Stress, motivation and the gut-brain axis: A focus on the ghrelin system and alcohol use disorder

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Abstract

Since its discovery, the gut hormone ghrelin has been implicated in diverse functional roles in the central nervous system. Central and peripheral interactions between ghrelin and other hormones, including the stress-response hormone cortisol, govern complex behavioral responses to external cues and internal states. By acting at ventral tegmental area dopaminergic projections and other areas involved in reward processing, ghrelin can induce both general and directed motivation for rewards, including craving for alcohol and other alcohol-seeking behaviors. Stress-induced increases in cortisol seem to increase ghrelin in the periphery, suggesting a pathway by which ghrelin influences how stressful life events trigger motivation for rewards. However, in some states, ghrelin may be protective against the anxiogenic effects of stressors. This critical review brings together a dynamic and growing literature, that is at times inconsistent, on the relationships between ghrelin, central reward-motivation pathways and central and peripheral stress responses, with a special focus on its emerging role in the context of alcohol use disorder.

Keywords

ghrelin; alcohol craving; stress; motivation; reward processing

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Introduction

Over the past two decades it has become apparent that the stomach-derived hormone ghrelin has central nervous system (CNS) effects that go beyond regulation of feeding and energy balance. Originally, the major effect of ghrelin was demonstrated to cause the release of growth hormone (GH) from the pituitary (Kojima et al., 1999). Subsequently, ghrelin was suggested to be involved in the central regulation of appetite, food intake, food reward and energy homeostasis (Nakazato et al., 2001). More recently, it has been shown that ghrelin's effects on appetite are also mediated peripherally; in fact, ghrelin's peripheral action on the vagus nerve acts to antagonize the appetite-suppressing effects of other gut hormones, including leptin and cholecystokinin (Ueno and Nakazato, 2016, Date, 2014). Indeed, ghrelin is the only circulating orexigenic (i.e., appetite stimulant) gut hormone produced by the periphery. While there are some reports that ghrelin is produced in the CNS, by the hypothalamus (Kojima et al., 1999), consistent evidence in support of this is largely lacking (Cabral, 2017). However, ghrelin expression has been observed in the kidney, lung, placenta, pancreas and testis (Volante et al., 2002a, Volante et al., 2002b, Tena-Sempere et al., 2002, Barreiro et al., 2002, Kamegai et al., 2001, Gualillo et al., 2001, Mori et al., 2000).

Peripheral ghrelin concentrations increase during fasting states and reduce following feeding or glucose administration in rats (Tschop et al., 2000). Plasma ghrelin concentrations increase two-fold immediately prior to a meal in humans, before dropping around one hour post-meal (Cummings et al., 2001). Intravenous ghrelin in humans increases food intake and self-reported appetite (Wren et al., 2001). Its role in meal initiation during states of hunger occur within a regular diurnal rhythm (Cummings et al., 2001).

While the mediation of energy regulation via increasing weight gain remains one of the primary neurophysiological effects of ghrelin (Nakazato et al., 2001), its involvement in reward processing and motivation-related neural signaling is also becoming more evident. Furthermore, ghrelin has been shown to be anxiogenic (i.e., producing anxiety) (Carlini et al., 2002, Asakawa et al., 2001a, Carlini et al., 2004) and elevates serum corticosterone in mice (Asakawa et al., 2001a) and cortisol in humans (Kluge et al., 2011, Lambert et al., 2011). These interactions have placed ghrelin between systems of autonomic and motivational drive, in response to a myriad of internal and external events and cues.

This critical review brings together a dynamic and growing literature, that is sometimes inconsistent, on the relationships between ghrelin, its CNS targets and its subsequent impact on behavioral drive, with a special focus on the emerging role of ghrelin in the interplay between craving and stress within the context of alcohol use disorder (AUD).

Physiology of Ghrelin

Ghrelin is a 28-amino acid peptide synthesized by specific endocrine cells, localized mainly in the stomach, and released into circulation. Ghrelin is derived from the proteolytic break down of precursor proteins, pre-pro-ghrelin and pro-ghrelin, a process that produces both ghrelin and obestatin; the latter biologically active peptide is less well known and may exert

opposing effects to ghrelin, although this is not confirmed (Rucinski et al., 2009, Gutierrez et al., 2008, Lagaud et al., 2007).

