Beyond appetite: Acylated ghrelin as a learning, memory and fear behavior-modulating hormone

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ABSTRACT

Although often referred to as a hunger hormone, recent evidence highlights a neuroprotective function of acylated ghrelin (AG) and a substantial role in the regulation of declarative and aversive memories as well as fear behavior. As such, in this review, we i) evaluate what specific stages and forms of memory, as well as which respective brain areas are affected by acylated ghrelin, ii) illustrate the plasticity-associated signaling pathways of AG in the hippocampus, also involving memory resolution-enhancing neurogenesis, iii) elucidate how the peptide modulates neurotransmitter systems (glutamate, γ-aminobutyric acid, dopamine, serotonin), iv) clarify the role of AG in conditioned taste aversion, novelty learning and the formation of spatial, recognition, auditory fear, contextual fear and passive avoidance memories in the hippocampus and amygdala as well as V) solve the mystery behind AG, its impact on the 5-HT system, the recently established link to post-traumatic stress disorder and the either fear-suppressing or fear-potentiating effects under neutral and acutely stressed conditions or chronic stress, respectively.

1. Introduction

As a major metabolic hormone, ghrelin is predominantly produced and released by gastric X/A-like cells (Date et al., 2000), although the peptide is also endogenously expressed in peripheral tissue and across the brain (Ferrini et al., 2009). In a series of catalytic steps, mature ghrelin is cleaved from its precursor proghrelin. Furthermore, the peptide may be acetylated at Ser3 by ghrelin O-acyltransferase, resulting in bioactive acylated ghrelin (AG) and its counterpart non-acylated ghrelin (DAG) (Yanagi et al., 2018). Once generated, AG and DAG are secreted into the blood stream in response to nutrient deficiency, fasting and energy shortage to encourage appetite and food-seeking behaviour (Cummings et al., 2001; Liu et al., 2008; Yanagi et al., 2018). Importantly, only AG, but not DAG, is capable of stimulating growth hormone secretagogue receptor type 1α (GHS-R1α) (Yanagi et al., 2018). Interestingly, GHS-R1α displays a remarkable basal activity of ~50 % (Damian et al., 2012), which may explain why ghrelin is genetically expendable (Sun et al., 2003). Moreover, GHS-R1α is capable to form heterodimers with other receptors, including dopamine receptor subtype 1 (D1R) and D2R (Jiang et al., 2006; Kern et al., 2012), somatostatin receptor subtype 5 (Park et al., 2012), serotonin (5-HT) receptor 2c (5-HT2cR) (Schellekens et al., 2013), melanocortin 3 receptor (Rediger et al., 2011, 2009) or GHS-R18, which abrogates GHS-R1α-signaling (Leung et al., 2007), thus affecting the downstream response.

Abbreviations: AG, acylated ghrelin; 5-HT, serotonin; 5-HTR, serotonin receptor; GH, growth hormone; γ-aminobutyric acid (GABA), growth hormone secretagogue receptor type 1α (GHS-R1α); post-traumatic stress disorder; LA, lateral nucleus of the amygdala; basolateral amygdalalCUTA, (BLA), dorsal raphe nucleus (DRN) conditioned taste aversion.

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the cornu ammonis (CA1), CA2, CA3 and the dentate gyrus (DG), to navigate long-term memory, food/reward behaviour, motivation, spontaneity, anxiety and other cognitive or emotional aspects (Fig. 1) (Abdalla, 2015; Date, 2012; Gnanapavan et al., 2002; Gun, Yu, Palyha, McKee, Feighner, Sirinathsinghji, Smith, VanderPloeg et al., 1997; Jiang et al., 2006; Yanagi et al., 2018). AG is thought to be the main mediator of the neuroprotective effects of caloric restriction (Bayliss et al., 2016), hence we have recently evaluated the potential of AG as a neuroprotective agent in Alzheimer’s disease (AD) and Parkinson’s disease (PD) (Reich and Holscher, 2020). Additionally, besides regulating feeding and food-related learning (Hsu et al., 2016; Serrenho et al., 2019), we highlight a substantial role of AG in the formation of various forms of memory and associated behaviors.

2. Synaptic plasticity and memory formation

Long-term potentiation (LTP) represents an interneuronal process that results in the permanent strengthening of synapses, thought to be the basis of memory formation. LTP is initiated by electrical impulses (action potentials) that arise due to mechanical high-frequency stimulation, the exposure to an environmental stimulus, or training in novel tasks. The latter lead to the opening of voltage-gated Ca$^{2+}$ channels (CaV$\alpha$), Ca$^{2+}$ influx, and the associated activation of various Ca$^{2+}$-sensitive modulators that mediate the presynaptic liberation of glutamate into the synaptic cleft. Glutamate subsequently diffuses towards and interacts with excitatory α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), N-methyl-D-aspartate receptors (NMDARs) and kainate receptors on postsynaptic neurons, resulting in depolarization. Moreover, NMDAR-associated ion channel opening induces the postsynaptic influx of Ca$^{2+}$, leading to the initiation of second messenger cascades that comprise kinases such as Ca$^{2+}$/calmodulin-dependent protein kinase (CaMK), CaMKII/IV, PKC, tyrosine kinase or extracellular signal-regulated kinase (ERK). In conjunction, the latter kinases mediate the phosphorylation and incorporation of AMPARs into postsynaptic terminals and navigate the remodeling of dendritic spines. Moreover, ERK- and cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)-signaling induce the key transcription factor cyclic adenosine monophosphate (cAMP)/cAMP response element-binding protein (CREB) and the transcription of immediate early genes (IEGs), for instance c-Fos, early growth response protein 1 (Egr-1) or activity-regulated cytoskeleton-associated protein (Arc), to enhance the synaptic strength (Byth, 2014; Chaaya et al., 2018; Deak and Sonntag, 2012; Warburton, 2014).

While the expression of IEGs may indicate plasticity, synaptic strength is electrophysiologically measured in form of total current flow, termed excitatory postsynaptic potential (EPSP), across postsynaptic membranes. Alternatively, γ-aminobutyric acid (GABA)-induced inhibitory postsynaptic potentials (iPSPs) may be assessed. Various events may facilitate EPSPs, and thus, drive LTP, such as the increased release of excitatory and decreased release of inhibitory neurotransmitters (GABA) at presynaptic terminals, the incorporation of further postsynaptic excitatory neurotransmitter receptors, the enhancement of neurotransmitter receptor affinities towards their substrates, or prolonged Ca$^{2+}$ channel opening times. Indeed, various excitatory and inhibitory neurotransmitters, including glutamate, GABA, dopamine, 5-HT, acetylcholine and noradrenaline (NA), their individual receptors as well as several ion channels cooperate in an intricate time-, context- and region-specific manner to regulate LTP and its counterpart long-term depression (LTD), which represents the selective weakening of synaptic strength (Chaaya et al., 2018; Deak and Sonntag, 2012; Palacios-Filardo and Mellor, 2019; Warburton, 2014).

On a cellular level, memory is generated by the synchronous firing of interconnected neurons during learning, leading to the stabilization of this neuronal network. While LTP and LTD in different brain regions drive distinct types of memory, see (Amin and Malik, 2013), the hippocampus imparts the formation of declarative long-term memories, including contextual/associative, semantic, episodic and spatial memories (Bird and Burgess, 2008). In a series of events, sensory information is transmitted from the (parahippocampal) perforant path and entorhinal cortex to DG granule cells, across the CA3 and towards CA1-located pyramidal neurons. The CA1 poses the final output station of the dorsal hippocampus, which projects to cortical fields to integrate memories or to initiate decision-making, while looping back to the perforant path to consolidate hippocampal input (Deak and Sonntag, 2012).

Importantly, hippocampal plasticity is thought to be a direct reflection of the ability to acquire and consolidate hippocampus-associated memories. Within the first 24 h, the hippocampus temporally retains the formed declarative information, yet, after approximately a week has passed, these memories eventually become hippocampus-independent. Indeed, other brain regions, such as the prefrontal cortex, mediate

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Fig. 1. Expression sites of GHS-R1α in the human brain. GHS-R1α is found at high densities in multiple hypothalamic nuclei (in particular the arcuate nucleus) to induce appetite, the hippocampal formation (CA1, CA2, CA3 and DG) to modulate declarative memory, the pituitary gland to affect hormone release and various midbrain sites (ventral tegmental area, dorsal raphe nuclei and more) to, amongst others, regulate dopamine and serotonin transmission (Airapetov et al., 2021). The shown relative mRNA expression levels (transcripts per kilobase million) were derived from the Human Protein Atlas (Uhlen et al., 2015).
memory expression and store consolidated memories in the long-term (Beck and Pourie, 2013; Deak and Sonntag, 2012).

Besides the hippocampus, the presence of emotional stimuli engages the amygdala to drive the formation of emotional memories. Traditionally, research on emotional memories falls into two main categories, including classical cue-based Pavlovian conditioning, commonly with rodents, or emotional episodic memories (Dunsmoor and Kroes, 2019). As relevant for the scope of this review, we will expand on fear memory formation through Pavlovian and contextual fear conditioning in Section 5.1.

Furthermore, the process of creating a memory involves several steps. In the acquisition phase (i), as occurring during the training of rodents in a behavioral paradigm, the induction of cellular plasticity (LTP) in selected brain regions creates an initial, yet labile memory. Subsequently, when training ceases and the animal is returned to its cage, the memory trace is consolidated (ii) through CREB-mediated protein synthesis into a stable long-term memory. Finally, retrieval (iii) describes the recollection of a previously stored memory (Abel and Lattal, 2001; de Quervain et al., 2017).

As a major objective this review, we will critically appraise the currently available animal and human data to illustrate the modulatory impact of AG on memory formation, the underlying brain regions, biochemistry and behavioral changes, with a focus on the hippocampus and amygdala. Hereafter, we will summarize the in vivo and, as available, clinical data to show a supportive role of AG in the formation of hippocampus-dependent memory under healthy and demented conditions (Section 3). This will be followed by the respective neurotransmitter-, plasticity- and neurogenesis-modulating effects of AG in the hippocampus (Section 4). Finally, we will explore the impact of AG on various forms of fear memory, conditioned taste aversion (CTA) as well hippocampus (Section 4). Finally, we will explore the impact of AG on various forms of fear memory, conditioned taste aversion (CTA) as well as the recently established clinical link to post-traumatic stress disorder (PTSD). Indeed, the context-dependent effects of AG on fear memory strength and PTSD involve a complex interplay between the ghrelin system, various amygdaloid nuclei, the hippocampus and serotonin-signaling (Section 5).

3. Acylated ghrelin enhances hippocampal memory

As presented below, animal studies advocate a learning and consolidation-enhancing effect of exogenously administered AG, synthetic ghrelin analogues as well as the ghrelin receptor GHS-R1α on hippocampus-dependent memory in wild-type and AD rodent models.

3.1. Ghrelin enhances spatial memory in wild-type animals

Various studies in healthy animals show that AG promotes the formation of hippocampus-dependent spatial memories. While these effects seem to be dose-dependent (inverse U-shaped), there is evidence for an acquisition and consolidation-improving outcome of AG treatment, GHS-R1α-modulating drugs and GHS-R1α itself on object recognition (Carlini et al., 2007, 2008; Atcha et al., 2009; Diano et al., 2006; Ribeiro et al., 2020) and spatial memory (Atcha et al., 2009; Chen et al., 2011; Toth et al., 2010; Davis et al., 2011; Tian et al., 2019).

The administration of intra-hippocampal AG post-training enhanced the consolidation of short-term (1 h) and long-term (24 h) object recognition memory memories (Carlini et al., 2007, 2008), while the pre-training administration of ghrelin analogues enhanced the novel object exploration time and index (preferential investigation of the novel over familiar objects), suggesting an acquisition-boosting effect of AG (Atcha et al., 2009). Similarly, the central injection of AG following training ameliorated undernutrition-induced deficits in 1 h and 24 h object recognition memory (Carlini et al., 2005). In turn, ghrelin-α mice exhibited impaired object recognition memory that could be restored with the initial daily infusion of AG for 14 days (Diano et al., 2006).

Notably, a preliminary report announced that the pre-training injection of a GHS-R1α inverse agonist weakened the recognition of novel and displaced objects (Luís F. Ribeiro et al., 2020). Therefore, both the tonic activity (~ 50% Gαq/11-coupling in the absence of ligands (Damian et al., 2012)) and stimulation of GHS-R1α improve the consolidation and, possibly, acquisition of spatial recognition memories.

In experimental paradigms that assess hippocampus-associated spatial learning and memory, the 4 day-long daily and pre-training peripheral injection of a ghrelin mimetic improved the training performance of rodents in the Atlantis Water Maze (Atcha et al., 2009), while dorsohippocampal AG strengthened spatial memory acquisition and retention in the Morris Water Maze (MWM) (Chen et al., 2011). However, the intra-CA1 infusion of low doses of AG (8 ng per hemisphere) prior to every other training day impaired spatial acquisition and memory retention (Zhao et al., 2014; Zhu et al., 2015). Notably, while the hormone augmented hippocampus-dependent passive avoidance memories when given at 100 ng or 150 ng, central injections of < 50 ng AG resulted in a trend for poorer performance. 200 ng AG, in fact, significantly decreased the inhibitory memory of healthy mice in the T-Maze foot shock avoidance paradigm. This implies that an inverse U-shaped relationship between the hippocampal concentrations of AG and memory exists (Diano et al., 2006). Interestingly, the microinjection of AG into the amygdala following the first MWM training day, in a GHS-R1α-dependent manner, led to enhanced performance on the second day. This proposes that the putative plasticity-suppressing effects of AG in the amygdala (Section 5.2.2) may facilitate the hippocampal consolidation of spatial memories (Toth et al., 2010).

On the other hand, reports concerning GHS-R1α-deficient mice are mixed. Two studies showed that the deletion of GHS-R1α impeded spatial acquisition across the test days and attenuated reference memories during the probe trial in the MWM (J. F. Davis et al., 2011; Tian et al., 2019). However, a separate study by (Albarran-Zeckler et al., 2012) found that GHS-R1α−/− mice did not show any differences in spatial learning compared to wild-type littermates, but displayed heightened spatial memory in the MWM when tested 7 days, but not 1 day, following training. First, to provide an explanation for the latter results, it must be considered that GHS-R1α has neuroprotective effects (Section 3.2) and that both receptor deletion or the age-associated complex formation of accumulating Aβ42 with GHS-R1α, which impairs the function of the ghrelin receptor, generate an AD-like phenotype that is more explicit with greater age (Tian et al., 2019). Indeed, (Tian et al., 2019) observed early deficits in spatial (MWM) memory formation and reductions in the hippocampal synapse densities in 4 month old GHS-R1α−/− mice, while 9 month old GHS-R1α−/− rodents further exhibited impaired hippocampal LTP-induction and postsynaptic CaMKII phosphorylation. What improved the spatial memory retention in 6 month old GHS-R1α−/− mice 7 days post training is a mystery, however.

To add to the puzzle, (Albarran-Zeckler et al., 2012) also observed reduced contextual fear memory strength, another form of hippocampus-dependent memory, 30 days after conditioning. As we explain in Section 5.2.7, long-term increases in the plasma AG levels beyond 8 days, as achieved with caloric restriction, prolong the hippocampal (contextual fear) memory recall by stimulating neurogenesis (Hornsby et al., 2016; Gu et al., 2012; Pan et al., 2012).

In conclusion, the evidence emphasizes a memory formation-enhancing role of AG and GHS-R1α in the hippocampus. How AG and GHS-R1α affect spatial memory retention and recall over a multi-week period following training, however, requires further investigations.

