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Review

Ghrelin-Mediated Hippocampal Neurogenesis: Implications for Health and Disease

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There is a close relationship between cognition and nutritional status, however, the mechanisms underlying this relationship require elucidation. The stomach hormone, ghrelin, which is released during food restriction, provides a link between circulating energy state and adaptive brain function. The maintenance of such homeostatic systems is essential for an organism to thrive and survive, and accumulating evidence points to ghrelin being key in promoting adult hippocampal neurogenesis and memory. Aberrant neurogenesis is linked to cognitive decline in ageing and neurodegeneration. Therefore, identifying endogenous metabolic factors that regulate new adult-born neurone formation is an important objective in understanding the link between nutritional status and central nervous system (CNS) function. Here, we review current developments in our understanding of ghrelin's role in regulating neurogenesis and memory function.

Highlights

There is a close relationship between cognitive performance and nutritional status, but the mechanisms underlying this relationship are not well understood.

The hormone, ghrelin, which is released during food restriction, triggers adaptive responses to improve learning and memory by increasing the formation of new neurones in the adult brain.

The birth of new neurones (neurogenesis) from neural stem cells in the adult mammalian brain is an important process involved in protecting against the age-related decline in cognitive function.

Activation of the hippocampal ghrelin receptor may be a viable therapeutic approach to stimulate neurogenesis and protect against age- and disease-related cognitive decline.

Ghrelin

The gut-derived hormone, ghrelin, was identified in 1999 as the natural ligand of the **growth hormone secretagogue receptor 1a (GHS-R1a)** (see Glossary) that stimulates the release of growth hormone (GH) from the pituitary gland [1]. An early discovery was its ability to stimulate feeding [2], resulting in the 'hunger hormone' name-tag. Ghrelin has subsequently been found to have numerous functions, including regulating gastric acid secretion and motility [3], pancreatic cell proliferation and apoptosis [4], sleep [5], and cardiovascular function [6]. More recently, ghrelin has been linked to central nervous system (CNS) function, including protecting neurones [7,8], regulating mood [9], and promoting neural plasticity via adult hippocampal neurogenesis (AHN) [10–12].

Ghrelin is predominantly produced by oxyntic cells of the stomach (as illustrated in Figure 1), with lower amounts generated in the pancreas [13], kidney [14], and placenta [15]. There are reports of low-level ghrelin mRNA and peptide expression in the hypothalamus [16]; however, subsequent studies unequivocally demonstrate that ghrelin is not produced in the brain [17,18]. Thus, ghrelin in the CNS derives from a peripheral source and is consistent with the ability of ghrelin to cross the blood–brain barrier (BBB) in a metabolic state-dependent manner [19,20] and bind its receptor in the brain [21,22].

Human ghrelin, encoded by the gene *GHRL*, located on chromosome 3p25-26, generates the preproghrelin peptide that is enzymatically processed to a mature 28 amino acid (aa) peptide [23]. The native peptide, **unacylated ghrelin (UAG)** (or des-acyl ghrelin), undergoes enzymatic modification in the endoplasmic reticulum by **ghrelin-O-acyl transferase (GOAT)** to generate **acyl-ghrelin** [24,25]. This involves the addition of a medium chain fatty acid (generally octanoic acid) at Serine residue 3 (Ser3), which is essential for it to bind and activate GHS-R1a signalling. Notably, GOAT expression has been observed in the hippocampus, where it is able to acylate ghrelin locally [26]. Acyl-ghrelin can also undergo de-acylation into UAG, mediated by **acyl-protein thioesterase 1 (APT1)** [27] or **butyrylcholinesterase** [28] to regulate its activity. The expression of APT1 in the brain has not been characterised, however, butyrylcholinesterase is expressed in the hippocampus [29]. As both acyl-ghrelin and UAG can cross the BBB [21], it is therefore possible that both peptides undergo enzymatic conversion within the hippocampus in a cell-specific manner. Whilst a receptor for UAG has not been identified, it has been shown to induce genome-wide expression changes in GHS-R1a knockout mice [30], suggesting the existence of an unknown UAG receptor.

GHS-R1a is a 366 aa, seven transmembrane domain, G protein-coupled receptor (GPCR) [31]. It belongs to the rhodopsin family of GPCRs, primarily interacting with $G\alpha_{q/11}$, with activation leading

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to the PLCβ/IP_3 signalling cascade. A splice isoform of the receptor, termed GHS-R1b, is a truncated 289 aa form with only five transmembrane domains, that lacks intracellular signalling ability [32]. GHS-R is expressed in several peripheral tissues, including the pancreas, lungs, myocardium, liver, intestine, and adipose tissue [33]. Expression in the brain is restricted to several specific regions, including the hypothalamus, cortex, midbrain (including the substantia nigra), pons, and medulla oblongata [34,35]. Most notably, GHS-R is highly expressed in the adult hippocampus, with initial reports of GHS-R1a mRNA expression [33,36] subsequently confirmed at the protein level [34]. More specifically, the use of genetically modified GHS-R–eGFP reporter mice demonstrated that GHS-R is most highly expressed in the **dentate gyrus (DG)** of the hippocampus [11,35]. This pattern of receptor localisation is thought to contribute to the regulation of a diverse array of ghrelin-related behaviours, including anxiety, stress-response, feeding, reward and learning and memory [37]. These behaviours are thought to involve distinct circuits; however, where circuitry may overlap (i.e., signalling stress- and learning-related stimuli via hippocampal circuits), distinct signalling may be partially mediated by the formation of GHS-R1a dimers with other GPCRs. For example, GHS-R forms a complex with the dopamine receptor subtype 1 (D1R), which enhanced dopamine signalling *in vitro* [38]. Interestingly, this led to a switch in the G protein coupled to the receptor from $G\alpha_q$ to $G\alpha_{i/o}$, suggesting that ghrelin is able to selectively enhance dopamine signalling in cells coexpressing these proteins. However, dimerisation is thought to occur in the absence of ghrelin, indicating **allosteric regulatory processes** [39] (Figure 2). GHS-R1a has also been shown to modulate serotonin signalling via dimerisation with 5-HT_{2C} receptors [40,41] and oxytocin receptors [42], whilst GHS-R1b forms **heterodimers** with GHS-R1a to diminish cell surface expression [32,43].

To add further complexity, GHS-R1a exhibits high constitutive activity, at least *in vitro* [44–46]. One potential explanation for this phenomenon is to attune a high signalling set-point to maintain energy state, necessary for survival [47]. Indeed, emerging evidence suggests ghrelin plays a role in survival, with increased levels of circulating ghrelin during energy insufficiency acting in a homeostatic manner to defend against hypoglycaemia (for review see Mani and Zigman [48]). For example, $GOAT^{-/-}$ mice, which lack acyl-ghrelin, show a marked increase in hypoglycaemia-induced mortality when exposed to a prolonged 48 h fast [49]. Alongside the glycaemic adaptations induced by acyl-ghrelin during energy deficit, it regulates important behavioural and physiological adaptations, beyond the stimulation of hunger, that may also play convergent roles in survival. These adaptive responses to energy deficit involve learning and memory [11], neuroprotection [8], and mood [9,50], suggesting that acyl-ghrelin is a key metabolic hormone linking nutritional state to improvements in CNS function. Here, we focus on the evidence for ghrelin-mediated action at a key learning brain region, the hippocampus. In particular, we discuss the most recent developments in our understanding of ghrelin's role in regulating the generation of new adult-born hippocampal neurones.

