Ghrelin mediated hippocampal neurogenesis – implications for health and disease

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Trends

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.

Glossary

Acyl-ghrelin (AG): 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 mechanism: 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 enzyme that hydrolyses many different choline-based esters, but can also de-acylate acyl-ghrelin, (AG) to form unacylated ghrelin (UAG). Endogenous esterase enzyme present in the plasma known to de-acylate acyl-ghrelin.

Caloric restriction (CR): 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 CR

cAMP response element binding protein (CREB): A transcription factor regulating BDNF expression and important in neuronal plasticity and memory.

Dentate gyrus (DG): A sub-region 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 α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR) receptors: Glutamate receptor and ion channels responsible for fast excitatory synaptic transmission. Important for synaptic plasticity and long-term potentiation **(**LTP).

Growth hormone secretagogue receptor (GHSR): A G-protein-coupled receptor (GPCR) also known as the ghrelin receptor. It is the only known receptor for acyl-ghrelin.

GHSR-eGFP mice: genetically mutated mice that co-express green fluorescent protein (GFP) with the Growth Hormone Secretagogue Receptor (GHS-R) gene

Heterodimers: A protein complex, consisting of two different proteins.

Hormesis: A bi-phasic dose-response to a drug or intervention, in which a low dose produces a beneficial effect and a high dose produces a harmful effect.

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 evaluate 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.

Un-acylated ghrelin (UAG): The un-acylated form of ghrelin (also known as des-acyl ghrelin (DAG)). Initially thought to be the inactive as it does not activate the ghrelin receptor. Subsequent research suggests it has physiological effects independent of GHSR.

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).

Abstract

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 CNS function. Here, we review current developments in our understanding of ghrelin's role in regulating neurogenesis and memory function.

Ghrelin

The gut-derived hormone, ghrelin, was identified in 1999 as the natural ligand of the growth hormone secretagogue receptor 1a (GHS-R1a) 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 pre-proghrelin peptide that is enzymatically processed to a mature 28 amino acid peptide[23]. The native peptide, unacylated-ghrelin (UAG or des-acyl ghrelin), undergoes enzymatic modification in the endoplasmic reticulum by ghrelin-O-acyltransferase (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 characterized, 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 knock-out mice[30], suggesting the existence of an unknown UAG receptor.

Figure 1. Summary of ghrelin production from the stomach.

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 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 GHSR-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 Gαq to Gαi/o, suggesting that ghrelin is able to selectively enhance dopamine signalling in cells co-

expressing these proteins. However, dimerization 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 dimerization 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].

Figure 2. Putative GHS-R1a signaling pathways.

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 & Zigman[48]). For example, $GOAT^{-/-}$ mice, which lack acyl-ghrelin, show a marked increase in hypoglycaemia induced mortality when exposed to a prolonged 48h 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 that 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 processing autobiographical 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 intra-hippocampal (i.h)[54] promoted memory 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 intra-hippocampal 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 two 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 intra-hippocampal 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. (2015) 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 intrahippocampal 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.

Table 1. Summary of studies on ghrelin and hippocampal function

Adult hippocampal neurogenesis (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 et al. 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 which 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 SGZ of the human DG[68,69] (see **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 episodic memory processing, whilst AHN in the caudal DG is linked with processing anxiety and stress-related memory[73].

Figure 3. Stages of adult hippocampal neurogenesis (AHN).

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 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.

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 (for review see Kempermann 2018[79]). (i.e prohibited use of the carcinogen, BrdU). 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 post-mortem human brain tissue. However, an increasing number of studies demonstrate that post-mortem human tissue, collected with short post-mortem 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.

Benefits of CR on brain function and adult hippocampal neurogenesis

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]. 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), a 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 two-weeks. Quantification of AHN revealed that two-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 to *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 the DG in wild-type but not ghrelin-ko 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 (see **Box 2**)

Acyl-ghrelin treatment increases adult hippocampal neurogenesis

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 (IP) 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 IP 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 one 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 GHSR-eGFP mice, either indirectly via an overnight fast, directly via IP injection (1mg/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 growth hormone (GH)[101] and consequent insulin growth factor-1, both of which can enter the brain to influence cognition and neurogenesis[102]. 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 independently from the somatotropic axis[103]. 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 promotes AHN[104]. Furthermore, vagal afferents, which express GHS-R1a that regulate ghrelin-mediated feeding[105], also support hippocampal BDNF expression and neurogenesis[106]. 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.

