UNIVERSIDAD DE GUANAJUATO

Nuclear expression of thyroid hormone receptor α1 in the population of the hippocampal neurogenic niche of the adult mouse

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There are far, far better things ahead than any we leave behind.

- *C. S. Lewis*

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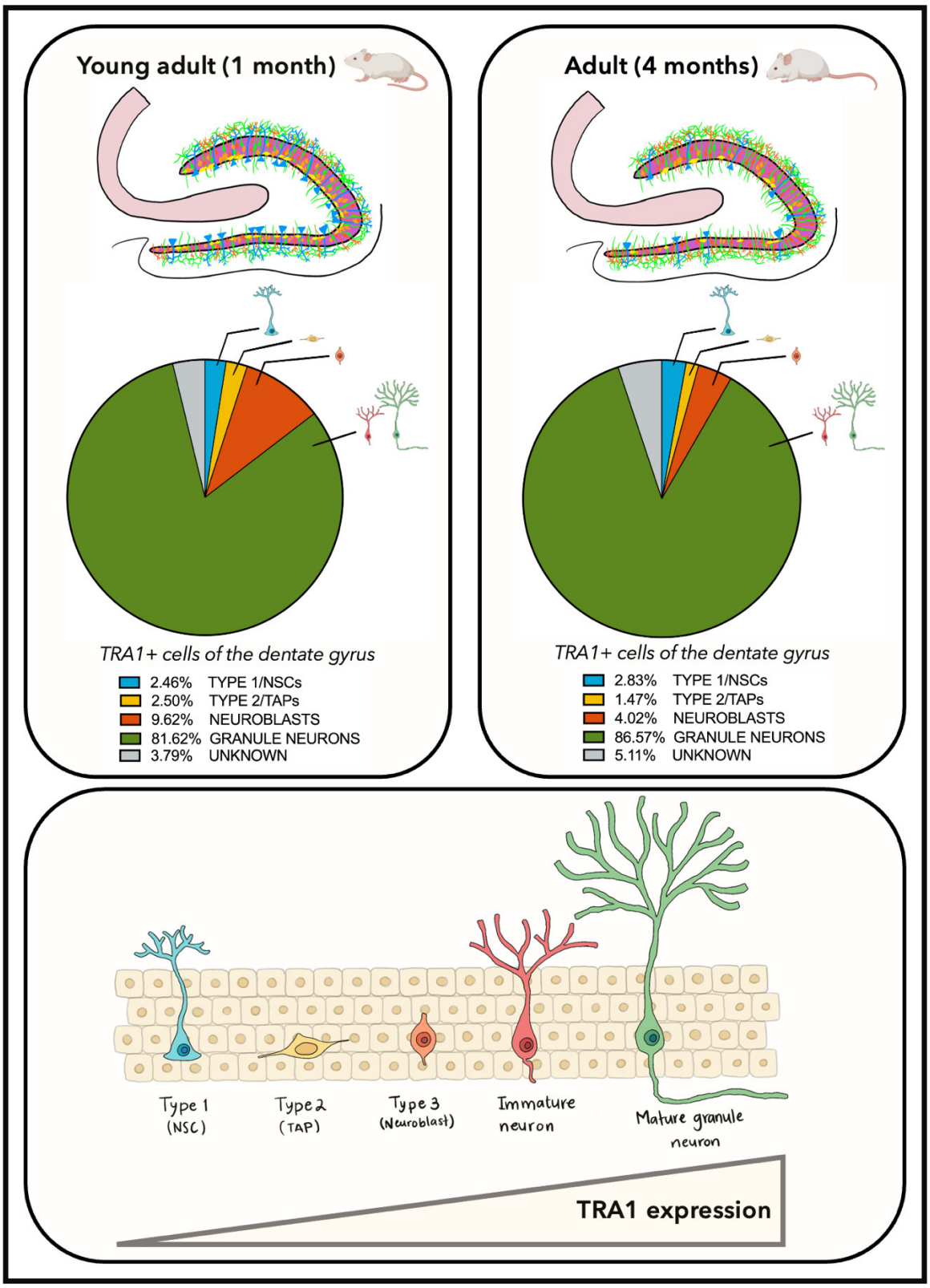
Graphical abstract



Abstract

Adult hippocampal neurogenesis (AHN) is a process that takes place in a specialized microenvironment called the subgranular zone (SGZ) localized in the dentate gyrus (DG) of the hippocampus. This neurogenic niche is of fundamental importance due to its involvement in higher cognitive functions, particularly in memory encoding, learning and mood regulation. In past studies, it has been demonstrated that hippocampal neurogenesis persists throughout life, even if it decreases with age. AHN has been found to be sensitive to perturbations in thyroid hormone (TH) signaling, with evidence supporting a key role for their effects through thyroid hormone receptors (TR) in the regulation of neural progenitor survival, neuronal differentiation, and maturation of postmitotic cells.

The thyroid hormone receptor alpha 1 (TRα1) has been found to be the major TR expressed in the brain, with a strong presence in the hippocampus. Through the nuclear action of TRα1, due to the presence of TH, the regulation of diverse aspects of stage specific hippocampal progenitor development occurs. Whether TRα1 is expressed in the nucleus of the different cells of the DG, from neural stem cells (NSCs) to mature granule neurons, has been topic of investigation, with multiple studies utilizing mutant murine models and TH treatments to try and resolve this.

To answer this question, and to identify if any changes occur in the TRα1 expression at different ages in adults, we used euthyroid mice from 1 and 4 months old, having used qPCR and immunohistochemistry to identify the expression of this receptor in the different cell populations of the DG. Together, our results reveal that from the available pool of proliferative cells, NSCs and transient amplifying progenitors (TAP), a small subpopulation expresses TRα1 and is maintained throughout aging. Furthermore, we corroborated that differentiated cells such as neuroblasts (NB) and granule neurons (GN) majorly expressed TRα1, though the overall pool of NB decreases and the pool of GN increases, respectively, with age.

Resumen

La neurogénesis en el hipocampo adulto (AHN) es un proceso que se lleva a cabo en un microambiente especializado denominado la zona subgranular (SGZ) localizada en el giro dentado (DG) del hipocampo. Este nicho neurogénico tiene fundamental importancia debido a que se encuentra involucrado en funciones cognitivas de orden mayor, particularmente en la codificación de memorias, el aprendizaje y la regulación del humor. Se han realizado estudios que demuestran que la neurogénesis en el hipocampo persiste a través de la vida, aun si disminuye durante el envejecimiento. Se ha encontrado que la AHN es sensible a perturbaciones ocasionadas por las hormonas tiroideas (TH), donde se ha encontrado evidencia de su efecto a través de los receptores a hormonas tiroideas (TR) en la regulación de la supervivencia de los progenitores neurales, la diferenciación neuronal, y la maduración de las células postmitóticas.

El receptor a hormonas tiroideas alfa 1 (TRα1) es el más abundante de los TR presentes en el cerebro, con una fuerte presencia en el hipocampo. A través del mecanismo de acción nuclear del TRα1, en presencia de la TH, ocurre la regulación de diferentes aspectos específicos del desarrollo de los progenitores hipocampales. Un tema de investigación en la época actual ha sido el definir si el TRα1 es expresado de manera nuclear en las diferentes poblaciones neuronales del giro dentado, desde las células madre neuronales (NSC) hasta las neuronas granulares (GN), donde múltiples estudios han utilizado modelos murinos mutantes y tratamientos tiroideos para dilucidar esta incógnita.

Para resolver esta pregunta, y para determinar si existen cambios en la expresión de TRα1 en diferentes etapas de la adultez, utilizamos ratones eutiroideos de 1 y 4 meses de edad, y las técnicas de RT-qPCR e inmunohistoquímica de fluorescencia para identificar la expresión de este receptor en las distintas poblaciones celulares del DG. Nuestros resultados revelan que, de la población disponible de células proliferativas, las NSCs y los progenitores amplificadores transitorios (TAP), una pequeña subpoblación expresa TRα1 y este nivel de expresión se mantiene a través de la edad. Adicionalmente, corroboramos que las células diferenciadas como los neuroblastos (NB) y las GN mayormente expresan TRα1 de manera nuclear, aunque el conjunto de NB decrementa y el conjunto de GN aumenta, respectivamente, a través de la adultez.

Introduction

Neurogenesis

For many years a central dogma of neuroscience was that neurogenesis, the process of generating new neurons, only occurred during development, stopping soon after birth, and thus, incapable of forming new neurons in the adult mammalian brain (Ramon y Cajal, 1928). It wasn't until the 1960s, that J. Altman reported, for the first time, that new neurons were present in different areas of the post-natal brain, such as the neocortex, the dentate gyrus (DG) of the hippocampus (HP), the olfactory bulb (OB), and the cerebellar cortex of different mammals (Altman, 1962). Throughout the next decades, this discovery was corroborated by different scientists such as Kaplan (Kaplan, 1981), and Gage and Kempermann (Gage et al., 1998; Kempermann & Gage, 1999), in adult rodents; Kornack and Rakic in macaque monkeys (Kornack & Rakic, 1999); Nottebohm in adult songbirds (Nottebohm, 2002); Reynolds and Weiss in the adult mouse striatum (Reynolds & Weiss, 1992); Kuhn with *in vivo* BrdU+ cells in adult rodents (Kuhn et al., 1996); and Erikkson in the DG of adult humans (Eriksson et al., 1998).

Together, this evidence has led to the conclusion that the brain does not have an immutable neuronal composition, with the concept moving towards a more plastic brain, where new neurons are continuously formed throughout adulthood in specific areas. The existence of adult human neurogenesis is controversial, and to this day, the scientific community has yet to reach a true verdict. The last two decades have been filled with discoveries regarding insights into the identity of neural stem cells (NSCs), the properties and cellular composition of neurogenic niches, the cellular dynamics, and the migratory behavior of newly generated cells, as well as the survival, integration, and functional relevance of these adult-born cells.

Neural stem cells

Neural stem cells (NSCs) possess two essential properties: self-renewal through cell division and the ability to generate differentiated progeny comprising neurons, astrocytes, and oligodendrocytes (Gage et al., 1998; Kilpatrick & Bartlett, 1993; Palmer

et al., 1997; Reynolds & Weiss, 1992). The proliferative capacity of NSCs significantly diminishes after development and through aging, coinciding with an increased prevalence of neurodegenerative disorders and age-related conditions (Figure 1). While only a limited number of neurogenic regions remain active in adulthood, they exhibit remarkable dynamism influenced by various physiological stimuli and pathological conditions (Urbán & Guillemot, 2014).

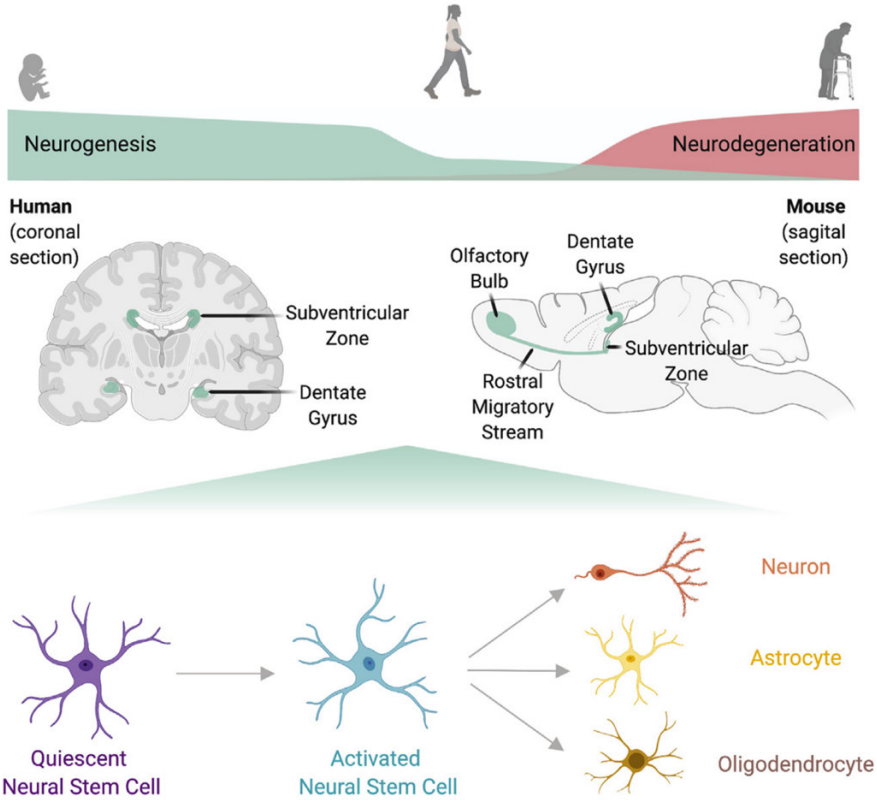


Figure 1. Neurogenesis during aging, the main neurogenic niches of the adult brain, and the neural lineages of NSCs

Throughout life, the ability of NSCs to proliferate declines sharply after development and continues to decline during aging, conversely the neurodegeneration incidence and age-related diseases increments. The most studied reservoirs of regenerative NSCs in adult mammalian brain are the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles. These niches contain quiescent NSCs (qNSCs) that, when active, produce actively proliferating NSCs (aNSCs) with the potential to differentiate into neurons, astrocytes and oligodendrocytes. Figure from (Navarro Negredo et al., 2020).

Among the neurogenic regions, the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus and the subventricular zone (SVZ) of the lateral ventricles (LV) are the most extensively studied in the adult mammalian brain (Alvarez-Buylla & Lim, 2004). These regions harbor quiescent NSCs (qNSCs) that can be activated to become actively proliferating NSCs (aNSCs). The aNSCs have the remarkable potential to differentiate into neurons, oligodendrocytes, or astrocytes (Navarro Negredo et al., 2020; Ribeiro & Xapelli, 2021).

Neurogenesis in the adult brain

Neurogenesis in the adult brain is regulated by a coordinated interplay of various signals that influence the behavior of neural stem cells (NSCs) and determine the diversity of neural and glial populations in the mature brain. During embryonic neurogenesis, the signals guide NSCs toward specific cell fate outcomes. In contrast, adult neurogenic regions exhibit more restricted potential, with stem cells primarily generating one or a few types of neurons, such as granule neurons in the subgranular zone (SGZ) or specific neuron types in the subventricular zone (SVZ) (Ming & Song, 2011; Zhao et al., 2008). Consequently, the factors and signals involved in subtype specification during embryonic development can exert more nuanced control over adult stem cell behavior (Urbán & Guillemot, 2014).

While certain transcription factors (TFs) demonstrate similar activity in both embryonic and adult hippocampal neurogenesis, such as Neurog2, Tbr2, and Prox1, some exhibit distinct effects during different stages of development and adulthood, including Tlx, CcnD2, and Ascl1 (Urbán & Guillemot, 2014). However, recent studies have revealed molecular-level differences in their functions between the developing and adult brains (Götz et al., 2016). For instance, Ascl1 plays a vital role in the proliferation, commitment, and differentiation of progenitors in the embryonic brain, but it is not necessary for DG development at this stage (Urbán & Guillemot, 2014). In the adult brain, Ascl1 continues

to be expressed in intermediate progenitors, and its increased degradation leads to a significant quiescence of NSCs in the DG (Harris et al., 2021).

Hippocampal neurogenic niche

The hippocampus is a crucial brain region involved in cognitive functions, such as learning, spatial memory, and spatial navigation (Masachs et al., 2021). It is situated in the medial temporal lobe and forms part of the limbic system associated with memory formation and emotions. Within the hippocampus, there are interconnected subregions, including the dentate gyrus, the CA1, CA2, and CA3 regions. Of particular interest is the DG, which exhibits a unique characteristic of adult neurogenesis. This neurogenesis occurs within a specialized microenvironment known as the subgranular zone (SGZ) (Li et al., 2009; Overall & Kempermann, 2018; Song et al., 2016).