The Hippocampus

The hippocampus (Figure 3), named from the Greek 'Hippokampos' due to its structural resemblance to the seahorse, is a major component of the limbic system that is essential to learning and memory. Studies investigating the role of the hippocampus in learning and memory were conducted on patients by William Scoville and Brenda Milner in 1957 [51]. One patient, suffering from an incurable form of epilepsy, underwent temporal lobectomy, which successfully reduced seizures but resulted in profound global amnesia. The memory dysfunction was due to the removal of a large part of the hippocampal formation and the surrounding cortical region. Indeed, subsequent studies in model organisms revealed the essential nature of the hippocampus to episodic memory [52]. This memory system is essential for processingautobiographical memories, remembering the 'what, where, and when' of events, thus providing spatial and contextual information for an organism to make important decisions relating to health and survival.

Ghrelin-Mediated Hippocampal Memory

Since 2002, accumulating evidence has indicated ghrelin's involvement in hippocampal memory formation in various animal models (Table 1). Initial investigations showed that a single administration of acyl-ghrelin, either intracerebrovascular (i.c.v.) [53] or intrahippocampal (i.h.) [54], promoted memory

Glossary

Acyl-ghrelin: a form of ghrelin that has been acylated by GOAT, which enables it to bind to GHS-R1a.
Acyl-protein thioesterase 1 (APT1): an endogenous enzyme known to de-acylate acyl-ghrelin.
Allosteric regulatory process: regulation of a protein or receptor independent of its active site.
Brain derived neurotrophic factor (BDNF): a growth factor in the brain, involved in cell proliferation, survival, learning, and memory.
Bromodeoxyuridine (BrdU): a synthetic thymidine analogue, incorporated into DNA during replication. It can be used as a marker of proliferation during a specific timeframe.
Butyrylcholinesterase: an endogenous esterase enzyme, present in the plasma, that hydrolyses many different choline-based esters, but can also de-acylate acyl-ghrelin to form un-acylated ghrelin (UAG).
Calorie restriction: a reduction of food intake (typically 30% less) in the absence of malnutrition.
Calorie restriction mimetics: endogenous or exogenous factors that mimic the effects of calorie restriction.
cAMP response element binding protein (CREB): a transcription factor regulating BDNF expression and important in neuronal plasticity and memory.
Dentate gyrus (DG): a subregion of the hippocampus where neurogenesis occurs.
Ghrelin-O-acyl transferase (GOAT): a member of the membrane bound O-Acyl transferase (MBOAT) family, the only enzyme known to acylate ghrelin.
GluA1-containing AMPA receptors: glutamate receptor and ion channels responsible for fast excitatory synaptic transmission. Important for synaptic plasticity and long-term potentiation (LTP). AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.
Growth hormone secretagogue receptor (GHS-R): a G protein-coupled receptor (GPCR) also known as the ghrelin receptor. It is the only known receptor for acyl-ghrelin.
GHS-R–eGFP mice: genetically mutated mice that coexpress

retention in rats, suggesting a consolidating effect on episodic memory. Later experiments revealed that acyl-ghrelin, when administered peripherally, enhanced hippocampal dendritic-spine synapse formation, increased **long-term potentiation (LTP)** (a physiological correlate of memory), and improved spatial memory [22]. Moreover, ghrelin knockout mice displayed reduced CA1 spine synapses and impaired spatial memory performance in the **novel object recognition (NOR)** task, which was rapidly recovered by peripheral treatment with acyl-ghrelin [22]. Electrophysiological analysis demonstrated that i.h. administration of acyl-ghrelin enhanced the excitability of the hippocampus, thereby facilitating the induction of LTP and improved memory in rats [55,56]. Moreover, i.c.v. injections of acyl-ghrelin for 2 weeks promoted hippocampal LTP and memory retention while ameliorating **synaptic plasticity** in a rat model of Alzheimer's disease (AD) [57]. This is consistent with the PI3K-dependent enhancement in LTP following i.h. injection of acyl-ghrelin [58]. Subsequent molecular analysis showed that the non-peptidyl GHS-R1a agonist, MK-0677, enhanced synaptic delivery of **GluA1-containing AMPA receptors**, consequently increasing excitatory synaptic transmission and plasticity [59]. Furthermore, acyl-ghrelin upregulated expression of the phospho-GluN2B [60] and the NR2B [61] subunit of the NMDA receptor to enhance release of the excitatory neurotransmitter, glutamate [61]. Kern *et al*. showed that GHS-R1a forms a heterodimer with the dopamine receptor DRD1 in a complex with $G\alpha_q$ *ex vivo*. To investigate this *in vivo*, mice treated with a DRD1 agonist into the DG showed enhanced hippocampal working memory, which was blocked by co-infusion of a GHS-R1a antagonist [39]. These tests indicate that DRD1-regulated behaviours depend on interactions between DRD1 and GHS-R1a in the DG.

There are several lines of evidence to support a role for acyl-ghrelin in protecting neurones, which may ultimately support improvements in cognitive function. Firstly, acyl-ghrelin rescued deficits in spatial memory in streptozotocin-induced diabetic rats [62]. More specifically, acyl-ghrelin increased **cAMP response element binding protein (CREB)** expression and led to the activation of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway and the promotion of **brain derived neurotrophic factor (BDNF)** expression in the hippocampus. Moreover, acyl-ghrelin treatment of i.h. amyloid-β injected mice, that mimic aspects of AD, rescued memory deficits [63,64], decreased neuroinflammation [64], and prevented the amyloid-β induced suppression of hippocampal LTP [64]. Similarly, in amyloid-β injected rats treated with acyl-ghrelin, there was a reduction in amyloid-β plaque deposition and attenuation of memory impairments due to increased AMP-activated protein kinase (AMPK) and glycogen synthase kinase (GSK) phosphorylation and decreased tau phosphorylation [65]. In a transgenic 5xFAD mouse model of AD, neuronal cell loss was prevented in the hippocampus of mice treated with acyl-ghrelin [66]. Notably, in the senescent **SAMP8 mice**, acyl-ghrelin increased baseline cognitive performance, suggesting that ghrelin signalling may preserve cognitive function in ageing [22]. Collectively, these data highlight an important role for ghrelin in hippocampal function under normal and disease conditions.

AHN

The process of AHN, as depicted in Figure 3, is an ongoing form of brain plasticity that combines expansion of the neural stem cell pool (to maintain the population) and differentiation, maturation, and functional integration of these newly formed neural cells. In 1962, Altman provided the first evidence of newly formed neurones in the postnatal rat hippocampus [67], termed 'adult hippocampal neurogenesis' (AHN). Since then, the use of **bromodeoxyuridine (BrdU)**, a synthetic analogue of thymidine that labels proliferating cells, has greatly facilitated studies on neurogenesis in model organisms. From the 1990s, researchers studying AHN have demonstrated the existence of new adult-born neurones in the sub-granular zone (SGZ) of the human DG [68,69] (Box 1). Notably, these nerve cells show temporal functional specificity to separate highly similar components of memories into distinct memory representations that are unique and less easily confused. This function, termed '**pattern separation**', is dependent on a reduced threshold for action potential firing when new adult-born neurones are 4–6 weeks of age [70]. A series of elegant loss-of-function [71] and gain-of-function [72] studies in mice confirmed the essential role of new adult-born neurones to pattern separation-dependent memory in mice. In addition, recent studies suggest a spatial functional specificity to neurogenesis, with new adult-born neurones in the rostral pole of the DG being important for

