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Central growth hormone action regulates neuroglial and proinflammatory markers in the hypothalamus of male mice

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Abstract

Growth hormone (GH) action in specific neuronal populations regulates neuroendocrine responses, metabolism, and behavior. However, the potential role of central GH action on glial function is less understood. The present study aims to determine how the hypothalamic expression of several neuroglial markers is affected by central GH action in male mice. The dwarf GH- and insulin-like growth factor-1 (IGF-1)-deficient *Ghrhr*$^{lit/lit}$ mice showed decreased mRNA expression of *Nes* (Nestin), *Gfap, Iba1, Adgre1* (F4/80), and *Tnf* (TNFα) in the hypothalamus, compared to wild-type animals. In contrast, transgenic overexpression of GH led to high serum GH and IGF-1 levels, and increased hypothalamic expression of *Nes, Gfap, Adgre1, Iba1*, and *Rax*. Hepatocyte-specific GH receptor (GHR) knockout mice, which are characterized by high serum GH levels, but reduced IGF-1 secretion, showed increased mRNA expression of *Gfap, Iba1, Tnf,* and *Sox10*, demonstrating that the increase in GH levels alters the hypothalamic expression of glial markers associated with neuroinflammation, independently of IGF-1. Conversely, brain-specific GHR knockout mice showed reduced expression of *Gfap, Adgre1*, and *Vim* (vimentin), indicating that brain GHR signaling is necessary to mediate GH-induced changes in the expression

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Conflict of interest

The authors declare no conflicts of interest.

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of several neuroglial markers. In conclusion, the hypothalamic mRNA levels of several neuroglial markers associated with inflammation are directly modulated by GHR signaling in male mice.

Keywords

astrocyte; cytokine; GH; IGF-1; microglia; neuroinflammation

1. Introduction

Growth hormone (GH) is a protein produced by somatotropic cells of the anterior pituitary gland. GH modulates numerous physiological functions, including protein synthesis, cellular proliferation, body growth, and metabolism [1]. GH receptor (GHR) is widely expressed in different tissues and most of the literature has focused on the action of GH in tissues such as the liver, adipose tissue, muscle, pancreas, and bone [1]. Since GHR signaling in the liver regulates the expression and secretion of insulin-like growth factor-1 (IGF-1), changes in GH secretion frequently produce similar alterations in the IGF-1 circulating levels [2]. Consequently, several of the physiological functions associated with GH are often attributed to the action of IGF-1 or at least partially [1].

Although the brain is not considered a classical target tissue of GH, evidence from animal models and humans also indicates that GH can control important neurological functions [3, 4]. GHR knockout mice are protected against aging-induced cognitive decline [5, 6]. In contrast, GH oversecretion impairs spatial learning and memory in middle-aged male mice [5]. However, these cognitive alterations may be indirectly mediated by GH's effects on insulin sensitivity, which in turn plays a critical role in regulating memory and other neurological functions [7]. It is worth mentioning that GHR knockout mice exhibit improved insulin sensitivity, whereas acromegalic mice are insulin resistant [8, 9]. GH can also have direct effects on the brain affecting cognitive and neurological functions. For example, acute GH treatment improves spatial learning and memory in young rats, old rats, and adult mice via changes in glutamate receptors [10]. Another study has found that GH enhances glutamatergic transmission in the hippocampus, helping to understand the effects of GH on memory and learning [11]. Restoration of hippocampal GH levels following chronic stress promotes stress resilience [12]. GHR recruits the signal transducer and activator of transcription 5 (STAT5) as its major intracellular pathway. Brain-specific STAT5 ablation impairs learning and memory in mice, reinforcing the importance of normal GHR/STAT5 signaling to maintain appropriate neurological functions [13]. GH action affects systemic and local IGF-1 levels [14]. Since IGF-1 also regulates synapse plasticity, neurotransmission, and cognition [11, 15–18], part of the effects of GH on the brain is thought to be mediated by IGF-1.

Recent studies have improved our knowledge about the mechanisms involved in GH regulation of brain functions [4]. Since GHR signaling recruits the STAT5 pathway, acute GH injections have been used to induce STAT5 phosphorylation (pSTAT5) to identify GH-responsive cells in the brain of rodents [19, 20]. The brain cells that exhibit GH-induced pSTAT5 are mostly neurons and the hypothalamus contains the largest population of GH-

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responsive cells [19]. Thus, it is clear that GH can act directly in the brain to regulate different cellular effects. Additional studies investigating the phenotype of mice carrying neuron-specific GHR ablation have observed that the absence of GH action in specific neuronal populations leads to neuroendocrine, metabolic, and behavioral dysfunctions [21–34].

Neuroglial cells play a fundamental role in brain homeostasis but the potential role of central GH action on glial function has been less studied. GH-deficient mice exhibit hypomyelination caused by impaired glial proliferation [35], although another study found that GH deficiency does not adversely affect myelination [36]. Adult-onset GH/IGF-1 deficiency decreases the survival of newborn oligodendrocytes [37]. GHR knockout mice exhibit smaller astrocytes [38]. GH treatment for one week in adult rats increases the expression of the glial fibrillary acidic protein (GFAP), an astrocyte marker, whereas vimentin expression (a radial glia marker) is decreased in the hypothalamus and hippocampus [39]. GH treatment induces the proliferation of neural precursors, neurogenesis, and gliogenesis in cultured rat cerebral cortical cells, possibly via IGF-1 [40]. Sadagurski et al. showed that the hypothalamus of 18-month-old Ames dwarf mice exhibits a decreased expression of GFAP, ionized calcium-binding adapter molecule 1 (Iba1; a microglial marker), and tumor necrosis factor-α (TNFα), suggesting that GH deficiency protects against aging-induced neuroinflammation [41].