The DG has a distinct V-shaped structure comprising three layers (Figure 2). The molecular layer (ML), the outermost first layer, primarily contains the dendrites of dentate granule cells and fibers from the perforant path originating in the entorhinal cortex. The second layer, the granule cell layer (GCL), consists of densely packed granule cells forming a thickness of approximately 6 to 8 neurons, surrounding the innermost layer. The third layer, called the hilus or polymorphic cell layer, encompasses various cell types, including mossy cells and GABAergic basket cells. The SGZ, a narrow band of cells, is located at the interface between the inner granule cell layer and the hilus of the DG. Within the SGZ, neural stem cells (NSCs) and transient amplifying progenitors (TAPs) can be found (Toni & Schinder, 2016).

The neurogenic niche consists of several components that interact and regulate the process of adult neurogenesis (Kempermann et al., 2015; Kozareva et al., 2019; Snyder & Drew, 2020), such as the differentiation between cellular groups and their mitotic capabilities. We will define two groups by their proliferative abilities, the mitotic pool,

which consists of the NSCs, TAPs, and neuroblasts (NB); and the post-mitotic group, that include the immature and mature granule neurons (GN).

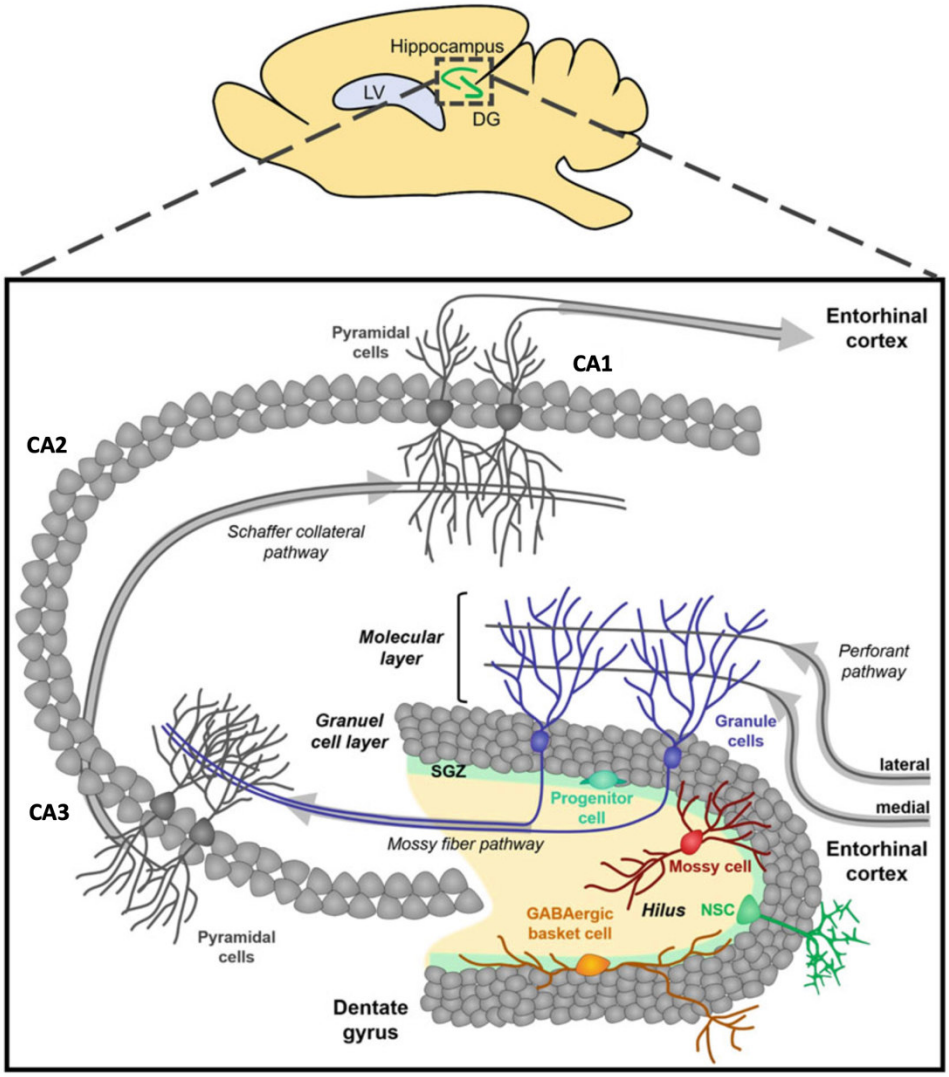


Figure 2. Schematic representation of the hippocampus, their structure, and circuits

The hippocampus is composed by the dentate gyrus (DG), the CA1, CA2 and CA3 regions. The CA 1-3 regions are composed by pyramidal cells, while the DG is divided in three layers, the molecular layer (ML) occupied by the dendrites of the granule cells and fibers of the perforant path; the granule cell layer (GCL/GL), which consists of densely packed granule cells; and the hilus that contains basket and mossy cells. Granule cells are connected synaptically via the trisynaptic circuit, where the main input is the performant path constituted by the axons coming from the lateral and medial entorhinal cortex. Then, granule neurons (GN) project mossy fibers to CA3 pyramidal cells, which afterwards project Schaffer collaterals to the CA1. Figure from (Calzà et al., 2021).

Hippocampal neural stem cells (NSCs), referred to as type 1 cells, are radial glial-like precursor cells. They possess astrocytic electrophysiological properties and express markers such as GFAP, Nestin, and Sox2. Type 1 cells have their cell bodies in the SGZ and extend processes that establish contact with both the molecular layer and blood vessels. Type 1 cells are typically quiescent and are maintained in this state by the release of GABA from GABAergic basket cells.

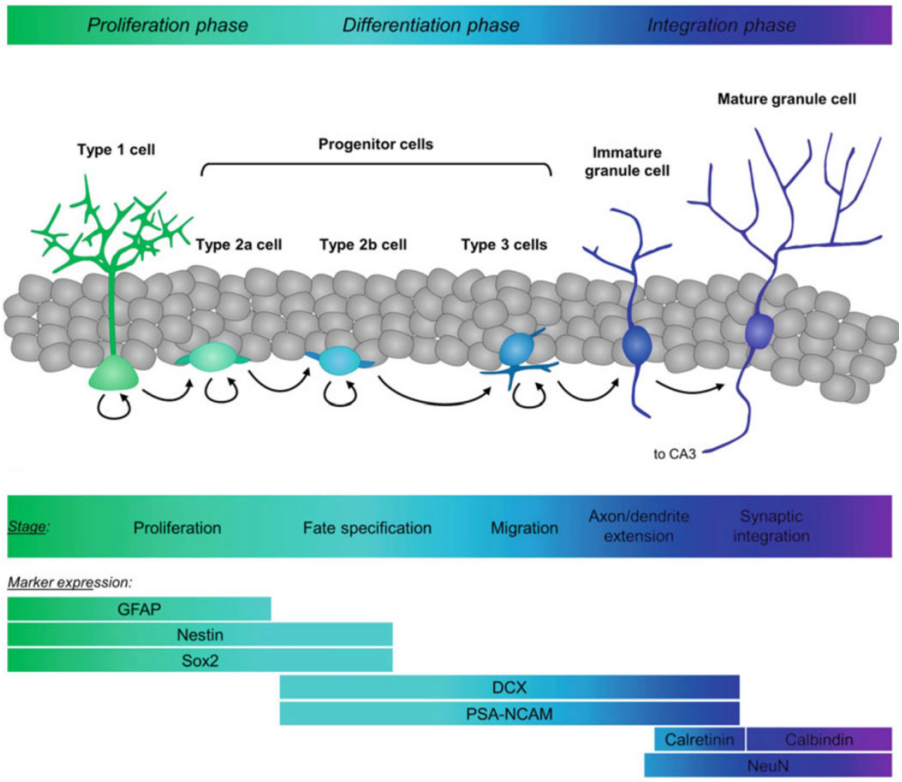


Figure 3. Schematic representation of adult neurogenesis in the DG, its cell types and the main markers expressed during this neurogenesis

The hippocampal neurogenesis can be represented by five developmental stages: first, proliferation by activated quiescent NSC (qNSCs) of non-radial precursors and transient amplifying progenitors/type 2a cells; second, fate specification that generates neuronal precursors/type 2b cells; third, short-distance radial migration of neuroblasts/type 3 cells through the granule cell layer; forth, dendrites and axon development by immature granule neurons; and fifth, the synaptic integration of adult born mature granule cells. GFAP, glial fibrillary acidic protein; DCX, doublecortin; PSA-NCAM, polysialylated neural cell adhesion molecule; NeuN, neuronal nuclei Figure from (Calzà et al., 2021).

Upon exiting quiescence, type 1 cells give rise to type 2 cells, which are transient amplifying progenitor cells (TAP). Unlike type 1 cells, type 2 cells exhibit high

proliferative activity and have a simpler morphology with horizontally oriented short processes. Type 2 cells can be further divided into two subsets: type 2a cells, which still express glial markers, and type 2b cells, which lack GFAP and express neuronal markers such as DCX and PSA-NCAM.

Neuroblasts or Type 3 cells, are generated from type 2b cells and display limited proliferative activity. They do not express Nestin or glial markers but instead express neuronal lineage markers, including DCX and PSA-NCAM. Type 3 cells exhibit diverse morphologies, lacking both radial and tangential processes. These cells migrate short distances radially to the granule cell layer, where they exit the cell cycle and differentiate into granule neurons. These neurons express neuronal markers such as NeuN and, depending on their level of maturation, calretinin for immature neurons, which are later replaced by calbindin for mature granule neurons.

Upon reaching the granule cell layer, newborn neurons continue to mature their processes. They extend their axons through the mossy fiber tract in the hilar region to establish connections with hippocampal CA3 neurons. Furthermore, they extend their dendrites towards the molecular layer, where they form synaptic contacts with axons from the entorhinal cortex.

Thyroid hormone regulation in the hippocampal neurogenic niche

The process of adult hippocampal neurogenesis is tightly regulated by intrinsic and extrinsic factors. It is influenced by systemic factors such as hormones, neurotransmitters, immune signals, and various environmental stimuli, including physical exercise, environmental enrichment, stress, and learning experiences (Navarro Negredo et al., 2020; Urbán & Guillemot, 2014).

Thyroid hormones (TH) (Figure 4), primarily thyroxine (T4) and triiodothyronine (T3) play a crucial role in the regulation of various physiological processes, including brain

development and function. TH are involved in neurogenesis during development, but their role in adult neurogenesis is still being explored. Likewise, they may also modulate various signaling pathways and factors involved in neurogenesis, including neurotrophins, growth factors, and transcription factors (Gothié et al., 2017, 2020; RD et al., 2012; Remaud et al., 2014; Saponaro et al., 2020; Schroeder & Privalsky, 2014).

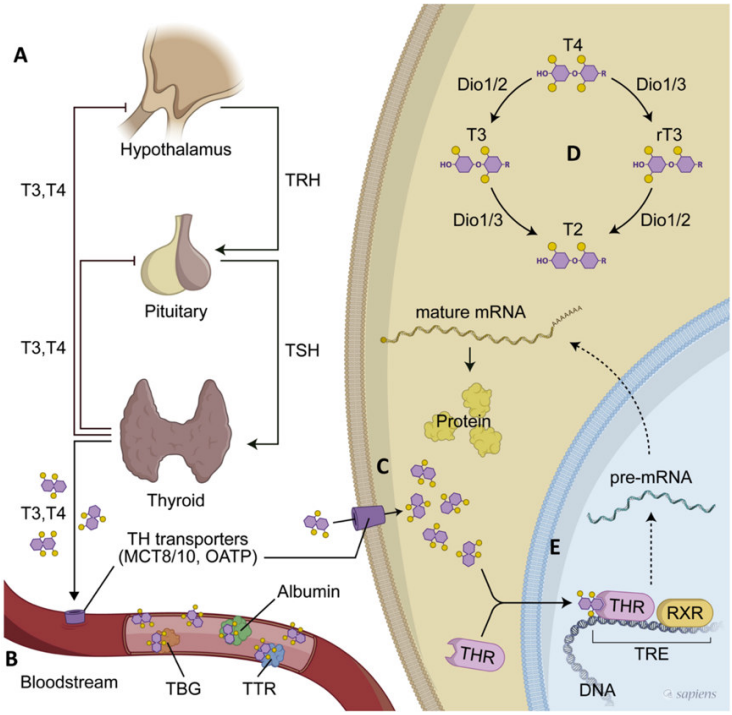


Figure 4. Graphic representation of the Hypothalamus-Pituitary-Thyroid (HPT) axis, TH blood transport, and TH action in the target cell, (Giannocco et al., 2021).

A. The hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the production and release of thyroid-stimulating hormone (TSH) in the anterior pituitary. TSH then binds to its receptor in the thyroid gland, triggering the production and release of thyroid hormones (T3 and T4). (B) These hormones are released into the bloodstream and, through negative feedback mechanisms, affect the hypothalamus and pituitary gland to regulate their production. During circulation, thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin help transport thyroid hormones. (C) Upon reaching the target tissues, transporters like monocarboxylate transporters (MCT8/10) and organic anion transporters polypeptides (OATP) facilitate the entry of T3 and T4 into cells. (D) Inside the cells, deiodinases (Dio) play a role in modifying the thyroid hormones based on the cell's requirements. There are three types of deiodinases: D1, D2, and D3, which convert T4 to T3, T3 to reverse T3 (rT3), and T3/rT3 to T2, respectively. T3 then binds to its specific thyroid hormone receptor (THR or TR), which interacts with the thyroid hormone response element (TRE) present in the DNA.

The expression and action of TH and its receptors TRα1 and TRβ1 (Figure 5) in the context of adult hippocampal neurogenesis have been the subject of extensive research

(Bernal, 2007; Bernal et al., 2015; Fanibunda et al., 2018; Kapoor et al., 2010, p. 20, 2015; Mayerl et al., 2020; Montero-Pedrazuela et al., 2006; Sánchez-Huerta et al., 2016). It has been observed that reduced levels of thyroid hormones, as seen in hypothyroidism, can lead to decreased neurogenesis in the hippocampus. Conversely, increased levels of thyroid hormones, as seen in hyperthyroidism, can enhance neurogenesis.

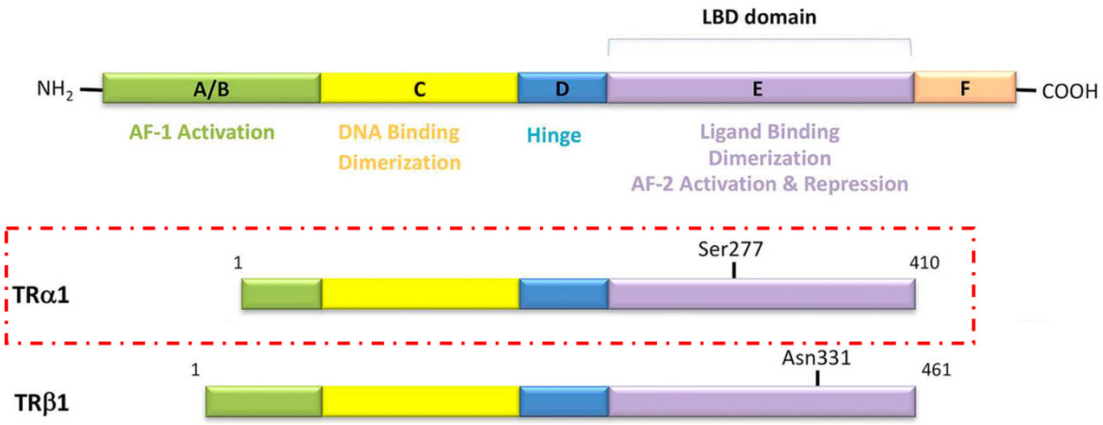


Figure 5. Domain organization of Thyroid Hormone Receptors (TRs) and functional regions along with the schematic representation of the thyroid hormone receptor alpha 1

Thyroid hormone receptors (TRs) are composed of four distinct domains that are conserved across the nuclear receptor superfamily. These domains include the N-terminal A/B domain, which plays a role in transactivation and contains the activation function-1 (AF-1) region; the DNA-binding domain (DBD) located in the central region and consisting of two zinc fingers; the C-terminal ligand-binding domain (LBD), which is responsible for ligand binding, dimerization interfaces, and contains the activation function-2 (AF-2); and a linker or hinge region connecting the LBD and DBD. The linker region contributes to various functions, such as DNA binding, activation and repression, ligand binding, and interactions with corepressors. (Mondal et al., 2016; Nascimento et al., 2006; Pawlak et al., n.d.; Zhang et al., 2018). Specifically, TRα1, a subtype of thyroid receptor, possesses AF-1 domains that are involved in the transcriptional response to thyroid hormones (Anyetei-Anum et al., 2018a; Oberste-Berghaus et al., 2000; Tomura et al., 1995). Figure from (Saponaro et al., 2020).