green fluorescent protein (GFP) with the growth hormone secretagogue receptor (GHS-R) gene.
Heterodimers: a protein complex, consisting of two different proteins.
Long-term potentiation (LTP): an important process in memory formation, resulting in a long-term increase in the strength of electrical activity or transmission between two neurones.
Neural stem progenitor cells (NSPCs): multipotent stem cells that reside in the brain with the capacity to differentiate into any neural cell type.
Novel object recognition (NOR): behaviour test of cognitive function that requires recognising novel from familiar objects. This is a hippocampal-dependent task.
Passive avoidance learning (PAL): behaviour test that evaluates memory and learning in rodents with neurological disorders. The rodents learn to avoid an environment in which an aversive stimulus was previously delivered.
Pattern separation: the ability to distinguish similar inputs into separate discrete outputs.
SAMP8 mice: a naturally occurring mouse model of accelerated ageing with symptoms of cognitive decline.
Spontaneous location recognition (SLR) task: behavioural test of cognitive function that requires the ability to discriminate similar but distinct spatial contexts. This is a hippocampal neurogenesis-dependent task.
Synaptic plasticity: the ability of a synapse to respond to signals, thereby enhancing or decreasing the strength of connection. Thought to be an important mechanism in learning and memory.
Unacylated ghrelin (UAG): the unacylated form of ghrelin [also known as des-acyl ghrelin (DAG)]. Initially thought to be inactive as it does not activate the ghrelin receptor. Subsequent research suggests it has physiological effects independent of GHS-R.
5xFAD mice: a transgenic mouse model of Alzheimer's disease (AD) expressing mutant versions of human amyloid precursor protein (APP) [Swedish (K670N/M671L), Florida (I716V), London (V717I)], and Presenilin 1 (PSEN1) (M146L and L286V).

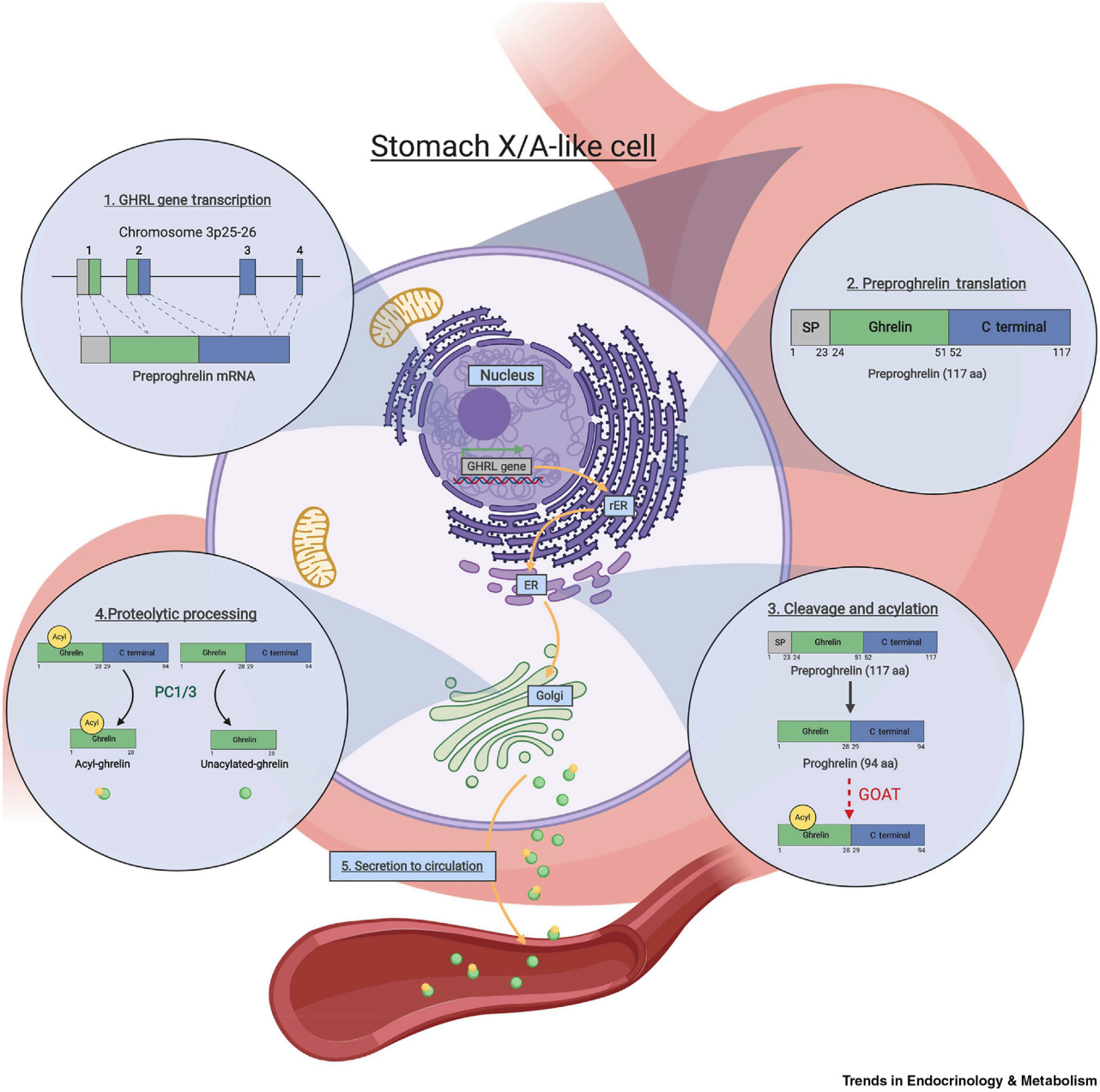

Figure 1. Summary of Ghrelin Production from the Stomach.
(1) Ghrelin gene (GHRL) is transcribed to preproghrelin mRNA. (2) Preproghrelin mRNA is translated to a 117 amino acid (aa) peptide in the rough endoplasmic reticulum (rER). (3) Preproghrelin peptide undergoes proteolytic cleavage to proghrelin in the ER and optional acylation by ghrelin-O-acyl transferase (GOAT). (4) Prohormone convertase (PC1/3) processes proghrelin to 28 amino acid mature ghrelin. (5) Acyl or unacylated ghrelin is secreted into the circulation. Abbreviation: SP, signal peptide.

episodic memory processing, whilst AHN in the caudal DG is linked with processing anxiety and stress-related memory [73].

The number of new adult-born neurones is regulated by several extrinsic factors. For example, aerobic exercise [74] and environmental enrichment [75] were shown to increase cell proliferation and