Although the studies described above indicate an action of GH on neuroglial cells, it is still unclear whether these effects occur through a direct action of GH on the central nervous system or if they are mediated by other factors such as IGF-1. Thus, the present study has the objective to investigate whether the hypothalamic expression of several neuroglial and inflammatory markers is affected by central GH action. For this purpose, distinct mouse models that have critical differences in the GH/IGF-1 axis as well as in GHR signaling in the brain were used. We chose to study the hypothalamus because it contains the largest number of cells directly responsive to GH in the entire nervous system of rodents [19, 20].

2. Materials and Methods

2.1. Animals

The "little" mouse that carries a spontaneous and null mutation in the gene encoding the GH-releasing hormone receptor (*Ghrhr*$^{lit/lit}$ mice; RRID: IMSR_JAX:000533, The Jackson Laboratory, Bar Harbor, ME, USA) was used to study the consequences of GH and IGF-1 deficiency. Mice carrying a transgene that leads to overexpression of bovine GH (bGH mice) [42] were used to investigate the consequences of increased GH and IGF-1 secretion. Hepatocyte-specific GHR knockout (Albumin ^{GHR}) mice were produced, as previously described [14], using GHR-flox [43] and Albumin-Cre mice (RRID: IMSR_JAX:003574, The Jackson Laboratory). Brain-specific GHR ablation (Nestin ^{GHR} mice) was achieved by breeding GHR-flox and Nestin-Cre mice (RRID: IMSR_JAX:003771, The Jackson Laboratory), as earlier described and validated [23, 26]. All mice used were adult males (3 to 4 months old) in the C57BL/6 background with ad libitum access to a regular rodent chow (2.99 kcal/g, 9.4% kcal derived from fat, 236 g protein/kg; Nuvilab CR-1, Quimtia, Brazil) and filtered water. The mutations were confirmed through polymerase chain reaction (PCR)

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using the DNA that had been previously extracted from the tail tip (REDExtract-N-Amp™ Tissue PCR Kit, MilliporeSigma, St. Louis, MO, USA). The control groups were composed of wild-type (when compared to *Ghrhr*$^{lit/lit}$ or bGH mice) or GHR$^{flox/flox}$ mice (when compared to Albumin ^{GHR} or Nestin ^{GHR} mice). This study was approved by the Ethics Committee on the Use of Animals of the Institute of Biomedical Sciences, University of Sao Paulo.

2.2. Tissue collection and hormone assessment

Three to four months old male mice were anesthetized with isoflurane, euthanized by decapitation, and the trunk blood was collected. The serum was obtained by centrifugation and stored at −80°C until the assessment of the serum concentration of IGF-1 using a commercially available enzyme-linked immunosorbent assay (ELISA) (#MG100; RRID: AB_2827989; R&D Systems, Minneapolis, MN, USA) and GH using an "in-house" ELISA, as described [26, 28, 30, 31, 44]. The lower limit of detection and the intra-assay and inter-assay coefficients of variation were, respectively, 0.0035 ng/mL, 4.3% and 6.0% for the IGF-1 assay and 0.04 ng/mL, 2.6% and 9.7% for the GH assay. Peroxidase reaction was used to label pSTAT5 immunoreactive cells in the brain of mice that received an intraperitoneal injection of porcine GH (20 μg/g; National Hormone and Pituitary Program, Torrance, Ca). The protocols for the perfusion, brain sectioning, and pSTAT5 immunostaining are detailed described in previous publications [19, 45, 46].

2.3. Hypothalamic mRNA expression

The entire hypothalamus was quickly removed after euthanasia according to the following anatomical references: rostrocaudal, 1 mm anterior to the optic chiasm and immediately posterior to the mammillary bodies; lateral, defined by the optic tract; and superior, the dorsal limit of the third ventricle. The hypothalamic RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA), and RNA quantity and quality were determined with an Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA; RRID: SCR_017317). RNA was incubated in DNase I RNase-free (MilliporeSigma), followed by reverse transcription to produce the cDNA using 2 μg of total RNA, Superscript II Reverse Transcriptase (Invitrogen), and random primers p(dN)6 (MilliporeSigma). Real-time PCR was performed using the 7500TM Real-Time PCR System (Applied Biosystems, Warrington, UK), SYBR Green Gene Expression PCR Master Mix (Applied Biosystems), and specific primers for target genes (Table 1). The data from *Ghrhr*$^{lit/lit}$ mice and their respective controls were normalized to *Ppia* expression, whereas the hypothalamic gene expression of the other mouse models was normalized to the geometric average of *Actb* and *Gapdh*. The expression of the reference genes was not different among the experimental groups ($P > 0.40$). Data were reported as fold changes compared to values obtained from each control group (set at 1.0). Relative quantification of mRNA was calculated by $2^{-\quad Ct}$.

2.4. Statistical analysis

The normality and homogeneity of the data were analyzed by the Shapiro-Wilk and F tests. If the data present a normal distribution, the differences between groups were analyzed by the unpaired two-tailed Student's t-test. Otherwise, the data were analyzed by the Mann-Whitney non-parametric test. All results were expressed as mean ± standard error of the

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mean, and only p values < 0.05 were considered to be statistically significant. Statistical analyses were performed using the Prism software version 8.4.3 (GraphPad, San Diego, CA).