3.2. Ghrelin rescues cognitive decline in Alzheimer’s disease in vivo models

Besides healthy animals, AG rescued cognition in multiple animal models of AD. In the latter, AG or ghrelin mimetics enhanced the performance of rodents in passive avoidance, novel object recognition, MWM, Barnes Maze and Y-Maze paradigms, signifying improvements in the acquisition of inhibitory avoidance, reference and spatial memory (Dhurandhar et al., 2013; Diano et al., 2006; Eslami et al., 2018; Kang...
Importantly, AG improved the cognitive function of the AD animals by ameliorating their cerebral pathology, including Aβ and Tau pathology, neuroinflammation, hyperglycemia, insulin resistance and the activation of GSK-3β (see (Reich and Holscher, 2020) for details). This, in turn, resulted in enhanced EPSPs across CA3-CA1 synapses (even in control mice), rescued from the Aβ-induced weakening of high-frequency-stimulated LTP (Islami et al., 2018; Santos et al., 2017), normalized brain acetylcholinesterase levels (Madhavadas et al., 2014) and prevented a decline in plasticity-associated p-CREB, CREB binding protein and p-300 levels plus the reduction in Egfr expression in the CA1 and DG regions (Bartolotti et al., 2016; Jeong et al., 2018). Likewise, AG-treatment upregulated CREB, p-CREB and brain-derived neurotrophic factor (BDNF) levels in streptozotocin-injected rats, improving cognition (Ma et al., 2011), while the intrahippocampal co-administration of AG preserved MWM spatial memory in response to a seizure-inducing GABA	extsubscript{A} receptor antagonist (Babri et al., 2015). On the other hand, ghrelin	extsuperscript{AC} mice showed attenuated spatial (recognition) memory, olfactory deficits and elevated micro- and astroglial immuno-reactivity in the hippocampus (V. Santos et al., 2017).

3.3. Association studies of ghrelin and cognition are misleading in humans

While there are currently no human studies that assess the outcome of administered AG or its analogues on memory or cognition, it was reported that the plasma ghrelin levels were inversely associated with neuropsychological test scores in non-demented elderly individuals, including auditory working memory, verbal recall and confrontation naming (Spitznagel et al., 2010). The investigation had a few major weaknesses, however, such as limited sample size (35 participants), the use of overweight patients (BMI 28.35 ± 4.60) with a clinical history of cardiovascular risk factors that accelerate cognitive decline, such as hypertension (42.9 %) and elevated cholesterol levels (37.1 %), and the lack of a healthy control group. Also, plasma ghrelin levels are chronically reduced during obesity (Rigamonti et al., 2002; Shiiya et al., 2002; Kern et al., 2015; Tschop et al., 2001), which presumably resulted in skewed findings (Spitznagel et al., 2010).

4. The ghrelin system modulates hippocampal plasticity

As described in Section 3, there is robust evidence that AG enhances hippocampal learning and memory consolidation in animals. Therefore, Section 4 aims to condense the underlying memory-facilitating mechanisms of AG. Table 1 provides an overview of studies that have investigated the plasticity-altering and biochemical effects of AG and its constitutively active receptor, GHS-R1α, in the hippocampus. Subsection 4.1 will explore these studies and elucidate the established, but also still enigmatic, role of the ghrelin system in the hippocampal regulation of glutamatergic, GABAergic and dopaminergic neurotransmission. We will also highlight play a considerable role of AG and GHS-R1α in the mesolimbic dopamine pathway, with implications on feeding behavior, locomotion and novelty learning.

4.1. Neurotransmitter systems

4.1.1. Acylated ghrelin promotes glutamatergic neurotransmission in the hippocampus

As illustrated in Fig. 2, both GHS-R1α and its ligand AG enhance glutamatergic neurotransmission in the dorsal hippocampus. Spatially, it was shown that GHS-R1α distributed across the cell body and dendrites of isolated hippocampal neurons and in the CA1, located in close proximity to hippocampal excitatory synapses (Ribeiro et al., 2014; Berrouet and Isokawa, 2018). Using in vivo monitoring under anesthesia, the hippocampal infusion of AG induced slowly raising and long-lasting (>4 h) postsynaptic plasticity in a phosphoinositide 3-kinase (PI3K)/Akt-dependent and NMDAR-independent manner in the rat DG, resulting in improved spatial memory. Of note, even though AG did not affect the expression of high frequency-stimulated LTP, the AG-driven and delayed activation of ERK1/2 (2 h) prevented a decline in these electrically evoked LTP (Chen et al., 2011). Similar to the latter investigation, AG did not elevate the magnitude of stimulus-induced (NMDAR-dependent) LTP in another ex vivo study. However, the application of AG lessened the LTP generation threshold in the DG, similarly resulting in GHS-R1α-dependent improvements in hippocampal passive avoidance memory consolidation (Gherzi et al., 2015). Besides the DG, there is evidence that AG or the GHS-R1α agonist MK-0677 dose-dependently elevated NMDAR-driven EPSPs across CA3-CA1 synapses (Ribeiro et al., 2014; Muniz and Isokawa, 2015), predominantly by increasing the synaptic AMPA/NMDA current ratio (Ribeiro et al., 2014) and facilitating LTP generation in the CA1 (Diano et al., 2006). Furthermore, AG dose-dependently stimulated the (4-amino-pyridine-evoked) presynaptic glutamate release by hippocampal synaptic boutons and in the DG in vivo, while enhancing the excitability of DG granule cells in the rat brain (Chen et al., 2011; Gherzi et al., 2015). This suggests that modifies the presynaptic glutamatergic space, which, as explained below, involves the modulation of AMPARs.

Mechanistically, AG regulates the synaptic incorporation of AMPARs in a temporal manner, which can be categorized into short-term and long-term effects (see also Fig. 2). In the short-term, the use of a ghrelin mimetic rapidly elevated PI3K/Akt activity in hippocampal slices (~30 min) and evoked the PI3K- and PKA-co-dependent phosphorylation of GluA1-AMPA subunits at Ser845, which promoted AMPAR surface exposition. Furthermore, simultaneous NMDAR channel activity was necessary to incorporate these exocytosed GluA1-AMPARs into synapses (Ribeiro et al., 2014). Notably, the Ser845-phosphorylation of GluA1-AMPARs was shown to be necessary for LTP execution in the CA1 (J.Y. Lee et al., 2010; H.K. Lee et al., 2010) and spatial memory retention in the MWM (Lee et al., 2003). In this context, hippocampal GHS-R1α receptor interactions provide an explanation for the non-canonical induction of cAMP/PKA-signaling and the PKA-mediated AMPAR at Ser845-phosphorylation by AG. Generally, the canonical activation of GHS-R1α involves Gαq/11-coupling, phospholipase C (PLC) activation and the simultaneous, PLC-mediated turnover of phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) as well as the generation of diacylglycerol (DAGly). IP3 further triggers the liberation of calcium (Ca^{2+}) from intracellular ER stores. Then, DAGly and the IP3-driven accumulation of Ca^{2+} induce protein kinase C (PKC). The induction of cAMP/PKA has been reported for GHS-R1α, yet is still under debate and conditional (Reich and Holscher, 2020; Yin et al., 2014). In this context, it was shown that GHS-R1α forms heteromers with D1R in neurons across the dorsal hippocampus (Jiang et al., 2006; Kern et al., 2015; Tian et al., 2019). AG and dopamine co-treatment triggered the heterodimerization of GHS-R1α and D1R, engaged Gαq/11 (instead of Gαq/11 by GHS-R1α) and induced a multi-fold synergistic augmentation of cAMP accumulation, as compared to dopamine/D1R-signaling alone (Jiang et al., 2006). Notably, AG also induces the mesolimbic transmission of dopamine from the VTA to the hippocampus (Section 4.1.3). Likewise, the association of GHS-R1α with its inactive splicing variant, GHS-R1β, was shown to induce Gαq/11-coupling (Navarro et al., 2016). In this case, as observed in primary hippocampal and striatal neurons, the presence of dopamine or AG, but not both, initiated the GHS-R1β necessitating GHS-R1β: GHS-R1β:G1R heteromers to activate protein kinase ζ (PKζ) and cAMP accumulation. There were no synergistic effects of AG and dopamine on cAMP accumulation anymore, however (Navarro et al., 2016). As such, AG may trigger the formation of GHS-R1α:GHS-R1β:D1R oligomers in the CA1, (CA3) or DG to drive non-canonical Gαq/11-evoked cAMP/PKA-signaling in the hippocampus.

In the long-term, prolonged ghrelin agonist treatment (5–20 h), in a GHS-R1α-dependent fashion, resulted in PKC activity, the activation of stargazin (which is phosphorylated by PKC, but also CaMKII (Tomita et al., 2015; Kunath et al., 2015; Madhavadas et al., 2014; Moon et al., 2011; Santos et al., 2017).
Table 1

Hippocampal effects of AG, GHS-R1α agonists, antagonists and inverse agonists, receptor deletion or GHS-R1α receptor interactions on plasticity as well as glutamatergic (AMPA receptors and NMDA receptors), GABAergic and dopaminergic neurotransmission.

| Actions of AG | Compounds | Study details | LTP & memory | Biochemical & histological observations | References |
|---------------|-----------|---------------|--------------|----------------------------------------|------------|
| AG and GHS-R1α antagonist (D-Lys3-GHRP-6) as well as GHS-R1α inverse agonist ([D-Arg1,D-Phe5, D-Trp7,8,Leu11] Substance P) | Ex vivo | Bath application to GHS-R1α−/−, GHS-R1α−/− or GHS-R1α−/− mouse hippocampal slices | • GHS-R1α- and dose-dependent augmentation of NMDAR-induced EPSPs by AG in CA1 pyramidal cells (rat hippocampal slices) | • AG binding to GHS-R1α across CA1 - CA3 | (Manitz and Isokawa, 2015) |
| | | | • GHS-R1α antagonism, in the absence of AG, weakened NMDAR current generation | | |
| AG | In vivo | Dorsohippocampal infusion into rats. LTP recording under anesthesia. | • NMDAR-independent, but GHS-R1α- and P38/Akt-dependent, increase in long-lasting LTP (EPSC slope and amplitude) in the DG (> 4 h). DAG used as negative control. | • AG increased Akt Ser473-phosphorylation (from 30 min post infusion and peak at 120 min) | (Chen et al., 2011) |
| | Ex vivo | Addition to rat hippocampal slices & isolated synaptosomes | • GHS-R1α-dependent reduction in the LTP generation threshold by AG in the DG | • AG dose-dependently potentiated stimulus (4-AP)-evoked presynaptic glutamate release by hippocampal synaptosomes | (Ghersi et al., 2015) |
| | | | • GHS-R1α-dependent improvement in hippocampus-associated inhibitory avoidance memory consolidation | • AG-induced ERK1/2 activation at 2 h, but not 1 h, maintained high-frequency-stimulated LTP | |
| AG | Ex vivo | Addition to rat hippocampal slices | N/A | • GHS-R1α predominantly located on soma and dendrites of pyramidal cells in area CA1 | (Bernout and Isokawa, 2016) |
| | | | | • GHS-R1α and Fyn-dependent, but NMDAR-, Ca2+ (CaMKII) and NR2B activity-independent, increase in NR2B-NMDAR phosphorylation at Tyr1336 (CA1) | |
| | | | | • Co-inhibition of Ca2+ release from internal stores, cAMP/PKA or casein kinase II augmented AG-induced NR2B-NMDAR phosphorylation (Tyr1336) | |
| AG | Ex vivo | Addition to rat hippocampal slices | N/A | • GHS-R1α predominantly located on soma, but also dendrites, in the CA1 | (Casillas and Isokawa, 2011) |
| | | | | • AG induced a GHS-R1α, NR2B-NMDAR and cAMP/(PKA)-dependent, but IP3/ Ca2+-independent, 4-fold increase in phospho-CREB | |
| | | | | • The acute presence of AG sustained F-actin polymerization and cytoskeletal reorganization in dendrites and spines of CA1 neurons | |
| AG | In vivo | Microinjection into the dorsal third ventricle close to the hippocampus in rats | Enhanced acquisition in the object location memory test by AG reversed with co-administration of D1R antagonist | | (Jacoby and Carrie, 2011) |

(continued on next page)
### Table 1 (continued)