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized 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 exhibit impaired AHN and cognition coupled with increased hippocampal Aβ deposits. Choi et al.[107] 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 two 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.

Box 2. Calorie restriction **mimetics – 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 calorie restriction mimetics (CRMs), defined as drugs or compounds that mimic the beneficial effects of calorie restriction[108]. Indeed, a similar approach is ongoing to identify exercise-mimetics[107,109]. 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.[110] 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[111]. 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[112]. Notably, the synthetic peptide GHS-R agonist, hexarelin, promoted the survival of new adult born hippocampal cells[113], 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.

Characterisation of GHS-R expression in the brain using a GHSR-eGFP reporter mouse[34], identified GHSR-eGFP$^+$/NeuN$^+$ immunoreactive cells in the mature DG neurone granule cell population[11]. These were in apposition to both type I (nestin$^+$) and type II (Sox2$^+$) NSPCs. GHSR-eGFP immunoreactivity was not detected in NSPCs. Furthermore, GHSR-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[114] and enhance pattern separation memory[97]. It is important to note that Chung et al.[115] 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-ko 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[116]. Moreover, cell type specific RNA-seq data from post-natal mouse brain, including the DG, illustrates the absence of GHS-R expression within NSPCs[117], supporting our findings obtained in GHSR-eGFP mice[11]. Together, these studies suggest that acyl-ghrelin is acting via a non cell-autonomous mechanism to support AHN (**figure 4**).

Figure 4. Ghrelin-mediated mechanism(s) regulating adult hippocampal neurogenesis (AHN).

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 *vs* dangerous environments, as a result of increased neurogenesis and enhanced pattern-separation, would improve the chances of successful re-feeding and survival. In humans, acyl-ghrelin enhances hippocampal signalling relevant to feeding[118,119] 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.

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.

Outstanding questions

1. How does ageing effect acyl-ghrelin mediated-AHN and can acyl-ghrelin restore AHN in aged mammals?
2. Which cellular and molecular mechanism(s) underpin acyl-ghrelin's pro-neurogenic effects?
3. 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?
4. Does acyl-ghrelin induced AHN mediate the anti-anxiety effect of calorie restriction?

5. Obesity has a negative effect on cognitive performance, is this mediated by a reduction in acyl-ghrelin and subsequent drop in neurogenic drive?
6. Does UAG modulate AHN and memory via an unidentified receptor?

References

1. Kojima, M. *et al.* Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* **402**, 656–660 (1999).
2. Tschöp, M., Smiley, D. L. & Heiman, M. L. Ghrelin induces adiposity in rodents. *Nature* **407**, 908–13 (2000).
3. Masuda, Y. *et al.* Ghrelin Stimulates Gastric Acid Secretion and Motility in Rats. **908**, 905–908 (2000).
4. Granata, R. *et al.* Acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of pancreatic beta-cells and human islets: involvement of 3',5'-cyclic adenosine monophosphate/protein kinase A, extracellular signal-regulated kinase 1/2, and phosphatidyl inosit. *Endocrinology* **148**, 512–29 (2007).
5. Szentirmai, E., Sun, Y., Smith, R. G., Kapa, L. & Krueger, J. M. The preproghrelin gene is required for the normal integration of thermoregulation and sleep in mice. *Proc. Natl. Acad. Sci.* **106**, 14069–14074 (2009).
6. Nagaya, N. & Kangawa, K. Ghrelin improves left ventricular dysfunction and cardiac cachexia in heart failure. *Curr. Opin. Pharmacol.* **3**, 146–151 (2003).
7. Andrews, Z. B. *et al.* Ghrelin Promotes and Protects Nigrostriatal Dopamine Function via a UCP2-Dependent Mitochondrial Mechanism. *J. Neurosci.* **29**, 14057–14065 (2009).
8. Bayliss, J. A. *et al.* Ghrelin-AMPK Signaling Mediates the Neuroprotective Effects of Calorie Restriction in Parkinson's Disease. (2016). doi:10.1523/JNEUROSCI.4373-15.2016
9. Lutter, M. *et al.* The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat. Neurosci.* **11**, 10–11 (2008).
10. Kent, B. A. *et al.* The orexigenic hormone acyl-ghrelin increases adult hippocampal neurogenesis and enhances pattern separation. *Psychoneuroendocrinology* (2015). doi:10.1016/j.psyneuen.2014.10.015
11. Hornsby, A. K. E. *et al.* Short-term calorie restriction enhances adult hippocampal

