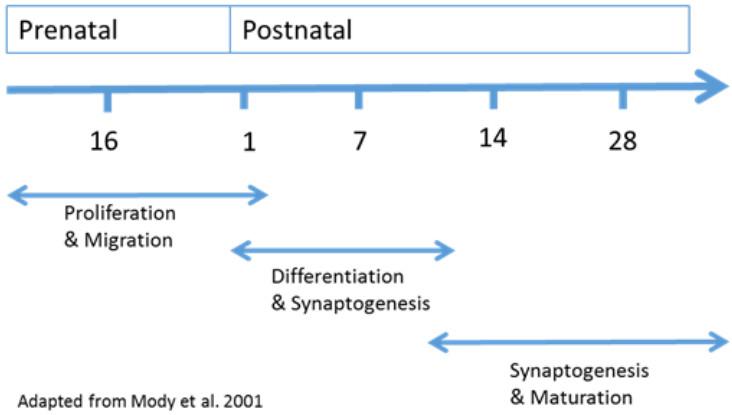
Sex	Evidence
Female	High
Male	High

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.

Key Event Description

Thyroid hormones control genes in the developing brain by classical ligand (T3) activation of thyroid receptors which leads to DNA binding and subsequent transcription and translation (for a review of TH rols in brain development see, Bernal 2015). 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 then 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).

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Event: 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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop: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
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
Aop:152 - Interference with thyroid serum binding protein transthyretin and subsequent adverse human neurodevelopmental toxicity (https://aopwiki.org/aops/152)	KeyEvent

Stressors

Name
Propylthiouracil

Name
Methimazole

Biological Context

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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.

Key Event Description

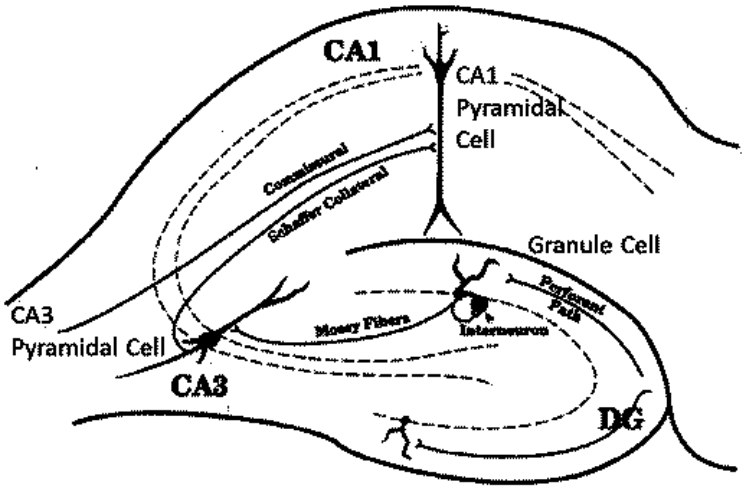
The hippocampus is a major brain region located in the medial temporal lobe in humans and other mammals (West, 1990). Developmentally it is derived from neuronal and glial cells in the neural tube and differentiates in the proencephalon and telencephalon. The hippocampus is a cortical structure, but 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; OCED, 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; Zgraggen et al., 2012). Similar techniques used in rodent stydies 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).

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Event: 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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	KeyEvent
Aop: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
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	KeyEvent
Aop: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 Context

Level of Biological Organization
Tissue

Organ term

Organ term
brain

Domain of Applicability

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	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10116)
mouse	Mus musculus	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=10090)

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Female	High
Male	High

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).

Key Event Description

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 areaCA1s 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 maintanence (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.

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List of Adverse Outcomes in this AOP

Event: 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
Aop:42 - Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	AdverseOutcome
Aop:65 - XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	AdverseOutcome
Aop:134 - Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	AdverseOutcome
Aop: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 Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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.

Key Event Description

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 (Eichenbaun, 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 (OCSPP 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 neurospychological 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 Significance of the AO

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 expsoures on cognitive function, often including spatially-mediated behaviors, is an intergral part of both EPA and OECD developmental neurotoxicity guidelines (USEPA, 1998; OECD, 2007).

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Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 309: Thyroperoxidase, Inhibition leads to TH synthesis, Decreased (https://aopwiki.org/relationships/309)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**adjacent**	**High**	**Low**
Thyroperoxidase inhibition leading to reduced young of year survival via anterior swim bladder inflation (https://aopwiki.org/aops/159)	**adjacent**		

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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.

Key Event Relationship Description

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 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).