| Actions of AG | Study details | LTP & memory | Biochemical & histological observations | References |
|---------------|---------------|--------------|----------------------------------------|-------------|
| **Actions of GHS-R1 agonists**<br>**Compound**<br>MK-0677 (and AG) | **Study details**<br>**In vivo** Addition to primary rat hippocampal neurons | **LTP & memory**<br>• Potentiated NMDA-induced EPSCs across CA3 - CA1 synapses<br>• Increased synaptic AMPA/NMDA ratios in CA1 neurons. Unaltered NMDA/GABA ratios (20 h treatment of hippocampal slices with MK-0677)<br>• No effect on ion channel conductance, resting membrane potential nor spontaneous action potential firing (CA1 and CA3 neurons) | **Biochemical & histological observations**<br>• GHS-R1 located at dendritic excitatory synapses; partial co-localization with postsynaptic PSD-95 and presynaptic Vglut1<br>• GHS-R1-mediated increase in surface exposition and synaptic levels of GluA1-AMAPRs after MK-0677 treatment (1 h in hippocampal neurons and 2 h in hippocampal slices).<br>• Rapid PEK activation (30 min)<br>• PEK- and PKA-co-dependent increase in GluA1-AMAPR phosphorylation at Ser<sup>845</sup> and NMDAR/voltage-gated sodium channel (activity)-dependent synaptic recruitment of GluA1-AMAPRs following MK-0677 treatment (acutely and after 20 h)<br>• GHS-R1α-induced increase in stargazin phosphorylation at Ser<sup>239/240</sup> (20 h)<br>• GHS-R1α-driven phosphorylation of GluA1-AMAPRs (Ser<sup>845</sup>) and stargazin (Ser<sup>239/240</sup>) unaffected by NMDAR/voltage-gated sodium channel antagonists, but synaptic incorporation of GluA1-AMAPRs blocked<br>• GHS-R1α-dependent upregulation of PKC activity and GluA1-AMAPR phosphorylation at Ser<sup>239/240</sup> by MK-0677 (5 h)<br>• CaMKII activity completely unaltered (30 min - 20 h)<br>• Synaptic GluA1-AMAPR recruitment by MK-0677 dependent on PKA activation (1 h) and maintained by PKC (20 h) in hippocampal slices. | **References**<br>(Ribeiro et al., 2014) |
| **Actions of GHS-R1α antagonists, inverse agonists (inhibitors of constitutive GHS-R1α activity) or receptor deletion**<br>**Compound**<br>GHS-R1α antagonist (D-Lys<sub>3</sub>-GHRP-6) | **Study details**<br>In vivo Intracerebroventricular post-training infusion in rats | **LTP & memory**<br>Deterioration in passive avoidance memory consolidation | **Biochemical & histological observations**<br>• Transcriptional downregulation of GluA1-AMARs, HT<sub>1</sub> and HT<sub>2</sub> levels in the hippocampus following infusion of D-Lys<sub>3</sub>-GHRP-6. No effect on NR1-NMDARs or CaMKII. | **References**<br>(Beheshti et al., 2020) |
| GHS-R1α antagonists (JMV2959, JMV3002, and BIM-28163) & D1R agonist (SKF81297) | **Study details**<br>In vivo Medium treatment of primary hippocampal neurons and GHS-R1α/D1R-overexpressing HEK293 cells | **LTP & memory**<br>D<sub>1</sub>R agonist-imported improvements in extinction memory consolidation and T-Maze working memory prevented by GHS-R1α antagonist JMV2959 | **Biochemical & histological observations**<br>• Presence of GHS-R1α/D1R heterodimers in the CA1 and, more abundant, CA3 and DG.<br>• Heterodimerization of D1R & GHS-R1α simulated with D1R agonists in primary mouse hippocampal neurons<br>• Ca<sup>2+</sup> mobilization following D<sub>1</sub>R agonist treatment absent in GHS-R1α<sup>−/−</sup> mouse hippocampal slices.<br>• Dopamine- or D<sub>1</sub>R agonist-induced Ca<sup>2+</sup> release via GHS-R1α/D<sub>1</sub>R heteromers GHS-R1α/D<sub>1</sub>R was mediated by non-canonical G<sub>αq</sub>/PLC/PIP2-signaling and independent of G<sub>αq</sub>/AC/PKA, G<sub>αq</sub>/G<sub>βγ</sub>, and PCK. The final Ca<sup>2+</sup> release correlated with the rate of GHS-R1α/D<sub>1</sub>R heteromer formation.<br>• Dose-dependent inhibition of Ca<sup>2+</sup> release across GHS-R1α/D<sub>1</sub>R heteromers by GHS-R1α antagonist JMV2959, but no effect of GHS-R1α antagonists on D<sub>1</sub>R agonist/D1R-induced cAMP accumulation.<br>• Suggested was a 50% equilibrium between D<sub>1</sub>R/D<sub>1</sub>R monomers (G<sub>αq</sub>/PLC- signaling) and GHS-R1α/D<sub>1</sub>R heteromers (G<sub>αq</sub>/PLC/P2-coupling)<br>• D<sub>1</sub>R agonist-elicited CaMKII phosphorylation and postsynaptic translocation (as driven by non-canonical GHS-R1α/D<sub>1</sub>R/ Gαq/PLC/P2/Ca<sup>2+</sup> -signaling) only in hippocampal slices. | **References**<br>(Kern et al., 2015) |
| **(continued on next page)**
| Compound                  | Study details                                                                 | LTP & memory | Biochemical & histological observations                                                                 | References                                                                 |
|---------------------------|-------------------------------------------------------------------------------|--------------|---------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| BBB-penetrant (AZ12861903) and non-BBB-penetrant (D-Arg1,D-Phe5,D-Trp7,9,Leu11)-substance P; SP-A) GHS-R1 inverse agonists, shRNA knockout of GHS-R1 (and GHS-R1 antagonist JM2959) | In vitro Application to primary or superelipctic pHluorin (SEP)-GluA1-expressing hippocampal neurons as well as a neuronal model of chemical LTP induction Ex vivo Addition to organotypic hippocampal slices | Intraperitoneal injection (pre-training) into mice                        | GHS-R1α/-/-, but not GHS-R1α+/+, hippocampal slices (GHS-R1α+/+D1R dependence). Changes in CaMKII-phosphorylation also inhibited with GHS-R1α antagonist. Increase in GluA1-AMPAR Ser234/235 phosphorylation by D1R agonist blocked with GHS-R1α antagonist, CaMKII inhibitor and in GHS-R1α-hippocampal slices (GHS-R1α:D1R dependence) D1R agonist-induced synaptic excytosis of GluA1-AMPARs and NR1-NMDARs, as well as synaptic actin rearrangements, absent in GHS-R1α-hippocampal neurons and inhibited with GHS-R1α antagonist in GHS-R1α-hippocampal slices (GHS-R1α:D1R dependence) Plasticity-associated S6 phosphorylation and gene expression (Nrx4a1, Nrx4a2, Nrx4c3, Nrk2, Arc and Zif268) elicited by D1R agonist dependent on GHS-R1α:D1R heteromers Dopamine-induced reduction in PPI (sensorimotor gating) disrupted in GHS-R1α-/+ mice (in vivo) Reduction in total surface GluA1-AMPARs, synaptic GluA1/GluA2-AMPARs and co-localization of AMPAR-PSD-95/AMPAR-vGlut1 upon blockage of constitutive GHS-R1α activity, but not with GHS-R1α agonist. The latter effect observed in older hippocampal neurons (15 or 20 DIV), but not younger neurons (7 DIV) with lower expression levels of GHS-R1α. Reduction in GluA1, as mediated by GHS-R1α inverse agonist binding, absent in hippocampal neurons with shRNA-silencing of GHS-R1α GHS-R1α inverse agonists encouraged surface distribution and lowered synaptic recruitment of GluA1/GluA2-AMPARs Glycine (NMDAR agonist) induced synaptic GluA1-AMPAR recruitment blocked with GHS-R1α inverse agonists Inhibition of constitutive GHS-R1α activity lesioned GluA1-AMPARs Ser234/235 and CaMKIV phosphorylation. No changes in GluA1-AMPARs Ser231, star-gazin Ser230/234 or Akt Ser473 phosphorylation. Ser234,GluA1-AMPAR phosphorylation by tonically active GHS-R1α mediated synaptic GluA1-AMPAR recruitment (hippocampal slices) Tonic activity of GHS-R1α inhibits CaV1 currents, including CaV1.3, and mainly CaV1.2, by reducing presynaptic CaV densities Inhibition of presynaptic GABA release, but not glutamate release, through tonic GHS-R1α activity and its interference with CaV2.3 currents | (Luis F. Ribeiro et al., 2020) |
| Deletion or overexpression of (mutant) GHS-R1α | In vitro primary hippocampal neurons (GHS-R1α-/- or GHS-R1α+/-) Ex vivo Mouse hippocampal slices (GHS-R1α-/- or GHS-R1α+/-) | No differences in resting membrane potential or LTP threshold between GHS-R1α-/- and GHS-R1α knockout hippocampal slices in DG granule cells and CA3 pyramidal neurons |                                                                                           | (Damonte et al., 2018) |
| Interactive effects of GHS-R1α | Study details | LTP & memory | Biochemical & histological observations                                                                 | References       |
| AG & dopamine | In vitro GHS-R1α and D1R co-expressing HEK293(AEQ17) cells or GHS-R1α-GFP and D1R co-expressing SK-N-SH cells IHC Ghsr-IRES-tauGFP mouse brain slices | N/A | Co-expression of GHS-R1α and D1R in the dorsal hippocampus, cortex, substantia nigra, ventral terminal area and midbrain Dopamine-induced cAMP accumulation, yet AG did not in, GHS-R1α/D1R co-expressing HEK293 cells. PKC | (Jiang et al., 2006) |
### Table 1 (continued)

| Compound | Study details | LTP & memory | Biochemical & histological observations | References |
|----------|---------------|--------------|----------------------------------------|------------|
| AG, and D1R agonist (SKF81297 and GHS-R1α antagonist (YIL781)) | In vitro addition to (mutant) GHS-R1β/D1R co-transfected HEK-293 T cells and primary rat hippocampal or striatal neurons | N/A | | (Navarro et al., 2016) |
| GHS-R1α agonist (MK-066) and dopamine agonist (SKF81297) | In vitro addition to (mutant) GHS-R1α/D1R-overexpressing HEK293T cells and primary mouse hippocampal neurons | N/A | | |
| Human post-mortem brain tissue of AD patients | | | | |
| • Impairment in stimulus-evoked EPSPs across CA3 – CA1 synapses in 9 month old GHS-R1α−/− mouse hippocampal slices. | | | | |
| • Deteriorated MWM spatial memory retention in 9 month old GHS-R1α−/− mice, resembling age-matched 5xFAD mice. | | | | |
| • Deficits in stimulus-elicited EPSPs across CA3 – CA1 synapses and mEPSCs (postsynaptic receptor conductance) in CA1 neurons in 4 month old 5xFAD mouse organotypic slices. The latter reversible with GHS-R1α and D1R agonist co-treatment, but not individual drug use. | | | | |
| • Weakened MWM spatial learning and reference memory in 5xFAD independent, multi-fold synergistic augmentation of cAMP accumulation by dopamine and AG co-treatment. | | | | |
| • AG induced Ca²⁺ mobilization in GHS-R1α and D1R-expressing HEK293-AEQ17 cells, but dopamine and dopamine/AG co-application had no effect. | | | | |
| • Non-canonical Gαi(o) coupling to GHS-R1α/D1R (instead of canonical Gαs/11 coupling to GHS-R1α) when bound to AG | | | | |
| • Co-application of dopamine and AG induced surface GHS-R1α/D1R heterodimerization. In the absence of any receptor agonists, GHS-R1α/GHS-R1α homodimer formation. | | | | |
| • Inverted U-shape association between relative ratio of GHS-R1β:GHS-R1α (0, 0.25, 0.5, 1.4, and 5) and plasma membrane exposition of GHS-R1α. | | | | |
| • Membrane localization of GHS-R1α, AG/GHS-R1α-signaling, Ca²⁺-mobilization, ARF6, arrestin-2 recruitment, ERK1/2 phosphor-ylation and inhibition of forskolin-driven cAMP accumulation were maximized at a 0.5 ratio of GHS-R1β:GHS-R1α and greater than in GHS-R1α uni-expressing cells. | | | | |
| • GHS-R1α and GHS-R1β capable of forming heterotrimers and heterotetramers | | | | |
| • Greater total expression of GHS-R1α and GHS-R1β and relative GHS-R1β/GHS-R1α ratio in striatal compared to hippocampal neurons. Transfection with GHS-R1β enhanced AG-driven activation of GHS-R1α and, in contrast to HEK293T cells, D1R-dependent cAMP accumulation in hippocampal neurons, but the inverse effect in striatal neurons. | | | | |
| • While GHS-R1α typically Gαs/11-coupled, GHS-R1β:GHS-R1α heteromers were Gαi/o-coupled. Association of GHS-R1β/GHS-R1α with D1R, as present in primary hippocampal/striatal neurons, induced switch from Gαs/11 to Gαi/o | | | | |
| • In the presence of GHS-R1α, GHS-R1β and D1R, Gαs/11-mediated and GHS-R1α/D1R-dependent increase in the dimerization of GHS-R1α, GHS-R1β and D1R and cAMP accumulation following dopamine or AG treatment. No synergistic effects of dopamine/AG on cAMP accumulation. | | | | |
| • GHS-R1β necessary for GHS-R1α/D1R receptor dimerization and AG-induced increase in cAMP | | | | |
| • Elevated GHS-R1α levels that correlated with soluble Aβ40 and Aβ42 levels in the hippocampus of AD patients and 5xFAD mice. | | | | |
| • Increased Aβ1-42/GHS-R1α complex formation and lessened GHS-R1α/D1R heterodimers, which inversely correlated with soluble Aβ40 and Aβ42 levels, observed in AD human brain tissue and in comparison to age-matched controls. Age-dependent increase of Aβ1-42/GHS-R1α complexes and GHS-R1α/D1R heteromers in 5xFAD mice (from 4 to 9 months). | | | | |
| • Association of Aβ1-42 with GHS-R1α (residues 42–116) and Aβ1-42/GHS-R1α complex formation (HEK293T cells) | | | | |
| • Oligomeric Aβ1-42 antagonized MK-0677-induced activation of GHS-R1α and GHS-R1α/D1R heterodimerization. D1R (continued on next page)
et al. 2005)), the stargazin-associated GluA1-AMPAR phosphorylation at Ser831 and the PKC-dependent synaptic maintenance of GluA1-AMPARs (Ribeiro et al., 2014). Notably, the Ser831 phosphorylation of GluR1-AMPARs reduces their activation threshold and improves channel conductance (Kristensen et al., 2011), whereas the phosphorylation of stargazin itself and its interaction with synaptic PDZ domain scaffold complexes, such as PSD-95, traps AMPARs in synapses (Opazo et al., 2010; Tomita et al., 2005). Interestingly, despite the fact that GHS-R1a/Gq/11/PLC/IP3 signaling both raises DAGly and releases Ca2+ from the ER stores (Reich and Holscher, 2020; Yin et al., 2014), the hippocampal activity of the Ca2+-sensitive CaMKII was unaltered over a 20 h period with a GHS-R1a agonist (Ribeiro et al., 2014), suggesting that the intracellular Ca2+ levels were unaltered. A possible reason might be that the tonic GHS-R1a activity and AG-binding elicited the endocytosis of N- and P/Q-type CaV3.2 in hippocampal neurons, hence limiting Ca2+ insteam (Damonte et al., 2018; Lopez Soto et al., 2015; Mustafa et al., 2017). Given that PKC may be jointly activated by DAGly and Ca2+ accumulation or DAGly alone (Miningou and Blackwell, 2020), long-term protein activity and Ca2+-sensitive signaling presumably maintains synaptic AMPARs and initiates CREB-driven plasticity gene synthesis in a Ca2+-independent manner, through the GHS-R1a/Gq/11/DAGly/PKC/stargazin pathway. Furthermore, Gq/11-induced PKC activity is linked to the stimulation of Raf, mitogen-activated protein kinase (MAPKK/MEK), extracellular signal-regulated kinases (ERK) and, ultimately, ERK-evoked translation of plasticity-enhancing transcription factors, such as CREB, in the hippocampus (Fig. 2) (Miningou and Blackwell, 2020). The fact that PKC is only activated by DAGly, and not both DAGly and Ca2+, may explain the delayed PKC activation (observed at 5 h and 20 h, but not acutely, ex vivo) (Ribeiro et al., 2014) and retarded downstream ERK1/2 activation (occurring at 2 h, but not 1 h, in the DG in vivo) (Chen et al., 2011) following AG treatment.

In agreement with the role of AG in AMPAR regulation, the i.c.v. microinjection of a GHS-R1a antagonist downregulated the transcriptional levels of GluA1-AMPARs in the rat hippocampus, worsening passive avoidance memory consolidation (Beheshi et al., 2020). Furthermore, the constitutive activity of GHS-R1a is involved. A pre-print paper of a study of a combination of hippocampal primary neurons and slice culture, reported that the use of GHS-R1a inverse agonists encouraged a more diffuse surface distribution of GluA1/GluA2-AMPARs, lessened their basal and glycine (NMDAR)-induced synaptic recruitment, weakened GluA1 phosphorylation at Ser845, decreased AMPA/NMDA current ratios at CA3 – CA1 synapses and worsened the murine acquisition of object memories following the i.p. injection of the BBB-penetrant GHS-R1a inverse agonist AZ12861903 (Ribeiro et al., 2020).

Synoptically, in the short-term, the AG-induced oligomerization of hippocampal GHS-R1a/GHS-R1a/D1R leads to the Gq/11/CAMP/PKA-driven GluA1-AMPAR-phosphorylation at Ser845, priming them for the NMDAR activity-induced recruitment to hippocampal synapses. In the long term, the delayed activation of the GHS-R1a/PKC/stargazin axis, the stargazin-induced phosphorylation of GluA1-AMPARs at Ser831 and the PKC-conveyed induction of ERK1/2 maintain the synaptic insertion of AMPARs. The latter effects of AG on AMPAR recruitment, in combination with enhancing stimulus-evoked presynaptic glutamate release (Chen et al., 2011; Gheresi et al., 2015), likely explain the reduction in the LTP generation threshold in the CA1 and DG (Diano et al., 2006; Gheresi et al., 2015), the in vivo increase in postsynaptic excitability in DG granule cells and the sustainment of long-lasting LTP in the DG (Chen et al., 2011) following AG treatment.

Besides AMPARs, AG modulates NR1- and NR2-NMDAR subunits (Fig. 2). The latter may be composed of seven subunits, including the obligatory NR1 and glutamate-interacting NR2A-D or, in some cases, NR3A. Typically, NMDARs are found in the form of NR1/NR2A/NR2B trimerodimers or NR1/NR2(A/B) heterodimers in the forebrain. Interestingly, GHS-R1a is not only located closely to dendritic NMDARs in pyramidal neurons, but also the bath application of AG enhanced the NMDAR activity-induced recruitment to hippocampal synapses. In the long term, the delayed activation of the GHS-R1a/PKC/stargazin axis, the stargazin-induced phosphorylation of GluA1-AMPARs at Ser831 and the PKC-conveyed induction of ERK1/2 maintain the synaptic insertion of AMPARs. The latter effects of AG on AMPAR recruitment, in combination with enhancing stimulus-evoked presynaptic glutamate release (Chen et al., 2011; Gheresi et al., 2015), likely explain the reduction in the LTP generation threshold in the CA1 and DG (Diano et al., 2006; Gheresi et al., 2015), the in vivo increase in postsynaptic excitability in DG granule cells and the sustainment of long-lasting LTP in the DG (Chen et al., 2011) following AG treatment.