neurogenesis and remote fear memory in a Ghsr-dependent manner. *Psychoneuroendocrinology* **63**, 198–207 (2016).

12. Elabi, O. F., Duskova, K., Davies, J. S. & Lane, E. L. The Impact of Ghrelin on the Survival and Efficacy of Dopaminergic Fetal Grafts in the 6-OHDA-Lesioned Rat. *Neuroscience* (2018). doi:10.1016/j.neuroscience.2018.10.045
13. Date, Y. *et al.* Ghrelin , a Novel Growth Hormone-Releasing Acylated Peptide , Is Synthesized in a Distinct Endocrine Cell Type in the Gastrointestinal Tracts of Rats and Humans *. **141**, (2000).
14. Kiyoshi, M. *et al.* Kidney produces a novel acylated peptide , ghrelin. *FEBS Lett.* **486**, 213–216 (2000).
15. Gualillo, O. *et al.* Ghrelin, a novel placental-derived hormone. *Endocrinology* **142**, 788–94 (2001).
16. Mondal, M. S., Date, Y., Yamaguchi, H., Toshinai, K. & Tsuruta, T. Identification of ghrelin and its receptor in neurons of the rat arcuate nucleus. **126**, 55–59 (2005).
17. Sakata, I. *et al.* Regulatory Peptides Characterization of a novel ghrelin cell reporter mouse. *Regul. Pept.* **155**, 91–98 (2009).
18. Furness, J. B. *et al.* Investigation of the presence of ghrelin in the central nervous system of the rat and mouse. *Neuroscience* **193**, 1–9 (2011).
19. Schaeffer, M. *et al.* Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc. Natl. Acad. Sci.* **110**, 1512–1517 (2013).
20. Banks, W. A., Burney, B. O. & Robinson, S. M. Effects of triglycerides, obesity, and starvation on ghrelin transport across the blood-brain barrier. *Peptides* **29**, 2061–2065 (2008).
21. Banks, W. A. Extent and Direction of Ghrelin Transport Across the Blood-Brain Barrier Is Determined by Its Unique Primary Structure. *J. Pharmacol. Exp. Ther.* **302**, 822–827 (2002).
22. Diano, S. *et al.* Ghrelin controls hippocampal spine synapse density and memory performance. *Nat. Neurosci.* **9**, 381–8 (2006).

23. Kojima, M. & Kangawa, K. Ghrelin : Structure and Function. *Physiol. Rev.* **85**, 495–522 (2005).

24. Gutierrez, J. a *et al.* Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 6320–6325 (2008).

25. Yang, J., Brown, M. S., Liang, G., Grishin, N. V & Goldstein, J. L. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. *Cell* **132**, 387–96 (2008).

26. Murtuza, M. I. & Isokawa, M. Endogenous ghrelin-O-acyltransferase (GOAT) acylates local ghrelin in the hippocampus. *J. Neurochem.* **144**, 58–67 (2018).

27. Satou, M., Nishi, Y., Yoh, J., Hattori, Y. & Sugimoto, H. Identification and characterization of acyl-protein thioesterase 1/lysophospholipase I as a ghrelin deacylation/lysophospholipid hydrolyzing enzyme in fetal bovine serum and conditioned medium. *Endocrinology* **151**, 4765–4775 (2010).

28. Schopfer, L. M., Lockridge, O. & Brimijoin, S. Pure human butyrylcholinesterase hydrolyzes octanoyl ghrelin to desacyl ghrelin q. *Gen. Comp. Endocrinol.* **224**, 61–68 (2015).

29. Darvesh, S., Grantham, D. L. & Hopkins, D. A. Distribution of Butyrylcholine ste rase in the Human Amygdala and Hippocampal Formation. **390**, 374–390 (1998).