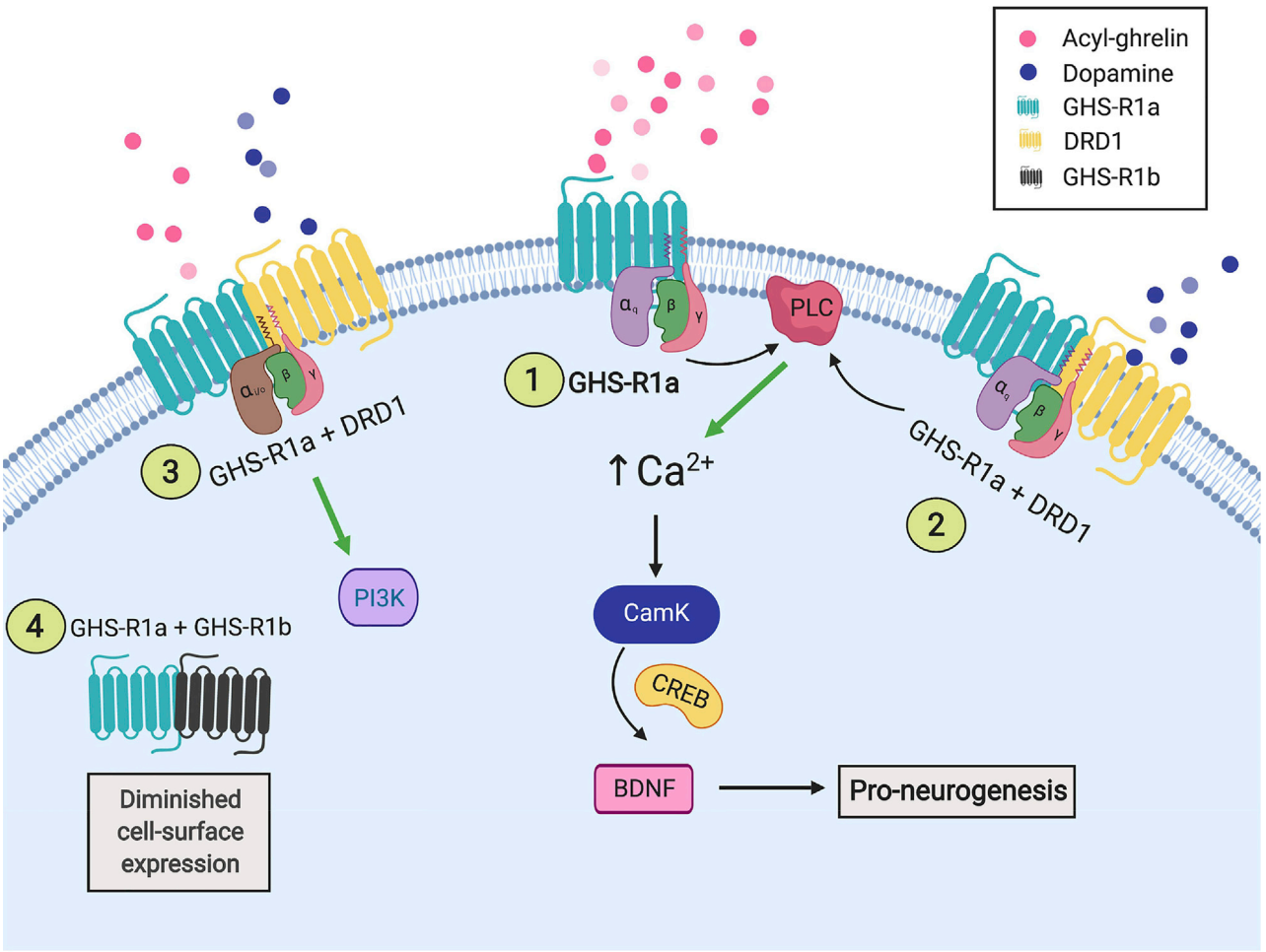

Figure 2. Putative Growth Hormone Secretagogue Receptor 1a (GHS-R1a) signalling pathways.
(1) Canonical GHS-R1a signalling via Gα_q leading to calcium release, CamKII phosphorylation, and activation of downstream targets such as cAMP response element binding protein (CREB)/brain derived neurotrophic factor (BDNF). (2) GHS-R1a dimerisation with the dopamine receptor DRD1 regulates activation in the absence of ghrelin [39]. (3) GHS-R1a dimerisation with DRD1 results in a switch of G protein-coupled Gα_q to G$\alpha_{i/o}$, leading to PI3K downstream signalling [38]. (4) GHS-R1a dimerisation with GHS-R1b results in diminished cell-surface expression [43].

survival of new adult-born neurones, respectively, in rodents. Conversely, stress [76] and ageing [77] negatively impact neurogenesis, demonstrating the remarkable plasticity of the process to factors that lay outside of the brain. Notably, alternate-day feeding [78] was shown to increase the survival of new adult-born cells in the adult DG, suggesting that factors responding to reduced calorie intake may regulate the hippocampal neurogenic niche. Here, we look at the impact of **calorie restriction** on hippocampal function and neurogenesis.

Benefits of Calorie Restriction on Brain Function and AHN

Calorie restriction, defined as a reduction in food intake without incurring malnutrition [83], increases lifespan and protects against obesity and cardiovascular disease (for review see Di Francesco *et al.* [84]). In addition, calorie restriction benefits the brain by preventing cognitive decline [85] and ameliorating neurodegeneration [86,87] in rodent models of disease. Remarkably, calorie restriction reduces brain atrophy in monkeys [83,88] and improves verbal memory scores in humans [89].

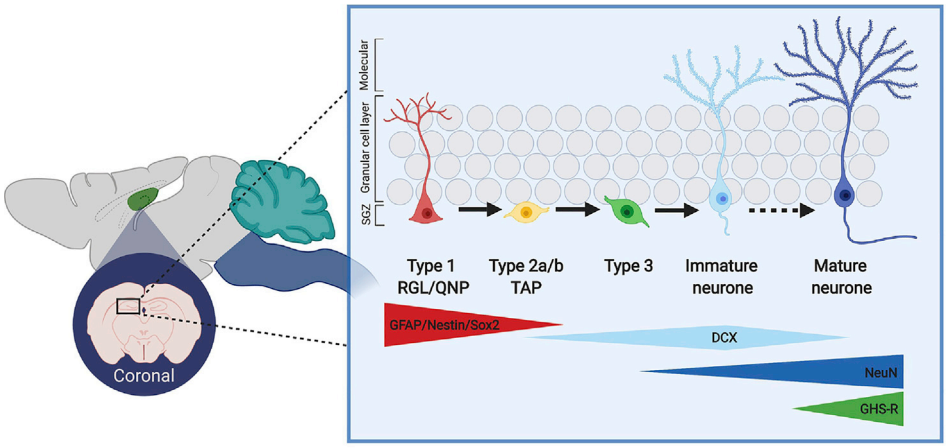

Figure 3. Stages of Adult Hippocampal Neurogenesis (AHN).
AHN comprises of sequential phases of neural stem progenitor cell (NSPC) proliferation and differentiation, leading to the generation of functionally integrated neurones within the hippocampal circuitry. There are distinct NSPC types: quiescent neural progenitor cells (QNPs) or 'type-1' NSPCs are slowly dividing and have a single radial process extending through the granular cell layer of the dentate gyrus. As such, they are referred to as radial glial-like (RGL) cells and express nestin and glial fibrillary acidic protein (GFAP). QNPs divide to produce highly proliferative type-2 transit-amplifying progenitors (TAPs) that express Sox2 and lack radial morphology. Subsequent type 2b cells can also express neuronal markers such as NeuroD1 and Prox1 (not shown), whilst type 3 neuroblasts are characterised by Dcx immunoreactivity and lack nestin expression. During the early postmitotic phase, cells undergo programmed cell death or begin to display enhanced dendritic extensions and express NeuN. During the late maturation phase (4–6 weeks of age), cells integrate into the hippocampal network and have reduced threshold for action potential firing, an important aspect of laying down similar but distinct memories (i.e., pattern separation function). Notably, growth hormone secretagogue receptor (GHS-R) expression has been characterised throughout the differentiation process and is only expressed in the mature neuronal population [11]. Abbreviation: SGZ, subgranular zone.