3. Results

3.1. *Ghrhr$^{lit/lit}$* mice show decreased hypothalamic expression of genes associated with neuroinflammation

To initially investigate the potential role of central GH action on the expression of neuroglial markers, the hypothalami of *Ghrhr$^{lit/lit}$* and wild-type male mice were collected and the mRNA expression was compared between these groups. The body weight of *Ghrhr$^{lit/lit}$* mice was 12.2 ± 0.7 g, compared to 23.5 ± 0.7 g in control mice ($P < 0.0001$). In accordance with the dwarfism exhibited by *Ghrhr$^{lit/lit}$* mice [25, 47], these animals showed suppressed serum GH and IGF-1 levels, compared to control mice (Figure 1). Hypothalamic *Ghr* expression was unchanged in *Ghrhr$^{lit/lit}$* mice. A decreased mRNA expression of *Nes* (nestin), *Gfap*, *Adgre1* (F4/80), *Iba1*, and *Tnf* (TNFα) was observed in the hypothalamus of *Ghrhr$^{lit/lit}$* mice, compared to control animals (Figure 1). Thus, GH and IGF-1 deficiency leads to decreased hypothalamic expression of genes associated with neuroinflammation.

3.2. Overproduction of GH and IGF-1 increases the hypothalamic expression of markers of cell proliferation, microglia, and neuroinflammation

To investigate whether GH overproduction affects the hypothalamic expression of genes associated with glial function, mice with transgenic overexpression of bovine GH were used. As expected, transgenic bGH mice exhibited increased serum GH and IGF-1 levels, compared to control animals (Figure 2). Furthermore, the body weight of bGH mice was 41.6 ± 1.2 g, compared to 28.9 ± 1.0 g in control mice ($P < 0.0001$). Hypothalamic *Ghr* expression was not different between the groups. Although S100 calcium-binding protein β (*S100b*), SRY-related HMG-box 10 (*Sox10*), and *Vim* (vimentin) mRNA levels were not affected by increased GH and IGF-1 oversecretion, bGH mice showed increased hypothalamic expression of *Nes, Gfap, Adgre1, Iba1,* and retina and anterior neural fold homeobox (*Rax*) compared to control animals (Figure 2). *Tnf* mRNA levels tended to be higher in the hypothalamus of bGH mice but this trend did not reach statistical significance ($p > 0.05$). Thus, increased GH/IGF-1 axis activity affects the brain expression of markers of cell proliferation, microglia, and neuroinflammation.

3.3. GHR-ablation in hepatocytes increases GH secretion and the hypothalamic expression of neuroglial markers associated with neuroinflammation

Enhanced GH secretion normally leads to increases in circulating IGF-1 levels since liver IGF-1 production and release are regulated by GHR signaling in hepatocytes [2, 44]. To determine the specific role of GH action on the expression of neuroglial markers, independently of IGF-1 levels, we generated mice carrying a hepatocyte-specific GHR ablation. Confirming earlier results [14], Albumin $^{\text{GHR}}$ mice showed very high serum GH levels, but a striking suppression in circulating IGF-1 concentration (Figure 3). Albumin $^{\text{GHR}}$ mice also exhibited a lower body weight (21.0 ± 1.0 g), as compared to control mice (23.5 ± 0.6 g; $P = 0.0425$). Hypothalamic *Ghr* expression remained unaltered in

Albumin ^{GHR} mice (Figure 3). Remarkably, similar to the findings observed in bGH mice, Albumin ^{GHR} mice displayed increased hypothalamic mRNA expression of *Gfap, Iba1,* and *Tnf* in comparison to control animals (Figure 3). Albumin ^{GHR} mice also exhibited a higher mRNA expression of *Sox10* (Figure 3). Therefore, increased GH secretion, independent of IGF-1 levels, is responsible for altering the expression of neuroglial markers associated with neuroinflammation.

3.4. Brain GHR signaling is necessary to induce GH-induced changes in the expression of neuroglial markers

The findings described above suggest that increased GH secretion affects the hypothalamic expression of neuroglial markers associated with neuroinflammation. To determine whether this effect depends on GHR signaling in the central nervous system or is indirectly regulated, a brain-specific GHR knockout mouse was produced. For this purpose, *Ghr* was ablated in nestin-positive cells. The phenotype of Nestin ^{GHR} mice has been described in recent studies and these mice exhibit increased GH secretion due to the loss of GH-negative feedback in the hypothalamus [23, 26]. The group of Nestin ^{GHR} mice used in the present study showed no difference in body weight 19.4 ± 0.6 g), compared to control mice (19.4 ± 0.5 g; $P = 0.9696$). Initially, we confirmed that the ability of a systemic GH injection to induce the activation of the GHR/STAT5 pathway in the hypothalamus of Nestin ^{GHR} mice was drastically reduced, compared to control animals (Figure 4a). Furthermore, *Ghr* mRNA expression in the hypothalamus of Nestin ^{GHR} mice is reduced by more than tenfold compared to the values found in control mice (Figure 4b). When the expression of genes associated with glial cells was analyzed, Nestin ^{GHR} mice showed reduced mRNA expression of *Gfap, Adgre1,* and *Vim*. In contrast, *Tnf* mRNA expression was increased in Nestin ^{GHR} mice, compared to control animals (Figure 4c). Thus, brain GHR signaling is necessary to induce GH-induced changes in the expression of neuroglial markers, although the upregulation of the proinflammatory cytokine *Tnf* seems to be indirectly regulated in Nestin ^{GHR} mice.