30. Delhanty, P. J. D. *et al.* Unacylated ghrelin rapidly modulates lipogenic and insulin signaling pathway gene expression in metabolically active tissues of GHSR deleted mice. *PLoS One* **5**, (2010).

31. Howard, A. D. *et al.* A Receptor in Pituitary and Hypothalamus That Functions in Growth Hormone Release. *Sciencecience* **273**, 974–977 (1996).

32. Leung, P. K. *et al.* The truncated ghrelin receptor polypeptide (GHS-R1b) acts as a dominant-negative mutant of the ghrelin receptor. *Cell. Signal.* **19**, 1011–1022 (2007).

33. Guan, X. *et al.* Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Mol. Brain Res.* **48**, 23–29 (1997).

34. Mani, B. K. *et al.* Neuroanatomical characterization of a growth hormone

secretagogue receptor-green fluorescent protein reporter mouse. *J. Comp. Neurol.* **3666**, 3644–3666 (2014).

35. Mani, B. K. *et al.* The role of ghrelin-responsive mediobasal hypothalamic neurons in mediating feeding responses to fasting. *Mol. Metab.* **6**, 882–896 (2017).
36. Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B. & Elmquist, J. K. Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J. Comp. Neurol.* **494**, 528–548 (2006).
37. Müller, T. D. *et al.* Ghrelin. *Mol. Metab.* **4**, 437–460 (2015).
38. Jiang, H., Betancourt, L. & Smith, R. G. Ghrelin Amplifies Dopamine Signaling by Cross Talk Involving Formation of Growth Hormone Secretagogue Receptor / Dopamine Receptor Subtype 1 Heterodimers. *Mol. Endocrinol.* **20**, 1772–1785 (2006).
39. Kern, A. *et al.* Hippocampal Dopamine/DRD1 Signaling Dependent on the Ghrelin Receptor. *Cell* **163**, 1176–1190 (2015).
40. Schellekens, H., Van Oeffelen, W. E. P. A., Dinan, T. G. & Cryan, J. F. Promiscuous dimerization of the growth hormone secretagogue receptor (GHS-R1a) attenuates ghrelin-mediated signaling. *J. Biol. Chem.* **288**, 181–191 (2013).
41. Schellekens, H. *et al.* Ghreli??s Orexigenic Effect Is Modulated via a Serotonin 2C Receptor Interaction. *ACS Chem. Neurosci.* **6**, 1186–1197 (2015).
42. Wallace Fitzsimons, S. E. *et al.* A ghrelin receptor and oxytocin receptor heterocomplex impairs oxytocin mediated signalling. *Neuropharmacology* 1–12 (2018). doi:10.1016/j.neuropharm.2018.12.022
43. Chow, K. B. S. *et al.* The truncated ghrelin receptor polypeptide (GHS-R1b) is localized in the endoplasmic reticulum where it forms heterodimers with ghrelin receptors (GHS-R1a) to attenuate their cell surface expression. *Mol. Cell. Endocrinol.* **348**, 247–254 (2012).
44. Holst, B. *et al.* High Constitutive Signaling of the Ghrelin Receptor — Identification of a Potent Inverse Agonist. **17**, 2201–2210 (2003).
45. Holst, B. *et al.* Common structural basis for constitutive activity of the ghrelin receptor

family. *J. Biol. Chem.* **279**, 53806–53817 (2004).

46. Damian, M. *et al.* High Constitutive Activity Is an Intrinsic Feature of Ghrelin Receptor Protein A STUDY WITH A FUNCTIONAL MONOMERIC GHS-R1a RECEPTOR RECONSTITUTED IN. **287**, 3630–3641 (2012).

47. Yanagi, S., Sato, T., Kangawa, K. & Nakazato, M. Review The Homeostatic Force of Ghrelin. *Cell Metab.* **27**, 786–804 (2018).