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Table 1 (continued)

| Compound | Study details | LTP & memory | Biochemical & histological observations | References |
|----------|---------------|--------------|----------------------------------------|-------------|
| AG       | mice alleviated with 30 day peripheral injections of both GHS-R1α and D1R agonists. | agonist/D1R-signaling unaltered by Aβ42 (HEK293T cells) | Decreased postsynaptic CaMKII Thr286, phosphorylation in 9 month old and GHS-R1α-/-, 5 × FAD, and and GHS-R1α-/-/5 × FAD mice compared to control mice. | (Ribeiro et al., 2014) |
|          |               |              | • AJ1-42 damaged synapses and blunted synaptogenesis by MK-0677 or a D1R agonist in hippocampal neurons. The co- | |
|          |               |              | activation of GHS-R1α and D1R protected from AJ1-42-driven synapse loss, induced GHS-R1α/D1R heterodimerization, disso- | |
|          |               |              | ciated AJ1/GHS-R1α complexes and restored GHS-R1α ligand sensitivity (hip- | |
|          |               |              | pocampal neurons and HEK293T cells) | |
|          |               |              | • 30 day co-injection of GHS-R1α/D1R agonists into 5xFAD mice alleviated CA1 | |
|          |               |              | synapse loss, heightened GHS-R1α/D1R heterodimer formation, lessened GHS- | |
|          |               |              | R1α/AJ complex levels plus increased | |
|          |               |              | birth of new neurons (DCX) in the DG. No change in total CA1 AJ1 plaque deposition, Tau burden nor Tau | |
|          |               |              | hyperphosphorylation. | |
Hippocampal effects of AG:
- NMDAR-mediated EPSPs \( \uparrow \) (CA1)
- LTP threshold \( \downarrow \) & LTP extension (DG)
- nNOS activity / NO production \( \downarrow \) (DG)
- iPSFs \( \uparrow \) (DG)
- Postsynaptic intracellular Ca\(^{2+}\) levels (CAMKII activity) unaltered
- Rag-1 levels \( \uparrow \) (DG)
- (Transient) synapse formation \( \uparrow \) (CA1)
- (Transient) dendritic spine density \( \uparrow \)
- IGF-1 levels \( \uparrow \)
- BDNF \( \uparrow \) (CA3)
- Egr-1 expression \( \uparrow \) (DG)
- Neurogenesis \( \uparrow \)

(caption on next page)
outcomes (Ghersi et al., 2015). Similarly, intermittent fasting, a condition that is linked to the systemic release of AG, led to the hippocampal threshold in the DG and impaired step-down memory consolidation, the former which raises the LTP generation (~10%) heightened the number of NR2B-NMDAR-positive neurons in the CA1 and DG in organotypic slices. Moreover, whilst NR2B antagonism raised the LTP generation threshold and prolonging LTP expression in an NMDAR-independent manner in the DG. The latter involve the enhancement of NR1 and NR2B phosphorylation in the CA1, whilst lowering the LTP generation threshold and prolonging LTP expression in an NMDAR-independent manner in the DG. The latter is induced by the interaction of either AG or dopamine with GHS-R1α/GHS-R1β-Rheteromers, leading to the exocytosis-promoting GluA1 phosphorylation at Ser831, which primes AMPRs for their activity-induced synaptic recruitment. PKA further phosphorylates NR1-NMDARs at Ser896 and activates Fyn, which evokes NR2B-NMDAR phosphorylation at Tyr1336, resulting in the enhanced surface exposition and synaptic anchoring of NMDARs. It is still enigmatic how AG triggers GluA1-AMPAR (Ser845) co-phosphorylation by elevating PI3K/Akt-signaling, possibly occurring indirectly through increasing the hippocampal and plasma IGF-1 levels (further discussed in the main text). In the long term (20 h), the binding of AG to GHS-R1α results in a receptor switch from Gαs/olf to Gαq/11 and canonical PLC/IP2/IP3/DAG/PKC-signaling, which stimulates starargin to enhance GluA1-AMPAR conductance (Ser831 phosphorylation) and synaptic AMPAR trapping, supports the synaptic localization of NMDARs (PKA-mediated NR1-NMDAR phosphorylation at Ser831) and creates an IREK/CREB to sustain long-term plasticity. Notably, the LTP threshold-reducing effects of AG have partially been associated with enhancing NO/nNOS activity in the DG. Further studies are necessary to investigate this phenomenon, since AG does not alter the intracellular Ca2+ levels and drives plasticity by elevating AMPAR, but not NMDAR, currents in the hippocampus. Rising hippocampal AG levels, for example in response to caloric restriction, or AG treatment further transiently augment the presynaptic vesicle density, spineogenesis and synapse formation, promote neurogenesis (and, thus, memory resolution and pattern separation in the DG; see Section 4.2.2) and elevate Rag-1, IGF-1 and BDNF levels in the hippocampus; all events that are associated with improved plasticity and the formation of declarative memories. Lastly, AG elicits the mesolimbic transmission of dopamine to the hippocampus, while inhibiting the plasticity-suppressing release of 5-HT at synaptic terminals. Finally, in postsynaptic neurons and the absence of GHS-R1α, the co-binding of AG and dopamine induces the oligomerization of GHS-R1α with D1R. Once assembled, AG-binding to the receptor oligomers augments dopamine-induced cAMP accumulation across D1R, while the interaction of dopamine with Gαs/olf-Rheteromers leads to non-canonical Gαq/11-signaling, ultimately enhancing memory consolidation. Notably, GHS-R1α further dimerizes with 5-HT2R-R (not shown) in the hippocampus, whereas the interaction of 5-HT with GHS-R1α/5-HT2R complexes abolishes hippocampal AG-signaling, reduces plasticity and interferes with the consolidation of hippocampus-dependent (i.e. spatial or passive avoidance) memories. Dashed lines indicate incompletely understood signaling pathways. Abbreviations: acylated ghrelin (AG); growth hormone secretagogue receptor type 1α (GHS-R1α/GHS-R1β); dopamine receptor subtype 1 (D1R); serotonin (5-HT); γ-amino butyric acid (GABA); γ-aminobutyric acid receptor (GABAR); serotonin receptor 2c (5-HT2c-R), voltage-gated Ca2+ channel (CaV); α-aminohexanoic acid (GABA), γ-aminobutyric acid receptor (GABAR); serotonin receptor 2c (5-HT2c-R), voltage-gated Ca2+ channel (CaV); α-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR); N-methyl-D-aspartate receptors (NMDAR); excitatory postsynaptic potential (EPSP); inhibitory postsynaptic potential (IPSPs); cyclic adenosine monophosphate (cAMP); cAMP response element-binding protein (CREB); protein kinase A (PKA); phosphoinositide 3-kinase (PI3K); phospholipase C (PLC); phosphatidylinositol 4,5-bisphosphate (PIP2); inositol trisphosphate (IP3); diacylglycerol (DAG) protein kinase C (PKC); neuronal nitric oxide synthase (nNOS); mitogen-activated protein kinase (MEK), extracellular signal-regulated kinases (ERK); early growth response protein 1 (Egr-1); insulin-like growth factor 1 (IGF-1); insulin-like growth factor 1 receptor (IGF-1R); brain derived neurotrophic factor (BDNF); recombination activating gene 1 (Rag-1); dentate gyrus (DG); ventral tegmental area (VTA); dorsal raphe nucleus (DRN).

bath application of AG modestly (~10%) heightened the number of NR2B-NMDA-positive neurons in the CA1 and DG in organotypic slices. Moreover, whilst NR2B antagonism raised the LTP generation threshold in the DG and impaired step-down memory consolidation, the administration of AG, which lowered the DG LTP generation threshold even in the absence of the NR2B antagonist, prevented these detrimental outcomes (Ghersi et al., 2015). Similarly, intermittent fasting, a condition that is linked to the systemic release of AG, led to the hippocampal upregulation of NR2B-NMDAR subunits, enhanced plasticity and improved spatial memory in vivo (Fontan-Lozano et al., 2007). Another study showed that exogenous AG, in a GHS-R1α-dependent manner, activated Fyn kinase, leading to the Fyn-dependent phosphorylation of NR2B at Tyr1336 in the CA1 (Berrout and Isokawa, 2018). Notably, Fyn-mediated NR2B phosphorylation at various Tyr residues prevents the endocytosis of synaptic NMDARs, thus sustaining LTP (W. Lu et al., 2014). As such, AG-binding to Gαs/olf-coupling GHS-R1i:GHS-R1α/D1R heteromers probably accounts for the induction of Fyn (Fig. 2). In summary, the evidence suggests that AG/GHS-R1α-signaling mildly stimulates the expression of the NMDAR subunits in both the CA1 and DG, whilst inducing the phosphorylation of NR1 via PKC at Ser896, and PKA at Ser896, as well as NR2B at Tyr1338 by Fyn, in the CA1. This local enhancement of NR1 and NR2B phosphorylation in the CA1, which promotes the surface presentation and synaptic recruitment of NMDARs (Scott et al., 2001; Lu et al., 2015; Przybylowski et al., 2005), might be partially responsible for the augmentation of classic NMDAR-induced EPSPs that were observed in response to AG or MK-0677 treatment in CA3 - CA1 synapses (Ribeiro et al., 2014; Muniz and Isokawa, 2015). Intriguingly, the evidence suggests that AG and its receptor drive plasticity in a distinct manner in area CA1 (NMDAR activity-dependent) versus the DG (NMDAR-independent). As explained earlier, AG boosted classic NMDAR-dependent LTP in the CA1 and required NMDAR activity for the synaptic recruitment of GluA1-AMPARs (Ribeiro et al., 2014; Muniz and Isokawa, 2015). However, the GHS-R1α agonist MK-0677 and tonic activity of GHS-R1α did not increase NMDA currents, but elevated AMPA conductivity, in CA1 synapses (Ribeiro et al., 2014; Ribeiro et al., 2020). This suggests that NMDAR activity is required as a signal to trigger the postsynaptic AMPAR incorporation, whereby AG/GHS-R1α-signaling facilitates this process by stimulating AMPAR surface presentation and locking synapse-rerecruited AMPARs in place. In stark contrast to area CA1, NMDAR antagonism did not affect the LTP-potentiating, LTP-extending nor LTP generation threshold-lowering effects of AG in the DG (Chen et al., 2011; Gheresi et al., 2015). Chen et al. (2011) denoted this phenomenon as ‘a new form of synaptic plasticity’ that is dependent on the atypical activation of PI3K-signaling following AG treatment, yet does not necessitate high frequency stimulation or NMDAR induction. PI3K plays a crucial role in the DG, with PI3K inhibitors blocking the local presynaptic glutamate release and execution of LTPs (Kelly and Lynch, 2000). In particular, a training-induced increase in BDNF/TbrkB/PI3K/Akt-signaling, as dependent on the BDNF-driven downstream activation of PI3K and ERK1/2 (Gottschalk et al., 1998), mediate hippocampal plasticity and spatial memory formation (Mizuno et al., 2003). Indeed, AG was shown to activate the PI3K/Akt pathway in DG-derived neural stem cells (Chung et al., 2013; Johansson et al., 2008). AG might also indirectly induce Akt/PI3K-signaling via IGF-1, given that a GHS-R1α agonist was shown to elevate IGF-1 synthesis in the hippocampus of healthy rodents (Frago et al., 2002; Dyer et al., 2016). Further knockdown evidence suggests that GHS-R1α located on afferent fibers of the vagus nerve, as activated by circulatory AG (Diet, 2012), influences the expression of the PI3K/Akt-inducing BDNF in, at least, area CA3, also affecting object recognition memory (Davis et al., 2020). Finally, the greater density of GHS-R1α:DI1R co-expressing neurons in the DG and CA3 compared to the area CA1 (Kern et al., 2015) might lead to subregional differences in AG-signaling that, possibly, alter the dependency on NMDAR activity in the CA1 and DG areas (Fig. 2).

4.1.2. Acylated ghrelin restricts neuroinhibitory GABA release in the hippocampus

AG and its cognate receptor further regulate GABAergic...
neurotransmission. Interestingly, it was revealed that the tonic activity of GHS-R1α dampens electric currents in CaV2.1 and CaV2.2. Indeed, the overexpression or deletion of GHS-R1α in primary hippocampal neurons confirmed that, predominantly by weakening CaV2.2 conductivity, GHS-R1α attenuated neurotransinhibitory GABA release in vitro, while decreasing iPSs in the granule cell layer of the DG ex vivo (Damon et al., 2018) (see also Fig. 2). In area CA1, however, (Ribeiro et al., 2014) saw no differences in NMDA/GABA currents after 20 h GHS-R1α agonist treatment in organotypic slices, suggesting that AG might only suppress the release of GABA acutely or, selectively, in the DG.

Likewise, in a GluA3-driven fashion, tonically active GHS-R1α suppressed N- and P/Q-type CaV2 currents by engaging CaVβ2 to trap CaVα1 subunits in the ER to reduce the surface expression of CaV2.1/2.2, but also CaV1 and CaV3, in primary hypothalamic neurons (Lopez Soto et al., 2015; Mustafa et al., 2017). Moreover, CaVα-channel inhibition correlated with the expression levels of GHS-R1α (Lopez Soto et al., 2015). AG-binding to GHS-R1α evoked a switch from GluA1 to GluA1/2, and GluR, equally resulting in the quenching of CaV2.1 and (preferentially) CaV2.2 currents in a CaVβ-dependent manner. Indeed, CaV2 blockade by constitutively active GHS-R1α, synergistically augmented by receptor interaction with AG, suppressed stimulated, but not spontaneous, iPSs in hypothalamic neurons (Lopez Soto et al., 2015). Given that GHS-R1α is expressed throughout the dorsal hippocampal formation (Guan et al., 1997; Zigman et al., 2006), GHS-R1α/AG-induced CaV2 internalization may attenuate GABA release not only in the DG (Damon et al., 2018), but throughout the hippocampus.

4.1.3. The ghrelin system potentiates dopamine transmission and novelty learning

AG poses a major component of the food/reward machinery that is navigated by the mesolimbic transmission of dopamine. Although the locus coeruleus is the primary source of dopamine for the dorsal hippocampus, the VTA also partially supplies the hippocampus with dopamine (McNamara and Dupret, 2017; Serrenho et al., 2019).

Generally, colicin restriction and the associated plasma release of AG, in a GHS-R1α-dependent fashion, stimulate orexin-expressing neurons in the lateral hypothalamus which, subsequently, induce VTA dopaminergic neurons. The latter was necessary to induce postprandial dopaminergic spikes in vivo (Cone et al., 2014). Furthermore, the expression of GHS-R1α has been confirmed for VTA neurons (Abizaid et al., 2006). Receptor activation by its ligand AG leads to the mesolimbic projection of dopamine from the VTA to the nucleus accumbens, stimulating food-seeking behavior, meal intake and, if given centrally, locomotion in a GHS-R1α-dependent manner (Abizaid et al., 2006; Cornejo et al., 2018; Jerlhag et al., 2007, 2012; Quarta et al., 2009; Skibicka et al., 2011, 2012). Moreover, GHS-R1α co-localized with cholinergic neurons in the laterodorsal tegmental area that projects towards the VTA (Dickson et al., 2010), whereby the AG-driven stimulation of locomotion, food intake and accumbal dopamine outflow necessitated nicotinic acetylcholine receptors (nACHRs) in the VTA (Dickson et al., 2010; Jerlhag et al., 2006, 2008). On the other hand, the pre-test and systemic utility of GHS-R1α antagonists prevented reward-induced dopamine release, locomotor induction and psychostimulant/alcohol-encouraged conditioned place preference (Engel et al., 2015; Jerlhag et al., 2010, 2009; Skibicka et al., 2011, 2012; Sustkova-Fiserova et al., 2014).