4. Discussion

In the present study, we used different mouse models to uncover the specific role of brain GHR signaling in controlling the hypothalamic expression of key glial and inflammatory markers. Briefly, our findings revealed that brain GHR signaling regulates the expression of astrocyte and microglia cell markers, which are frequently associated with neuroinflammation. This regulation is independent of circulating IGF-1 levels.

Previous studies have provided compelling evidence that GH deficiency improves several metabolic and neurological functions during aging. In addition, different GH-deficient animal models show a remarkable increase in longevity, particularly GHR-deficient mice [48]. Among the beneficial consequences of decreased GH action during the lifespan, GH-deficient dwarf mice exhibit increased insulin sensitivity, despite presenting a higher percentage of body adiposity, compared to wild-type animals [9, 48]. Furthermore, these mice are protected against aging-induced cognitive decline [5, 6] and neuroinflammation [41]. GHR knockout mice are also resistant to developing obesity-induced hypothalamic

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inflammation [49]. In contrast, GH oversecretion decreases lifespan and favors the development of insulin resistance and diabetes mellitus [48]. These numerous metabolic and developmental features in the animals that exhibit alterations in GH secretion represent confounding factors in the determination of whether the effects on the central nervous system are directly caused by changes in GHR signaling or via indirect mechanisms. Moreover, intrinsic alterations in IGF-1 circulating levels can also produce neurological consequences. Therefore, the main objective of the present study was to isolate the effects induced by GHR signaling in the brain from these potential confounding factors.

Using the *Ghrhr*$^{lit/lit}$ mice that exhibit a profound deficiency of GH and IGF-1, we observed reduction in the expression of several glial markers associated with neuroinflammation. In accordance with our findings, a previous study showed decreased expression of GFAP, Iba1, and TNFα in the hypothalamus of Ames dwarf mice, which are also GH and IGF-1 deficient [41]. Young adult GHR knockout mice have normal Iba1 but decreased GFAP expression in the hypothalamus as compared to wild-type animals [49].

Transgenic bGH mice show increased GH secretion and consequently higher circulating IGF-1 levels compared to wild-type animals. *Nes* expression was increased in the hypothalamus of bGH mice. On the other hand, *Ghrhr*$^{lit/lit}$ mice exhibited reduced *Nes* expression. The physiological importance of changes in *Nes* expression is unclear, but nestin is a well-established marker of stem cells that originate new neurons and glial cells in the brain [50]. Since GH and IGF-1 stimulate cell proliferation in numerous tissues, the increased *Nes* expression in bGH mice may reflect the proliferative action of these hormones. Albumin $^{\text{GHR}}$ mice also show increased GH secretion, but reduced serum IGF-1 levels. Considering that hypothalamic *Nes* expression was normal in Albumin $^{\text{GHR}}$ mice, it is possible that the increased IGF-1 secretion plays a significant role in stimulating *Nes* expression in the brain.

bGH mice also exhibited increased *Rax* expression in the hypothalamus. Rax is a transcription factor involved in cell differentiation in the retina and hypothalamus [51]. Rax expression has been used as a marker of tanycytes in the hypothalamus of adult animals [51, 52]. Thus, the simultaneous increase in circulating levels of GH and IGF-1 possibly led to the increased *Rax* expression, which may also reflect a stimulatory action of these hormones on cell proliferation. Conversely, Albumin $^{\text{GHR}}$ mice showed increased expression of *Sox10 mRNA*. Sox10 is a transcription factor involved in cell differentiation and neural development. Sox10 is particularly important for some glial cells since Sox10 activity directs neural stem cells toward the oligodendrocyte lineage [53], which could affect axonal myelination. A decreased number of Sox10-positive cells was observed in neural crest derived stem cells treated with IGF-1 [54].

Notably, increased expression of *Gfap, Adgre1,* and *Iba1* was observed in the hypothalamus of bGH mice. *Tnf* also showed a numerical (not significant) increase in bGH mice. GFAP is a typical marker of astrocytes, whereas F4/80 (encoded by the *Adgre1* gene) and Iba1 expressions are found in microglial cells. However, the expression of GFAP, F4/80, and Iba1 is frequently associated with conditions of neuroinflammation and brain injury [55, 56]. The proinflammatory profile found in the hypothalamus of bGH mice is in agreement with

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the cognitive and metabolic dysfunctions exhibited by this mouse model [48]. This effect involves microglial cells since F4/80 and Iba1 expression increases in reactive microglia, which has an M1-like (proinflammatory) profile [56]. Importantly, when the hypothalamus of Albumin ^{GHR} mice was analyzed, a similar proinflammatory pattern was observed (e.g., increased expression of *Gfap, Iba1*, and *Tnf*. Comparing the differences in the GH/IGF-1 axis between bGH and Albumin ^{GHR} mice, it is evident that the increased GH secretion played a dominant role in stimulating the proinflammatory profile observed in the hypothalamus of bGH and Albumin ^{GHR} mice, whereas circulating IGF-1 levels were irrelevant to this response. However, we cannot rule out the possibility that GH-induced IGF-1 local production may have stimulated the proinflammatory response observed in the hypothalamus. Nonetheless, this effect requires GHR signaling since Nestin ^{GHR} mice show an opposite phenotype, associated with reduced expression of *Gfap* and *Adgre1* as well as a tendency towards a reduction in *Iba1* mRNA levels. Taken together, to our best knowledge, our study is the first to provide direct evidence that GH presents a GHR-dependent proinflammatory effect on the mouse hypothalamus.