One aspect of TH signaling in the DG is the role of thyroid hormone receptor alpha 1 (TRα1) (Figure 5, red box) in regulating the proliferation, differentiation, and survival of neural stem cells (NSCs) and progenitor cells. Contrary to expectations, some studies have shown that adult-onset hypothyroidism in rodents does not change the proliferative activity of type 1 NSCs in the subgranular zone (SGZ) of the DG (Desouza et al., 2005; RD et al., 2012; Sánchez-Huerta et al., 2016). Similarly, TRα1 knockout (TRα1−/−) mice lacking TRα1 and TRα2 overexpression (TRα2−/−) mice did not show

alterations in the proliferation of SGZ-NSCs (Kapoor et al., 2010). These findings suggest that type 1 SGZ-NSCs are not responsive to TH and that TRα1 might not play a major role in their proliferation.

In contrast, studies using thyroidectomized rats, which lack TH, have shown a decrease in the number of proliferating cells in the hippocampal progenitor cell population (Montero-Pedrazuela et al., 2006). This effect was rescued by supplying thyroxine (T4) and triiodothyronine (T3), the active form of TH, to the drinking water, indicating that TH is necessary for the maintenance of normal proliferation of hippocampal progenitor cells.

The role of TRβ, another subtype of thyroid hormone receptor, in SGZ-NSC turnover has also been investigated. Studies in TRβ knockout (TRβ KO) mice have shown an increase in SGZ-NSC self-renewal, suggesting that unliganded TRβ isoforms or T3 acting through TRβ may repress SGZ-NSC turnover (Kapoor et al., 2011, 2015).

Regarding the differentiation stage of neurogenesis, the action of T3 and TRα1 becomes more prominent (Figure 6). T3 acting through TRα1 initiates the differentiation of type 2b transient amplifying progenitor cells (TAPs) into doublecortin (DCX)-positive neuroblasts (Kapoor et al., 2010). Neuroblasts are located near the hippocampal blood vessel network, allowing for blood-brain barrier (BBB)-mediated T3 uptake. Neuroblasts express monocarboxylate transporter 8 (MCT8), the primary TH transporter in this stage, and its deletion has been shown to impair neuroblast differentiation and granule cell genesis (Mayerl et al., 2020).

Moreover, TH, through TRs, influences the survival of granule cells by regulating the expression of pro- and anti-apoptotic factors. The expression of TRβ is higher compared to TRα1 and TRα2 in this context (Desouza et al., 2005; Guadaño-Ferraz et al., 2003; Kapoor et al., 2010, 2011; Mayerl et al., 2020). Additionally, the local conversion of thyroxine (T4) to T3 by the enzyme type 2 deiodinase (DIO2) is crucial for generating

sufficient T3 levels and promoting cell differentiation and maturation (Bárez-López & Guadaño-Ferraz, 2017). DIO2 knockout mice with decreased hippocampal T3 content exhibit emotional alterations, suggesting the importance of local DIO2 in hippocampal neurogenesis.

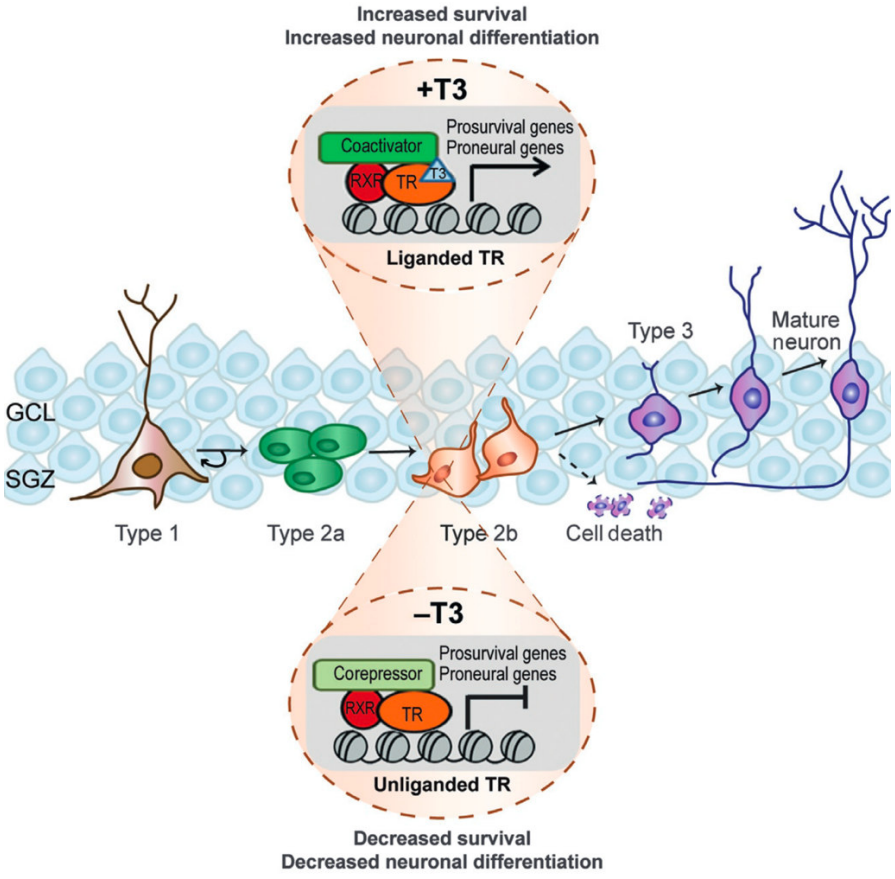


Figure 6. Putative mechanism of thyroid hormone action on adult hippocampal progenitors

The process that undergoes the quiescent neural progenitor in the subgranular zone (SGZ) to a mature neuron integrated into the granule cell layer (GCL). Research suggests that the mechanism primarily operates in Type 2b hippocampal progenitors. When thyroid hormone (T3) is present, activated thyroid hormone receptors (TRs) form heterodimers with retinoid-X-receptor (RXR) and recruit coactivators to specific target genes. This recruitment enhances gene transcription associated with cell survival and neuronal differentiation. Consequently, TH promotes cell survival and facilitates their progression toward becoming neurons within the hippocampal neurogenic niche. On the other hand, in the absence of thyroid hormone, inactive unliganded TRs recruit corepressors to promoters containing thyroid hormone response elements (TREs) of genes related to cell survival and proneural factors. This recruitment leads to gene repression, resulting in reduced progenitor survival and impaired neuronal differentiation. Figure from (Fanibunda et al., 2018).

Overall, the available evidence suggests that TH and its receptors play complex roles in regulating different stages of adult hippocampal neurogenesis. While type 1 SGZ-NSCs may not be responsive to TH, TH is necessary for the proliferation of hippocampal progenitor cells. T3 acting through TRα1 promotes the differentiation of neuroblasts, and TRβ may play a role in repressing SGZ-NSC turnover. The expression and action of TH transporters, such as MCT8 and MCT10, are critical for TH uptake during neurogenesis (Anyetei-Anum et al., 2018b; Deure et al., 2010; Friesema et al., 2003; Mayerl et al., 2020).

Additionally, TRα1 is expressed in granule neurons and may be found in the differentiated cells of the DG, also, it plays a significant role in mediating the actions of thyroid hormones in this region. TH regulates neurogenesis, synaptic plasticity, memory processes, and network activity within the DG. Identifying and understanding the precise mechanisms and expression patterns of TRα1 in all the cell types within the DG is crucial for unraveling the complex interplay between thyroid hormones and hippocampal functions. Further research is needed to understand the role of thyroid hormones in adult neurogenesis and their significant implications for brain health, cognitive function, and potential therapeutic interventions for neurological disorders.

It has been elusive to detect which types of cells within the DG express TRα1 without utilizing transgenic mice or thyroid treatments, but at a basal state. Due to the difficulty of obtaining reliable protein staining with the antibodies available, the findings have been mainly described in terms of mRNA expression and utilizing KO/KI mice.

In this work, the main goal was finding out what cellular populations within the dentate gyrus of the adult mouse express TRα1 in a euthyroid state and if any changes occur in the expression of TRα1 in these populations during adulthood, specifically between 1 month (1M) and 4 months (4M) old mice. It's important to note that these descriptions provide a general overview of the characteristics of the DG in adult mice, the precise

patterns, and levels of thyroid hormone expression in the DG at 1 month and 4 months of age may vary depending on various factors, including the specific experimental conditions and individual variability. Deeper knowledge is needed to elucidate the exact mechanisms and functions of thyroid hormone signaling in the DG during different developmental stages.

Thus, our hypothesis was that the cells of the DG – Type 1/NSCs, Type 2/TAPs, Type 3/NB, immature granule neurons, and mature granule neurons, present an expression of TRα1, though it increases as they differentiate and mature. To answer this, we decided to utilize male adult mice, and focused on the nuclear TRα1 found in the cells within the SGZ and the GCL of the DG. Likewise, we employed molecular biology techniques to identify *Tra1* mRNA and TRα1 protein expression, with additional support of image processors and statistical analysis to establish all differences found in the TRα1 expression in the different populations and at both ages presented above.

Material and Methods

Animals

All procedures were conducted according to the principles and procedures described in Guidelines for Care and Use of Experimental Animals and validated by local and national ethical committees, and in accordance with the Mexican normative NOM-003-ZOO-1994, NOM-033-ZOO-1995, NOM-051-ZOO-1995, NOM-087-ECOL-1995. Male mice were kept under standard conditions (22°C, 50% humidity, 12 h light/dark cycle), where food and water were available *ad libitum*.

	Weight (g)	
	1M	**4M**
Mouse 1	29	42
Mouse 2	30	33
Mouse 3	31	40
Mouse 4	30	39
Mouse 5	27	39
Mouse 6	29	38
Mouse 7	28	40
Mouse 8	26	37
Mouse 9	26	33
Mouse 10	29	
Mouse 11	25	
Mouse 12	28	
N	12	9
Mean	28.1666667	37.8888889
SD	1.85047087	3.10017921
SEM	0.53418493	1.03339307
Theoretical weights (g)	(21-30)	(36-47)

Table 1. Representative weights of 1- and 4-months old mice used for IHC, strain OF-1

Preparation and sectioning of mouse brain tissue

OF-1 adult mice of 1 and 4 months of age, were transcardially perfused with phosphate-buffered saline (PBS), followed by 4% and 8% paraformaldehyde (PFA), and postfixed for 24-48 h in PFA 1% at 4°C. Brains were cryoprotected in 30% sucrose at 4°C and embedded in OCT, optimal cutting temperature compound (Tissue Tek) and stored at -20°C. Brains were sectioned in a coronal plane at 35 mm using a cryostat. The entire rostral-caudal extent of the hippocampus was collected in a 12-well plate.

RT-qPCR

Hippocampi of ten young adults (1 month, creating 5 pools of two mice each) and 5 adults (4 months, creating five pools of one mouse each) were dissected under a stereomicroscope, snap-frozen in dry ice, and stored (-20°C) until processed. RNA tissue extractions were conducted using TRIzol Reagent following the manufacturer's instructions. Chloroform was used to extract the aqueous phase and isopropanol to complete the extraction. RNAs integrity and quality were corroborated by an agarose 1% gel with SYBR green. RNA concentration and quantification were analyzed with Nanodrop.

A PCR (Q5 High-Fidelity PCR Kit, New England Biolabs, E0555) followed by a 3% agarose gel with SYBR green was used to determine the right annealing temperature for each pair of primers. 60°C for Sox2 and Trα1, and 64°C for Nestin, DCX, NeuN. The following primers (Table 2) were chosen to generate the PCR fragments.

Primers	Forward 5'-3'	Reverse 5'-3'	Reference
Sox2	AACGGCAGCTACAGCATGATGC	CGAGCTGGTCATGGAGTTGTAC	(Cao et al., 2022; Qin et al., 2020)
Nestin	AGGCTGAGAACTCTCGCTTGC	GGTGCTGGTCCTCTGGTATCC	(Isaksen et al., 2020; Joseph et al., 2016)
Dcx	CTGACTCAGGTAACGACCAAGAC	TTCCAGGGCTTGTGGGTGTAGA	(Alfonso et al., 2015; Azim et al., 2012; Di Marco et al., 2020)

NeuN	CACCACTCTCTTGTCCGTTTGC	GGCTGAGCATATCTGTAAGCTGC	(Konno et al., 2019; Sung Kang et al., 2020; Zhang (张广芬) et al., 2021)
Tra1	GGCAGTTATCTTGTCCCTTT	CAAGTAAGCACAGACGACTA	(Mayerl et al., 2020)

Table 2. Primers utilized in the RT-qPCR

2 µg of each RNA sample was transcribed into cDNA. 1 µl of cDNA was employed in one qPCR reaction. qPCR reactions were performed in duplicate for each sample using Maxima Probe/ROX qPCR Master Mix (2X) (Thermo Fisher Scientific) on a Rotor-Gene 3000 (Corbett Research), following manufacturer recommendations.

In RT-qPCR, RNA was isolated, amplified and transcribed into cDNA as described above. The cycle threshold (Ct's) was averaged and normalized (ΔCt) against the geometric mean of a housekeeping gene (β-actin). Variations of expression were quantified by the ΔΔCt method (Livak & Schmittgen, 2001). Analysis of relative gene expression data was done using real-time quantitative PCR and the 2(-ΔΔCt) Method. All data represent mean +SEM.

Antibodies and immunofluorescence

At least 3 sections per combination were stained and analyzed. For experiments requiring the detection of fixation-sensitive antigens, free-floating sections were subjected to heat-mediated antigen retrieval in sodium citrate solution (10 mM, pH 6.0) at 95°C for 15 min. Following incubation at 95°C, sections were blocked in 5% normal donkey serum diluted in PBS-Triton X-100 (0.1%) and 4% bovine serum albumin (BSA) for 2 hours. Sections were then incubated overnight at 4°C with primary antibodies diluted in a blocking buffer. Afterwards, they were washed 3 times in PBS 1X, for 5 min and incubated with fluorescent secondary antibodies at room temperature for 2 h.

Sections were stained with Hoechst and mounted onto microscope slides with ProLong Gold antifade reagent (Thermo Fisher Scientific).

The following primary antibodies were used: chicken polyclonal anti-GFAP (1/500; Invitrogen, PA1-10004), goat polyclonal anti-SOX2 (1/500; R&D Systems, AF2018), rat monoclonal anti-Nestin (1/400; ABCAM, AB81462), chicken polyclonal anti-DCX (1/200; ABCAM, AB153668), rat monoclonal anti-NeuN (1/500; ABCAM, AB279297), chicken polyclonal anti-Calretinin (1/200; Novus Biologicals, NBP2-50029), chicken polyclonal anti-Calbindin (1/200; Novus Biologicals, NBP2-50028SS), rabbit polyclonal anti-TRα1 (1/300; Rockland, 600-401-A96).