Intriguingly, the AG-driven stimulation of the mesolimbic VTA pathway boosts the formation of hippocampus-dependent memories. A study showed that the microinjection of AG in close proximity to the hippocampus, in a D1R-dependent manner, enhanced memory acquisition in the novel object task (Jacoby and Currie, 2011). Indeed, photo-stimulation revealed that VTA dopaminergic neurons project towards the CA1 to stabilize food reward/location memories during training and improve spatial recall in a crossword-like maze (McNamara et al., 2014). Furthermore, the intra-VTA injection of a GHS-R1α antagonist impeded the hippocampus-mediated consolidation of passive avoidance memories (Behehsi and Aslani, 2018). Another study found that the joint administration of ineffective doses of AG (intra-CA1) and nicotine (subcutaneous) could, synergistically, prevent morphine-driven impairments in the consolidation of passive avoidance memories (Nazarri-Serenjeh et al., 2019). Thus, AG engages the dopaminergic-cholinergic reward axis in the VTA to potentiate hippocampus-dependent novelty (spatial) learning and the consolidation of passive avoidance memories.

Indeed, GHS-R1α augments hippocampal plasticity in cooperation with D1R (see Fig. 2). In the absence of ghrelin, GHS-R1α hetero-dimerized with D1R in neurons across multiple brain regions, including the hippocampal CA1–3 and DG, but also the cortex, midbrain, substantia nigra and the VTA (Jiang et al., 2006; Kern et al., 2015; Tian et al., 2019). Typically, the interaction of dopamine with D1R leads to recruitment of Gαi, intracellular cAMP amassing and PKA activation; all processes that have been associated with hippocampal long-term memory (Abel et al., 1997; Huang and Kandel, 1995). Following GHS-R1α:D1R heterodimer formation, however, the downstream signaling of dopamine is altered, leading the coupling of Gαi to D1R (as opposed to Gαi), PLC and IP3 activation, intracellular Ca2+ release, the induction of CaMKII, the CaMKII-orchestrated phosphorylation of AMPAR subunits at sites associated with synaptic plasticity, synaptic rearrangements, the exocytosis of glutamate receptors and the expression of early hippocampal plasticity markers (Kern et al., 2015). Notably, the intra-AG infusion of a D2R agonist post extinction training enhanced the consolidation of extinction memories, while the pre-test application of these drugs heightened food location working memory in the T-Maze, suggesting that hippocampal dopamine-signaling improved spatial memory retrieval. However, the latter memory improvements by the dopamine agonist were abolished by the co-administration of a GHS-R1α antagonist or in response to GHS-R1α/- knockout, likely by interfering with GHS-R1α:Gαi heterodimers (Kern et al., 2015). Besides dopamine, AG also acts as a ligand for hippocampal GHS-R1α:D1R heterodimers, which, at least in the absence of GHS-R1β, amplifies dopaminergic signaling. In vitro, the co-presence of dopamine and AG induced GHS-R1α:D1R heterodimerization, while the binding of AG to GHS-R1α:D1R complexes recruited Gαi/0 (instead of the canonical coupling of Gαi/0 to GHS-R1α) to synergistically potentiate dopamine/D1R-driven cAMP accumulation (Jiang et al., 2006) (Fig. 2). Notably, recent post-mortem investigations discovered that Aβ directly interacts with amino acid residues 42–116 of GHS-R1α in the hippocampus of AD patients, which both desensitized GHS-R1α to AG and impaired the association of GHS-R1α with D1R. In turn, the 30 day co-injection of AG and dopamine analogues opposed the loss of GHS-R1α:D1R heterodimers, prevented Aβ1–42-GHS-R1α complex formation, lessened synapse loss, restored stimulation-induced LTP and raised neurogenesis in the CA1, resulting in enhanced spatial memory acquisition and reference memory in 5xFAD animals in the MWM (Tian et al., 2019). As such, being impaired in AD, the hippocampal interaction of GHS-R1α with D1R facilitates plasticity and declarative memory.

4.2. Other plasticity- and memory-related processes

Besides modulating neurotransmission, AG exerts other plasticity-facilitating effects in the hippocampus that will be presented below (Section 4.2.1). Furthermore, the neurogenesis-enhancing properties of AG strengthen hippocampal memory through an LTP-independent process, known as hippocampal pattern separation (Section 4.2.2).

4.2.1. Ghrelin induces transient structural changes in the hippocampus

Indeed, studies in healthy rodents demonstrated that peripherally administered AG traversed the BBB, diffused into the dorsal hippocampus CA1, CA3 and DG, interacted with GHS-R1α, upregulated CA1 spine density and strengthened CA1 EPPSPs. In contrast, ghrelin-null mice displayed lower spine synapse numbers in the CA1 (Diano et al., 2006). Strikingly, the cerebral deficits observed in GHS-R1α knockout mice,
including decreased synapse density in the CA1, weakened stimulus-induced LTP and EPSPs across CA3-CA1 synapses and even Aβ burden, paralleled those in the 5xFAD animal model (Tian et al., 2019). This implies that both GHS-R1α and ligand-receptor interactions modulate the hippocampal architecture to facilitate plasticity.

In the DG, the intra-hippocampal infusion of AG dose-dependently increased the generation of nitric oxide (NO) by neuronal NO synthase (nNOS), lessened the hippocampal threshold to trigger LTP and improved passive avoidance memory consolidation. Furthermore, the hippocampal LTP threshold across all rodent groups, as lowered by AG, was inversely correlated to memory retention. However, while a NOS inhibitor prevented the memory enhancement bestowed by AG, NO synthase blockade could only partially reverse the AG-evoked decrease in the LTP threshold (Carlini et al., 2010). This suggests that AG facilitates LTP induction in the DG by NO/NO-independent means as well, likely by augmenting glutamatergic and weakening GABAAergic neurotransmission (see Fig. 2).

Generally, the activity of the Ca\(^{2+}\)-sensitive NOS is coordinated by the intracellular Ca\(^{2+}\) levels, and its induction is linked to the enhancement of presynaptic vesicle recycling, glutamate release and LTPs in excitative neurons of the hippocampus and cortex (Hardingham et al., 2013). As we argue in Section 4.1.1, AG-signaling does not appear to alter the intracellular Ca\(^{2+}\) levels in hippocampal neurons, however. Furthermore, AG-driven LTP potentiation was NMDAR-dependent in the CA1 (Ribeiro et al., 2014; Muniz and Isokawa, 2015), but not DG (Chen et al., 2011; Ghersi et al., 2015). Even in the CA1, AG and GHS-R1α boosted AMPA, as opposed to NMDA, currents (Ribeiro et al., 2014; Ribeiro et al., 2020). Thus, it is unlikely, albeit not impossible, that AG induces NMDAR channel opening to elevate the intracellular Ca\(^{2+}\) levels and nNOS activity in DG neurons. Indeed, the mechanisms underlying AG and GHS-R1α-induced plasticity in the DG, such as non-canonical PI3K/Akt activation, receptor interactions or, possibly, the engagement of IGF-1 and BDNF, require further studies.

AG was further shown to modulate synapses. In a GHS-R1α-mediated manner, AG increased the density of polymerized actin in rat hippocampal slices (Berrout and Isokawa, 2012). This indicates that AG rearranged dendritic spines to generate additional synapses, as observed in the hippocampus in vivo (Diano et al., 2006). In contrast, both the withdrawal of AG and GHS-R1α antagonism showed that these dendritic and synaptic changes are transient (Berrout and Isokawa, 2012). Similarly, in the cortex, in a GHS-R1α- and dose-dependent fashion, AG boosted synapse densities, network outgrowth and activity (Stoyanova and le Feber, 2014; Stoyanova et al., 2013).

Interestingly, besides boosting synaptic vesicle and postsynaptic membrane receptor formation, AG elicited the expression of recombination activating gene 1 (Rag-1) in the CA3 region of wild-type mice (Wang et al., 2013). Although Rag-1 is primarily expressed by T- and B-lymphocytes, there is evidence that Rag-1, at least in part, contributes to the formation of spatial memory (Cashman et al., 2003; Fang et al., 2013; Marin and Kipnis, 2013).

In summary, when systematically released upon fasting, circulating AG diffuses across the BBB into the hippocampus to induce transient dendritic actin polymerization, spine and synapse formation in area CA1, leading to greater stimulus-elicted LTP. In the DG, a yet poorly understood activation of nNOS by AG seems to lower the local LTP generation threshold. Finally, the AG-evoked expression of Rag1 might play a role in hippocampal memory.

4.2.2. Ghrelin facilitates neurogenesis and hippocampal pattern separation

Another mechanism that is associated with improved declarative memory formation is the enhancement of neurogenesis, and thus, hippocampal pattern separation by AG. Generally, in response to hippocampal activity, neuronal progenitor cells are born in the granular layer of the DG. These immature neurons subsequently mature, differentiate and are integrated as granule neurons into the DG. It has been proposed that plasticity mainly occurs in newborn DG neurons, allowing the preservation of already learned information that is stored in mature granule neurons. Although newborn neurons encode less detailed and more redundant information compared to mature DG granule neurons, their presence enhances memory resolution, hence facilitating the distinction of homogenous information (Aimone et al., 2011). Importantly, these immature DG neurons, whose total quantities are dependent on the rate of neurogenesis, contribute to hippocampal pattern separation. The latter describes the ability to discriminate and retain highly similar spatial, temporal or contextual information as well as temporally adjacent events as distinct memories (Aimone et al., 2010, 2011; Deng et al., 2010). For instance, the targeted elimination of immature DG cells, even though LTP in the CA1 area was not affected, resulted in learning deficits in the hippocampus-dependent trace conditioning task, which necessitates the association of a temporally separated CS and US (Shors et al., 2001). Similarly, the loss of neurogenesis led to impairments in the distinction of closely, but not more widely separated, stimuli (Cléland et al., 2009). Lack of neurogenesis also impaired long-term object recognition memory (48 h, but not 1 h or 24 h) (Pan et al., 2012) and, due to impeded allocentric discrimination, spatial relational memory formation and long-term memory retention in the MWM (Dupret et al., 2008; Jessberger et al., 2009; Pan et al., 2012). However, the ablation of memory resolution-enhancing immature DG neurons did not affect more simple forms of spatial navigation, such as the habituation to a new environment or contextual fear conditioning (Dupret et al., 2008). Notably, ~4-week-old and highly plastic adult-born DG granule cells were shown to facilitate hippocampal recall following training, implying that elevating the pools of these immature DG neurons with neurogenesis supports memory conservation (Gu et al., 2012).

Both cell culture and in vivo studies support that AG boosts neurogenesis in the DG. In vitro, the application of AG augmented the proliferation, but not differentiation, of primary adult rat hippocampal neural stem cells via the GHS-R1α-dependent induction of the PI3K/Akt/mTOR/p70s6K pathway, ERK1/2 and STAT3 activation as well as the inactivation of GSK-3β. The treatment with AG also heightened the transcriptional levels of GHS-R1α by these cells, suggesting the presence of a positive feedback loop (Chung et al., 2013). On the other hand, a synthetic ghrelin analogue rescued both apoptosis, necrosis and caspase-3 induction by stimulating PI3K/Akt- and ERK1/2-signaling in isolated and growth-factor deprived adult rat hippocampal progenitor neurons (Johansson et al., 2008). Thus, AG induces the non-canonical, Gα-associated activation of the PI3K/Akt and ERK1/2 pathways in DG neural stem cells, leading to enhanced proliferation (PI3K/Akt and Jak2/STAT3) and survival (PI3K/Akt and ERK1/2).

In vivo, both mice and dwarf rats were shown to display GHS-R1α-expressing immature neuroblasts in the granule cell layer of the DG (Hornsby et al., 2016; Li et al., 2013; Moon et al., 2009). Furthermore, the application of AG enhanced neurogenesis in healthy rodents (Hornsby et al., 2016; Kent et al., 2015; Zhao et al., 2014), dwarf rats (Li et al., 2013), an AD (Moon et al., 2014) as well as a PD animal model (Elabi et al., 2018). Indeed, while the daily intraperitoneal treatment with AG for 8 days promoted the numbers of proliferating (BrDU) and total (DCX) DG neuroblasts in adult mice, the antibody-mediated depletion of plasma ghrelin for the same time period resulted in the inverse outcome (Moon et al., 2009). Indeed, the neurogenesis-heightening effects of extended caloric restriction, which elevates the plasma and hippocampal AG levels, were dependent on AG (Kim et al., 2015). Similarly, the daily peripheral AG injection for 2 weeks, to mimic food restriction-associated physiological levels of AG, boosted neurogenesis in the DG of healthy rats (Kent et al., 2015). On the other hand, the knockout of GHS-R1α did not alter the DG morphology or basal rate of neurogenesis, suggesting compensatory effects (Hornsby et al., 2016). However, in contrast to their wild-type littermates, GHS-R1α−/− mice exhibited slower proliferation and exacerbated progenitor neuronal loss in the ventral DG in response to chronic social defeat stress (Walker et al., 2015). As discussed earlier, these pro-proliferative and neuroblast-preserving effects of AG and its receptor
are supported by the activation of the neuroprotective PI3K/Akt pathway (and others) in cultured primary hippocampal progenitor neurons (Johansson et al., 2008; Chung et al., 2013). Interestingly, the acute injection of AG or overnight fasting further increased the transcription of neurogenic transcription factor (Egr-1) in the DG, while prolonged caloric restriction encouraged the differentiation of progenitor into mature DG neurons in wild-type, but not GHS-R1α knockout, mice (Hornby et al., 2016). Egr-1 regulates the survival and differentiation of newborn neurons, their integration into the hippocampal learning circuit, dendrite and spine outgrowth as well as the expression of GluA1-AMPARs and several chloride ion channels, enabling immature DG neurons to receive glutamatergic and GABAergic input by other neurons (Veyrac et al., 2013). In opposition to the latter study, (Kent et al., 2015) observed no improvements in DG neuroblast differentiation following the 14 day i.p. administration of AG. Since the animals were culled 14 days after the final AG injection, any differentiation-enhancing effects might have faded by the time the rodents were biochemically assessed. Besides Egr-1, despite the fact that AG drove neurogenesis in IGF-1-deficient dwarf rats (Li et al., 2013), AG is a well-known, potent stimulator of the GH/IGF-1 axis, leading to the prompt release of the neurogenesis-stimulating and BDNF-penetrant IGF-1 (Khatib et al., 2014; Nass et al., 2011; Nieto-Estevez et al., 2016; Pan and Kastin, 2000). Moreover, controversially, even though GHS-R1α-exhibiting immature DG neurons have been identified in vivo (Hornby et al., 2016; Li et al., 2013; Moon et al., 2009), it was postulated that GHS-R1α is confined to adult DG granule cells, stimulating these to release the neurogenesis-promoting BDNF into the neurogenic niche (Buntwal et al., 2019). Peripheral AG might also stimulate the hippocampal production of BDNF across the vagus nerve (Davis et al., 2020). Caloric limitation enhanced the hippocampal production of BDNF in both control and ghrelin−/− mice, however, implying that BDNF release is ghrelin-independent (Kim et al., 2015). Synaptically, prolonged AG/GHS-R1α-signaling for 2 weeks, either achieved through regular AG injections or caloric restriction, stimulate the birth, proliferation, survival and possibly differentiation of immature DG neurons in vivo. Besides direct effects, AG likely supports neurogenesis by upregulating Egr-1 and IGF-1 levels in the DG, whilst the impact of AG on BDNF is questionable.

Importantly, AG-induced neurogenesis has been linked to the enhancement of hippocampal pattern separation and memory. Daily and 14-day-long peripheral injections of AG, to maintain plasma levels that would be anticipated in response to fasting, increased the numbers of DCX-positive young granule cells and augmented allocentric pattern separation in the spontaneous location recognition test (Kent et al., 2015). Similarly, the pre-training infusion of AG into dwarf rats, for a period of 28 days, encouraged neurogenesis, alternation behavior in the Y-maze as well as novel object exploration times, suggesting neurogenesis- and hippocampal pattern separation-associated improvements in spatial memory resolution and the distinction of (close) objects (Li et al., 2013). As such, AG promotes the birth and proliferation of DCX-positive immature neurons in the DG that enhance hippocampal memory resolution and pattern separation, shown to facilitate allocortical spatial memory and object discrimination in rodents. These neurogenesis-imparted memory improvements are independent of the plasticity-enhancing effects of AG in the hippocampus.