However, increased *Tnf* expression was observed in the hypothalamus of Nestin ^{GHR} mice. The expression of proinflammatory cytokines in the brain precedes the increased mRNA levels of *Gfap* and *Iba1* following a systemic lipopolysaccharide-induced immune challenge [55]. A high-fat diet also promotes inflammation in the hypothalamus [57]. Notably, the increased hypothalamic expression of proinflammatory cytokines is observed after one day of high-fat diet intake, whereas microglia changes are observed later (after 3 days) [57]. Thus, an unknown factor is possibly stimulating the expression of proinflammatory cytokines in the brain of Nestin ^{GHR} mice, but under the conditions studied (e.g., age, diet), the astrocytes and microglial cells have not yet been polarized to an M1-like phenotype, explaining their reduced expression of *Gfap* and *Adgre1*. It is also possible that part of the microglia in the brain of Nestin ^{GHR} mice was not affected by GHR ablation because in vivo microglia repopulation is primarily derived from residual microglial cells and not from nestin-positive cells [58]. Thus, the proinflammatory action of GH could induce the polarization of non-nestin-derived microglial cells, leading to an increase in *Tnf* expression in the hypothalamus of Nestin ^{GHR} mice. A recent study showed that nestin-Cre transgenic mice exhibit an abnormal microglial activation, characterized by an enhanced expression of GFAP and Iba1, suggesting “Cre toxicity” [59]. Other problems have been reported with this mouse model because the nestin-Cre transgene also contains a GH minigene which induces the expression of human GH in the brain [60, 61]. Consequently, the pituitary gland of nestin-Cre mice produces less GH due to negative feedback induced by the transgenic GH expression [60, 61]. Thus, the increased expression of neuroinflammatory markers in the brain of nestin-Cre transgenic mice can be explained by the here-described proinflammatory action of central GHR signaling. However, since our study uses nestin expression to drive GHR ablation, the brain of Nestin ^{GHR} mice is intrinsically protected against the undesired effects of GH transgenic expression.

In conclusion, the gene expression analysis in the hypothalamus of several mouse models that present distinct profiles in GH and IGF-1 secretion or brain GHR signaling suggests that glial cells are regulated by these growth factors. The expression of several neuroglial markers, especially those associated with neuroinflammation, is directly modulated by

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central GHR signaling. Finally, our study was performed exclusively in male mice. Thus, some caution is needed in extrapolating our findings to females because marked physiological, endocrine, and metabolic sex differences have been reported in mice and humans [62, 63].

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Highlights

- GH-deficient mice show decreased brain expression of *Nes, Gfap, Iba1, Adgre1,* and *Tnf*
- GH overexpression increases brain expression of *Nes, Gfap, Iba1, Adgre1,* and *Rax*
- Liver-specific GHR ablation increases the mRNA levels of *Gfap, Iba1,* and *Tnf*
- Increased GH secretion causes hypothalamic inflammation, independent of IGF-1 levels
- Brain GHR signaling is necessary to modify the expression of several glial markers

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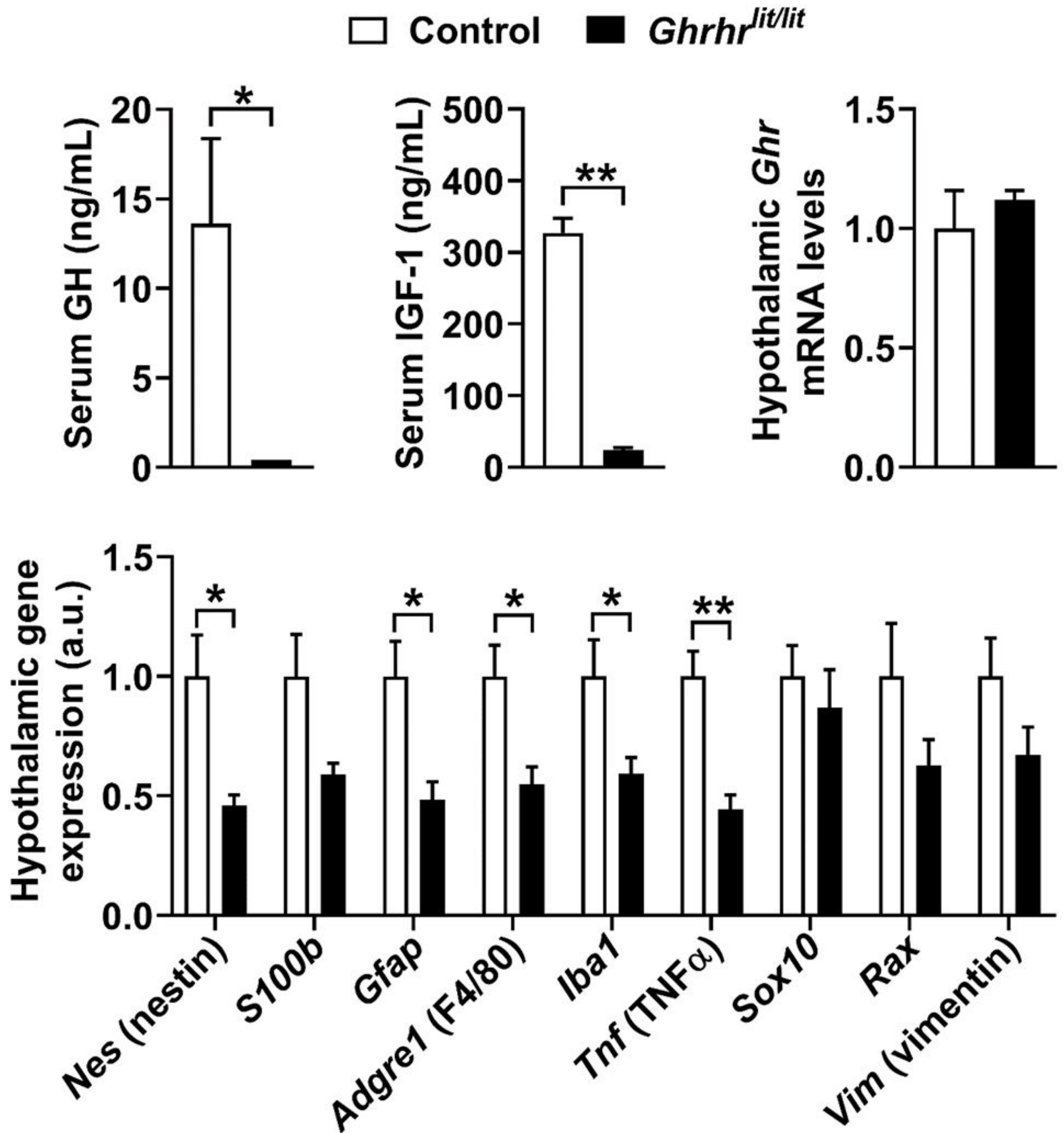