The secondary antibodies utilized were, donkey anti-chicken AF488 (1/400; Jackson ImmunoResearch, AB_2340375), donkey anti-rabbit AF647 (1/600; ABCAM, AB150075), donkey anti-goat AF555 (1/800; ThermoFisher Scientific, A32816), donkey anti-rat AF555 (1/800; ThermoFisher Scientific, A48270), donkey anti-rat AF488 (1/400; ABCAM, AB150153).

Each cell type will be identified by their identity markers representative of each lineage, as shown in Figure 3, with the specific combinations presented in Table 3.

Cell type	**Identity markers**	
Neural stem cells	GFAP	Sox2
Transient amplifying progenitors	Nestin	Sox2
Neuroblasts	DCX	
Maturing neuroblats	DCX	NeuN
Immature granule neurons	NeuN	
Mature granule neurons	NeuN	Calbindin

Table 3. Combination of identity markers for each cell type of the DG

Marker positive cells (GFAP, Sox2, Nestin, DCX, NeuN, TRα1, and Calbindin) in the SGZ were counted and normalized to the area of the SGZ using ImageJ and QuPath. For the supplemental figures 1-4, GFAP+/SOX2+/TRα1+ and Nestin+/SOX2+/TRα1+, cells

were counted and expressed as percentage of the total density of all SOX2+ cells, in DCX+/NeuN+/TRα1+, cells were counted and expressed as percentage of the total density of all DCX+ cells, and in Cb+/NeuN+/TRα1+, they were counted and expressed as percentage of the total density of all NeuN+ cells, respectively, to account for inter-animal variability. In the immunohistochemistry studies, 2 to 5 images were analyzed per combination, depending on the available sections. All data represent mean +SEM.

Statistical significance between groups and ages was determined by Two-way ANOVA and Šídák's multiple comparisons test. Differences were considered significant when $p < 0.05$ and marked as follows $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****).

Results

The DG has an heterogenous population; thus, we quantified the expression of *Tra1* mRNA and specific identity markers of different neuronal lineages of the DG to compare their expression at different stages of adulthood, i.e., 1 month (1M), and 4 months (4M) old, utilizing RT-qPCR. We utilized *Sox2* mRNA to identify pluripotent cells, such as NSCs; *Nestin* mRNA for TAPs; *Dcx* mRNA for neuroblasts; and *Neun* mRNA for granule neurons. Then, we identified the cells expressing the TRα1 protein in this region by labeling the markers (Table 3) corresponding to neural stem cells (NSCs), transient amplifying progenitors (TAPs), neuroblasts (NBs), and granular neurons (GNs) by IHC. Triple immunostaining was performed in brain tissue slices of young-adult (1M) and adult mice (4M), to see if there was an inherent difference in their expression of TRα1 (Figure 7).

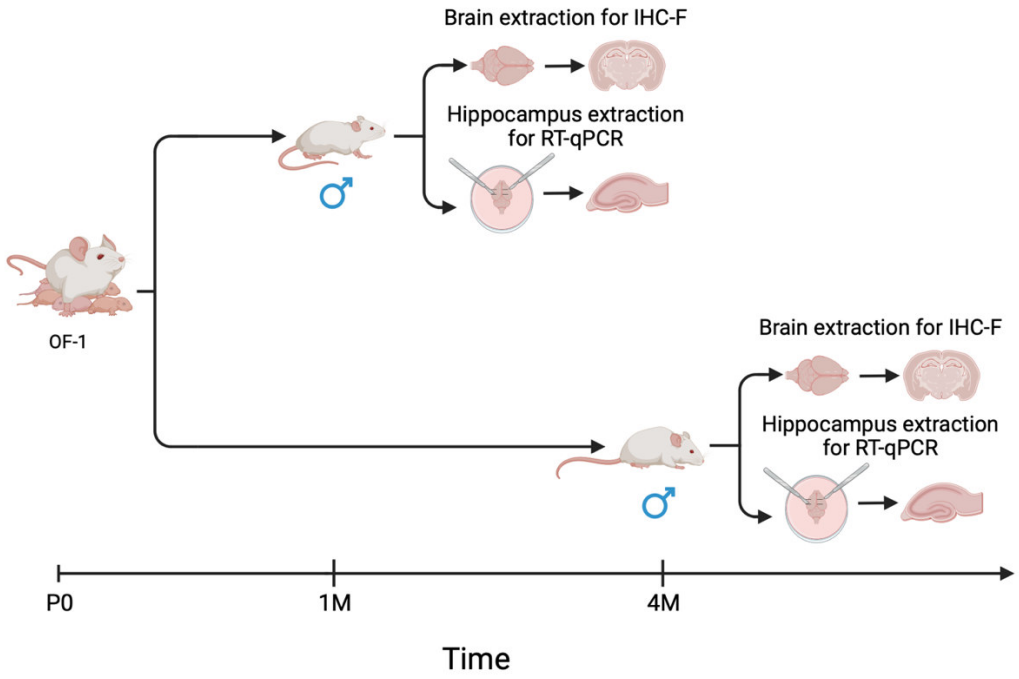


Figure 7. Timeline and schematic representation of the experimental procedure utilized for tissue extraction from the brains of young-adult (1 month, 1M) and adult mice (4 months, 4M)

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Expression of TRα1 in the mitotic cells of the dentate gyrus of adult mice

To determine if there were changes in the expression of NSCs in the DG, we quantified the *Sox2* mRNA in the hippocampus of young adults (1M) and adults (4M) (Figure 8B). We noticed that there was greater variability in the expression of adults than in the younger mice. While some of the dispersion in the data could be attributed to the learning curve in the tissue obtention process, it is interesting to note that the overall *Sox2* expression in the adults is higher than the younger age group, even if there are no significant differences between the expressions.

The expression of *Sox2* mRNA was expected to be lower in adults (4M) as seen in the protein expression (Figure 8C), since it is known that NSCs decrease drastically during adulthood, though it could be credited to a higher degree of truncated processes in the protein synthesis. A similar case resulted from the expression of *Tra1* mRNA, where there is both a larger expression and higher dispersion in the data obtained from adults than from the younger mice, though no statistical differences were shown.

Where *Sox2* expression indicates the presence of pluripotent or proliferative cells such as NSCs or type 2a cells, the expression of *Tra1* is more commonly found in postmitotic cells, such as granule neurons, though according to our hypothesis, NSCs and proliferative cells could also express this transcript. The data presented indicated that the overall expression of *Tra1* mRNA seems to be maintained during both stages of adulthood.

When we analyzed the markers for protein expression of NSCs, we divided the cells in four groups (Figure 8C). The first group were all the SOX2 positive cells that were present within the GL, specifically in the SGZ. From there, we identified two more groups, SOX2+/TRα1+ cells which were classified as proliferative cells and, SOX2+/GFAP+ that were identified as NSCs. From this last group, we found a subpopulation of NSCs that expressed TRα1, which was our fourth group.

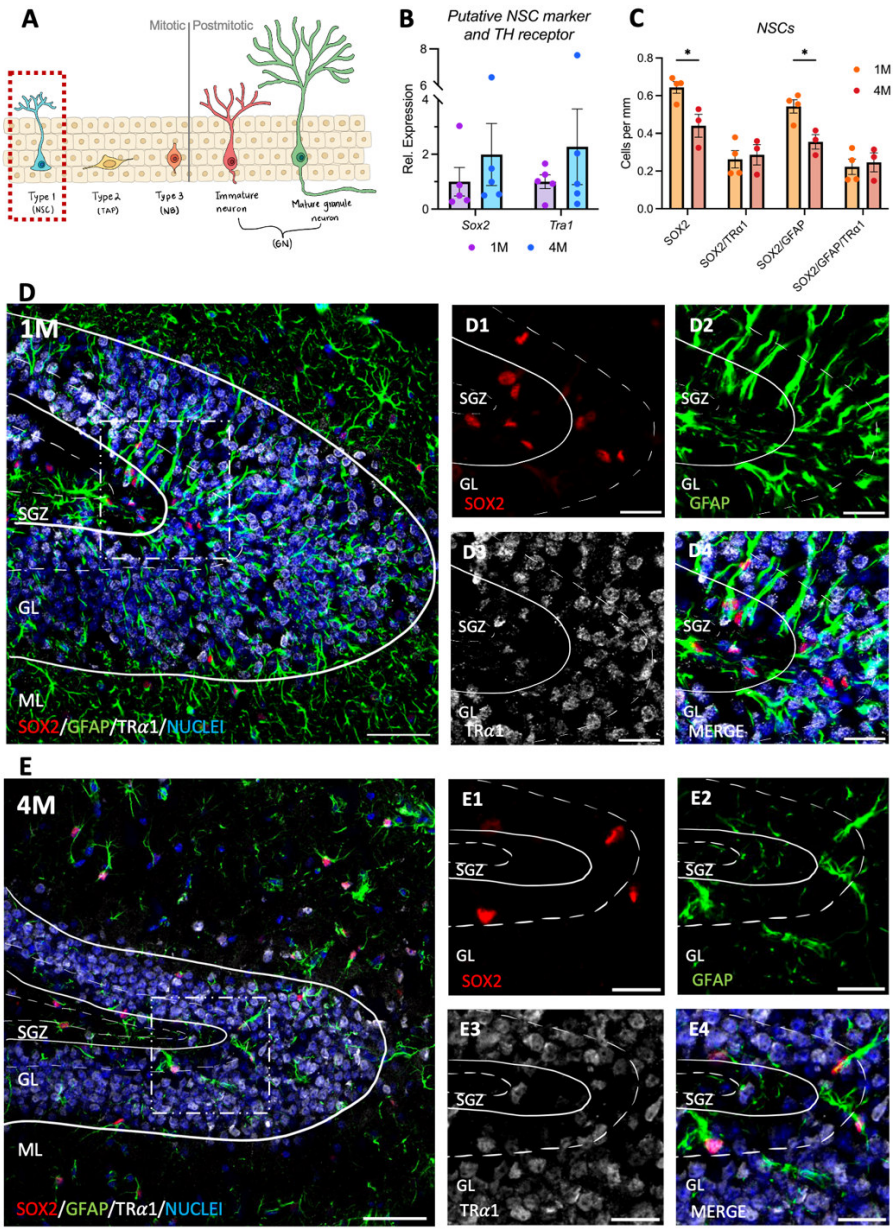

Figure 8. A limited pool of NSCs in the adult DG expressed TRα1 and it is maintained even when the pluripotent pool of cells decreases throughout aging

TRα1 expression was analyzed in the NSC population. A representative illustration of the target cells (Type 1/NSCs, red box in A). Relative mRNA expression of SRY-box 2 (Sox2) and thyroid hormone receptor alpha (Tra1) was analyzed by RT-qPCR. (B). Transcript levels were normalized to β-actin expression and transcript expression at 1 month (n = 5). A scatter dot plot with group means + SEM is shown. (C) Density of different groups of SOX2+ positive cells, TRα1/SOX2, SOX2/GFAP, SOX2/GFAP/TRα1 at different stages of adulthood, i.e., 1 month and 4 months, were analyzed by multiple comparisons and a two-way ANOVA, p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). Cell count (C) from n =3 individual samples, N= 4 and N = 3 for 1M and 4M, respectively. Scatter dot plot with group means + SEM are shown. Microphotography of DG region from 1-month (D) and 4 months (E) old mice. Nuclei were stained with Hoechst (blue). Scale (D-E) 50 μm, (D1-D4, E1-E4) 20 μm. SGZ, subgranular zone; GL, granule cell layer; ML, molecular layer; NSC, neural stem cells.

As we established before, NSCs have been documented to decrease with age, though there is little to no data about their expression of TRα1. We found that both SOX2+ cells and NSCs decrease significantly between young adults and adults ($p \leq 0.05$, Figure 8D, 8D1-4, 8E, 8E1-4), even if the overall pool of SOX2+/TRα1+ cells show no changes between ages. A similar result is seen in the small subpopulation of NSCs that expressed TRα1, which persisted throughout aging. This last result has led us to believe that it may be a small pool of actively proliferating NSCs (aNSCs) that are expressing TRα1, though further investigation is needed to elucidate this. With this data we could partially confirm a portion of our hypothesis, were we established that the hippocampal NSCs expressed TRα1, even if it is only a small subpopulation.

From a qualitative perspective, we observed two things: one, GFAP+ cells in the molecular layer (ML) appear to have an absence of nuclear TRα1 expression, and two, a decrease of the astrocyte-like cells (GFAP+) in the molecular layer of the adult (4M) mice compared with the young-adult mice (1M) (Figure 8D, 8E).

After the NSCs, we focused on the transient amplifying progenitors (TAP), which are a group that maintains their proliferative and mitotic abilities. For this group, we analyzed *Nestin* mRNA as an identity marker for progenitor cells in both age groups, 1 and 4 months old, respectively (Figure 9B). Our results show that at both ages, *Nestin* has a similar level of relative expression, without significant differences. This could indicate that after the documented decrease of these neural progenitors in the post-natal brain, the expression of this transcript is maintained during adulthood.

Subsequently, we analyzed the protein expression of the lineage markers characteristic of TAPs, SOX2 and Nestin. We divided the cells in four groups: the first one contained all the SOX2+ cells, then we subdivided that group into two, SOX2+/TRα1+ cells which were classified as proliferative cells, and SOX2+/Nestin+ cells that were characterized as TAPs; our fourth group consisted of a subpopulation of TAPs which expressed nuclear TRα1 (Figure 9C). We found no significant changes in the overall expression of these four groups during different stages of adulthood.