Chromosome 3 encodes the *GHRL* gene for pre-pro-ghrelin, which is acylated by the ghrelin O-acyl transferase (GOAT) enzyme, also found predominantly in the stomach (Gutierrez et al., 2008, Yang et al., 2008). This acylation is crucial for ghrelin's action on the growth-hormone secretagogue receptor 1a (GHS-R1a) and its physiological effects. It has been suggested that, in its acylated form, human ghrelin can cross the blood-brain barrier in a complex and bidirectional manner (Banks et al., 2002) that is influenced by fasting states and obesity (Banks et al., 2007). The GHS-R1a is found primarily in the hypothalamus and pituitary in both rodent and human populations (Kojima et al., 1999, Howard et al., 1996) (Figure 1), which causes the release of GH. Furthermore, recent work characterizes the liver-expressed antimicrobial peptide 2 (LEAP2) as an endogenous ghrelin receptor antagonist (Ge et al., 2017).

In plasma, ghrelin is also found in its unacylated form with higher prevalence than the acylated form, however unacylated ghrelin (UAG) does not act on the GHS-R1a (Broglia F, 2003, Torsello A, 2002). On this basis, the GOAT enzyme has been suggested to be a 'master switch' for the ghrelin system, acylating it into its 'active' form (Romero et al., 2010). There is also evidence that UAG can antagonize the actions of ghrelin (Gauna et al., 2005, Broglia et al., 2004, Broglia et al., 2003), or act independently of ghrelin on non-metabolic functions (for review, see: (Delhanty et al., 2012)). Whether UAG holds physiological actions remains controversial, especially as its putative receptor has not been identified.

The GHS-R1a is a G-protein coupled receptor that is located on chromosome locus 3q26.31 (Howard et al., 1996). Genetic variations in the *GHS-R* have been posited to influence growth, stature, obesity and AUD (Suchankova et al., 2017, Baessler et al., 2005, Hai-Jun Wang, 2004). In the hypothalamus, the GHS-R1a is particularly expressed in neuropeptide Y (NPY) and agouti gene related protein (AGRP) neurons, whose activation is thought to be the initial neural basis for the orexigenic effects of ghrelin (Hewson and Dickson, 2000, Cummings et al., 2001) (Figure 1). Indeed, attenuation of NPY or AGRP signaling blocks the orexigenic effects of ghrelin in rats (Asakawa et al., 2001b, Nakazato et al., 2001). GHS-R1a expression is also observed in the hippocampus, thalamus, brainstem nuclei and the centrally-projecting Edinger-Westphal (EW) nucleus that regulates stress responses, feeding and alcohol intake (May et al., 2008, Kaur and Ryabinin, 2010) and on dopaminergic neurons of the substantia nigra and ventral tegmental area (VTA) involved in motivational drive (Zigman et al., 2006, Guan et al., 1997, Mitchell et al., 2001). An inactive isoform of this receptor (GHS-R1b) is also distributed throughout the brain and peripheral organs and has largely been thought to lack a function. However, recent evidence suggests that it may directly mediate the expression and functioning of the GHS-R1a, thereby facilitating ghrelin-induced signaling (Navarro et al., 2016).

Ghrelin, Reward and Motivation

While the homeostatic, ingestion-initiating effects of ghrelin are well-established, its role in hedonic feeding, mediated by reward-motivation systems, is also becoming apparent (Zheng et al., 2009, Malik et al., 2008). As mentioned, GHS-R1as are expressed on the VTA, a key node involved in reward processing and motivational drive (Zigman et al., 2006). VTA dopaminergic afferents to the ventromedial striatum, including the medial nucleus accumbens shell (O'Donnell et al., 1999, Ikemoto, 2007, Bolam et al., 2000), can act as a 'Go' signal for foraging or exploration in rodents (Chambers and Potenza, 2003). In parallel, the substantia nigra provides a dopaminergic input to the dorsal striatum, including caudate and putamen, for the initiation and execution of motor programs and motivation in rodents and humans (Chambers et al., 2001, Chambers and Potenza, 2003, Bolam et al., 2000). Triggering motivation for food or feeding behaviors, via this mesolimbic dopaminergic pathway, is therefore an expected locus for the motivational effects of ghrelin. However, striatal dopamine has been shown to encode more general rewarding properties of stimuli, as well as an unexpected presence or absence of reward, and their magnitude across species (Schultz et al., 1992, Schultz et al., 1997, Tobler et al., 2005, O'Doherty et al., 2002), representations that drive appetitive behaviors more generally. Environmental stimuli that can trigger release of dopamine to the ventral striatum in rodents include natural rewards (food, sex) and rewards related to alcohol and drugs of abuse (Wise, 2004, Wise, 1988, Ikemoto, 2007). Furthermore, stimuli that simply predict these rewards may also engender the subjective pleasure associated with the reward themselves, by activating the VTA dopaminergic projection (Berridge, 2012, Berridge and Robinson, 2003, Schultz, 2007, Schultz, 1998).