Figure 1.

Ghrhr$^{lit/lit}$ mice *(n = 4-5)* show decreased serum GH and IGF-1 levels, and hypothalamic expression of genes associated with inflammation, compared to control animals ($n = 5$-6). * $p < 0.05$; ** $p < 0.01$.

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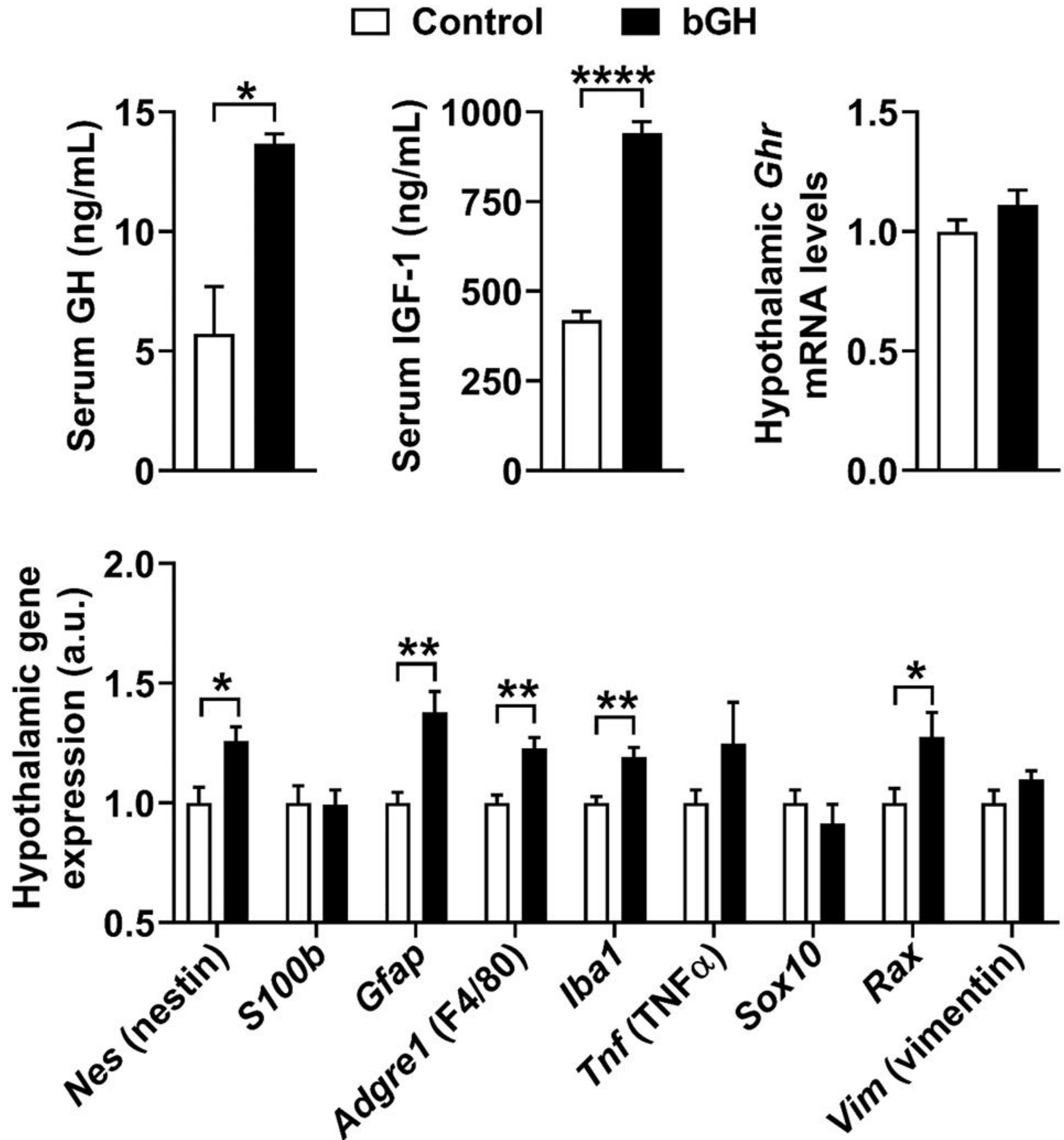

Figure 2.