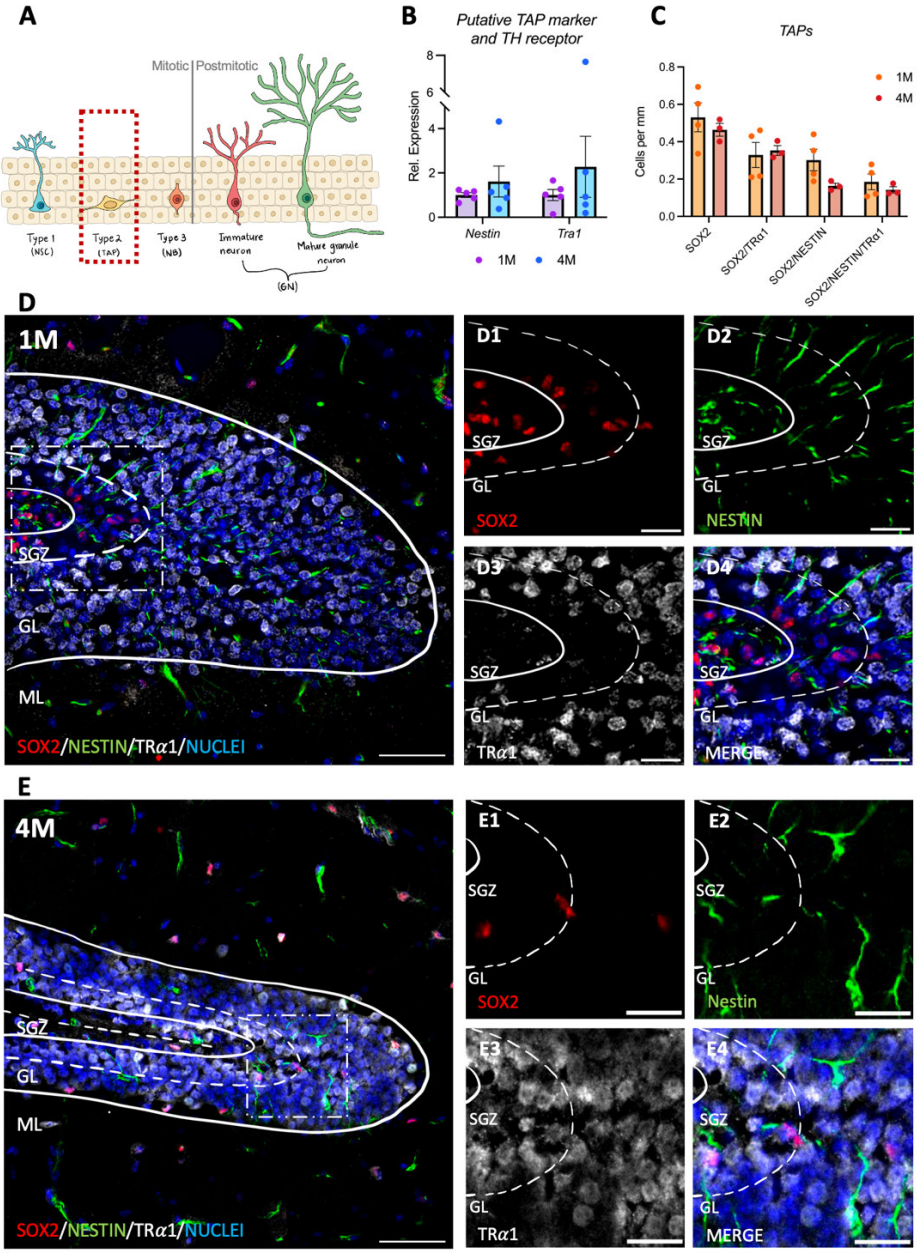

Figure 9. Expression of TH nuclear receptor alpha 1 on transient amplifying progenitor cells is maintained during adulthood in the hippocampal neurogenic niche

TRα1 expression was analyzed in the TAP population. A representative illustration of the target cells (Type2/TAPs, red box in A). Relative mRNA expression of neuroepithelial stem cell protein (Nestin) and thyroid hormone receptor alpha (Tra1) was analyzed by RT-qPCR (B). Transcript levels were normalized to β-actin expression and transcript expression at 1 month (n = 5, individual samples per group). A scatter dot plot with group means + SEM is shown. (C) Density of different groups of SOX2+ positive cells, TRα1/SOX2, SOX2/NESTIN, SOX2/NESTIN/TRα1 at different stages of adulthood, i.e., 1 month and 4 months, were analyzed by multiple comparisons and a two-way ANOVA, p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). Cell count (C) from n =3 individual samples, N= 4 and N = 3 for 1M and 4M, respectively. Scatter dot plot with group means + SEM are shown. Microphotography of DG region from 1-month (D) and 4 months (E) old mice. Nuclei were stained with Hoechst (blue). Scale (D-E) 50 μm, (D1-D4, E1-E4) 20 μm. SGZ, subgranular zone; GL, granule cell layer; ML, molecular layer; TAP, transient amplifying progenitors.

Though the statistical analysis presented no difference between both age groups, we observe a higher expression of proliferative cells within the young adults compared to adults (Figure 9D, 9D1-4, 9E, 9E1-4). With the results presented, we could moderately corroborate our hypothesis in which a subpopulation of TAPs express TRα1 and, subsequently, that this expression is maintained at both stages of adulthood.

Overall, from the mitotic cell pool within this niche, we identified three populations that expressed SOX2 and subsequently, compared them utilizing the total density of SOX2 from each age as their corresponding total (Figure S1, S2). From the NSCs combination (Figure S1), the young adult mice (1M) present a significant decrease in the quantity of SOX2+/TRα1+ ($p \leq 0.0001$) and, from the available NSCs pool, approximately less than half is positive to TRα1 ($p \leq 0.001$). These expression patterns remain mostly unchanged in the adult mice (4M), though with a diminished difference ($p \leq 0.05$ and $p \leq 0.01$, respectively). As a result, we could presume that from the available pool of proliferative cells, and consequently the pool of NSCs, only a small portion expressed nuclear TRα1.

The same process was executed regarding the other proliferative cell group belonging to the mitotic pool, the Type 2 cells (Figure S2). In this comparison, we found that the overall population of SOX2+/TRα1+ and SOX2+/NESTIN+ declined in both young adult ($p \leq 0.0001$ and $p \leq 0.0001$, respectively) and adult mice ($p \leq 0.01$ and $p \leq 0.0001$, respectively). A noted difference was in the subpopulation of SOX2+/NESTIN+ with a TRα1 expression, which dropped in the young adult (1M) ($p \leq 0.05$) but was maintained in the adults (4M). These results indicate that from the available TRα1+ cells, there is a small population of mitotic cells, from both NSCs and Type 2 cells, that expressed this receptor, and it appears that this pool is maintained throughout adulthood without significative changes.

The final group we analyzed from the mitotic population were the neuroblasts (NB), which have minimal proliferative capabilities, though they migrate short distances radially to the granule cell layer, where they exit the cell cycle and differentiate. For this cellular type, we examined *Dcx* mRNA, a microtubule associated protein characteristic in the NB lineage. Our results present a slight increase in the transcript expression in the

older age group (4M) compared to the younger one (1M), even if no significant differences were found (Figure 10B). These findings may suggest that the mRNA accessible for neuroblasts is limited but available throughout adulthood.

The results from the *Dcx* mRNA expression differ from the results exhibited by the markers for protein expression of NBs, *Dcx* is slightly higher in adults, whereas the DCX protein expression in adults significantly decreases from the young adults ($p \leq 0.0001$) (Figure 10C). As stated above, NBs have minimal ability to proliferate and are mainly migrating through the granule cell layer (GL) to differentiate into immature granule neurons. Thus, we divided the NBs into four groups: the first, consisted of all DCX+ cells in the SGZ and GL; then we identified a group of DCX+/TRα1+ cells, and another of DCX+/NeuN+ cells which were characterized as maturing neuroblasts; and finally, the DCX+/NeuN+/TRα1+ cells that were a subpopulation of maturing NBs (Figure 10C).

The expression of NBs is significantly higher in young adult (1M) than in adults (4M) (Figure 10D, 10E). This could be explained by the known decrease of NBs throughout aging (Babcock et al., 2021; Kapoor et al., 2010). As observed in the other populations of mitotic cells (NSCs and TAPs), not all the NBs exhibit a TRα1 expression and is only a subpopulation of this lineage that does (Figure 10D, 10D1-4, 10E, 10E1-4). The expression of TRα1+ migrating neuroblasts is maintained at similar levels in both ages (Figure 10C), though it could be attributed to the presence of NeuN in these cells as they are in the process of exiting the cell cycle and differentiating into immature granular neurons.

To proceed, we then compared the three previous subgroups to the total density of DCX+ cells from each age as their corresponding total (Figure S3). We noticed that in both ages, the subpopulation that is positive to TRα1 is composed of the majority of the neuroblasts ($p < 0.001$ and $p > 0.01$ for 1M and 4M, respectively), and a small subgroup of mature neuroblasts ($p < 0.0001$) that also express TRα1 is maintained similar levels throughout aging.

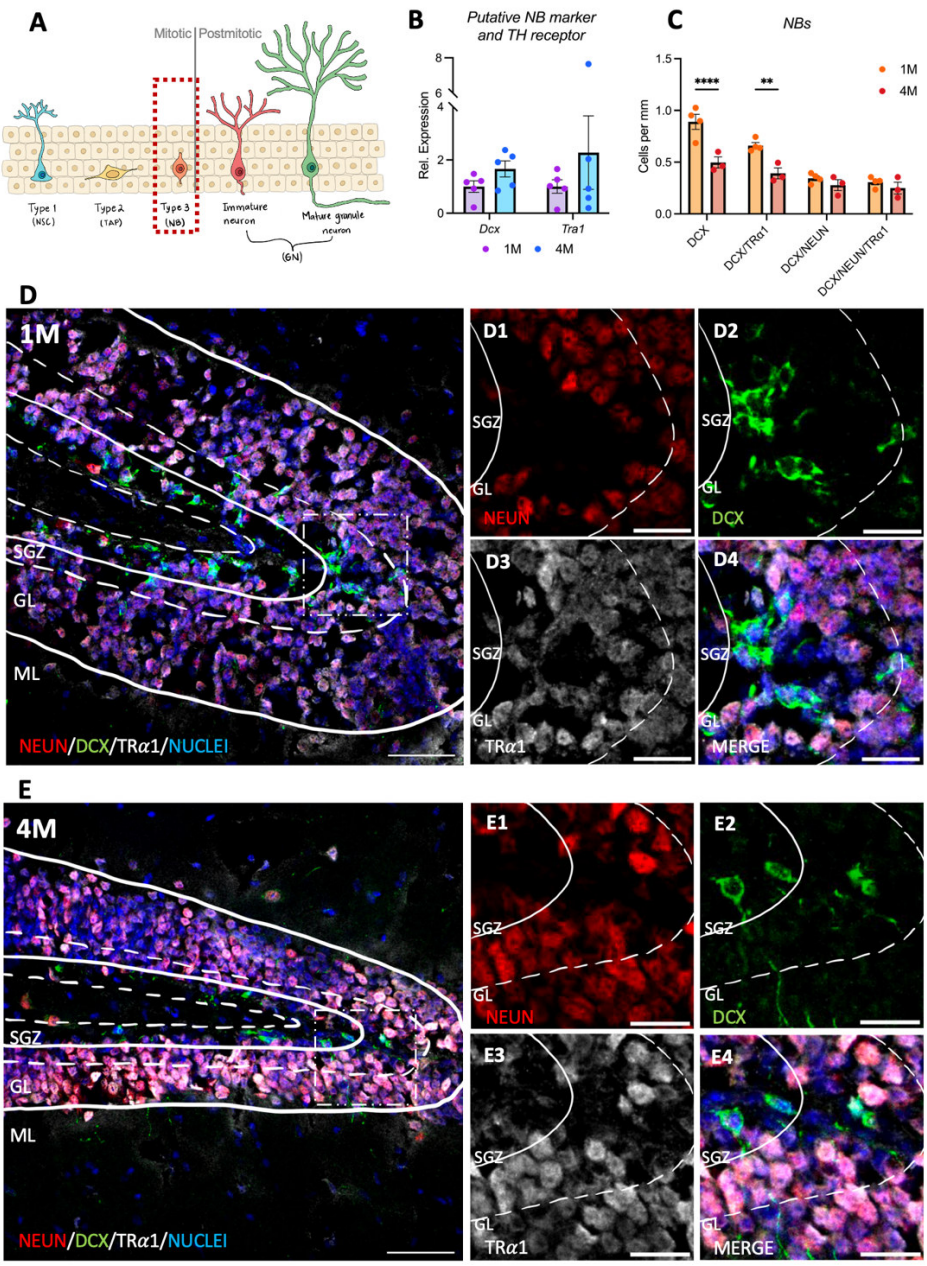

Figure 10. A decrease in nuclear TRα1+ migrating neuroblasts occurs throughout adult life

TRα1 expression was analyzed in the neuroblast population. A representative illustration of the target cells (Type 3/NB, red box in A). Relative mRNA expression of microtubule-associated doublecortin (Dcx) and thyroid hormone receptor alpha (Tra1) was analyzed by RT-qPCR (B). Transcript levels were normalized to β-actin expression and transcript expression at 1 month (n = 5, individual samples per group). A scatter dot plot with group means + SEM is shown. (C) Density of different groups of DCX+ positive cells, TRα1/DCX, DCX/NEUN, DCX/NEUN/TRα1 at different stages of adulthood, i.e., 1 month and 4 months, were analyzed by multiple comparisons and a two-way ANOVA, p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). Cell count (C) from n =3 individual samples, N= 4 and N = 3 for 1M and 4M, respectively. Scatter dot plot with group means + SEM are shown. Microphotography of DG region from 1-month (D) and 4 months (E) old mice. Nuclei were stained with Hoechst (blue). Scale (D-E) 50 μm, (D1-D4, E1-E4) 20 μm. SGZ, subgranular zone; GL, granule cell layer; ML, molecular layer; NB, neuroblasts.

Nuclear expression of TRα1 in the postmitotic cell pool of the DG

To assess if there were changes in the postmitotic population of the DG, we quantified *Neun* mRNA expression in the hippocampal area to identify the granule neurons at different stages of adulthood (1M and 4M). In the data obtained, we observed no statistically significant difference in the expression of *NeuN* between ages (Figure 11B). These findings may suggest that the mRNA available for granule neurons is maintained at parallel levels throughout adulthood.

Afterwards, we investigated whether the postmitotic cells within the DG expressed TRα1 and if it changed during adulthood, i.e., in 1M and 4M old mice. We identified the postmitotic population by the lineage marker NeuN, a neuronal nuclear protein, present in hippocampal granule neurons. Immature granule neurons (IGN) were classified by the expression of NEUN+/CALBINDIN-, and mature granule neurons (MGN) by NEUN+/CALBINDIN+ markers. From our data, we can observed that the overall expression of granule neurons increases with aging (Figure 11C), as stated in previous studies where the DG has been known to produce adult-born dentate granule cells (abDGCs) throughout the lifespan (McHugh et al., 2022), which maintains and replenishes the available pool of the niche.

We analyzed four groups of granule neurons: the first, encompasses both IGN and MGN known as a group as GN; the second is the GN that expressed TRα1; the third group is comprised of the MGN; while the fourth group is the subpopulation of MGN that expressed TRα1. From our data, we can discern that the incidence of colocalization between GN (NEUN+ cells) with TRα1 is nearly a 100% (Figure 11C, S4).

The overall population of GN increases with age ($p \leq 0.01$), though the MGN pool is maintained at similar levels at both stages of adulthood, however it must be mentioned that the results from the last two subgroups (MGN) of the young adults (1M) are preliminary results ($n = 2$). Hence, we may conclude that the population of granule neurons increases throughout adulthood, the subpopulation of MGN seems to be maintained throughout aging, and the entirety of both groups expresses nuclear TRα1.