48. Mani, B. K. & Zigman, J. M. Ghrelin as a Survival Hormone. *Trends Endocrinol. Metab.* **28**, 843–854 (2017).

49. Zhao, T.-J. *et al.* Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 7467–72 (2010).

50. Walker, A. K. *et al.* The P7C3 class of neuroprotective compounds exerts antidepressant efficacy in mice by increasing hippocampal neurogenesis. *Mol. Psychiatry* 1–9 (2014). doi:10.1038/mp.2014.34

51. Scoviille, W. B. & Milner, B. Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiat* **20**, 11–21 (1957).

52. Burgess, N., Maguire, E. A. & Keefe, J. O. The Human Hippocampus and Spatial and Episodic Memory. **35**, 625–641 (2002).

53. Carlini, V. P. *et al.* Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem. Biophys. Res. Commun.* **299**, 739–43 (2002).

54. Carlini, V. P. *et al.* Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochem. Biophys. Res. Commun.* **313**, 635–641 (2004).

55. Carlini, V. P. *et al.* Ghrelin induced memory facilitation implicates nitric oxide synthase activation and decrease in the threshold to promote LTP in hippocampal dentate gyrus. *Physiol. Behav.* **101**, 117–23 (2010).

56. Carlini, V. P., Ghersi, M., Schiöth, H. B. & de Barioglio, S. R. Ghrelin and memory: differential effects on acquisition and retrieval. *Peptides* **31**, 1190–3 (2010).

57. Eslami, M., Sadeghi, B. & Goshadrou, F. Chronic ghrelin administration restores hippocampal long-term potentiation and ameliorates memory impairment in rat model of Alzheimer ' s disease. 1–11 (2018). doi:10.1002/hipo.23002

58. Chen, L. *et al.* Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. **33**, 266–275 (2011).

59. Ribeiro, L. F. *et al.* Ghrelin triggers the synaptic incorporation of AMPA receptors in the hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E149-58 (2014).

60. Berrout, L. & Isokawa, M. Ghrelin upregulates the phosphorylation of the GluN2B subunit of the NMDA receptor by activating GHSR1a and Fyn in the rat hippocampus. *Brain Res.* **1678**, 20–26 (2018).

61. Ghersi, M. S. *et al.* Ghrelin increases memory consolidation through hippocampal mechanisms dependent on glutamate release and NR2B-subunits of the NMDA receptor. *Psychopharmacology (Berl).* **232**, 1843–1857 (2015).

62. Ma, L.-Y. *et al.* Ghrelin-Attenuated Cognitive Dysfunction in Streptozotocin-induced Diabetic Rats. *Alzheimer Dis. Assoc. Disord.* **25**, 352–363 (2011).

63. Moon, M. *et al.* Ghrelin ameliorates cognitive dysfunction and neurodegeneration in intrahippocampal amyloid-β1-42 oligomer-injected mice. *J. Alzheimer's Dis.* **23**, 147–159 (2011).

64. Santos, V. V *et al.* Acyl ghrelin improves cognition, synaptic plasticity deficits and neuroinflammation following amyloid beta (Aβ1-40) administration in mice. *J. Neuroendocrinol.* 1–11 (2017). doi:10.1111/jne.12476

65. Kang, S., Moon, N. R., Kim, D. S., Kim, S. H. & Park, S. Central acylated ghrelin improves memory function and hippocampal AMPK activation and partly reverses the impairment of energy and glucose metabolism in rats infused with ??-amyloid. *Peptides* **71**, 84–93 (2015).

66. Moon, M., Cha, M. Y. & Mook-Jung, I. Impaired hippocampal neurogenesis and its enhancement with ghrelin in 5XFADl. *J. Alzheimer's Dis.* **41**, 233–241 (2014).

67. Altman, J. Are New Neurons Formed in the Brains of Adult Mammals ? *Science (80-.).* 1127–1128 (1962).

68. Eriksson, P. S. *et al.* Neurogenesis in the adult human hippocampus. *Nat. Med.* **4**, 1313–7 (1998).

69. Spalding, K. L. *et al.* Dynamics of hippocampal neurogenesis in adult humans. *Cell* **153**, 1219–1227 (2013).

70. Ge, S., Yang, C. hao, Hsu, K. sen, Ming, G. li & Song, H. A Critical Period for Enhanced Synaptic Plasticity in Newly Generated Neurons of the Adult Brain. *Neuron* **54**, 559–566 (2007).