Transgenic bGH male mice ($n = 6$-8) show increased serum GH and IGF-1 levels and changes in the hypothalamic expression of several neuroglial markers, compared to control animals ($n = 7$-8). * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$.

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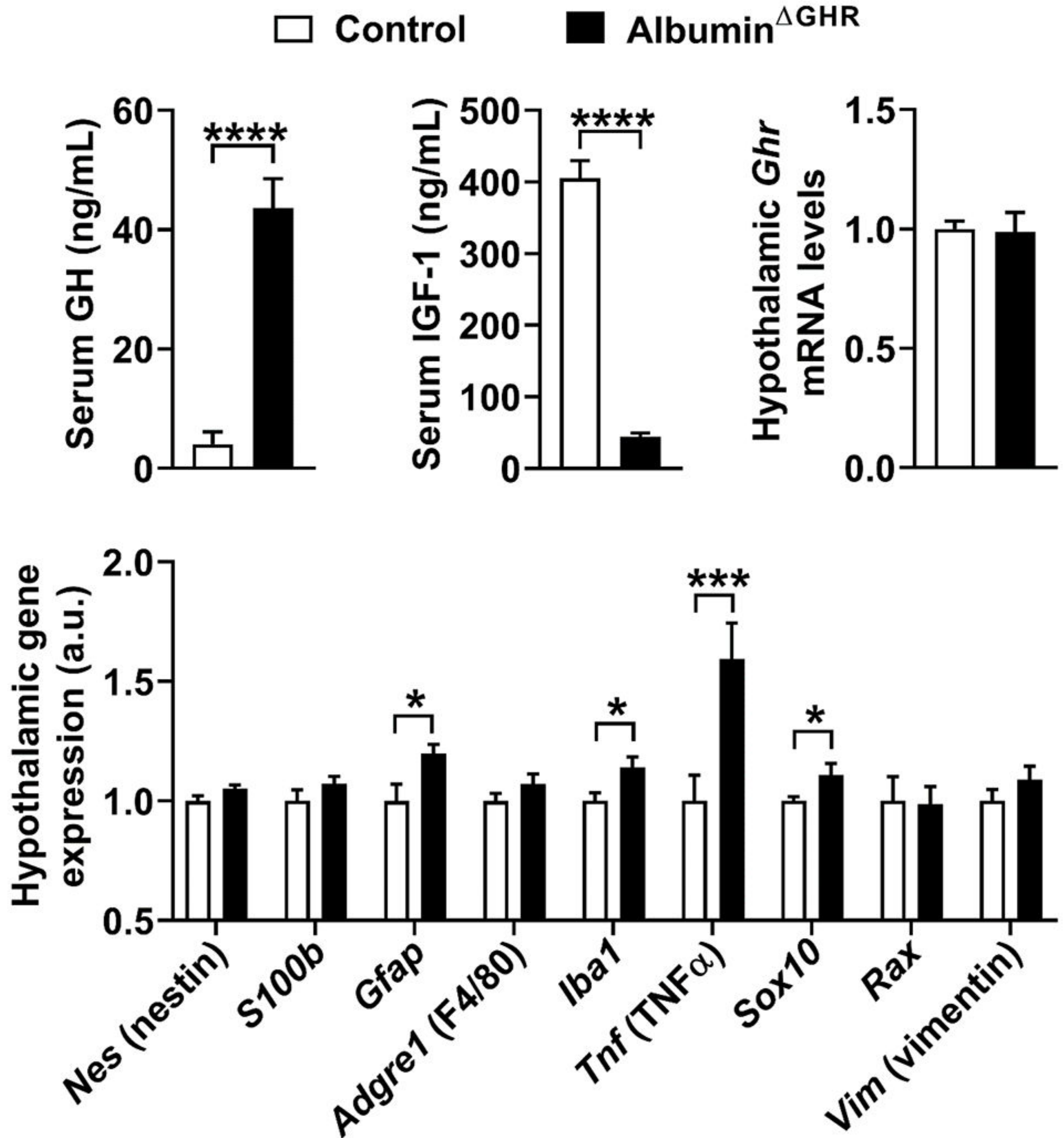

Figure 3.

Albumin GHR male mice ($n = 8$-9) show increased serum GH but reduced IGF-1 levels, and changes in the hypothalamic expression of several neuroglial markers, compared to control animals ($n = 8$-9). * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$.