Evidence Supporting this KER

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 systhesis of TH. TPO catalyzes several reactions, including the oxidation of iodide, nonspecific iodination of tyrosyl residues of thyroglobulin (Tg) to form monoiodotyrosyl (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 Evidence
Empirical support for this KER is strong. There are several papers that have measured alterations in TPO and subsequent effects on TH synthesisTaurog et al. (1996) showed decreased guicaol 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 (guicaol 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) using data using data from studies of iodine deficiency and chemicals that inhibit NIS. 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.
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> *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 guicaol 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 and 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.

It is important to note that data from studies on genistein highlight this uncertainty. Doerge and colleagues have demonstrated that for this compound up to 80% TPO inhibition did not result in decreased serum T4 in rats (Doerge and Chang, 2002). This is not consistent with other prototypical TPO inhibitors (e.g., PTU, MMI). It remains to be determined, if for some presently unknown reason, that genistein is an outlier or not. This again points to the need for quantitative modeling of the relationship between TPO inhibtion and downstream KEs.

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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.

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

AOPs Referencing Relationship

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9606)

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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 ares 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).

Key Event Relationship Description

Thyroid hormones (THs), thyroxine (T4) and triiodothyronine (T3) are synthesized by NIS and 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).

Evidence Supporting this KER

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 (serum T4).

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

Empirical Evidence
There is limited direct evidence supporting the relationship between decreased TH synthesis and lowered circulating hormone levels during development. Lu and Anderson (1994) assessed the time course of TH synthesis, measured as thyroxine secretion rate, analyzed in non-treated pregnant rats and correlated it with serum T4 levels. However, while empirical data is scarce, it is widely accepted dogma the TPO or NIS inhibition leads to declines in serum TH levels. 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 (or NIS inhibition) will lead to decreased serum TH concentrations due to inadequate synthesis. 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 131-I 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 of decreased TH synthesis on serum hormones is similar across all ages. Good evidence for the temporal relationship comes from thyroid system modeling of the impacts of iodine deficiency and NIS inhibition (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 are available demonstrating correlations between thyroidal TH and serum TH concentrations during gestation and lactation during development (Gilbert et al., 2013). These data were used to develop a rat quantitative biologically-based dose-response model for iodine deficiency (Fisher et al., 2013), which support the role of NIS activity inhibition in relation to TH levels (T3 and T4) in serum.

Uncertainties and 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.

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

AOPs Referencing Relationship

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

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	High
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).

Key Event Relationship Description

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 expresses 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 OATP), monocarboxylate transporter 8 (MCT8) (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 (MCT8) into neurons (Visser et al., 2011). Compared to the wildtype, a mouse MCT8 knockout model was shown to have decreased plasma T4, decreased uptake of T4 into the brain, and decreased brian T3 concentrations, as well as increased cortical diodinase Type 2 activity and increased plasma T3 concentrations (Mayerl et al., 2014; Barez-Lopez et al., 2016) .

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 eventually 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).

Evidence Supporting this KER

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 lifestages 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 Evidence

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 are 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 completely restore tissue TH levels to control levels.
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> *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 and Inconsistencies

The fact that decreased serum TH results in lower brain TH concentrations is well accepted. However, there is Limited data is available that demonstrates that changes in local deiodination in 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

The role of cellular transporters represents an additional uncertainly. In addition, future work on cellular transport mechanisms and deiodinase activity is likley to inform addition of new KEs and KERs between serum and brain T4.

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Relationship: 746: T4 in neuronal tissue, Decreased leads to Hippocampal gene expression, Altered

(https://aopwiki.org/relationships/746)

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**adjacent**	**Moderate**	**Low**
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	**adjacent**	**Moderate**	**Low**

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	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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 (see text above).

Key Event Relationship Description

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 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.

Evidence Supporting this KER

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 Evidence

The empirical support for this KER is moderate. Many in vitro studies have demonstrated a relationship between hormone concentraionsTH 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 and 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).

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**adjacent**	**Moderate**	**Low**
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	**adjacent**	**Moderate**	**Low**

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	High	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	High
Female	High

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.

Key Event Relationship Description

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.

Evidence Supporting this KER

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 Evidence

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/Nsfm 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/Nsfm 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.

Neuroregluin-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 and 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. Significant data gaps also exist for basic fetal hippocampal development.

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**adjacent**	**Moderate**	**Low**
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	**adjacent**	**Moderate**	**Low**

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	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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.

Key Event Relationship Description

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.

Evidence Supporting this KER

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 Evidence
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).

Neuroregluin-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 synapogenesis, 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-b 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-b 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 (Bruel-Jungerman et al., 2007, Bruel-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 and 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)

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**adjacent**	**High**	**Moderate**
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	**adjacent**	**Moderate**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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.