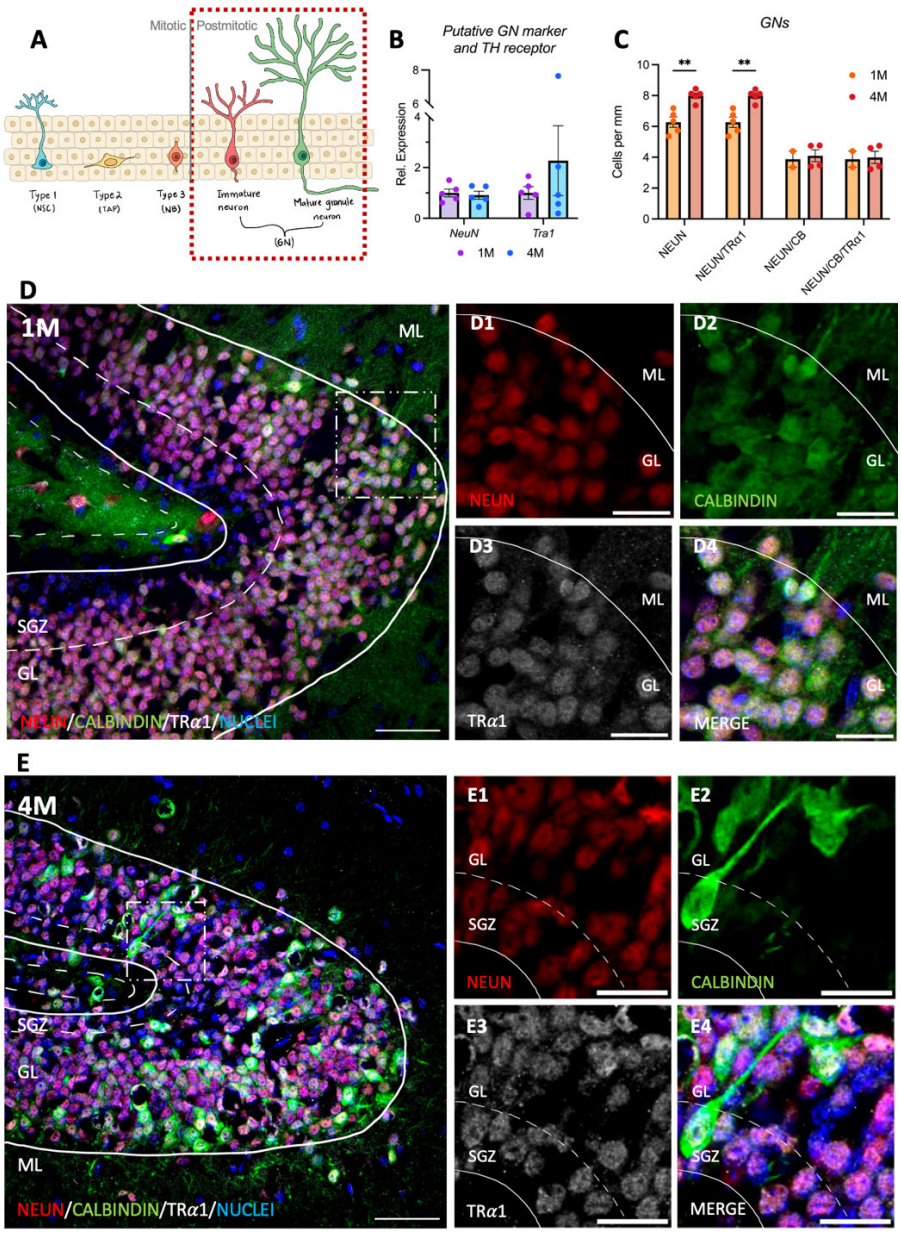

Figure 11. Expression of TH nuclear receptor alpha 1 on granular neurons during adult hippocampal neurogenesis

TRα1 expression was analyzed in the granule neurons of the DG. A representative illustration of the target cells (GN, red box in A). Relative mRNA expression of neuronal nuclear protein (NeuN) and thyroid hormone receptor alpha (Tra1) was analyzed by RT-qPCR (B). Transcript levels were normalized to β-actin expression and transcript expression at 1 month (n = 5, individual samples per group). A scatter dot plot with group means + SEM is shown. (C) Density of different groups of NeuN+ positive cells, TRα1/NeuN, NeuN/CB, NeuN/CB /TRα1 at different stages of adulthood, i.e., 1 month and 4 months, were analyzed by multiple comparisons and a two-way ANOVA, p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). Cell count (C) from n ≤4 individual samples, N= 5 and N = 5 for 1M and 4M, respectively. Scatter dot plot with group means + SEM are shown. Microphotography of DG region from 1-month (D) and 4 months (E) old mice. Nuclei were stained with Hoechst (blue). Scale (D-E) 50 μm, (D1-D4, E1-E4) 20 μm. SGZ, subgranular zone; GL, granule cell layer; ML, molecular layer; GN, granule neurons, CB, calbindin.

Analyzing the results by groups instead of ages (Figure S4), we found that all discernible NEUN+ cells expressed TRα1, and the subgroup of mature granule neurons followed a similar pattern, without comparable changes at different ages. We did find a significant difference between the density of NEUN+ cells and the pool of MGN ($p < 0.0001$, Figure S4), from which the available pool of post-mitotic cells, approximately half are mature granule neurons, while the remaining percentage may consist in maturing neuroblasts and immature granule neurons.

Discussion

Identification of nuclear thyroid hormone receptors in the hippocampal neurogenic niche

In this work, we focused on identifying the cellular lineages within the DG of young adult (1M) and adult (4M) mice that expressed nuclear TRα1 and discerning the changes in the expression of this receptor caused by aging.

Alpha and beta TR mRNA and TRs are detected in the brain during embryogenesis (Bárez-López & Guadaño-Ferraz, 2017; Préau et al., 2015), before endogenous TH is synthetized. Previous studies suggest that TH act on neural progenitors before the activation of the thyroid gland, mainly through maternal TH that is transferred to the embryo via the placenta (Moog et al., 2017). It has been recorded that Type 2/TAPs and NB/Type 3 cells are at the most thyroid hormone sensitive stage of the overall DG during adult neurogenesis (Gothié et al., 2017; Kapoor et al., 2015; RD et al., 2012; Remaud et al., 2014; Vancamp et al., 2020).

Tilting the TH balance towards either end outside of the normal TH range (hypo- or hyperthyroidism) negatively affects early brain development with adverse long-term effects in the adult brain, as well as cognitive function. In the hippocampal niche, studies from adult-onset (AO) perturbations of TH levels and TR isoform-specific mutant mouse models have been described, e.g., AO hyperthyroidism and TRα1 -/- mice expressed an increase in the number of immature and post-mitotic neuroblasts without

affecting the proliferation of early non-committed progenitors within the SGZ, while in hypothyroid mice a decrease of these cells was noted; on the other hand, TRβ1 -/- mice presented an increase in the number of NSCs and non-committed progenitor cells without affecting the post-mitotic cells (Ambrogini et al., 2005; Desouza et al., 2005; Fanibunda et al., 2018; Kapoor et al., 2010, 2011; Montero-Pedrazuela et al., 2006; RD et al., 2012).

Past research utilizing a knock-in TRα1-GFP mouse model (Kapoor et al., 2010; Wallis et al., 2010) has revealed that TRα1 was expressed in both NeuroD and DCX positive progenitors in the SGZ and granule cell layer (GCL) within the DG. These results along with further research, indicate that within the neurogenic niche of the adult hippocampus, TRα1 is predominantly expressed by postmitotic progenitors destined to acquire a neuronal fate (Kapoor et al., 2015; RD et al., 2012). Analysis of TRα1 transcript has shown this TR to be predominantly expressed in the NSC, NB, and GN populations (Mayerl et al., 2020).

TRα1 has been mostly detected in the neuronal lineage, especially in postmitotic subventricular progenitors rather than in committed proliferating progenitors (López-Juárez et al., 2012). It has been suggested that T3 through TRα1 enhances neurogenic effects while inhibiting gliogenesis and promoting NSCs commitment towards neuronal fate in the SGZ (Chen et al., 2012; Gothié et al., 2020).

With our findings, we corroborate that *Tra1* mRNA is present in the overall HP throughout adulthood as similarly noted in (Mayerl et al., 2020), though no significant changes are notable in the relative expression of this transcript within the studied ages (1M and 4M). We tried to neutralize the variability of the TH levels affecting TRA1 expression by utilizing euthyroid mice without any previous treatment. This experimental approach guided us to our findings that NSCs, TAPs, NBs, and GNs express TRα1, though at different densities. A small subpopulation of progenitor cells, NSCs and TAPs, express TRα1, and even though the overall mitotic pool decreases with age as seen in previous studies (Babcock et al., 2021; Encinas et al., 2011; Martín-Suárez et al., 2019;

Seib & Martin-Villalba, 2014; White et al., 2020), this small sub-pool of TRα1+ cells is maintained at the relative same density in young adult (1M) and adult (4M) mice.

Furthermore, this information has led us to believe that the mitotic cells that are actively producing cells of the neuronal lineage may be the ones that present this TRα1 nuclear expression, though further investigation is needed to corroborate this hypothesis. Interestingly, there is a significant difference in the density of TRα1+ NBs in young adult (1M) and adult (4M) mice, with the latter age presenting a decrement in their population. The population of DCX+/NEUN- we assumed to be migrating NBs, which we separated from the pool of maturing NBs (DCX+/NEUN+). This last pool majorly expressed TRA1 and had even numbers in their overall density between ages.

Finally, for the granule neurons, almost in their entirety they expressed TRα1, as has been seen previously (Kapoor et al., 2010). Through our findings, we established that the pool of these post-mitotic cells increased their density in adult mice (4M) compared to young adult mice (1M), even if the mature granule neurons remained without discerning significant changes in their population.

Conclusions

The neurogenic niche of the hippocampus has been studied since decades past and to this day there is still so much that hasn't been discovered. This structure is of vital importance for all the roles it plays, such as learning, memory, and mood management, but it is also likely to be affected by neurological and psychiatric disorders. Thyroid hormones (TH) are known to affect the growth, development, and metabolism of this niche, through genomic and non-genomic methods of action. TRα1 is a TH receptor that, in the presence of TH, acts as a nuclear activator of proneural transcription in the dentate gyrus (DG).

It has been discovered that progenitor cells of the DG are the most susceptible to changes in the TH levels, though it hasn't been elucidated which cells from this niche specifically

express TRα1 in euthyroid models, and if this expression changes at different times during adulthood. The results of this work provide evidence that NSCs, TAPs, NBs, and granule neurons, all express TRα1. It must be mentioned that only a small sub-pool of mitotic cells and neuroblasts present this expression, however, it appears to be maintained at the different points of adulthood that we studied. Furthermore, most post-mitotic cells such as the immature and mature granule neurons express this receptor, and it seems that this pool increases throughout aging.

The presented data are limited, nevertheless, they have open new research paths from which future investigations may be carried out. Some questions that arise are, does this TRα1 expression pattern is similar in other murine models? Does it change in post-natal brains? Does it change in models of neurodegenerative disorders? Do TRβ1 follows the same configuration?

This new available evidence is a step in the right direction to expound the foremost vital functions of TH and TRs and their interactions within the neurogenic microenvironment of the dentate gyrus, and their overall actions in the central nervous system (CNS), especially in the adult mammalian brain.

References

Alfonso, J., Penkert, H., Duman, C., Zuccotti, A., & Monyer, H. (2015). Downregulation of Sphingosine 1-Phosphate Receptor 1 Promotes the Switch from Tangential to Radial Migration in the OB. *The Journal of Neuroscience*, *35*(40), 13659–13672. https://doi.org/10.1523/JNEUROSCI.1353-15.2015

Altman, J. (1962). Are new neurons formed in the brains of adult mammals? *Science (New York, N.Y.)*, *135*(3509), 1127–1128. https://doi.org/10.1126/science.135.3509.1127

Alvarez-Buylla, A., & Lim, D. A. (2004). For the Long Run: Maintaining Germinal Niches in the Adult Brain. *Neuron*, *41*(5), 683–686. https://doi.org/10.1016/S0896-6273(04)00111-4

Ambrogini, P., Cuppini, R., Ferri, P., Mancini, C., Ciaroni, S., Voci, A., Gerdoni, E., & Gallo, G. (2005). Thyroid Hormones Affect Neurogenesis in the Dentate Gyrus of Adult Rat. *Neuroendocrinology*, *81*(4), 244–253. https://doi.org/10.1159/000087648

Anyetei-Anum, C. S., Roggero, V. R., & Allison, L. A. (2018a). Thyroid hormone receptor localization in target tissues. *Journal of Endocrinology*, *237*(1), R19–R34. https://doi.org/10.1530/JOE-17-0708

Anyetei-Anum, C. S., Roggero, V. R., & Allison, L. A. (2018b). Thyroid hormone receptor localization in target tissues. *Journal of Endocrinology*, *237*(1), R19–R34. https://doi.org/10.1530/JOE-17-0708

Azim, K., Raineteau, O., & Butt, A. M. (2012). Intraventricular injection of FGF-2 promotes generation of oligodendrocyte-lineage cells in the postnatal and adult forebrain. *Glia*, *60*(12), 1977–1990. https://doi.org/10.1002/glia.22413

Babcock, K. R., Page, J. S., Fallon, J. R., & Webb, A. E. (2021). Adult Hippocampal Neurogenesis in Aging and Alzheimer's Disease. *Stem Cell Reports*, *16*(4), 681–693. https://doi.org/10.1016/j.stemcr.2021.01.019

Bárez-López, S., & Guadaño-Ferraz, A. (2017). Thyroid Hormone Availability and Action during Brain Development in Rodents. *Frontiers in Cellular Neuroscience*, *11*. https://www.frontiersin.org/articles/10.3389/fncel.2017.00240

Bernal, J. (2007). Thyroid hormone receptors in brain development and function. *Nature Clinical Practice Endocrinology & Metabolism*, *3*(3), 249–259. https://doi.org/10.1038/ncpendmet0424

Bernal, J., Guadaño-Ferraz, A., & Morte, B. (2015). Thyroid hormone transporters—Functions and clinical implications. *Nature Reviews Endocrinology*, *11*(7), 406–417. https://doi.org/10.1038/nrendo.2015.66

Calzà, L., Aloe, L., & Giardino, L. (2021). *Recent Advances in NGF and Related Molecules The Continuum of the NGF "Saga": The Continuum of the NGF "Saga."* https://doi.org/10.1007/978-3-030-74046-7

Cao, L., Huang, M.-Z., Zhang, Q., Luo, Z.-R., Zhang, Y., An, P.-J., Yang, L.-L., Tan, W., Wang, C.-Q., Dou, X.-W., & Li, Q. (2022). The neural stem cell properties of Pkd2l1+ cerebrospinal fluid-contacting neurons in vivo. *Frontiers in Cellular Neuroscience*, *16*, 992520. https://doi.org/10.3389/fncel.2022.992520

Chen, C., Zhou, Z., Zhong, M., Zhang, Y., Li, M., Zhang, L., Qu, M., Yang, J., Wang, Y., & Yu, Z. (2012). Thyroid Hormone Promotes Neuronal Differentiation of Embryonic Neural Stem Cells by Inhibiting STAT3 Signaling Through TRα1. *Stem Cells and Development*, *21*, 2667–2681. https://doi.org/10.1089/scd.2012.0023

Desouza, L. A., Ladiwala, U., Daniel, S. M., Agashe, S., Vaidya, R. A., & Vaidya, V. A. (2005). Thyroid hormone regulates hippocampal neurogenesis in the adult rat brain. *Molecular and Cellular Neurosciences*, *29*(3), 414–426. https://doi.org/10.1016/j.mcn.2005.03.010

Deure, W. M. van der, Peeters, R. P., & Visser, T. J. (2010). Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic

variation in these transporters. *Journal of Molecular Endocrinology*, *44*(1), 1–11. https://doi.org/10.1677/JME-09-0042

Di Marco, B., Crouch, E. E., Shah, B., Duman, C., Paredes, M. F., Ruiz de Almodovar, C., Huang, E. J., & Alfonso, J. (2020). Reciprocal Interaction between Vascular Filopodia and Neural Stem Cells Shapes Neurogenesis in the Ventral Telencephalon. *Cell Reports*, *33*(2), 108256. https://doi.org/10.1016/j.celrep.2020.108256

Encinas, J. M., Michurina, T. V., Peunova, N., Park, J.-H., Tordo, J., Peterson, D. A., Fishell, G., Koulakov, A., & Enikolopov, G. (2011). Division-Coupled Astrocytic Differentiation and Age-Related Depletion of Neural Stem Cells in the Adult Hippocampus. *Cell Stem Cell*, *8*(5), 566–579. https://doi.org/10.1016/j.stem.2011.03.010

Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.-M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*, *4*(11), Article 11. https://doi.org/10.1038/3305

Fanibunda, S. E., Desouza, L. A., Kapoor, R., Vaidya, R. A., & Vaidya, V. A. (2018). Thyroid Hormone Regulation of Adult Neurogenesis. In *Vitamins and Hormones* (Vol. 106, pp. 211–251). Elsevier. https://doi.org/10.1016/bs.vh.2017.04.006

Friesema, E. C. H., Ganguly, S., Abdalla, A., Fox, J. E. M., Halestrap, A. P., & Visser, T. J. (2003). Identification of Monocarboxylate Transporter 8 as a Specific Thyroid Hormone Transporter*. *Journal of Biological Chemistry*, *278*(41), 40128–40135. https://doi.org/10.1074/jbc.M300909200

Gage, F. H., Kempermann, G., Palmer, T. D., Peterson, D. A., & Ray, J. (1998). Multipotent progenitor cells in the adult dentate gyrus. *Journal of Neurobiology*, *36*(2), 249–266. https://doi.org/10.1002/(SICI)1097-4695(199808)36:2<249::AID-NEU11>3.0.CO;2-9