Delineating the molecular pathways underlying the beneficial effects of calorie restriction remains an important objective. Currently, these mechanisms are not fully understood, however, calorie restriction is known to increase the level of BDNF in the hippocampus of adult mice [90]. BDNF is crucial for the survival of neurones during development and for improving memory function [91]. More recently, calorie restriction was shown to induce hippocampal CREB in mice [92]. CREB regulates Sirtuin1 (SIRT1), an NAD^+-dependent histone deacetylase that has a key role in neuronal plasticity and memory. In addition, calorie restriction (or the pharmacological activation of SIRT1) promoted SIRT1 activity in the hippocampus, leading to preserved memory functions, delayed synaptic loss, and attenuated onset of neurodegeneration [87]. While these studies show that calorie restriction can affect the molecular machinery of hippocampal cells involved in learning and memory, it does not address how these neurones sense nutritional state during calorie restriction. Moreover, the impact of these calorie restriction paradigms on hippocampal neurogenesis were not addressed; however, an increasing number of studies are starting to examine this relationship in more detail. For example, mice exposed to a very low calorie/low protein fasting mimicking diet had increased numbers of new immature DG neurones and improved cognitive performance [93].

Given that circulating acyl-ghrelin is increased during calorie restriction, we have previously investigated circulating acyl-ghrelin as a key metabolic hormone linking nutritional state with hippocampal neurogenesis and memory [11]. To determine whether calorie restriction promotes the generation of new mature adult-born hippocampal neurones and whether acyl-ghrelin signalling is required for this effect, we exposed adult wild type and GHS-R-null mice to a 30% reduction in food intake for 2 weeks. Quantification of AHN revealed that 2 weeks of calorie restriction led to a significant 52% increase in the number of new adult-born neurones, specifically in the rostral DG of wild type mice, compared

Model	Sex	Methods	Outcome: cellular	Outcome: behavioural	Refs
Wistar rats age: not specified.	M	i.c.v. of acyl-ghrelin (0.3, 1.5, and 3 nmol/μl) one injection, 7 days after surgery.		Increased memory retention (OF, EPM, SDT).	[53]
Wistar rats age: not specified.	M	i.h. of acyl-ghrelin 0.3, 1.5, and 3 nmol/μl one injection, 7 days after surgery.		Increased memory retention and anxiogenesis (EPM, SDT).	[54]
$Ghr^{-/-}$ and $Ghsr^{-/-}$ mice on c57/Bl6 background, CD-1 mice (4 months of age), 3-month- old Sprague-Dawley rats, SAMP8 mice (4 and 12 months).	M	i.c.v. of acyl-ghrelin (0, 50, 75, 100, 150, 200 ng per injection). s.c. of acyl-ghrelin (10 mg/kg) or LY444711 (5 mg/kg). Minipump of acyl-ghrelin (3.5 mg/kg).	Increased dendritic spine synapses (c57/Bl6 and $Ghr^{-/-}$ mice), LTP (CD-1 mice).	Increased learning and memory: spontaneous alternation plus maze task, NOR, T-maze footshock avoidance, and SDT.	[22]
Adult rat hippocampal progenitor cells (AHPs).	ns	Hexarelin, acyl-ghrelin and Unacyl-ghrelin at 1, 3, 10, 30 μM for 24 hours.	Hexarelin and acyl-ghrelin increased proliferation. Hexarelin protected against apoptosis and necrosis.		[95]
Lister hooded rats	M	Ghrelin receptor agonists 3 mg/kg (GSK894490A oral, CP-464709-18 s.c.) for 0.5, 1, 2, 3, or 6 hours.		Improved performance in the NOR and Atlantis water maze tests.	[111]
8-week-old C57Bl/6 mice	M	i.p. ghrelin (80 μg/kg) daily for 8 days.	Ghrelin increased the number of $BrdU^+$ and Dcx^+ cells in the GCL of the DG.		[96]
Wistar rats	M	i.h. or i.c.v. of ghrelin (0.03, 0.3, 3 nmol/μl). Ghrelin administered 15 min before the behavioural tests.		Improved long-term memory (SDT and EPM).	[56]
Wistar rats	M	I.h. injection of ghrelin (0.03, 0.3, 3 nmol/ μl) 30 min before the quantification of NOS activity or immediately after training in the SDT.	Ghrelin increased NOS activity and LTP.	Ghrelin increased long-term memory (SDT).	[55]
i.h. unilateral injection in ICR mice (3 μl of Aβ oligomers). HT22 cells incubated for 24 h with 1 μM of Aβ oligomers in presence/absence of acyl-ghrelin (10–100 nM).	M	i.p. of acyl-ghrelin 80 μg/kg, 30 min before surgery and daily for 7 days.	Ghrelin decreased Aβ-induced microgliosis, attenuated neuronal loss, and prevented Aβ oligomer-associated synaptic degeneration in mice injected with Aβ oligomers.	Ghrelin rescued memory deficits (assessed by spontaneous alternation Y maze task and PAL).	[63]
8-week-old streptozotocin-induced diabetic rats.	ns	i.c.v. injection of acyl-ghrelin (200 ng) daily for 7 days.	Increased expression of BDNF, CREB, and p-CREB by ERK1/2 pathway in the hippocampus.	Improved cognitive ability (assessed by MWM).	[62]

Table 1. Summary of Studies Investigating Ghrelin or Ghrelin Mimetic Treatment and Hippocampal Function[a]

Model	Sex	Methods	Outcome: cellular	Outcome: behavioural	Refs
2-month-old Wistar rats	M	i.h. injection of ghrelin (1 nM) once or for 4 days.	Enhanced LTP in the DG is PI3K-dependent.	Promoted spatial memory (MWM).	[58]
8–9-week-old ghrelin KO mice	M	i.p. injections of ghrelin (80 μg/kg) daily for 8 days.	Decreased $PCNA^+$, $Ki67^+$, $BrdU^+$ cells in DG, decreased proportion of $BrdU^+/DCX^+$ and $BrdU^+/NeuN^+$ cells in ghrelin KO mice, restored to WT levels by ghrelin replacement.	Ghrelin administration reversed memory impairment in ghrelin KO mice (Y-maze, NOR).	[99]
2–3 month-old GH-deficient spontaneous dwarf rats	M	i.p. injections of rat acyl-ghrelin (80 μg/kg) daily for 8 days or acyl-ghrelin administration (50 μg/kg per day for 28 day) with s.c. osmotic minipumps for the Y-maze task and NOR test.	Increased $PCNA^+$, $BrdU^+$, and DCX^+ cells.	Acyl-ghrelin administration reversed memory impairment in GH-deficient spontaneous dwarf rats (Y-maze, NOR).	[102]
Adult rat (age 43–55 days) hippocampal NSCs		Cells treated with acyl-ghrelin (1 nM to 10 mM) for 48 hours.	Increased proliferation of NSCs by activating MEK/ERK1/2, PI3K/Akt and Jak2/STAT3 pathways.		[114]
2-month-old 5xFAD mice	F	i.p. injections of acyl-ghrelin (80 μg/kg) every 2 days for 30 days.	Restoration of impaired Dcx, HH3, and calretinin in 5xFAD to WT levels.		[66]
8–10-week-old C57BL/6NCrlVr mice	M	i.p. rat acyl-ghrelin (80 μg/kg) for 8 days or dorsal CA1 infusion (8 ng in 0.5 μl) 20 min before MWM training sessions.	i.p. (but not intra-CA1) increased $BrdU^+$ cell number and proportion of $BrdU^+/DCX^+$ and $BrdU^+/NeuN^+$ cells. Ghrelin does not affect recruitment of new neurons into spatial memory networks.	Intra-CA1 infusion of ghrelin impairs spatial memory formation (assessed by MWM).	[98]
Wistar rats	M	i.h. injections of ghrelin (3 nmol/μl) for 7 days.	Increased hippocampal glutamate release Increased NR2B-NMDA receptor subunit expression.	Increased memory consolidation (via SDT).	[61]
10-week-old Tg-APPSwDI (C57BL/6 background) mice	M	Fed control diet, high glycaemic index (HGI) diet or HGI diet + ghrelin agonist (LY444711).	No effect on Aβ plaque load or microglia activation.	HGI diet + ghrelin agonist improved cognition (tested using MWM).	[110]
3-month-old male C57BL6/J wild type mice	M	JMV2959 (2 μg/side) delivered (0.5 μl/side) bilaterally at a rate of 0.5 μl/2 min.	GHS-R1a and DRD1 forms heteromers in a complex with Gaq, DRD1-induced hippocampal memory is regulated by allosteric DRD1:GHS-R1a interactions.	Hippocampal memory increased (contextual fear conditioning and T-maze task).	[39]