Key Event Relationship Description

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 itirrefutable that the hippocampus is essential for specific types of cognition abilities. Decades of animal research has reinforced this assertion.

Evidence Supporting this KER

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; Panjo 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 Evidence
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 asspatial 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 and 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-Garcia, 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.

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List of Non Adjacent Key Event Relationships

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

AOPs Referencing Relationship

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

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Male	High

Sex	Evidence
Female	High

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.

Key Event Relationship Description

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 section 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.

Evidence Supporting this KER

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 Evidence
The majority of research in support of this KER involve exposure to known TPO inhibitors and measurement of serum hormones. There are a 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; Sundaresh 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 a., 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 (Wishe 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 a., 2013).

Uncertainties and 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). The relationship between maternal and fetal levels of hormone following chemically-induced TPO inhibiton has not been well characterized and may differ based on kinetics. Reductions in serum TH in the fetus, in rat and human is derived a chemical's effect on the maternal thyroid gland as well as the fetal thyroid gland.

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**non-adjacent**	**High**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

Most of the data available has come from rodent models.

Key Event Relationship Description

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 (TRα and TRβ). 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.

Evidence Supporting this KER

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 Evidence

The empirical support for this KER is strong. A global transcriptome analysis of primary cerebrocoritical 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 + ClO4	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 + ClO4	Fetal GD17	Morte et al., 2010
Sema7a	Mouse- Cortex	MMI + ClO4	Fetal GD17	Morte et al., 2010
RC3 (Neurogranin)	Rat- Hippocampus, Cortex	MMI	Fetus-GD16	Dowling and Zoeller, 2001
Camk4	Mouse-cortex	Thyroidectomy, MMI + ClO4	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 and 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.

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**non-adjacent**	**High**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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

Key Event Relationship Description

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 (TRα and TRβ) 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.

Evidence Supporting this KER

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 Evidence
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 congenitaly hypothyroid children born to euthroid 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 it 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 euthroid 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 and 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. In one of the few dose-response studies assessing hippocampal anatomy, alterations in simple guidenline metrics of linear morphometry and volume of hippocampal subfields following developmental exposure to the PTU were largely restricted to the high dose group, despite alterations in downstream KEs of hippocampal physiology and cognitive function. This may result from inadequacy of the assessment tools or the timing of the observations. Similarly, in chemically induced serum hormone reductions of comparable magnitude as those induced by PTU or MMI, observations of hippocampal morphology are not always seen (PTU vs ETU or mancozeb, European Commission, 2017). Consideration of the sensitivity of neuroanatomical and neurobehavioral method used, as well as chemical kinetics that drive the reduction of maternal, fetal, or neonatal TH reduction, may be key to understanding these discrepancies.

More data is needed that link limited (small) decrements in serum TH to specific hippocampal anatomical changes. The role of direct fetal TPO inhibition contribution to fetal TH and subsequent changes to hippocampal structure and subsequent downstream KEs in humans is a knowledge gap.

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**non-adjacent**	**Moderate**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Sex Applicability

Sex	Evidence
Male	High
Female	High

Most of the data to support this KER are derived from rodent studies.

Key Event Relationship Description

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 transport of primarily T4 from serum, with subsequent conversion to T3 in the

astrocytes by deiodinase and transfer to nuclear receptors within the neuron. This is followed by TH dependent gene transcription that influences hippocampal structural development and subsequent physiological function.

Evidence Supporting this KER

The weight of evidence for this indirect relationship is moderate. A wide variety studies have been performed in several labs in which thyroid hormone reductions in serum induced by chemicals/treatments, acting at a variety of target sites to disrupt hormonal status, is coincident with altered hippocampal physiology and/or plasticity. These include inhibition of TPO, NIS, dietary insufficiencies of iodine, and upregulation of liver catabolism, NIS inhibition, or dietary manipulation of iodine. Most of the data available is from the model TPO inhibitors, PTU and MMI, and this data documents enduring hippocampal physiological impairments in adult offspring following a period of transient serum TH insufficiencies in the pre- and post-natal period. Serum hormones are reported for the neonate and the dam at the termination of exposure, and recovery of hormonal status in the adult has been demonstrated in a number of studies despite the persistence of the hippocampal deficit. A few laboratories have reported dose-dependent effects at less than maximal hormone depletion.

Biological Plausibility
The biological plausibility of this KER is rated as strong. The relationship is consistent with the known biology of how TH control development of hippocampal physiology.