Giannocco, G., Kizys, M. M. L., Maciel, R. M., & de Souza, J. S. (2021). Thyroid hormone, gene expression, and Central Nervous System: Where we are. *Seminars in Cell & Developmental Biology*, *114*, 47–56. https://doi.org/10.1016/j.semcdb.2020.09.007

Gothié, J.-D., Demeneix, B., & Remaud, S. (2017). Comparative approaches to understanding thyroid hormone regulation of neurogenesis. *Molecular and Cellular Endocrinology*, *459*, 104–115. https://doi.org/10.1016/j.mce.2017.05.020

Gothié, J.-D., Vancamp, P., Demeneix, B., & Remaud, S. (2020). Thyroid hormone regulation of neural stem cell fate: From development to ageing. *Acta Physiologica*, *228*(1), e13316. https://doi.org/10.1111/apha.13316

Götz, M., Nakafuku, M., & Petrik, D. (2016). Neurogenesis in the Developing and Adult Brain—Similarities and Key Differences. *Cold Spring Harbor Perspectives in Biology*, *8*(7), a018853. https://doi.org/10.1101/cshperspect.a018853

Guadaño-Ferraz, A., Benavides-Piccione, R., Venero, C., Lancha, C., Vennström, B., Sandi, C., DeFelipe, J., & Bernal, J. (2003). Lack of thyroid hormone receptor alpha1 is associated with selective alterations in behavior and hippocampal circuits. *Molecular Psychiatry*, *8*(1), 30–38. https://doi.org/10.1038/sj.mp.4001196

Harris, L., Rigo, P., Stiehl, T., Gaber, Z. B., Austin, S. H. L., Masdeu, M. del M., Edwards, A., Urbán, N., Marciniak-Czochra, A., & Guillemot, F. (2021). Coordinated changes in cellular behavior ensure the lifelong maintenance of the hippocampal stem cell population. *Cell Stem Cell*, *28*(5), 863-876.e6. https://doi.org/10.1016/j.stem.2021.01.003

Isaksen, T. J., Fujita, Y., & Yamashita, T. (2020). Repulsive Guidance Molecule A Suppresses Adult Neurogenesis. *Stem Cell Reports*, *14*(4), 677–691. https://doi.org/10.1016/j.stemcr.2020.03.003

Joseph, C., Nota, C., Fletcher, J. L., Maluenda, A. C., Green, A. C., & Purton, L. E. (2016). Retinoic Acid Receptor γ Regulates B and T Lymphopoiesis via Nestin-Expressing Cells in the Bone Marrow and Thymic Microenvironments. *The Journal of Immunology*, *196*(5), 2132–2144. https://doi.org/10.4049/jimmunol.1501246

Kaplan, M. S. (1981). Neurogenesis in the 3-month-old rat visual cortex. *The Journal of Comparative Neurology*, *195*(2), 323–338. https://doi.org/10.1002/cne.901950211

Kapoor, R., Fanibunda, S. E., Desouza, L. A., Guha, S. K., & Vaidya, V. A. (2015). Perspectives on thyroid hormone action in adult neurogenesis. *Journal of Neurochemistry*, *133*(5), 599–616. https://doi.org/10.1111/jnc.13093

Kapoor, R., Ghosh, H., Nordstrom, K., Vennstrom, B., & Vaidya, V. A. (2011). Loss of thyroid hormone receptor beta is associated with increased progenitor proliferation and NeuroD positive cell number in the adult hippocampus. *Neuroscience Letters*, *487*(2), 199–203. https://doi.org/10.1016/j.neulet.2010.10.022

Kapoor, R., van Hogerlinden, M., Wallis, K., Ghosh, H., Nordstrom, K., Vennstrom, B., & Vaidya, V. A. (2010). Unliganded thyroid hormone receptor alpha1 impairs adult hippocampal neurogenesis. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *24*(12), 4793–4805. https://doi.org/10.1096/fj.10-161802

Kempermann, G., & Gage, F. H. (1999). New nerve cells for the adult brain. *Scientific American*, *280*(5), 48–53. https://doi.org/10.1038/scientificamerican0599-48

Kempermann, G., Song, H., & Gage, F. H. (2015). Neurogenesis in the Adult Hippocampus. *Cold Spring Harbor Perspectives in Biology*, *7*(9). https://doi.org/10.1101/cshperspect.a018812

Kilpatrick, T. J., & Bartlett, P. F. (1993). Cloning and growth of multipotential neural precursors: Requirements for proliferation and differentiation. *Neuron*, *10*(2), 255–265. https://doi.org/10.1016/0896-6273(93)90316-J

Konno, D., Nakayama, R., Tsunoda, M., Funatsu, T., Ikegaya, Y., & Sasaki, T. (2019). Collection of biochemical samples with brain-wide electrophysiological recordings from a freely moving rodent. *Journal of Pharmacological Sciences*, *139*(4), 346–351. https://doi.org/10.1016/j.jphs.2019.02.006

Kornack, D. R., & Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *Proceedings of the National Academy of Sciences*, *96*(10), 5768–5773. https://doi.org/10.1073/pnas.96.10.5768

Kozareva, D. A., Cryan, J. F., & Nolan, Y. M. (2019). Born this way: Hippocampal neurogenesis across the lifespan. *Aging Cell*, *18*(5), e13007. https://doi.org/10.1111/acel.13007

Kuhn, H. G., Dickinson-Anson, H., & Gage, F. H. (1996). Neurogenesis in the dentate gyrus of the adult rat: Age-related decrease of neuronal progenitor proliferation. *Journal of Neuroscience*, *16*(6), 2027–2033. https://doi.org/10.1523/JNEUROSCI.16-06-02027.1996

Li, Y., Mu, Y., & Gage, F. H. (2009). Development of neural circuits in the adult hippocampus. *Current Topics in Developmental Biology*, *87*, 149–174. https://doi.org/10.1016/S0070-2153(09)01205-8

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

López-Juárez, A., Remaud, S., Hassani, Z., Jolivet, P., Pierre Simons, J., Sontag, T., Yoshikawa, K., Price, J., Morvan-Dubois, G., & Demeneix, B. A. (2012). Thyroid Hormone Signaling

Acts as a Neurogenic Switch by Repressing Sox2 in the Adult Neural Stem Cell Niche. *Cell Stem Cell*, *10*(5), 531–543. https://doi.org/10.1016/j.stem.2012.04.008

Martín-Suárez, S., Valero, J., Muro-García, T., & Encinas, J. M. (2019). Phenotypical and functional heterogeneity of neural stem cells in the aged hippocampus. *Aging Cell*, *18*(4), e12958. https://doi.org/10.1111/acel.12958

Masachs, N., Charrier, V., Farrugia, F., Lemaire, V., Blin, N., Mazier, W., Tronel, S., Montaron, M.-F., Ge, S., Marsicano, G., Cota, D., Deroche-Gamonet, V., Herry, C., & Abrous, D. N. (2021). The temporal origin of dentate granule neurons dictates their role in spatial memory. *Molecular Psychiatry*, *26*(12), Article 12. https://doi.org/10.1038/s41380-021-01276-x

Mayerl, S., Heuer, H., & Ffrench-Constant, C. (2020). Hippocampal Neurogenesis Requires Cell-Autonomous Thyroid Hormone Signaling. *Stem Cell Reports*, *14*(5), 845–860. https://doi.org/10.1016/j.stemcr.2020.03.014

McHugh, S. B., Lopes-dos-Santos, V., Gava, G. P., Hartwich, K., Tam, S. K. E., Bannerman, D. M., & Dupret, D. (2022). Adult-born dentate granule cells promote hippocampal population sparsity. *Nature Neuroscience*, *25*(11), Article 11. https://doi.org/10.1038/s41593-022-01176-5

Ming, G.-L., & Song, H. (2011). Adult neurogenesis in the mammalian brain: Significant answers and significant questions. *Neuron*, *70*(4), 687–702. https://doi.org/10.1016/j.neuron.2011.05.001

Mondal, S., Raja, K., Schweizer, U., & Mugesh, G. (2016). Chemistry and Biology in the Biosynthesis and Action of Thyroid Hormones. *Angewandte Chemie International Edition*, *55*(27), 7606–7630. https://doi.org/10.1002/anie.201601116

Montero-Pedrazuela, A., Venero, C., Lavado-Autric, R., Fernández-Lamo, I., García-Verdugo, J. M., Bernal, J., & Guadaño-Ferraz, A. (2006). Modulation of adult hippocampal

neurogenesis by thyroid hormones: Implications in depressive-like behavior. *Molecular Psychiatry*, *11*(4), 361–371. https://doi.org/10.1038/sj.mp.4001802

Moog, N. K., Entringer, S., Heim, C., Wadhwa, P. D., Kathmann, N., & Buss, C. (2017). Influence of maternal thyroid hormones during gestation on fetal brain development. *Neuroscience*, *342*, 68–100. https://doi.org/10.1016/j.neuroscience.2015.09.070

Nascimento, A. S., Dias, S. M. G., Nunes, F. M., Aparício, R., Ambrosio, A. L. B., Bleicher, L., Figueira, A. C. M., Santos, M. A. M., Neto, M. de O., Fischer, H., Togashi, M., Craievich, A. F., Garratt, R. C., Baxter, J. D., Webb, P., & Polikarpov, I. (2006). Structural Rearrangements in the Thyroid Hormone Receptor Hinge Domain and Their Putative Role in the Receptor Function. *Journal of Molecular Biology*, *360*(3), 586–598. https://doi.org/10.1016/j.jmb.2006.05.008

Navarro Negredo, P., Yeo, R. W., & Brunet, A. (2020). Aging and Rejuvenation of Neural Stem Cells and Their Niches. *Cell Stem Cell*, *27*(2), 202–223. https://doi.org/10.1016/j.stem.2020.07.002

Nottebohm, F. (2002). Neuronal replacement in adult brain. *Brain Research Bulletin*, *57*(6), 737–749. https://doi.org/10.1016/S0361-9230(02)00750-5

Oberste-Berghaus, C., Zanger, K., Hashimoto, K., Cohen, R. N., Hollenberg, A. N., & Wondisford, F. E. (2000). Thyroid Hormone-independent Interaction between the Thyroid Hormone Receptor β2 Amino Terminus and Coactivators*. *Journal of Biological Chemistry*, *275*(3), 1787–1792. https://doi.org/10.1074/jbc.275.3.1787

Overall, R. W., & Kempermann, G. (2018). The Small World of Adult Hippocampal Neurogenesis. *Frontiers in Neuroscience*, *12*, 641. https://doi.org/10.3389/fnins.2018.00641

Palmer, T. D., Takahashi, J., & Gage, F. H. (1997). The Adult Rat Hippocampus Contains Primordial Neural Stem Cells. *Molecular and Cellular Neuroscience*, *8*(6), 389–404. https://doi.org/10.1006/mcne.1996.0595

Pawlak, M., Lefebvre, P., & Staels, B. (n.d.). General Molecular Biology and Architecture of Nuclear Receptors. *Current Topics in Medicinal Chemistry*, *12*(6), 486–504.

Préau, L., Fini, J. B., Morvan-Dubois, G., & Demeneix, B. (2015). Thyroid hormone signaling during early neurogenesis and its significance as a vulnerable window for endocrine disruption. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, *1849*(2), 112–121. https://doi.org/10.1016/j.bbagrm.2014.06.015

Qin, T., Yuan, Z., Yu, J., Fu, X., Deng, X., Fu, Q., Ma, Z., & Ma, S. (2020). Saikosaponin-d impedes hippocampal neurogenesis and causes cognitive deficits by inhibiting the survival of neural stem/progenitor cells via neurotrophin receptor signaling in mice. *Clinical and Translational Medicine*, *10*(8), e243. https://doi.org/10.1002/ctm2.243

Ramon y Cajal, S. (1928). *Degeneration and regeneration of the nervous system*. Clarendon Press.

RD, K., Desouza, L., Nanavaty, I., Kernie, S., & Vaidya, V. (2012). Thyroid Hormone Accelerates the Differentiation of Adult Hippocampal Progenitors. *Journal of Neuroendocrinology*, *24*, 1259–1271. https://doi.org/10.1111/j.1365-2826.2012.02329.x

Remaud, S., Gothié, J.-D., Morvan-Dubois, G., & Demeneix, B. A. (2014). Thyroid Hormone Signaling and Adult Neurogenesis in Mammals. *Frontiers in Endocrinology*, *5*, 62. https://doi.org/10.3389/fendo.2014.00062

Reynolds, B. A., & Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science (New York, N.Y.)*, *255*(5052), 1707–1710. https://doi.org/10.1126/science.1553558

Ribeiro, F. F., & Xapelli, S. (2021). An Overview of Adult Neurogenesis. In L. Calzà, L. Aloe, & L. Giardino (Eds.), *Recent Advances in NGF and Related Molecules: The Continuum of the*

NGF "Saga" (pp. 77–94). Springer International Publishing. https://doi.org/10.1007/978-3-030-74046-7_7

Sánchez-Huerta, K., García-Martínez, Y., Vergara, P., Segovia, J., & Pacheco-Rosado, J. (2016). Thyroid hormones are essential to preserve non-proliferative cells of adult neurogenesis of the dentate gyrus. *Molecular and Cellular Neurosciences*, *76*, 1–10. https://doi.org/10.1016/j.mcn.2016.08.001

Saponaro, F., Sestito, S., Runfola, M., Rapposelli, S., & Chiellini, G. (2020). Selective Thyroid Hormone Receptor-Beta (TRβ) Agonists: New Perspectives for the Treatment of Metabolic and Neurodegenerative Disorders. *Frontiers in Medicine*, *7*. https://www.frontiersin.org/article/10.3389/fmed.2020.00331

Schroeder, A., & Privalsky, M. (2014). Thyroid Hormones, T3 and T4, in the Brain. *Frontiers in Endocrinology*, *5*. https://www.frontiersin.org/article/10.3389/fendo.2014.00040

Seib, D. R. M., & Martin-Villalba, A. (2014). Neurogenesis in the Normal Ageing Hippocampus: A Mini-Review. *Gerontology*, *61*(4), 327–335. https://doi.org/10.1159/000368575

Snyder, J. S., & Drew, M. R. (2020). Functional neurogenesis over the years. *Behavioural Brain Research*, *382*, 112470. https://doi.org/10.1016/j.bbr.2020.112470

Song, J., Olsen, R. H. J., Sun, J., Ming, G., & Song, H. (2016). Neuronal Circuitry Mechanisms Regulating Adult Mammalian Neurogenesis. *Cold Spring Harbor Perspectives in Biology*, *8*(8), a018937. https://doi.org/10.1101/cshperspect.a018937

Sung Kang, M., Ho Lee, J., Song, S.-J., Shin, D.-M., Jang, J.-H., Hyon, S.-H., Won Hong, S., Hun Lee, J., & Han, D.-W. (2020). Graphene oxide-functionalized nanofibre composite matrices to enhance differentiation of hippocampal neuronal cells. *Materials Advances*, *1*(9), 3496–3506. https://doi.org/10.1039/D0MA00733A

Tomura, H., Lazar, J., Phyillaier, M., & Nikodem, V. M. (1995). The N-terminal region (A/B) of rat thyroid hormone receptors alpha 1, beta 1, but not beta 2 contains a strong thyroid

hormone-dependent transactivation function. *Proceedings of the National Academy of Sciences*, *92*(12), 5600–5604. https://doi.org/10.1073/pnas.92.12.5600

Toni, N., & Schinder, A. F. (2016). Maturation and Functional Integration of New Granule Cells into the Adult Hippocampus. *Cold Spring Harbor Perspectives in Biology*, *8*(1), a018903. https://doi.org/10.1101/cshperspect.a018903

Urbán, N., & Guillemot, F. (2014). Neurogenesis in the embryonic and adult brain: Same regulators, different roles. *Frontiers in Cellular Neuroscience*, *8*, 396. https://doi.org/10.3389/fncel.2014.00396