5. Aversive memory formation and the ghrelin system

5.1. Contextual and auditory fear conditioning

Besides the hippocampus-mediated formation of spatial memories and novelty learning, ample evidence suggests that AG and GHS-R1α mediate aversive and fear memory formation as well as fear behavior in vivo, with a recently discovered link to PTSD in humans. As a foundation, this section provides an overview of the different forms of fear conditioning and the engaged brain networks in rodents.

Fear conditioning is coordinated by a brain circuit that involves the prefrontal cortex (PFC), amygdala and hippocampus, as portrayed in Fig. 3. Of these brain areas, the hippocampus retains the context of the fear memory, whereas the amygdala encodes incoming fear stimuli, mediates their long-term consolidation and induces defensive behaviors. Furthermore, once formed, fear memories appear to be stored in the amygdala across the entire lifespan. Besides the amygdala and hippocampus, efferent inputs from the PFC regulate the amygdaloid activity (Gale et al., 2004; Homberg, 2012; Isaacs, 2015; Phelps, 2004).

To generate fear memories in animals, various paradigms may be employed. During classical (cued) fear conditioning, an amygdala-dependent process, a (CS), such as a tone or light cue, is paired with an unconditioned stimulus (US), typically a foot shock. Following training, the rodents are re-exposed to the CS and the initiation of fear, expressed as freezing, is scored. In the contextual fear paradigm, the animal undergoes inescapable foot shocks in a dedicated conditioning chamber. Therefore, when re-tested the next day, the animal may memorize the contextual (hippocampal) association that the chamber triggers a foot shock (US). However, recent studies indicate that contextual fear conditioning also involves the separate acquisition of hippocampus-independent foot shock (US) memories. Lastly, hippocampus-dependent passive avoidance training implements both contextual fear conditioning and instrumental learning. In this case, the rodents have to choose between a bright and a foot shock-promoting dark compartment (step-through) or avoid descending onto a foot shock-triggering platform (step-down) (Homberg, 2012; Huff et al., 2016; Ogren and Stiedl, 2013; Qi et al., 2018).

The simplified serial model (depicted in Fig. 3) stipulates that the CS (such as a certain tone) and US (foot shock) are generated in the auditory cortex (thalamus) and the somatosensory cortex, respectively, and transmitted to the lateral nucleus of the amygdala (LA), a subnucleus of the basolateral amygdala (BLA). Additionally, LA neuronal activity is co-modulated by sensory input from the thalamus. In the LA, an association between the CS and US is formed and the resulting fear memory is consolidated. Subsequently, LA neurons stimulate the central nucleus of the amygdala (CA) that, by consulting the periaqueductal grey, hypothalamus and brain stem, triggers the expression of fearful behavior. In opposition to auditory fear, contextual fear memories are created by relaying the hippocampal context that surrounds a fear memory to the LA. As recently postulated, initial plasticity in the BLA may generate a contextual fear memory trace (the ‘foot shock memory’) that is projected across the entorhinal cortex and ventral hippocampus to the dorsal hippocampus. Finally, following enhanced plasticity in the CA1 and CA3 regions, the contextual fear memory undergoes consolidation (Chaaya et al., 2018). Lastly, the ventromedial PFC (vmPFC) functions as a gatekeeper, enabling the BLA/CA-mediated expression of fear, once the respective CS or the context of a fear memory is presented to the animal (de Quervain et al., 2017; Ehrlich et al., 2009; Homberg, 2012; Parsons et al., 2006).

It was shown that NMDAR-signaling in the LA was mandatory for the learning of both auditory and contextual fear (Rodrigues et al., 2001). Likewise, in a foot shock- and context-separating passive avoidance paradigm, the post-training administration of a muscarinic cholinergic agonist into the BLA augmented the consolidation of both foot shock and contextual fear memories (Malin and McGaugh, 2006). As such, amygdaloid plasticity is implicated in the formation of both cued and contextual fear memories.

On the other hand, while hippocampal inactivation does not affect auditory fear memories, the hippocampus is necessary for the acquisition (CA1), consolidation (CA3) and retention of contextual fear memories for a period of up to 2 weeks (Anagnostaras et al., 1999; Daumas et al., 2005; Kim and Fanselow, 1992; Maren et al., 1997; Phillips and LeDoux, 1992). In the long-term, however, the contextual fear memory is transferred and permanently stored in the BLA (Gale et al., 2004). Indeed, intra-hippocampal treatment with a muscarinic cholinergic receptor agonist promoted the consolidation of passive avoidance, but not foot shock, memories (Malin and McGaugh, 2006), while the hippocampal infusion of an NMDAR antagonist impeded
contextual, but not auditory, fear acquisition (Bast et al., 2003). Likewise, mouse strains with superior NMDAR activity in the dorsal hippocampus acquired context-associated passive avoidance memories more easily (Baarendse et al., 2008). Indeed, contextual fear conditioning upregulated memory-facilitating ryanodine receptors and ER Ca\textsuperscript{2+} channels 5–29 h post training, strengthening EPSCs in CA1 pyramidal neurons (More et al., 2018; Trifilieff et al., 2006). Contextual fear training also induced a biphasic stimulation of ERK\textsubscript{1/2}/CREB-signaling 0–1 h and 9–12 h thereafter (More et al., 2018). This implies that contextual fear memory formation co-requires hippocampal and amygdaloid plasticity.

5.2. Ghrelin modulates amygdala-dependent forms of aversive memory in vivo

The literature emphasizes a role of AG in regulating anxiety as well as the stress-associated formation, but also extinction, of various amygdala-dependent forms of aversive memory in animals, including CTA, passive avoidance, auditory fear and contextual fear memory. Beneath, the available evidence is presented and linked to the local effects of AG in the amygdaloid subnuclei.

5.2.1. Ghrelin’s anxiolytic and anxiogenic effects are unrelated to fear memory

Dependent on the context, AG may exert anxiolytic or anxiogenic responses. As summarized elsewhere, systemic or central administrations of AG, in most cases, trigger anxiety in unstressed rodents (Fritz et al., 2020; Morris et al., 2018). Importantly, the association between anxiety, fear and fear memories has to be clarified. Anxiety may be defined as an unspecific and long-lasting expression of fear due to a previously learned fear context. In other words, to be placed into a foot shock-inducing conditioning chamber (context) may render the animal anxious. In contrast, fear is the acute display of fear in response to a fear cue (CS) (Homberg, 2012). It has been emphasized that anxiety, per se, is not necessarily linked to the increased formation of fear memories (Ogren and Stiedl, 2013). For instance, an anxious mouse strain (DBA/2J) acquired fear memories worse than their less anxious counterparts (C57BL/6J). Instead, the learning of contextual fear (passive
avoidance) memories was dependent on LTP-induction in the dorsal hippocampus (Baarendse et al., 2008; Ogren and Stiedl, 2013). This implies that, at least, the formation of contextual fear memories and the display of anxiety are separate phenomena.

5.2.2. Ghrelin exerts diverse direct effects on various amygdaloid subnuclei

Generally, the amygdala is anatomically separated into multiple subnuclei that are composed of excitatory pyramidal/principal neurons and inhibitory GABAergic interneurons. As one of these amygdaloid nuclei, the BLA consists of the LA, the basal nucleus of the amygdala (BA) and the accessory basal nucleus. While the LA projects to the basal and accessory basal nuclei, it is also connected to the medial nucleus of the amygdala (MA) and the CA (see (Tsvetkov et al., 2015) for the amygdaloid classifications and connections) (Bocchio et al., 2016). Studies revealed that GHS-R1α is present in the ventrolateral and medial divisions of the LA, in the posteroverentral division of the MA (Alvarez-Crespo et al., 2012) and the CA (Cruz et al., 2013).

In the LA, AG appears to be neuroinhibitory. It was shown that the bath application of AG diminished the frequencies of EPSCs in LA-located pyramidal neurons (Alvarez-Crespo et al., 2012). However, AG dose-dependently potentiated the firing rate of (unidentified) neuronal populations in the LA ex vivo, while intra-LA infused AG enhanced LA neuron spike frequency in a GHS-R1α-mediated manner in vivo. Unfortunately, it was neither clarified whether excitatory principal neurons or GABAergic interneurons express GHS-R1α, nor which type of neuron showed increased activity (Song et al., 2013). However, both the microinjection of AMPAR or NMDAR antagonists as well as AG into the LA blocked the acquisition of CTA (Song et al., 2013). Therefore, it is more likely that AG suppressed LA plasticity by inducing GABAergic LA interneurons.

In the CA, the superfusion of rat slices with AG synergized with ethanol to augment iPSs in the CA, whereas a GHS-R1α antagonist attenuated these. Therefore, constitutive and ligand-induced GHS-R1α-signaling enhance GABAergic transmission in the CA (Cruz et al., 2013). Notably, the CA exclusively consists of GABAergic interneurons. By projecting to areas such as the hypothalamus and brainstem, the CA stimulates anxiety-like and ethanol withdrawal behavior (Jie et al., 2018), possibly contributing to the anxiogenic effects of AG (Fritz et al., 2020; Morris et al., 2018).

5.2.3. Ghrelin-signaling in the amygdala restricts conditioned taste aversion

Interestingly, AG-signaling in the LA, a subnucleus of the BLA, modulates CTA. Generally, CTA serves to memorize what types of flavor or smell are safe to ingest or potentially hazardous (nauseating). CTA generally is present as a form of declarative memory, is conveyed by the hippocampus (Bird and Burgess, 2004). Amongst the hippocampus, amygdala and DRN, the hippocampal structure, while the hippocampus, is of lesser importance (see (Welzl et al., 2001)).

The pre-training infusion of AG into the LA attenuated the acquisition, but also the extinction, of CTA by rats, as evident 24 h later (Song et al., 2018, 2013; Zhu et al., 2013). However, the post-training or pre-test administration of AG did not interfere with the consolidation or recall of CTA (Song et al., 2013). Since CTA acquisition and recall were dependent on NMDAR and AMPAR activation in the LA (Song et al., 2013), AG presumably suppresses plasticity in LA neurons, as discussed in Section 5.2.2. On the other hand, the intra-LA application of a GHS-R1α antagonist abolished the constitutive activity of the receptor, enhanced the acquisition of CTA (Li et al., 2018), whilst GHS-R1α antagonism prevented the blockade of CTA extinction by AG (Song et al., 2018). Resembling CTA, when neonatal chicks had to learn choosing between melanlantranilate-coated or neutral beads, the intracerebroventricular administration of AG following training impeded the development of melanlantranilate-avoiding memories. This suggests that AG might also impede the consolidation of CTA (Carvajal et al., 2009).

Given the latter findings and considering the orexigenic function of AG, the acylated hormone may restrict the adoption of food adversities. However, AG does not interrupt CTA recall nor extinction to guarantee that the hungry animal avoids the consumption of food that was previously identified as nauseating.

5.2.4. The cerebral microinjection of ghrelin enhances passive avoidance memory

Indeed, AG-signaling in various brain regions enhances context-retainment in passive avoidance paradigms. The post training central or targeted microinjection of AG into the hippocampus or CA1, amygdala or BLA and dorsal raphe nucleus (DRN) enhanced the consolidation of passive avoidance memories in the step-down (1 h and 24 h later) (Carlini et al., 2002, 2004, 2007; Carlini, Gherli et al., 2010; Carlini, Perez et al., 2010; Diano et al., 2006; Gherli et al., 2011; Toth et al., 2009), step-through (24 h, 48 h and 72 h later) (Goshadrou et al., 2013) and T-Maze foot shock avoidance paradigms (1 week later) (Diano et al., 2006). Interestingly, the administration timing is crucial, since intra-hippocampal AG only augmented the consolidation of passive avoidance memories when given immediately after training, but not with a delay of 15 or 60 min (Gherli et al., 2015). In turn, the central application of a GHS-R1α antagonist dose-dependently attenuated memory consolidation, while partially blocking acquisition, in the step-through trial (Bebeshti and Shahrokhii, 2015).

Amongst the hippocampus, amygdala and DRN, the hippocampal administration of AG enhanced passive avoidance memory most potently (Carlini et al., 2004), in agreement with the hippocampal dependence of this form of learning (Ogren and Stiedl, 2013) and AG’s plasticity-enhancing effects in the hippocampus (see Section 4 and Fig. 3). Indeed, the hippocampal threshold to induce LTP, as lowered by the hippocampal post training administration of AG, was inversely correlated to the latency times in the step-down test (Carlini, Perez et al., 2010). Moreover, the CA1 microinjection of AG ameliorated the morphine-induced deficits in inhibitory avoidance memories (Nazar-Serenjeh et al., 2019), presumably by restoring the morphine-driven impairments in the release of glutamate (Guo et al., 2005). Notably, AG did not enhance the acquisition or retrieval of passive inhibitory memories (Carlini, Gherli et al., 2010). Instead, although intra-hippocampal injections of AG enhanced 1 h short-term passive avoidance memories in earlier studies (Carlini et al., 2007; Carlini, Perez et al., 2010), it was specified that AG selectively drives the consolidation of hippocampal long-term memories (Carlini, Gherli et al., 2010). Besides the hippocampus, intra-BLA administered AG, in a GHS-R1α and BLA-dependent manner, enhanced the consolidation of passive avoidance memories (Goshadrou and Ronaghi, 2012; Toth et al., 2009), whereas the central, BLA, DG or VTA injection of a ghrelin antagonist following training attenuated the performance of rats in the passive avoidance paradigm 24 h later (Bebeshti and Aslani, 2018; Beheshti et al., 2020).

Even though GHS-R1α-signaling in the BLA improved passive avoidance memory (Bebeshti and Aslani, 2018; Goshadrou and Ronaghi, 2012; Toth et al., 2009), the application of the lesion-inducer lidocaine showed that the BLA is expendable for the consolidation of passive inhibitory memories (Goshadrou and Ronaghi, 2012; Tomaz et al., 1992). Instead, the contextual consolidation of passive avoidance memories, a form of declarative memory, is conveyed by the hippocampus (Bird and Burgess, 2008). Notably, the US (foot shock)-induced stimulation of the amygdala, as occurring during fear conditioning, transiently suppresses c-Fos activity and LTPs in the CA1, interfering with contextual fear learning (Dai et al., 2008; Waider et al., 2019). As such, it is possible that the microinjection of AG into the BLA restricts the local neuronal activity, hence favoring hippocampal plasticity and the formation of inhibitory avoidance memories. This concept is supported by the fact that systemic AG diminished c-Fos activity in the BLA (Hornby et al., 2016), while intra-amygdaloid AG boosted the consolidation of hippocampus-dependent spatial memories in the MWM (Toth et al., 2010).

Taken together, the hippocampal administration of AG or microinjection into the BLA, which might improve hippocampal activity,
facilitates the context consolidation of passive avoidance memories by enhancing local glutamatergic signaling (Section 4.1.1 and Fig. 2), transiently inducing synaptogenesis (Section 4.2.1) and, possibly, enhancing dopamine transmission by the VTA (Section 4.1.3). The timing is critical, however, and AG must be given immediately post training, in combination with the US-context pairing during conditioning, to observe these consolidation-enhancing effects. The dopaminergic and transient synaptic effects of AG might explain why AG further enhanced 1 h short-term passive avoidance memory in some studies.