71. Clelland, C. D. *et al.* A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* **325**, 210–3 (2009).

72. Sahay, A. *et al.* Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **472**, 466–70 (2011).

73. Kheirbek, M. Dorsal vs Ventral Hippocampal Neurogenesis: Implications for Cognition and Mood. *Neuropsychopharmacology* **36**, 372–3 (2011).

74. van Praag, H., Kempermann, G. & Gage, F. H. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat. Neurosci.* **2**, 266–270 (1999).

75. Kempermann, G., Kuhn, H. G. & Gage, F. H. More hippocampal neurons in adult mice living in an enriched environment. *Nature* **386**, 493–495 (1997).

76. Gould, E., Tanapat, P., McEwen, B. S., Flugge, G. & Fuchs, E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 3168–3171 (1998).

77. Kuhn, H. G., Dickinson-Anson, H. & Gage, F. H. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J. Neurosci.* **16**, 2027–2033 (1996).

78. Lee, J., Duan, W., Long, J. M., Ingram, D. K. & Mattson, M. P. Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression,

in the dentate gyrus of rats. *J. Mol. Neurosci.* **15**, 99–108 (2000).

79. Kempermann, G. *et al.* Human Adult Neurogenesis: Evidence and Remaining Questions. *Cell Stem Cell* **23**, 25–30 (2018).

80. Sorrells, S. F. *et al.* Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature* (2018). doi:10.1038/nature25975

81. Boldrini, M. *et al.* Human Hippocampal Neurogenesis Persists throughout Aging. *Cell Stem Cell* 589–599 (2018). doi:10.1016/j.stem.2018.03.015

82. Moreno-jiménez, E. P. *et al.* Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer ' s disease. *Nat. Med.* **25**, 554–560 (2019).

83. Mattison, J. A. *et al.* Calorie restriction improves health and survival of rhesus monkeys. *Nat. Commun.* **8**, 1–12 (2017).

84. Francesco, A. Di, Germanio, C. Di, Bernier, M. & Cabo, R. De. A time to fast. **775**, 770–775 (2018).

85. Halagappa, V. K. M. *et al.* Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* **26**, 212–20 (2007).

86. Maswood, N. *et al.* Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson ' s disease. *Proc. Natl. Acad. Sci.* 6–11 (2004).

87. Gräff, J. *et al.* A dietary regimen of caloric restriction or pharmacological activation of SIRT1 to delay the onset of neurodegeneration. *J. Neurosci.* **33**, 8951–60 (2013).

88. Colman, R. J. *et al.* Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science (80-.).* **373**, 201–204 (2009).

89. Witte, A. V, Fobker, M., Gellner, R., Knecht, S. & Floel, A. Caloric restriction improves memory in elderly humans. *Proc. Natl. Acad. Sci.* **106**, 1255–1260 (2009).

90. Lee, J., Seroogy, K. B. & Mattson, M. P. Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. *J. Neurochem.* **80**,

539–547 (2002).

91. Czuba, E., Was, M., Steliga, A. & Morys, J. BDNF : A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. 579–593 (2018). doi:10.1007/s10571-017-0510-4

92. Fusco, S. *et al.* A role for neuronal cAMP responsive-element binding (CREB)-1 in brain responses to calorie restriction. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 621–6 (2012).

93. Brandhorst, S. *et al.* A Periodic Diet that Mimics Fasting Promotes Multi-System Regeneration, Enhanced Cognitive Performance, and Healthspan. *Cell Metab.* **22**, 86–99 (2015).

94. Kim, Y. *et al.* Ghrelin is required for dietary restriction-induced enhancement of hippocampal neurogenesis : lessons from ghrelin knockout mice. *Endocr. J.* **62**, 269–275 (2015).

95. Johansson, I. *et al.* Proliferative and Protective Effects of Growth Hormone Secretagogues on Adult Rat Hippocampal Progenitor. 2191–2199 (2008). doi:10.1210/en.2007-0733

96. Moon, M., Hwang, L. & Park, S. Ghrelin Regulates Hippocampal Neurogenesis in Adult Mice. *Endocr. J.* **56**, 525–531 (2009).

97. Bekinschtein, P. *et al.* BDNF in the dentate gyrus is required for consolidation of “pattern-separated” memories. *Cell Rep.* **5**, 759–768 (2013).