Vancamp, P., Butruille, L., Demeneix, B. A., & Remaud, S. (2020). Thyroid Hormone and Neural Stem Cells: Repair Potential Following Brain and Spinal Cord Injury. *Frontiers in Neuroscience*, *14*, 875. https://doi.org/10.3389/fnins.2020.00875

Wallis, K., Dudazy, S., van Hogerlinden, M., Nordström, K., Mittag, J., & Vennström, B. (2010). The thyroid hormone receptor alpha1 protein is expressed in embryonic postmitotic neurons and persists in most adult neurons. *Molecular Endocrinology (Baltimore, Md.)*, *24*(10), 1904–1916. https://doi.org/10.1210/me.2010-0175

White, C. W., Fan, X., Maynard, J. C., Wheatley, E. G., Bieri, G., Couthouis, J., Burlingame, A. L., & Villeda, S. A. (2020). Age-related loss of neural stem cell O-GlcNAc promotes a glial fate switch through STAT3 activation. *Proceedings of the National Academy of Sciences*, *117*(36), 22214–22224. https://doi.org/10.1073/pnas.2007439117

Zhang, J., Roggero, V. R., & Allison, L. A. (2018). Chapter Three—Nuclear Import and Export of the Thyroid Hormone Receptor. In G. Litwack (Ed.), *Vitamins and Hormones* (Vol. 106, pp. 45–66). Academic Press. https://doi.org/10.1016/bs.vh.2017.04.002

Zhang (张**广芬**), G.-F., Chen (陈**少瑞**), S.-R., Jin (**金道忠**), D., Huang (**黄玉**莹), Y., Chen (陈红), H., & Pan (**潘惠麟**), H.-L. (2021). A2δ-1 Upregulation in Primary Sensory Neurons

Promotes NMDA Receptor-Mediated Glutamatergic Input in Resiniferatoxin-Induced Neuropathy. *The Journal of Neuroscience*, *41*(27), 5963–5978. https://doi.org/10.1523/JNEUROSCI.0303-21.2021

Zhao, C., Deng, W., & Gage, F. H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell*, *132*(4), 645–660. https://doi.org/10.1016/j.cell.2008.01.033

Supplemental Figures

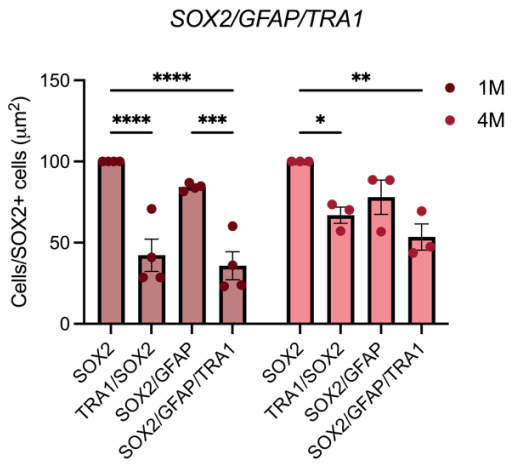

Figure S 1. A small subpopulation of pluripotent cells (NSCs) of the DG expressed TRα1 in young adult (1M) and adult mice (4M).

Two-way ANOVA analysis normalized to the total density of SOX2 from each age (1M and 4M), was used to compare the identified subpopulations of SOX2+ cells. Cell count from n =3 individual samples, N= 4 and N = 3 for 1M and 4M, respectively. Scatter dot plots with group mean + SEM are shown, p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). NSCs, neural stem cells.

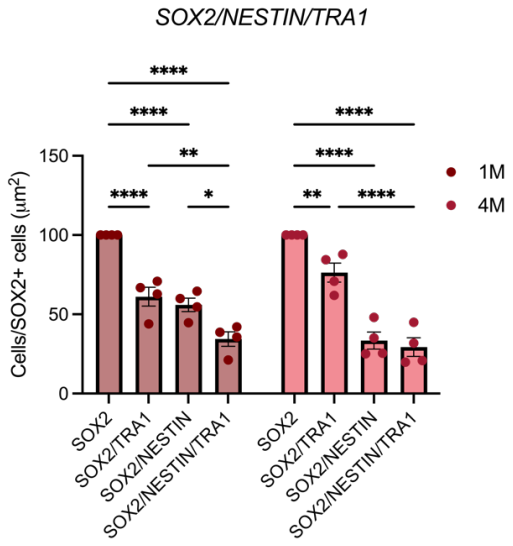

Figure S 2. A decline of TAPs expressing TRα1 is observed in the DG of adult mice (4M) compared to young adult ones (1M).

Two-way ANOVA analysis normalized to the total density of SOX2 from each age (1M and 4M), was used to compare the identified subpopulations of SOX2+ cells. Cell count from n =3 individual samples, N= 4 and N = 3 for 1M and 4M, respectively. Scatter dot plots with group mean + SEM are shown, p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). TAPs, transient amplifying progenitors.

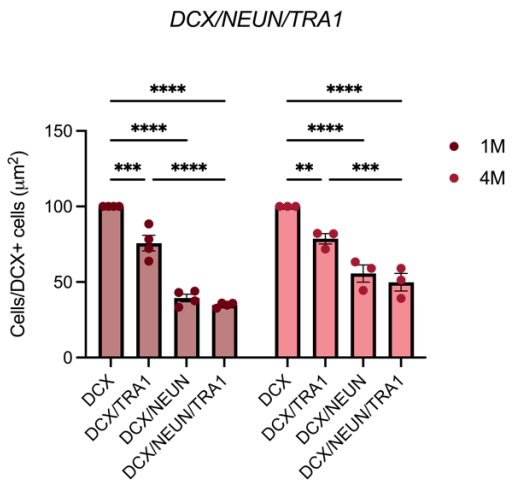

Figure S 3. A subpopulation of TRα1+ neuroblasts is identified in the adult hippocampal niche with an increase of maturing NB observed in older ages.

Two-way ANOVA analysis normalized to the total density of DCX from each age, was used to compare the identified subpopulations of DCX+ cells Cell count from n =3 individual samples, N= 4 and N = 3 for 1M and 4M, respectively. Scatter dot plot with group means + SEM are shown, p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). NB, neuroblasts.

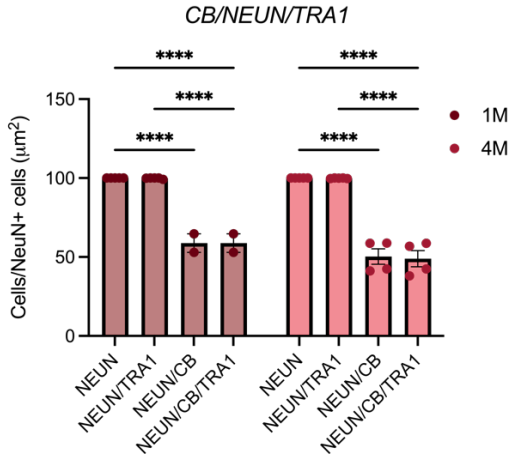

Figure S 4. Immature and mature granule neurons of the dentate gyrus expressed TRα1 throughout adulthood.

Two-way ANOVA analysis normalized to the total density of NEUN from each age, was used to compare the identified subpopulations of NeuN+ cells. Cell count from n ≤4 individual samples, N= 5 and N = 5 for 1M and 4M, respectively. Scatter dot plot with group means + SEM are shown., p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), p ≤ 0.0001 (****). CB, calbindin.

Abbreviations and acronyms

1M	1 month
4M	4 months
abDGCs	Adult-born dentate granule cells
AO	Adult-onset
AF-1	Activation function-1
AF-2	Activation function-2
AHN	Adult hippocampal neurogenesis
aNSCs	Actively proliferating neural stem cells
BBB	Blood-brain-barrier
CA	Cornus ammonis
Cb	Calbindin or vitamin D-dependent calcium-binding protein
CNS	Central nervous system
DBD	DNA-binding domain
DCX	Microtubule-associated doublecortin
DG	Dentate gyrus
DIO	Deiodinase
GCL/GL	Granule cell layer
GFAP	Glial fibrillary acidic protein
GN	Granular neurons
HP	Hippocampus
HPT	Hypothalamus-Pituitary-Thyroid
IHC	Immunohistochemistry
KO	Knockout
LBD	C-terminal ligand-binding domain
LV	lateral ventricles
MCT10	Monocarboxylate transporter 10
MCT8	Monocarboxylate transporter 8
ML	Molecular layer
NB	Neuroblasts
Nestin	Neuroepithelial stem cell protein
NeuN	Neuronal nuclear protein
NSC	Neural stem cells
OATP	Organic anion transporters polypeptides
OB	Olfactory bulb
PSA-NCAM	Polysialylated neural cell adhesion molecule
qNSCs	Quiescent neural stem cells
RXR	Retinoid-X-receptor
SGZ	Subgranular zone
SOX2	SRY-box 2
SVZ	Subventricular zone
T3	Triiodothyronine

T4	Thyroxine
TAP	Transient amplifying progenitors
TBG	Thyroxine-binding protein
TF	Transcription factors
TH	Thyroid hormones
TR/THR	Thyroid hormone receptors
Tra1	Thyroid hormone receptor alpha 1 (mRNA)
TRα1	Thyroid hormone receptor alpha 1 (protein)
TRα2	Thyroid hormone receptor alpha 2
TRβ	Thyroid hormone receptor beta
TREs	Thyroid hormone response elements
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
TTR	Transthyretin
Type 1 cells	Neural stem cells
Type 2 cells	Transient amplifying progenitors
Type 3 cells	Neuroblasts

UNIVERSIDAD DE GUANAJUATO
DIVISIÓN DE CIENCIAS NATURALES Y EXACTAS
DEPARTAMENTO DE FARMACIA

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Guanajuato, Gto. 16 de octubre de 2023
Asunto: Carta aceptación de tesis

Dr. Davis Yves Ghislain Delepine
Director de la División de Ciencias e Ingenierías
Campus León
Universidad de Guanajuato

Dr. David Delepine:

Por medio de la presente, informo a usted que he leído el manuscrito de tesis de la estudiante de la Maestría en Ciencias Aplicadas de la DCI, Ing. Biomédico EDNA AHTZA ESPARZA ARELLANO, la cual lleva por título: "Nuclear expression of thyroid hormone receptor α1 in the population of the hippocampal neurogenic niche of the adult mouse".

Después de haber realizado mis comentarios, la estudiante realizó las correcciones pertinentes. Por lo anterior doy mi consentimiento para que EDNA AHTZA ESPARZA ARELLANO, defienda de manera oral su tesis de Maestría en la fecha que sus directores de tesis juzguen conveniente.

Sin más por el momento, y agradeciendo las atenciones a la presente, me despido enviando un cordial saludo.

Atentamente

Clara Alba Betancourt

Dra. Clara Alba Betancourt
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Dr. Davis Yves Ghislain Delepine
Director de la División de Ciencias e Ingenierías
Campus León
Universidad de Guanajuato

León, Gto., 23 de octubre del 2023.

Dr. David Delepine:

Por medio de la presente, informo a usted que he leído el manuscrito de tesis de la estudiante de la Maestría en Ciencias Aplicadas de la DCI, Ing. Biomédica EDNA AHTZA ESPARZA ARELLANO, la cual lleva por título: "Nuclear expression of thyroid hormone receptor α1 in the population of the hippocampal neurogenic niche of the adult mouse".

Después de haber realizado mis comentarios, el estudiante realizó las correcciones pertinentes. Por lo anterior doy mi consentimiento para que el estudiante defienda de manera oral su tesis de Maestría en la fecha que sus directores de tesis juzguen conveniente.

Sin más por el momento, y agradeciendo las atenciones a la presente, me despido enviando un cordial saludo.

Atentamente

Valeria Piazza

Dra. Valeria Piazza
Profesora-Investigadora

Querétaro, Qro., 23 de octubre de 2023

Dr. David Yves Ghislain Delepine
Director de la División de Ciencias e Ingenierías
Campus León
Universidad de Guanajuato

Asunto: Voto Aprobatorio

Dr. David Delepine:

Por medio de la presente, informo a usted que he revisado el manuscrito de tesis que lleva por título: "Nuclear expression of thyroid hormone receptor α1 in the population of the hippocampal neurogenic niche of the adult mouse", que presenta la alumna **EDNA AHTZA ESPARZA ARELLANO,** para obtener el grado de Maestra en el programa de **Maestría en Ciencias Aplicadas**. En la calidad de Jurado, considero que la tesis cumple con los requisitos establecidos y emito mi **VOTO APROBATORIO** para que el trabajo sea presentado en el examen de grado.

Sin más por el momento, y agradeciendo las atenciones a la presente, me despido enviando un cordial saludo.

Atentamente

Dra. Rocío Brenda Anguiano Serrano
Jurado de Examen
Instituto de Neurobiología, UNAM (Campus Juriquilla)

BUAP

Asunto: aprobación de trabajo de tesis
Puebla, Pue., 6 de noviembre del 2023

Dr. David Delepine
Director de la División de Ciencias e Ingenierías
Campus León
Universidad de Guanajuato

En mi carácter de revisor del trabajo de tesis de licenciatura presentado por la alumna **Edna Ahtza Esparza Arellano,** para optar por el grado de Maestra en Ciencias Aplicadas con el trabajo titulado:
"Nuclear expression of thyroid hormone receptor α1 in the population of the hippocampal neurogenic niche of the adult mouse"
me permito comunicarle que después de haber revisado y discutido el manuscrito, considero que dicha tesis reúne los requisitos y méritos suficientes para poder continuar con sus trámites de solicitud de examen de grado.

Agradezco mucho su atención y aprovecho para enviarle un saludo cordial.

Atentamente

"Pensar Bien, Para Vivir Mejor"

Ma. del Carmen Cortés
Profesor-Investigador Titular "C" T.C.
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UNIVERSIDAD DE GUANAJUATO

León, Guanajuato, a 23 de agosto de 2023
Oficio Núm. SAC-131/2023
Asunto: Asignación de Jurado

Dra. Rocío Brenda Anguiano Serrano
Instituto de Neurobiología, UNAM
Dra. Ma. del Carmen Cortés
Instituto de Fisiología, BUAP
Dra. Clara Alba Betancourt
División de Ciencias Naturales y Exactas, UG
Dra. Valeria Piazza
Centro de Investigaciones en Óptica
PRESENTES

En base al Artículo 68 del Estatuto Académico y en atención a la solicitud que presenta la Dra. Silvia Alejandra López Juárez, Directora, y el Dr. Víctor Hugo Hernández González, Co-director, para la defensa del trabajo de tesis que, bajo el título: "*Nuclear expression of thyroid hormone receptor a1 in the population of the mouse hipocampal neurogenic niche*", defenderá el/la **C. Edna Ahtza Esparza Arellano**, estudiante de **Maestría en Ciencias Aplicadas,** de esta División, se hace la siguiente asignación de Jurado:

Dra. Clara Alba Betancourt	Presidenta
Dra. Valeria Piazza	Secretaria
Dra. Ma. del Carmen Cortés	Vocal
Dra. Rocío Brenda Anguiano Serrano	Suplente

Sirva la presente para solicitar al(a la) estudiante entregar su documento de tesis a la brevedad posible al Jurado designado para que, dentro del plazo que marca el Artículo 68 del Estatuto Académico, sus sinodales se pronuncien por escrito sobre la aprobación del documento.

Sin más por el momento, aprovecho la ocasión para enviarles un cordial saludo.

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CAMPUS LEÓN
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DR. DAVID YVES GHISLAIN DELEPINE
DIRECTOR DE LA DIVISIÓN DE CIENCIAS E INGENIERÍAS

C. p. Estudiante
Dra. Silvia Alejandra López Juárez, Directora de tesis y Dr. Víctor Hugo Hernández González, Co- Director de tesis
Archivo de Secretaría Académica