5.2.5. Systemic ghrelin suppresses the consolidation of auditory fear memories

Although AG promotes the consolidation of the hippocampus-dependent fear context in the passive avoidance trial (Section 5.2.4), the opposite effect is seen during amygdala-dependent auditory fear memory. A study showed that the systemic or intra-BLA application of a ghrelin analogue post conditioning blocked the retention (Harmatz et al., 2017), or showed a trend towards impaired (Meyer et al., 2014), auditory fear memories in non-stressed rodents, whereas the peripheral injection of a GHS-R1α antagonist achieved the opposite result (Harmatz et al., 2017). Furthermore, the baseline circulatory ghrelin levels were inversely correlated to long-term (48 h) auditory fear memory strength. Of note, the ghrelin analogue did not affect the acquisition or retrieval of auditory fear, nor modulate contextual fear, suggesting that AG interferes with the amygdala-imparted consolidation of auditory fear (Harmatz et al., 2017). Indeed, a study showed that daily and 14 day-long intraperitoneal injections of AG, to maintain plasma levels that would be anticipated following caloric restriction, attenuated c-Fos induction in the BLA (Hornsby et al., 2016), the hub for auditory fear formation (Homberg, 2012).

When released upon fasting, one of AG’s many functions entails the stimulation of appetite and foraging behavior (Yanagi et al., 2018). Therefore, it was proposed that AG prevents the formation of auditory (cue-based) fear memories in the absence of stress or life-threatening situations, because fear would interfere with the seeking of food (Harmatz et al., 2017).

5.2.6. Chronic stress-driven increases in plasma ghrelin potentiate auditory fear

It must be emphasized that the stress state of the animal influences auditory fear consolidation by AG. It is generally accepted that chronic, but not necessarily acute stress, augments the plasma secretion of AG (Fritz et al., 2020; Morris et al., 2018). Indeed, immobilization stress for at least 5 days heightened the plasma levels of ghrelin in rodents, even more in adrenalectomized littermates. Comparable to the 5 day intra-BLA or systemic administration of AG, these chronically immobilized PTSD mice displayed an increase in long-term auditory fear memory. Although the PTSD mice showed elevated plasma corticosterone levels, only the injection of a GHS-R1α antagonist, but not adrenalectomy, weakened the retention of auditory fear memories (Meyer et al., 2014). Interestingly, another study discovered that a single injection of a ghrelin analogue only impaired the consolidation of auditory fear memories in non-, yet not chronically, stressed rodents (Harmatz et al., 2017).

In contrast to the auditory fear memory-suppressing outcome in the absence of or upon acute stress, this implies that the chronic stress-driven plasma secretion of AG, independent of the stress-associated secretion of HPA hormones, exacerbates the amygdaloid consolidation of cued (auditory) fear memories. For an in-depth explanation, please see Sections 5.3.5 and 5.3.6.

5.2.7. Ghrelin prolongs the recall of contextual fear memories

Regrettably, in vivo studies that investigate the effects of AG-treatment in the contextual fear paradigm are lacking. Nonetheless, when contextual fear conditioning was preceded by two weeks of caloric restriction (to enhance the plasma AG levels), neurogenesis as well as contextual fear retention in these starved wild-type mice were elevated in a GHS-R1α-dependent manner 12 days after training, as compared to ad libitum-fed mice. However, contextual fear memory was unaffected during the acquisition phase or following 1 or 8 days in calorically restricted rodents. Given that AG acts as an appetitestimulating hormone, it was speculated that caloric restriction, raising the blood AG levels, simultaneously promotes fear context recall to heighten the likelihood of survival of the hungry animal (Hornsby et al., 2016). In agreement with the latter study, the genetic knockdown of GHS-R1α did not affect contextual fear acquisition and consolidation (24 h), yet it reduced long-term (30 day) contextual fear retention (Albarran-Zeckler et al., 2012). In this context of these findings, the selective post training optogenetic inhibition of highly plastic, ~4 week-old adult-born DG granule cells, but not immature DG neurons of other ages, diminished hippocampus-associated spatial (MWM) and contextual fear memory retrieval (Gu et al., 2012). The genetic attenuation of neurogenesis also deteriorated remote passive avoidance memory 21 days following conditioning (Pan et al., 2012). Therefore, the limited evidence proposes that long-term increases in plasma AG and the associated enhancement of neurogenesis in the DG (see Section 4.2.2) enlarge the pool of ~4 week-old, memory retrieval-facilitating DG immature granule neurons, thus extending the retention (recall period) of remote contextual fear memories.

5.2.8. Acylated ghrelin augments the extinction of fear memories

Generally, memories are not erased per se. Instead, extinction necessitates the acquisition of a novel extinction memory that is preferentially recollected instead of a previous fear memory. It has been postulated that novel extinction memories are produced by the joint engagement of the hippocampus, vmPFC and BLA. Once the new extinction memory is formed, the hippocampus-stimulated vmPFC inhibits the LA, preventing the expression of fear when the CS is presented. Therefore, the incoming CS (tone) in the amygdala is paired with a non-fearful context in the hippocampus, replacing the original CS (tone)-US (foot shock) association that was formed by the auditory/somatosensory cortex and amygdala (Fig. 3). Notably, besides the auditory cortex, the auditory thalamus relays tone-based stimuli (CS) to the amygdala (de Quervain et al., 2017;erry et al., 2010; Homberg, 2012; Parsons et al., 2006). Importantly, the acquisition of extinction memories necessitated amygdaloid plasticity, including NMDAR- and ERK-signaling, in the BLA (Falls et al., 1992;erry et al., 2006; Lin et al., 2003; Lu et al., 2001) and LA (Sotres-Bayon et al., 2007).

Interestingly, AG enhanced the extinction of fear in an unexpected manner. When mice underwent auditory fear conditioning, two sessions of extinction training and a final fear memory trial, food-deprived animals acquired and retained extinction memories more effectively. However, both extinction learning and retention could be prevented by the intra-LA infusion of a GHS-R1α antagonist (Huang et al., 2016). Thus, the plasma secretion of AG during caloric restriction, confirmed to be independent of glucocorticoids, mediated fear extinction by acting in the LA, possibly through inhibiting plasticity in this amygdaloid subnuclei (see 5.2.2.). In line with the extinction-promoting effects of high plasma AG, (Hornsby et al., 2016) reported that, contrary to wild-type mice, GHS-R1α−/− rodents showed poor contextual fear extinction over a period of 12 weeks. Notably, in the context of extinction, some studies suggest that classical Pavlovian conditioning triggers and sustains fear by transiently augmenting glutamatergic neurotransmission and auditory (CS) input across thalamus-LA synapses, whereas fear extinction might involve the targeted weakening of these synapses (Chalm and Huganir, 2010; Kim et al., 2007). Strangely, food deprivation and intra-LA administered AG impaired LTD at thalamus-LA synapses in a GHS-R1α-dependent fashion (Huang et al., 2016). Thus, AG does not facilitate fear extinction by blocking auditory or sensory (CS) input into the LA. Rather, by inhibiting LTD, AG supports the thalamic CS transmission to the LA. Nevertheless, this mechanism may ease extinction by selectively potentiating the relay of the CS (such as a tone), but not the
US (i.e. foot shock), to the LA. This, possibly, facilitates the pairing of the CS with a new, non-fearful context in the hippocampus. Indeed, (De la Casa, 2013) demonstrated that caloric restriction, which raises the circulatory AG levels, increased the attention of animals towards the CS (tone), preventing latent inhibition during auditory fear conditioning.

Mechanistically, AG improves the formation of extinction memories in cooperation with dopamine-signaling and via indirect effects in the hippocampus. Post training injections of a D₁R agonist into the CA1 encouraged the extinction of both contextual fear and passive avoidance memories, whereas the use of a D₁R antagonist weakened contextual fear extinction (Fiorenza et al., 2012). Strikingly, an intra-DG administered dopamine agonist promoted the consolidation of extinction memories, while the application of a GHS-R1α antagonist, by interfering with hippocampal GHS-R1α:D₁R heterodimers, cancelled these effects (Jiang et al., 2006; Kern et al., 2015). Finally, as related to the pro-neurogenic effects of AG, mice with genetic deficits in neurogenesis displayed deteriorated contextual fear extinction (Pan et al., 2012).

As such, AG-driven mesolimbic dopamine-signaling (Section 4.1.3), likely in combination with improvements in hippocampal plasticity (Section 4), as well as the AG-induced potentiation of neurogenesis and memory resolution in the DG (Section 4.2.2) facilitate the formation of extinction memories to abolish fear. How LA-administered AG, which seemingly decreases local plasticity, boosts extinction memory requires further studies, however. In conjunction, similar to chronic selective serotonin reuptake inhibitor (SSRI) treatment (Deschaux et al., 2011), the AG-induced stimulation of 5-HT neurons in the DRN and the ensuing release of 5-HT in the amygdala (Hansson et al., 2014; Ogaya et al., 2011) may further suppress fear return after extinction training (Deschaux et al., 2011; Homberg, 2012).

5.2.9. Summary: the role of acylated ghrelin and GHS-R1α in the formation of aversive memories

- While conditional, an increase in the systemic or cerebral AG levels typically induces anxiety. These effects are unrelated to aversive memory formation, however.
- GHS-R1α is expressed in the LA, MA and CA. AG-signaling likely suppresses LA plasticity by stimulating GABAergic interneurons in the LA, while augmenting (GABAergic) activity in the CA to drive anxiety. Despite the fact that GHS-R1α is not present in the BLA, the injection of AG into this amygdaloid nuclei inhibited auditory fear, while enhancing passive avoidance memories. This suggests that locally administered AG suppresses BLA activity, which appears to favor hippocampal activity.
- The micro-infusion of AG into the LA inhibits the acquisition, extinction and, potentially, consolidation of CTA.
- When administered into the hippocampus or amygdala (BLA) immediately after training, AG improves 1 h short-term working memory and the consolidation of hippocampus-dependent passive avoidance memories, as mediated by an enhancement in hippocampal plasticity (Section 4).
- The systemic or BLA administration of AG following training prevents auditory fear memory consolidation. Moreover, the plasma AG levels are inversely correlated to auditory fear memory strength. However, under conditions of chronic stress, which raises the circulatory AG levels, AG promotes auditory fear memory consolidation and reactivation (further explored in Section 5.3).
- Several systemic AG treatments, such as during caloric restriction, prolonged contextual fear reactivation by stimulating neurogenesis and, thus, facilitating hippocampal recall. However, in contrast to passive avoidance memory, the effects of AG injections on contextual fear memory formation are understudied.
- AG boosts auditory and contextual fear memory extinction by enhancing the acquisition and consolidation of extinction memories. This involves AG-imparted improvements in hippocampal plasticity (Section 4), mesolimbic dopamine-signaling (Section 4.2.1) and neurogenesis (Section 4.2.2). Since AG, unintuitively, enhances the CS relay towards the LA (Fig. 3), this might facilitate the pairing of the CS with a non-fearful context in the hippocampus.

5.3. Ghrelin and its memory-modulating interaction with the serotonin system

The highly complex serotonin system is not only implicated in hippocampal memory formation, but it also regulates the expression of fear as well as fear memory formation. As such, this section illustrates the hippocampal and amygdaloid interaction of AG- and serotonin-signaling in memory and fear, as physiologically influenced by feeding. Furthermore, the biological mechanisms that allow AG to function as a fear memory-suppressor under acute stress, but fear memory-potentiator during chronic stress and PTSD, will be elucidated.

5.3.1. Serotonin controls the expression of cued and contextual fear

According to a popular theory, the transmission of 5-HT suppresses aversive thinking, mitigating the outcome of punishing events (Homberg, 2012). However, 5-HT also drives the acute display of cue-based fear. Generally, during classical Pavlovian conditioning, the US (foot shock) activates neuronal activity across the CA and DRN, whereas fear-potentiated startle selectively induces c-Fos in neurons of the dorsal region of the DRN and the medial subdivision of the CA (Spannuth et al., 2011). Regarding 5-HT, in vivo studies have revealed that the extra-cellular 5-HT levels in the amygdala, but also the vmPFC and nucleus accumbens, rose within 30–40 min following the US (foot shock) during conditioning or CS (tone) exposure on the test day (Inoue et al., 1993; Yokoyama et al., 2005). Moreover, immobilization stress for 20 min stimulated the expression of the HPA effector corticotropic-releasing factor (CRF) by the CA, leading to the CRF receptor-evoked transmission of 5-HT from the DRN to the CA to induce freezing (Forster et al., 2006; Merali et al., 1998; Merlo Pich et al., 1995; Mo et al., 2008). On the other hand, the gradual release of 5-HT in the vmPFC terminated stress/CRF/US-triggered cue-based fear display (Forster et al., 2006; Inoue et al., 1993; Kawahara et al., 1993).

Interestingly, the use of 5-HT-elevating drugs confirmed that 5-HT navigates auditory and contextual fear display differently. While the acute systemic treatment with SSRIs prior to the test session potentiated auditory freezing in response to the CS (tone) (Burghardt et al., 2007), it attenuated the expression of fear when it was learned in a contextual paradigm (Hashimoto et al., 1996, 2009; Montezinho et al., 2010; Muraki et al., 2008; Nishikawa et al., 2007; Santos et al., 2006). In the latter, the selective pre-test microinjection of a SSR1 into the amygdala or hippocampus, respectively, were both sufficient to block contextual fear expression (Inoue et al., 2004; Montezinho et al., 2010).

5.3.2. Ghrelin stimulates serotonin transmission by the dorsal raphe nuclei

5-HT originates from nine distinct, 5-HT-producing raphe nuclei in the brain stem. To varying degrees, these raphe nuclei innervate other brain regions throughout the CNS (Burke and Heisler, 2015; Wang and Aghajanian, 1977). Immunohistochemical investigations showed that GHS-R1α is prevalent in the DRN and median raphe nuclei (Guan et al., 1997). Besides upregulating a multitude of 5-HTRs in the DRN (Hansson et al., 2014), AG depolarized ~75% of DRN-resident 5-HT neurons in rat slice preparations (Ogaya et al., 2011), resulting in the amygdaloid accumulation of 5-HT in vivo (Hansson et al., 2014). The DRN almost exclusively innervates the amygdala (Hale and Lowry, 2011), explaining the AG-evoked release of 5-HT in this brain area (Hansson et al., 2014).

Nonetheless, AG restricts the projection of 5-HT to selected brain areas. Indeed, AG blocked the release of 5-HT by hypothalamic synaptosomes (Brunetti et al., 2002), while intra-CA1-infused AG completely abolished the liberation of 5-HT in the rodent hippocampus (Ghersi et al., 2011). Similarly, prolonged caloric reduction, a condition that favors the plasma secretion of ghrelin, for 6 days or 4–5 weeks diminished 5-HT pools in the hypothalamus and hippocampus in vivo (Haiden et al., 2013).
and Haleem, 2000; Haleem, 2009; Jahng et al., 2007). Given that 5-HT impedes hippocampus-dependent passive avoidance, contextual fear and spatial memory formation, while antagonizing AG-induced plasticity in the hippocampus (Section 5.3.3), AG favors hippocampal plasticity (Section 4) and declarative memory (Section 3) by restricting 5-HT neurotransmission towards the hippocampus.

5.3.3. Feeding regulates antagonistic ghrelin- and serotonin-signaling to affect memory

While AG modulates serotonergic neurotransmission (5.3.2), it has been implied that 5-HT antagonizes AG both at a functional and physiological level in the hypothalamus and hippocampus. In the hypothalamus, 5-HT2cR activation diminishes the expression of the orexigenic peptides NPY and agouti-related peptide (AgRP) (Choi et al., 2006; Heisler et al., 2006), whereas AG boosts appetite by stimulating NPY/AgRP neurons (Currie et al., 2010; Yanagi et al., 2018). Additionally, 5-HT interacts with 5-HT2cR to stimulate the firing of anorexigenic pro-opiomelanocortin neurons and induce appetite-suppressing alpha-melanocyted-stimulating hormone/melanocortin 4 receptor-signaling in the hypothalamus, see (Lam et al., 2010; Heisler et al., 2002; Lam et al., 2008; Tiligada and Wilson, 1989). Likewise, from a physiological standpoint, the prolonged absence of food triggers the gastric secretion of AG (Cummings et al., 1989). Likewise, from a physiological standpoint, the prolonged absence of food triggers the gastric secretion of AG (Cummings et al., 2001; Liu et al., 2008; Yanagi et al., 2018), whereas the ingestion of food promotes the release of 5-HT in the hypothalamus to extinguish appetite (Orosco and Nicolaidis, 1992; Rouch et al., 1999; Schwartz et al., 1989; Voigt et al., 1998) and the plasma secretion of ghrelin (Nonogaki et al., 2006).