Table 1. *Continued*

Model	Sex	Methods	Outcome: cellular	Outcome: behavioural	Refs
Sprague Dawley rats Aβ injected into lateral ventricle	M	Acyl-ghrelin, unacyl-ghrelin or saline (i.c.v. 0.5μl/h, 4.8 nmol/24 h) for 14 days.	Acyl-ghrelin decreased Aβ plaques deposition.	Acyl-ghrelin prevented memory impairment (passive avoidance test and MWM) in AD rats.	[65]
Lister hooded rats	M	i.p. injection of acyl-ghrelin (10 μg/ml) for 14 days.	Increased AHN ($BrdU^+$/$NeuN^+$, $BrdU^+$, Dcx^+).	Enhanced pattern separation performance via SLR.	[10]
8–10-week- old ghrelin-KO mice	M	Every-other-day feeding for 3 months.	Increased survival of new cells in WT mice, but not in ghrelin-KO mice.		[94]
10–12-week-old ghrelin KO & WT mice treated with $Aβ_{1-40}$	F	i.c.v. injections of acyl-ghrelin (0.3 mg/kg) for 7 days.	Mice showed neuroinflammation (increased number of GFAP- and Iba1-positive cells in the rostral hippocampus). Acyl-ghrelin prevented decreased LTP (induced by $Aβ_{1-40}$ treatment).	Ameliorated memory deficits (NOR and modified Y-maze tests).	[64]
Sprague-Dawley rats, GHS-$R^{-/-}$ mice	M	Hippocampal slice culture: acyl-ghrelin (100 nM).	Upregulation of phospho-GluN2B subunit of the NMDA receptor.		[60]
Adult albino Wistar rats (5 μl of Aβ1–42 into LV for 7 days).	F & M	i.c.v. injection of acyl-ghrelin (200ng) for 2 weeks.	Ameliorated Aβ-induced synaptic plasticity impairment, restoring LTP.	Enhanced memory retention and performance (PAL).	[57]
3-month-old 5xFAD mice	M	i.p. injection of MK-0677 (5 mg/kg) for 3 weeks.	Reduced accumulation of Aβ, neuroinflammation, synaptic loss, and neuronal death. Increased pCREB levels in the DG.		[109]
Sprague Dawley rats, 6-OHDA lesion	M	i.p. injections of saline, acyl-ghrelin (10 mg/kg, 50 mg/kg) or ghrelin agonist JMV-2894 (160 mg/kg) for 8 weeks.	Acyl-ghrelin increased Dcx^+ cell number in the DG.	No motor difference between the groups treated with acyl-ghrelin or JMV-2894 compared with control.	[12]

Table 1. Continued

[a]Abbreviations: EPM, elevated plus maze; GCL, granule cell layer; KO, knockout; LV, left ventricle; MWM, Morris water maze; NOR, novel object recognition; NOS, nitrous oxide; ns, not specified; NSC, neural stem cell; 6-OHDA, 6-hydroxydopamine; OF, open field; **PAL, passive avoidance learning**; s.c., subcutaneous; WT, wild type.

with *ad libitum* fed wild type mice. In this study, the caudal DG did not respond to the calorie restriction stimulus, suggesting that calorie restriction may be particularly relevant to rostral DG circuits underlying learning and memory. Notably, there was no increased AHN in GHS-R-null mice in either rostral or caudal pole, demonstrating that the beneficial effect of calorie restriction on AHN was mediated by GHS-R. In addition, the increase in new adult-born neurone number seemingly contributed to enhanced remote contextual fear conditioning in the calorie restricted wild type mice [11]. However, their precise contribution needs to be further examined under conditions that place greater emphasis on pattern-separation performance. Nonetheless, these findings confirm the essential role of GHS-R in mediating the beneficial effects of calorie restriction on enhancing AHN. In support of this, a 3 month every-other-day feeding protocol increased the survival of new adult-born cells in

Box 1. Controversy over Human AHN?

Several studies have confirmed the generation and important function of new adult-born mature neurones in the adult rodent hippocampus to spatial memory performance [71,72]. However, technical limitations have resulted in some contradictory studies investigating the existence of AHN in humans (i.e., prohibited use of the carcinogen, BrdU) (for review see Kempermann [79]). For example, a recent study failed to detect AHN in aged human brain [80]. The field has been hampered by the limited collection and inconsistent preservation of postmortem human brain tissue. However, an increasing number of studies demonstrate that postmortem human tissue, collected with short postmortem interval and appropriate fixation procedures, reveal the existence of new adult-born neurones within the DG. Recently, Boldrini *et al.* [81] showed that AHN occurred in humans in the eighth decade of life, despite a decline in the quiescent stem cell pool, angiogenesis, and neuroplasticity. Moreover, Moreno-Jiménez *et al.* [82] described an improved protocol for the identification of new adult-born neurones in the human hippocampus and confirmed their presence in aged human hippocampus, which progressively decreased in AD patients. These studies demonstrate that the AHN paradigm exists in humans and that it is modulated by environmental factors (i.e., age, disease), as observed in rodent studies. These findings suggest that AHN may be modulated to prevent cognitive decline in humans.

the DG in wild type but not ghrelin-knockout mice [94], suggesting that ghrelin signalling is important for the survival of new hippocampal cells. Collectively, these studies highlight that acyl-ghrelin is a critical metabolic hormone linking peripheral nutritional state to hippocampal neurogenesis and cognitive performance. We speculate that acyl-ghrelin agonists represent putative **calorie restriction mimetics** (Box 2).

Acyl-Ghrelin Treatment Increases AHN

As ghrelin is seemingly unique in its ability to communicate peripheral energy status to the brain via the activation of Neuropeptide Y (NPY) and Agouti-related protein (AGRP)-dependent pathways, it is not unreasonable to suggest it plays an important role in mediating the beneficial effects of calorie restriction. Here, we discuss evidence for exogenous acyl-ghrelin regulating AHN. Johansson *et al.* initially described the proliferative effect of ghrelin on adult rat hippocampal progenitor cells [95]. Subsequently, experiments in mice showed that daily intraperitoneal injection (i.p.) of acyl-ghrelin at supra-physiological doses (80 μg/kg) for 8 days increased the number of newly generated cells, labelled by BrdU, in the hippocampus [96]. We later examined the longer-term effects of elevated acyl-ghrelin within the physiological range, using doses that mimic 24-hour fasting (10 μg/kg/day i.p. for 14 days) [10]. The total number of both immature neurones (Dcx^+) and new adult-born neurones ($BrdU^+/NeuN^+$) were significantly increased in the DG after acyl-ghrelin treatment. The increase in AHN resulted in enhanced performance in the **spontaneous location recognition (SLR) task**, a neurogenesis-dependent pattern separation-dependent test [97]. Notably, the behavioural test took place 1 week after the final injection of acyl-ghrelin, suggesting that it had a long-term effect to support memory. These findings are consistent with other studies reporting ghrelin-mediated increases in AHN [12,98,99].