Empirical Evidence
Empirical support for this indirect KER is rated as strong. Empirical data from studies that measure serum TH concentrations and then assess alterations in synaptic function in the hippocampus have come from several laboratories. This work has employed in vivo, ex vivo and in vitro preparations from developmentally exposed animals.

Most of the in vivo neurophysiological assessments have been performed in the dentate gyrus. Excitatory and inhibitory synaptic transmission were reduced by PTU in a dose-dependent fashion (Gilbert and Sui, 2006; Gilbert et al., 2007; Gilbert, 2011). Serum T4 decrements in dams and pups were positively correlated with the synaptic impairments. Serum T4 and hippocampal excitatory transmission were also reduced in pups from dams exposed to perchlorate (Gilbert et al., 2008) and iodine deficiency (Gilbert et al., 2013). However, serum T4 reductions induced by the complex PCB mixture, A1254, were associated with increases not decrements in excitatory response amplitudes (Gilbert et al., 2003).

Impaired synaptic transmission and plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD) have been reported using in vitro and ex vivo preparations (Sui and Gilbert, 2003; Sui et al., 2005; 2007; Gilbert and Sui, 2006; Gilbert and Paczkowski, 2003; Gilbert, 2011; Taylor et al., 2008; Vara et al., 2002), Dong et al., 2005; Gilbert, 2003; 2004; 2011; Gilbert et al., 2016;), .

In many studies these observations have been reported under conditions of severe hypothyroidism induced primarily by TPO-inhibitors MMI and PTU or severe iodine deficiency (Vara et al., 2002; Dong et al., 2005). In others, researchers produced graded degrees of TH insufficiency in dams and pups by administering varying doses of PTU, perchlorate, or dietary iodine deficiency, and reported dose-dependency of the observed effects. This work has provided increased confidence in the relationship between TH insufficiency and functional impairment of the hippocampus, and the specificity of the observed effects to be mediated by TH insufficiency (Gilbert and Sui, 2006; Gilbert, 2011; Gilbert et al., 2013; 2016).

As described in the KER entitled “Hippocampal anatomy, altered leads to Hippocampal Physiology, Altered”, there is dynamic reciprocal interplay between neuroanatomy and physiology, particularly evident in the developing nervous sytem, making it difficult to parse the effects of one independently of the other (Kozorovitsky et al., 2012). In the *in vitro* studies of Westerholz et al (2010; 2013), T3-induced increase in GABAgeric synapses is activity-dependent, in that the anatomical changes described required both spontaneous electical activity in the network in addition to thyroid hormone. The electrophysiological competence of that emerging synaptic network was similarly dependent upon the hormone stimulation in addition to the growth of the GABAergic neurons. In this manner, TH can directly influence the formation of emerging cortical networks. Although demontrated using cortical neurons, it is expected that very similar processes occur in the developing neural networks of the hippocampus.

> *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 physiological function in the dentate gyrus (Gilbert 2011; Sanchez-Huerta et al., 2015). Rescue experiments have not been performed in developmental hypothyroid models. In *in vitro* studies, temporal specificity of the influence of T3 on network activity has been demonstrated (Westerholz et al., 2013).
>
> *Dose-Response Evidence:* There are several reports on the dose-dependent nature of the correlation between serum THs and changes in hippocampal physiology albeit from a limited number of laboratories (Taylor et al., 2008; Gilbert et al., 2007; 2016; Gilbert 2011; Gilbert and Sui, 2006).

Uncertainties and 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. However, most studies have been performed under conditions of severe hypothyroidism induced primarily by TPO-inhibitors MMI and PTU, or severe iodine deficiency. 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).

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

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/42)	**non-adjacent**	**High**	**Moderate**
XX Inhibition of Sodium Iodide Symporter and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/65)	**non-adjacent**	**High**	**Moderate**
Sodium Iodide Symporter (NIS) Inhibition and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (https://aopwiki.org/aops/134)	**non-adjacent**	**High**	**Low**

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

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

Life Stage Applicability

Life Stage	Evidence
During brain development	High

Sex Applicability

Sex	Evidence
Male	High
Female	High

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

Key Event Relationship Description

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. Clearly the brain circuitry controlling cognitive function is complex and is not solely accomplished by the functionality of the hippocampus. However, it is well documented that 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.

Evidence Supporting this KER

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 hypothyoidism 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; Axelstand etal., 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 Evidence
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 and 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. A major uncertainty is the precise relationship between the degree, timing and duration of serum TH changes that leads to these behavioral deficits.

Inconsistencies may also exist for chemicals other than classical TPO inhibitors that may reduce serum TH and induce impairments in cognitive function, but through action on other endocrine systems, or via direct action on the brain in the absence of an intervening endocrine action.

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