Generally, the memory- and plasticity-associated effects of 5-HT in the dorsal hippocampus are region-specific. In the DG, acutely applied SSRIs weakened Arc expression, implying that 5-HT impedes DG plasticity (Ravinder et al., 2013). A study showed that a 5-HT2cR agonist blocked mossy fiber-CA3-relayed LTPs and LTDs, but also LTDs at perforant path-DG synapses, proposing that 5-HT may restrict glutamatergic input to the CA3 region (Twarkowski et al., 2016). Interestingly, the optogenetic stimulation of 5-HT release in the hippocampal CA1 region, in a 5-HT2cR-dependent manner, was shown to potentiate CA3-CA1 synaptic transmission and the retrieval of spatial memory (Kemp and Manahan-Vaughan, 2005; Teixeira et al., 2018). In the CA1, a neuroinhibitory role of 5-HT is apparent. 5-HT treatment hyperpolarized principal neurons in a 5-HT1bR-stimulated and regulated the spontaneous activity of GABAergic interneurons in a 5-HT1bR-conveyed manner in the CA1, thus blocking stimulation-driven LTDs and NMDA receptor activation in this region (Corradetti et al., 1992; Staibli and Otaky, 1994). Likewise, the utility of SSRIs showed that elevated extracellular levels of 5-HT impair LTP expression in CA1 pyramidal neurons (Igelstrom and Heyward, 2012; Mnie-Filali et al., 2006). Moreover, DRN-mediated 5-HT transmission induced hippocampal CA1/CA3 GABAergic interneurons, hyperpolarized a small subset of CA1 pyramidal neurons and had no effect on CA3 excitatory neurons (Varga et al., 2009). 5-HT also induced 5-HT1bR receptors on CA1 pyramidal neurons, leading to the postsynaptic enhancement of AMPAR-mediated LTP at tempororomamic (TA)-CA1 synapses. Importantly, the latter stimulation of TA-CA1 synapses via 5-HT1bR/5-HT2cR seems to interfere with the consolidation of spatial memory, since a 5-HT1bR antagonist augmented long-term spatial memory (Cai et al., 2013). On the other hand, the genetic lack of 5-HT1bR augmented c-Fos neuronal activity in the CA1 and DG regions, supporting a plasticity-suppressing function of 5-HT. Moreover, US exposure (foot shocks) impeded LTDs, elevated LTDs and strengthened c-Fos induction in GABAergic CA1 interneurons only in wild-type, but not 5-HT-deficient, rodents. Thus, 5-HT-depleted rodents exhibited the enhanced acquisition, recall and 10 day-retainment of contextual fear (Dai et al., 2008a; Waider et al., 2019), although displaying worsened spatial memory recollection (Dai et al., 2008b). Synoptically, 5-HT-signaling impedes hippocampal plasticity in the CA1 and DG, but not CA3, interfering with the learning and consolidation of hippocampus-dependent spatial, passive avoidance and contextual fear memories (Cai et al., 2013; Carlini et al., 2007; Dai et al., 2008a; Gersh et al., 2011; Waider et al., 2019). 5-HT, however, enhances CA3-CA1-mediated spatial memory retrieval (Dai et al., 2008b; Teixeira et al., 2018).

Importantly, 5-HT further counteracts AG-signaling, interfering with both appetite and hippocampus-associated spatial and contextual fear memory. As reversible with 5-HT2cR antagonists, it was unraveled that GHS-R1α formed heterodimers with 5-HT2cR in primary rat hippocampal and hypothalamic neurons, which resulted in the blockade of GHS-R1α-mediated Ca2+-signaling. Moreover, the dimerization of GHS-R1α and 5-HT2cR navigated appetite, since 5-HT2cR blockers augmented food intake, whereas 5-HT2cR agonists dampened feeding (Schellekens et al., 2015). Notably, even though AG stimulates 5-HT transmission by the DRN (Hansson et al., 2014; Ogaya et al., 2011), AG hampers the projection of 5-HT towards the hypothalamus and hippocampus (Brunetti et al., 2002; Gersh et al., 2011) (see also Fig. 2). As confirmed in vivo, the intra-CA1 infusion of AG not only lowered the hippocampal 5-HT levels, but also enhanced the consolidation of passive avoidance memories. Furthermore, the hippocampal 5-HT pools were inversely correlated to the escape latencies, suggesting that 5-HT abolishes AG’s plasticity and consolidation-boosting benefits in the hippocampus (Gersh et al., 2011). Indeed, the intra-hippocampal administration of a SSRI blunted the enhancement of passive avoidance and object recognition memory by AG (Carlini et al., 2007).

These findings support the concept that locally available AG inhibits the synaptic release of 5-HT in the CA1 and DG, thus facilitating plasticity and the formation of hippocampus-dependent forms of memory, such as spatial or passive avoidance memories (see Sections 3.1 and 5.2.4).

5.3.4. Ghrelin modifies the amygdaloid expression of serotonin receptors

Interestingly, AG elevated the amygdaloid transcription of 5-HT1bR, 5-HT2cR, 5-HT5aR and 5-HT4R, whereas GHS-R1α knockout mice exhibited the reduced transcription of HT5aR, 5-HT2cR and 5-HT4R (Hansson et al., 2014). Generally, round GABAergic interneurons exert feedforward inhibition onto pyramid-shaped principal neurons (~80%) to regulate the generation of amygdaloid LTPs. In this context, the region- and cell-specific stimulation of various 5-HTR subtypes modulates neuronal firing in the BLA (see (Bocchio et al., 2016)) to either enhance or impair plasticity, anxiety and fear memories (Homberg, 2012; Lesch and Waider, 2012).

5.3.5. Ghrelin mitigates auditory fear memory formation upon acute stress

Interestingly, greater circulatory levels of AG were inversely correlated to long-term auditory fear memory strength, while the systemic post training injection of a ghrelin analogue suppressed the consolidation of auditory fear memories (Harmatz et al., 2017). These results propose that AG functions as an auditory fear memory-mitigating factor when facing acute stress.

These fear memory-weakening effects seem to be based on three aspects. First, it has been implied that the binding of AG to GHS-R1α-expressing LA neurons suppresses principal neuron activity and plasticity (Section 5.2.2) (Alvarez-Crespo et al., 2012; Song et al., 2013). Similar to CTA, principal LA neurons mediate the learning and consolidation of cue-based (auditory) fear (Goosens and Maren, 2004; Monsey et al., 2011; Tipps et al., 2018). And indeed, AG suppressed the acquisition of CTA, which was dependent on an increase in LA plasticity (see Section 5.2.3) (Song et al., 2013).

Second, AG evokes 5-HT transmission from the DRN to the amygdala (Section 5.3.2). Generally, the acute systemic treatment with SSRIs, which increase extracellular 5-HT levels, enhances auditory fear acquisition and expression, whereas their chronic administration impairs auditory fear learning by rodents (as compiled by (Burghardt and Bauer, 2013)). In fact, acutely given SSRIs inhibit 5-HT neurotransmission and the terminal release of 5-HT by the raphe nuclei by activating...
autoinhibitory 5-HT₃A Rs. In contrast, the prolonged exposure to SSRIs desensitizes 5-HT₁A Rs, which gradually enhances anti-depressive 5-HT output by raphe projections (Blier and Bergeron, 1995; Burghardt and Bauer, 2013; Gardier et al., 1996; Gray et al., 2013). Furthermore, DRN-induced 5-HT release inhibited the amygdaloïd neuronal activity ex vivo (Wang and Aghajanian, 1977), while chronically administered SSRIs reduced amygdaloïd activity in patients (Arce et al., 2008) and NR2B-NMDAR levels in the rodent BLA (Burghardt et al., 2013). Notably, 5-HT was hypothesized to function as a high-pass filter in the BLA, only allowing the transmission of sufficiently strong fear stimuli (Bocchio et al., 2016; Yamamoto et al., 2012). Since higher AG plasma levels were correlated to reduced auditory fear memory strength (Harmsmatz et al., 2017), long-term increases in AG may elevate the basal rate of 5-HT release in the amygdala, similar to SSRIs, interfere with plasticity and fear memory formation. Indeed, the 14 day-long treatment with AG reduced c-Fos activity in the BLA (Hornsby et al., 2016).

Third, the upregulation of several 5-HTRs by AG (Section 5.3.5) may contribute to the anxiogenic (see (Morris et al., 2018)(Fritz et al., 2020)) (5-HT₂A/R) and fear extinction-blocking (Huang et al., 2016) (5-HT₂A/R) effects of AG. Of note, 5-HT₂A Rs counteract 5-HT₃A Rs to augment auditory fear consolidation (Matsushita et al., 2009; Takeda et al., 2017). Although to be confirmed, AG may further attenuate auditory fear consolidation by elevating the amygdaloïd 5-HT₁A/R:5-HT₃A R transcription ratio.

5.3.6. Chronic stress inverts ghrelin’s fear memory-attenuating effects

Curiously, animal studies indicate that chronic stress reverses AG’s suppressive effects on auditory fear memory formation. Indeed, chronic immobilization stress prevented the attenuated consolidation of auditory fear by the systemically given ghrelin analogue MK-677 (Harmsmatz et al., 2017). Furthermore, the daily systemic injection of MK-677 for 5 days prior to conditioning, to reproduce the plasma levels of AG during chronic stress, did not affect fear acquisition, yet augmented the long-term retention of auditory fear memories in a HPA axis-dependent manner (Meyer et al., 2014). In this context, AG treatment can selectively potentiate the HPA axis-mediated plasma release of glucocorticoids or cortisol (a human variant of glucocorticoids) by a yet to be fully characterized mechanism (Fritz et al., 2020; Morris et al., 2018), whereas glucocorticoids, in turn, are known to boost fear memory consolidation (see (de Quervain et al., 2017)). Importantly, chronic, but not acute, stress provokes multi-fold increases in the plasma AG levels, lasting up to years (Malik et al., 2020; Meyer et al., 2014; Yousufzai et al., 2018). Intriguingly, when GHS-R1α was systemically blocked during chronic immobilization stress and until fear conditioning took place, the PTSD mice did not show enhanced long-term auditory fear memories anymore (Meyer et al., 2014; Yousufzai et al., 2018). Therefore, chronic stress evokes long-term increases in the blood AG levels that, in a GHS-R1α-mediated and glucocorticoid-independent manner, encourage fear memory formation during PTSD in vivo.

There is also evidence for a role of AG in augmenting PTSD in humans. Indeed, the plasma levels of AG were correlated to PTSD in a recent clinical study, accounting for 76.3 % of the PTSD severity. Although circulatory cortisol was co-elevated in PTSD patients, it had a...
neuroendocrine signaling in the hypothalamus, whereas the local 5-HT release is heightened upon feeding (likely as a consequence of lowered AG levels, which inhibit the hypothalamic 5-HT release) to induce anorexigenic hypothalamic neurons. A similar antagonistic function is seen in the hippocampus, where AG-signaling blocks 5-HT transmission to facilitate plasticity and memory formation in the CA1 and DG, whereas the liberation of 5-HT in these brain regions and the inhibitory heterodimerization of GHS-R1α with 5-HT2cRs (Bobker and Williams, 1989; Cheng et al., 1998; Homberg, 2012), ghrelin resistance in the amygdala can exacerbate 5-HT desensitization (Murrough et al., 2011) and BLA stimulation (Holmes, 2008) during PTSD.

5.3.7. Synopsis of the interplay between the ghrelin and serotonin system under acute and chronic stress

- **GHS-R1α** is expressed in 5-HT neurons in the DRN, which project 5-HT to the amygdala in response to AG. However, local AG inhibits the synaptic release of 5-HT in the hypothalamus and hippocampus.
- AG is released upon food restriction to stimulate appetite in the hypothalamus, whereas the local 5-HT release is heightened upon feeding (likely as a consequence of lowered AG levels, which inhibit the hypothalamic 5-HT release) to induce anorexigenic hypothalamic neurons. A similar antagonistic function is seen in the hippocampus, where AG-signaling blocks 5-HT transmission to facilitate plasticity and memory formation in the CA1 and DG, whereas the liberation of 5-HT in these brain regions and the inhibitory heterodimerization of GHS-R1α with 5-HT2cRs attenuate learning and memory consolidation.
- Greater AG plasma levels suppress auditory fear memory consolidation and retention. In contrast, chronic stress, which provokes massive increases in circulatory AG, enhances auditory fear memory retention in rodents and PTSD symptoms in humans, independent of the stimulatory effects of AG on glucocorticoid/cortisol release by the HPA axis.
- Acute cerebral 5-HT release enhances auditory (cued) fear expression and amygdaloid/hippocampal 5-HT projection blocks contextual fear display, whereas chronically elevated 5-HT levels in the CNS, such as following SSRI treatment, suppress both auditory and contextual fear. Given that AG induces amygdaloid 5-HT transmission, targeted studies are necessary to investigate if this affects fear memory expression.
- Chronic stress might reverse the suppressive effects of AG on fear memory by stimulating GH expression in the BLA and inducing amygdaloid ghrelin resistance. The latter seemingly disinhibits the LA and leads to the loss of AG-evoked 5-HTR expression in the amygdala, especially neuroinhibitory 5-HT1aRs, possibly resulting in amygdaloid 5-HT desensitization and exacerbated fear display.

6. Conclusion

The presented studies support a fundamental role of AG in the formation of declarative and aversive memories, whilst influencing feeding-associated, but also inquisitive and fear behavior. An increase in plasma AG levels, as occurring during fasting, caloric restriction or direct hormone administration, result in the diffusion of BBB-penetrant AG into the brain. The constitutively active ghrelin receptor, GHS-R1α, as well as receptor stimulation by AG boost hippocampal synaptic plasticity (Fig. 2) and the birth of memory resolution-enhancing progenitor granule neurons in the DG, leading to the improved formation of spatial and object recognition memories as well as the consolidation of passive avoidance memories. By stimulating mesolimbic dopamine transmission in the VTA, which involves the dimerization of GHS-R1α with D1R in the hippocampus, AG not only prompts foraging behavior and locomotion, but also hippocampal reward and novelty learning. Presumably, the latter memory-enhancing effects of AG are mechanisms to allow the hungry mammal to remember its environment more vividly, including food-rich, fruitless or dangerous spots. Moreover, the plasma secretion of AG is responsible for the neuroprotective effects of caloric restriction (Bayliss et al., 2016), thus ameliorating age-associated neurodegeneration and cognitive deficits during AD (Reich and Holscher, 2020).

On the other hand, the intricate effects of AG in the amygdala, which involve the modulation of the 5-HT system, suppress the acquisition, but not extinction, of CTA, likely to avoid that the hungry animal starves to death due to adopting food adversities, yet does not consume nauseating food. Furthermore, even though typically anxiogenic, high AG levels suppress the consolidation and reactivation of auditory fear memories, whilst extending the recall period of contextual fear memories under non- and acutely stressed conditions. These appear to be means to support food-seeking in a low risk manner. Chronic stress, however, alters AG-signaling in the amygdala, induces amygdaloid ghrelin resistance and reverses the fear memory-mitigating effects of AG, turning the hormone into a biomarker for PTSD (see Fig. 4). As such, beyond appetite, we highlight the underappreciated role of AG in modulating memory and, especially, fear behavior.

Declaration of interests

The authors have no financial or commercial conflicts of interest.

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Author Contributions

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22

N. Reich and C. Hölzer

Neuroscience and Biobehavioral Reviews 143 (2022) 104952

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