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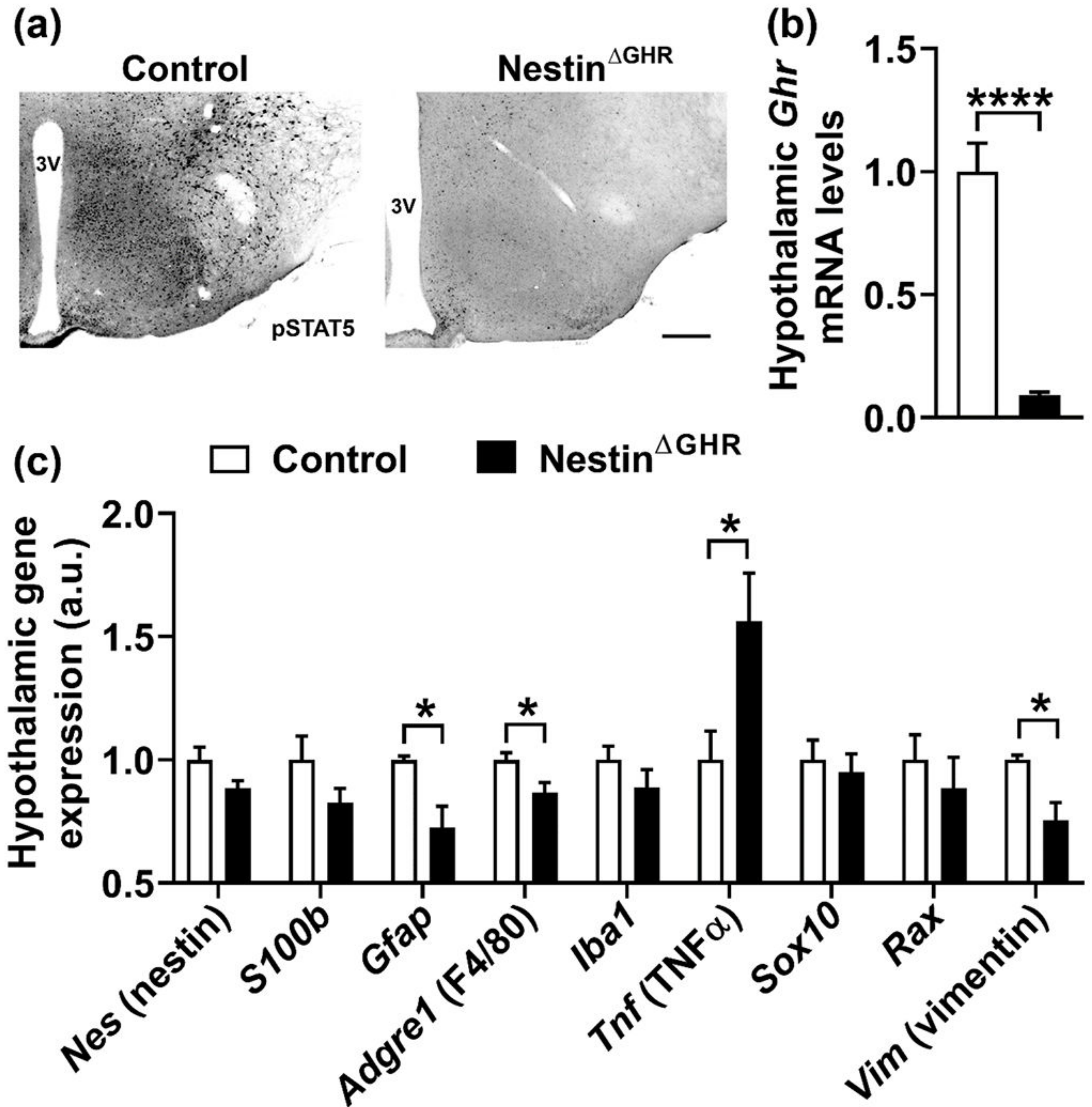

Figure 4.

Brain GHR signaling is necessary to induce GH-induced changes in the expression of neuroglial markers, **(a)** Nestin ^{GHR} male mice show a blunted responsiveness to GH in the brain since a systemic GH injection cannot induce pSTAT5 immunoreactivity in comparison with GH-treated control mice. Abbreviation: 3V, third ventricle. Scale bar = 200 μm. **(b)** Nestin ^{GHR} mice ($n = 5$) exhibit reduced hypothalamic *Ghr* expression, compared to control

animals ($n = 5$). **(c)** Nestin ^{GHR} mice present alterations in the hypothalamic expression of neuroglial markers. * $p < 0.05$; **** p 0.0001.

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Table 1.

Primer sequences.

Target gene	Gene function	Forward primer	Reverse primer
Actb (β-actin)	Reference gene	gctccggcatgtgcaaag	catcacaccctggtgccta
Adgre1 (F4/80)	“Reactive” microglia. Neuroinflammation	tgggatgcataatcgctgct	cctcagaacccacagtgtcc
Gapdh	Reference gene	gggtcccagcttaggttcat	tacggccaaatccgttcaca
Gfap	Astrocyte marker. Neuroinflammation	gctagccctggacatcgaga	ccccttctttggtgcttttgc
Ghr	Growth hormone receptor	atcaatccaagcctggggac	acagctgaatagatcctgggg
Iba1	“Reactive” microglia. Neuroinflammation	cagggatttgcagggaggaaa	gtttggacggcagatcctca
Nes (nestin)	Stem cell marker	cccttagtctggaagtggct	cttcagcttggggtcaggaaag
Ppia	Reference gene	tatctgcactgccaagactgagt	cttcttgctggtcttgccattcc
Rax	Transcription factor and tanycyte marker	tcccggagtacgaagctact	ggttgcgtcgatgcttcttc
S100b	Astrocyte marker	atggttgccctcattgatgtct	acttcctgctccttgatttcctcc
Sox10	Cell differentiation. Oligodendrocyte marker	acgcagaaagctagccgac	cactttcgttcagcaacctccag
Tnf (TNFα)	Proinflammatory cytokine	atggcctccctctcatcagt	tggtttgctacgacgtggg
Vim (vimentin)	Radial glia and tanycyte marker	ggctgcgagagaaattgcagg	aggtcaagacgtgccagaga