To further examine the mechanisms of ghrelin on AHN, we increased acyl-ghrelin levels in adult **GHS-R–eGFP mice**, either indirectly via an overnight fast, directly via i.p. injection (1 mg/kg), or a combination of both overnight fast and acyl-ghrelin injection. Here, all three treatment paradigms induced the expression of the immediate early gene, early growth response-1 (Egr-1) in DG neurones [11]. Egr-1 expression in mature DG neurones is an important gene involved in the selection, maturation, and functional integration of new-born neurones into the DG network [100]. However, hippocampal cell proliferation was unchanged and is consistent with previous studies investigating the impact of calorie restriction on hippocampal cell proliferation [78,94].

It is likely that ghrelin supports AHN via several distinct pathways, including via stimulation of GH [1] and consequent insulin growth factor-1, both of which can enter the brain to influence cognition and neurogenesis [101]. However, in spontaneous dwarf rats, which lack circulating GH, ghrelin's ability to enhance AHN and cognitive performance remained intact, suggesting that it can promote AHN

Box 2. Calorie Restriction Mimetics (CRMs): A Promising Option to Treat Disease?

A primary obstacle to benefiting from the myriad effects of calorie restriction is the need to adhere to diets that limit the intake of food. Furthermore, individuals diagnosed with chronic age-related neurodegenerative conditions, such as Alzheimer's and Parkinson's disease, often report weight loss and reduced appetite, suggesting impaired energy balance pathways that may not fully benefit from calorie restriction. Considerable effort is on-going to develop CRMs, defined as drugs or compounds that mimic the beneficial effects of calorie restriction [107]. Indeed, a similar approach is ongoing to identify exercise-mimetics [106,108]. We suggest acyl-ghrelin mimetics as putative CRMs to enhance cognition. Recent findings identify MK-0677, a synthetic GHS-R agonist that crosses the BBB, as a potential therapeutic cognitive enhancer as it promoted the accumulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor on excitatory hippocampal synapses and improved long-term potentiation (LTP) [59]. Moreover, Jeong *et al.* [109] showed that MK-0677 ameliorated Aβ deposition, neuroinflammation, and neurodegeneration in the 5xFAD mouse model of AD. In the APPSwDI mouse model of AD the GHS-R agonist, LY444711, improved spatial memory performance [110]. Other GHS-R agonists also improved memory and cognition (in particular, GSK894490A and CP-464709-18), crossed the BBB, and promoted object recognition and spatial memory [111]. Notably, the synthetic peptide GHS-R agonist, hexarelin, promoted the survival of new adult-born hippocampal cells [112], suggesting that it may promote AHN. These results provide compelling evidence that synthetic GHS-R agonists may form a novel class of CRMs. Further studies are required to determine the effect of these putative CRMs on AHN and cognition, as well as possible negative effects on metabolism, in models of ageing and disease.

independently from the somatotropic axis [102]. Additionally, ghrelin may modulate the neurogenic niche via extra-hippocampal neural circuitry. For example, GHS-R1a in the entorhinal cortex is activated by acyl-ghrelin and calorie restriction [11] and the entorhinal cortex promotes AHN [103]. Furthermore, vagal afferents, which express GHS-R1a that regulate ghrelin-mediated feeding [104], also support hippocampal BDNF expression and neurogenesis [105]. Other mechanisms, such as the phenomenon of ligand-independent GHS-R signalling [39], have not been studied in the context of neurogenesis. Collectively, current data suggests that physiological levels of acyl-ghrelin increase AHN by promoting neurone differentiation, maturation, and survival in the neurogenic niche of the DG. Further studies are necessary to examine the DG-specific, GHS-R1a-dependent, and ghrelin-independent GHS-R1a effects on AHN.

Acyl-Ghrelin Enhances AHN in Neurodegenerative Disease Models

AD is an age-related neurodegenerative disorder characterised by progressive neurone loss and deterioration of cognitive function. Hippocampal neurogenesis is impaired in AD and is thought to contribute to cognitive decline. The 5xFAD transgenic mouse model of AD exhibits impaired AHN and cognition coupled with increased hippocampal Aβ deposits. Choi *et al.* [106] used this model to demonstrate that aerobic exercise reduced Aβ levels, promoted AHN, and improved learning and memory performance. Mimicking the beneficial effects of exercise using genetic and pharmacological tools also promoted AHN and cognition, albeit without reducing Aβ levels. Notably, they show that suppressing AHN increased hippocampal neurone loss in 5xFAD but not wild type mice. These data suggest that AHN plays a fundamental role in the memory deficit and neurodegeneration observed in this model of AD. Notably, acyl-ghrelin treatment (i.p.) of **5xFAD mice**, at a dose of 80 μg/kg every 2 days for 30 days, increased the number of cells expressing markers of immature neurones (Dcx and calretinin) without affecting Aβ levels [66]. These data suggest that elevating peripheral acyl-ghrelin is sufficient to enhance neurogenesis in this model of AD. In addition, as neurogenic deficits are associated with several neurodegenerative diseases such as Parkinson's disease and dementia with Lewy bodies, it has been suggested that increasing AHN in these conditions may ameliorate the progression in cognitive decline or even restore cognition. Further studies are warranted to determine whether acyl-ghrelin can promote the generation of new mature adult-born neurones to rescue learning and memory deficits in models of neurodegeneration.

Characterisation of GHS-R expression in the brain using a GHS-R–eGFP reporter mouse [34], identified GHS-R–eGFP$^+$/NeuN$^+$ immunoreactive cells in the mature DG neurone granule cell