98. Zhao, Z. *et al.* Ghrelin administration enhances neurogenesis but impairs spatial learning and memory in adult mice. *Neuroscience* **257**, 175–85 (2014).

99. Li, E. *et al.* Ghrelin directly stimulates adult hippocampal neurogenesis: implications for learning and memory. *Endocr. J.* (2013). doi:10.1507/endocrj.

100. Veyrac, A. *et al.* Zif268/egr1 gene controls the selection, maturation and functional integration of adult hippocampal newborn neurons by learning. *Proc. Natl. Acad. Sci.* **110**, 7062–7067 (2013).

101. Kojima, M. *et al.* Ghrelin is a growth-hormone-releasing acylated peptide from

stomach. *Nature* **402**, 656–60 (1999).

102. Aberg, M. A., Aberg, N. D., Hedbäcker, H., Oscarsson, J. & Eriksson, P. S. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *J. Neurosci.* **20**, 2896–903 (2000).

103. Li, E., Kim, Y., Kim, S. & Park, S. Ghrelin-induced hippocampal neurogenesis and enhancement of cognitive function are mediated independently of GH/IGF-1 axis: lessons from the spontaneous dwarf rats. *Endocr. J.* **60**, 1065–75 (2013).

104. Stone, S. S. D. *et al.* Stimulation of entorhinal cortex promotes adult neurogenesis and facilitates spatial memory. *J. Neurosci.* **31**, 13469–84 (2011).

105. Date, Y. *et al.* The role of the gastric afferent vagal nerve in Ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* **123**, 1120–1128 (2002).

106. O'Leary, O. F. *et al.* The vagus nerve modulates BDNF expression and neurogenesis in the hippocampus. *Eur. Neuropsychopharmacol.* **28**, 307–316 (2018).

107. Choi, S. H. *et al.* Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer's mouse model. *Science (80-.).* **361**, (2018).

108. Madeo, F., Carmona-Gutierrez, D., Hofer, S. J. & Kroemer, G. Caloric Restriction Mimetics against Age-Associated Disease: Targets, Mechanisms, and Therapeutic Potential. *Cell Metab.* **29**, 592–610 (2019).

109. Li, S. & Laher, I. Exercise Pills : At the Starting Line. *Trends Pharmacol. Sci.* **36**, 906–917 (2015).

110. Jeong, Y. O. *et al.* Mk-0677, a ghrelin agonist, alleviates amyloid beta-related pathology in 5XFAD mice, an animal model of Alzheimer's disease. *Int. J. Mol. Sci.* **19**, (2018).

111. Kunath, N. *et al.* Ghrelin agonist does not foster insulin resistance but improves cognition in an Alzheimer's disease mouse model. *Sci. Rep.* **5**, 11452 (2015).

112. Atcha, Z. *et al.* Cognitive enhancing effects of ghrelin receptor agonists. *Psychopharmacology (Berl).* **206**, 415–27 (2009).

113. Barlind, A. *et al.* Growth Hormone & IGF Research The growth hormone

secretagogue hexarelin increases cell proliferation in neurogenic regions of the mouse hippocampus. *Growth Horm. IGF Res.* **20**, 49–54 (2010).

114. Ma, D. K. *et al.* Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science* **323**, 1074–7 (2009).
115. Chung, H., Li, E., Kim, Y., Kim, S. & Park, S. Multiple signaling pathways mediate ghrelin-induced proliferation of hippocampal neural stem cells. *J. Endocrinol.* **218**, 49–59 (2013).
116. Ratcliff, M. *et al.* Calorie restriction activates new adult born olfactory-bulb neurones in a ghrelin-dependent manner but acyl-ghrelin does not enhance sub-ventricular zone neurogenesis. *J. Neuroendocrinol.* (2019).
117. Zeisel, A. *et al.* Molecular architecture of the mouse nervous system. *Cell* **174**, 999–1014 (2018).
118. Malik, S., McGlone, F., Bedrossian, D. & Dagher, A. Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior. *Cell Metab.* **7**, 400–409 (2008).
119. Han, J. E. *et al.* Ghrelin Enhances Food Odor Conditioning in Healthy Humans : An fMRI Study. *CellReports* **25**, 2643-2652.e4 (2018).