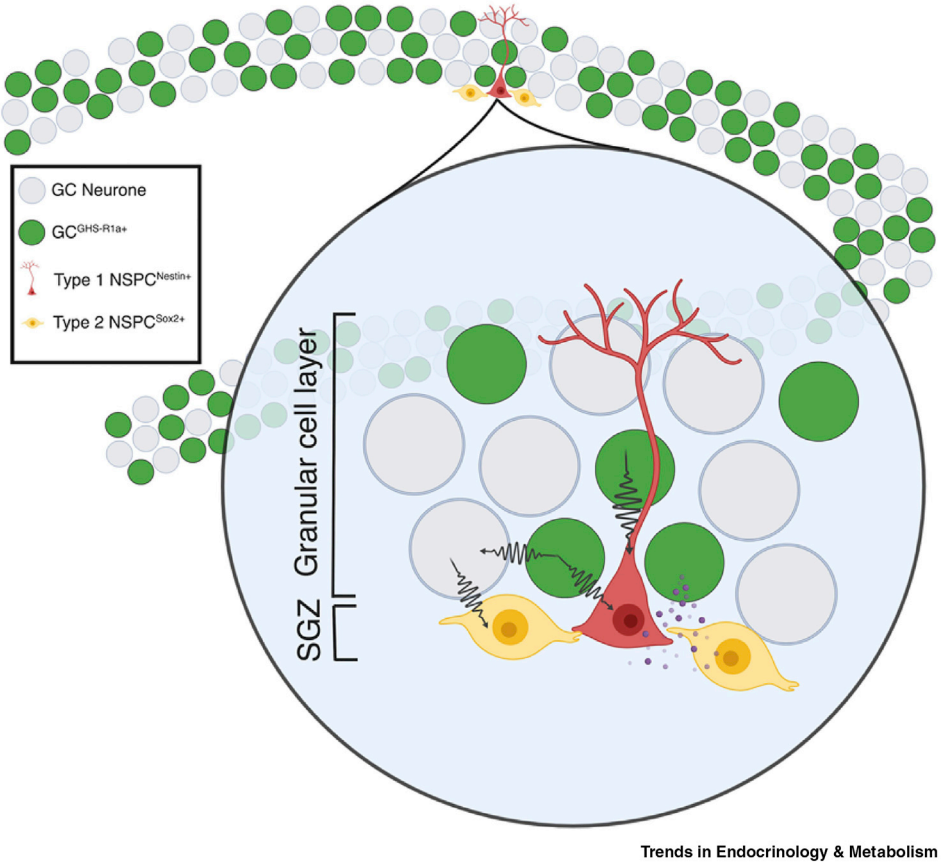

Figure 4. Ghrelin-Mediated Mechanism(s) Regulating Adult Hippocampal Neurogenesis (AHN).
The ghrelin receptor, [growth hormone secretagogue receptor (GHS-R)] (green), is highly expressed throughout the hippocampal dentate gyrus (DG) granular cell layer (GC). GHS-R is expressed in mature GC neurones but not in neural stem progenitor cells (NSPCs) that reside within the subgranular zone (SGZ) region of the DG. Non-cell autonomous pathways, including the release of soluble pro-neurogenic factors (illustrated by vesicles) and activity-induced stimulation of NSPCs (illustrated by arrows) are highlighted as putative mechanisms of acyl-ghrelin-mediated AHN.

population [11]. These were in apposition to both type I (nestin$^+$) and type II (Sox2$^+$) **neural stem progenitor cells (NSPCs)**. GHS-R–eGFP immunoreactivity was not detected in NSPCs. Furthermore, GHS-R–eGFP was not observed in proliferating (Ki67$^+$) cells within the SGZ. These findings suggest a non-cell-autonomous cellular mechanism for acyl-ghrelin-mediated AHN, possibly via diffusible neurotrophic factors such as BDNF, which is known to promote neurogenesis [113] and enhance pattern separation memory [97]. It is important to note that Chung *et al.* [114] previously reported GHS-R1a expression in these nestin$^+$ stem cells *in vitro*, using both Western blot and immunocytochemistry and acyl-ghrelin enhanced proliferation via ERK1/2 and Akt pathways. However, these *in vitro* studies were conducted without anti-GHS-R1a antibody validation on GHS-R-knockout cells or tissues, an important control considering that generating antibodies to specific GPCRs is notoriously difficult. We recently confirmed that a GHS-R1a antibody lacked specificity using brain tissue from GHS-R-null mice [115]. Moreover, cell type-specific RNA-seq data from postnatal mouse brain, including the DG, illustrates the absence of GHS-R expression within NSPCs [116], supporting our findings obtained in GHS-R–eGFP mice [11]. Together, these studies suggest that acyl-ghrelin is acting via a non-cell-autonomous mechanism to support AHN (Figure 4). (See Figure 5.)

Key Figure

Acyl-Ghrelin Stimulates Adult Hippocampal Neurogenesis

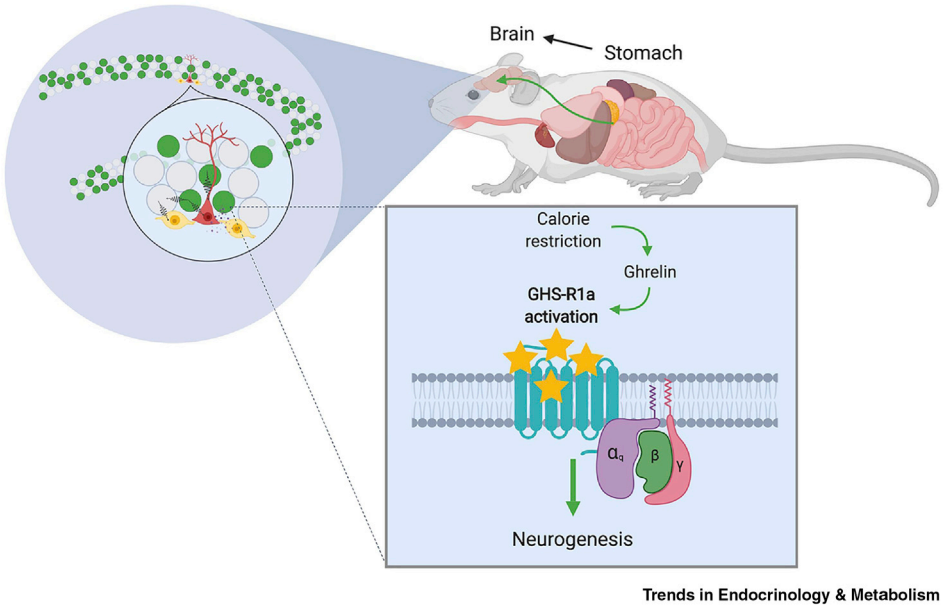

Figure 5. The hormone acyl-ghrelin is released from the stomach during calorie restriction and crosses the blood–brain barrier to regulate the hippocampal neurogenic niche. Activation of the acyl-ghrelin receptor, growth hormone secretagogue receptor (GHS-R), in the hippocampal dentate gyrus enhances adult neurogenesis and learning. This signalling pathway may offer a novel therapeutic approach to ameliorate age-related cognitive decline and dementia.

Outstanding Questions

How does ageing effect acyl-ghrelin-mediated-AHN and can acyl-ghrelin restore AHN in aged mammals?

Which cellular and molecular mechanism(s) underpin acyl-ghrelin's pro-neurogenic effects?

Are new adult-born hippocampal neurones, generated in response to acyl-ghrelin, particularly important to the formation and maintenance of memories relating to feeding behaviour?

Does acyl-ghrelin-induced AHN mediate the anti-anxiety effect of calorie restriction?

Obesity has a negative effect on cognitive performance; is this mediated by a reduction in acyl-ghrelin and subsequent drop in neurogenic drive?

Does UAG modulate AHN and memory via an unidentified receptor?

Concluding Remarks and Future Perspectives

Together, these findings indicate that the calorie restriction-mediated increase in plasma acyl-ghrelin, with subsequent enhancements in AHN, may confer important survival advantages to an organism. For example, this gut-derived signal for hunger increases the number of new adult-born hippocampal neurones, thereby improving discrimination of similar but distinct environments. This function would enhance the ability to remember precise locations of palatable food, particularly during periods of shortage. Similarly, the enhanced ability to distinguish safe versus dangerous environments, as a result of increased neurogenesis and enhanced pattern-separation, would improve the chances of successful refeeding and survival. In humans, acyl-ghrelin enhances hippocampal signalling relevant to feeding [117,118] and with recent studies validating the existence of hippocampal neurogenesis that is impaired with age and disease [81,82] (mimicking observations in experimental animal models) it will be important to assess the role of acyl-ghrelin on pattern-separation processes in humans (see Outstanding Questions).

Together, the findings extend our understanding of how adult brain plasticity is regulated by endocrine factors of nutritional state and suggest that the ageing brain is acutely sensitive to circulating factors such as ghrelin. Next, delineating the underlying mechanisms of ghrelin-mediated AHN is necessary to develop new potential treatments for age-related cognitive decline and diseases linked with impaired AHN.

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