Interestingly, ghrelin itself can directly induce the release of dopamine within the rodent nucleus accumbens, specifically via activating VTA neurons (Abizaid et al., 2006, Jerlhag et al., 2006, Skibicka et al., 2011) (Figure 1). Ghrelin's activation of the VTA – nucleus accumbens pathway increases both general motivation, in the form of enhanced locomotor activity (Jerlhag et al., 2006), as well as motivation for *preferred* rewards, like sucrose and high fat foods in rodents (King et al., 2011, Skibicka et al., 2011, Perello et al., 2010, Skibicka et al., 2012). Indeed, acute bilateral intra-VTA administration of ghrelin increases consumption of rewarding food, but not standard chow in rats (Egecioglu et al., 2010). In humans, recent evidence demonstrates that higher circulating ghrelin concentrations during satiety are associated with higher ventral striatal activation during the anticipation of food rewards (Simon et al., 2017), linking endogenous ghrelin concentrations and CNS reward anticipation signaling. Ghrelin itself can therefore activate reward processing pathways that are designed to drive motivated reward-seeking behavior.

Ghrelin-induced motivation for rewarding food seems to be at least partially mediated by dopamine receptor signaling. For example, 6-Hydroxydopamine lesions of the VTA suppress ghrelin's ability to elicit food-reinforced behavior in rats (Weinberg et al., 2011). In mice, the GHS-R1a has been shown to form a heteromeric complex with dopamine D2 receptors in the hypothalamus that is crucial for the influence of D2 on food intake (Kern et al., 2012). There is also evidence that GHS-R1a and dopamine D1 receptors are co-expressed in the hippocampus, VTA, substantia nigra and other cortical regions in mice

(Jiang et al., 2006). In rats, D1 receptor antagonism attenuates ghrelin-enhanced food reward self-administration (Overduin et al., 2012).

D1 receptor activation in the ‘direct pathway’ of the basal ganglia is implicated in reward-related incentive learning that directs approach behaviours for certain stimuli (“wanting”) (Beninger and Miller, 1998, Zhang et al., 2009). Therefore, the role of ghrelin in motivational drive may be mediated by a D1 receptor mechanism. This distinction between “wanting” and “liking” is important, as motivation for rewards (“wanting”) can occur without the simultaneous enjoyment of them (Robinson and Berridge, 1993, Robinson and Berridge, 2001, Everitt and Robbins, 2005). This “wanting”, that can be divorced from subjective pleasure, can lead to maladaptive appetitive or approach behaviors that persist despite a lack of reward, a phenomenon commonly observed in addictive behaviors in both rodents and humans (Robinson and Berridge, 1993, Robinson and Berridge, 2001, Everitt and Robbins, 2005). Interestingly, one study demonstrated that ghrelin increased motivation for food, but not “liking”; or the valuation of the food in rats (Overduin et al., 2012). Furthermore, ghrelin seems to directly activate the cholinergic input to the VTA from the laterodorsal tegmental area (Jerlhag et al., 2006), which, together with the VTA dopamine projection to nucleus accumbens, acts as a cholinergic-dopaminergic reward link. Activation of this pathway stimulates reward-related rodent locomotor activity and further implicates ghrelin in increasing incentive motivation or “wanting” (Dickson et al., 2011, Jerlhag et al., 2006).

Ghrelin also acts via opioid pathways. For example, ghrelin microinjection into the rodent VTA increases motivation for sucrose (Skibicka et al., 2011), which is blocked by the μ -opioid receptor antagonist naltrexone (Skibicka et al., 2012). This effect suggests that ghrelin also acts via opioid pathways in the VTA to mediate motivation for sucrose rewards.

Finally, a dispersed network of both subcortical and cortical regions has been associated with the central effects of ghrelin. Ghrelin administered intravenously to healthy, human volunteers increases desire for high energy food (Goldstone et al., 2014), mediates motivation for rewards and increases the neural response to food pictures in the substantia nigra, VTA, amygdala, orbitofrontal cortex, anterior insula and striatum (Malik et al., 2008). Post-meal reductions in endogenous ghrelin, in healthy humans, are associated with diminished responses to palatable food in the midbrain, amygdala, hippocampus, insula and orbitofrontal cortex (OFC) (Sun et al., 2014). These regions are implicated in several processes that drive motivated behavior: salience detection for the control of appetitive behaviors (amygdala) (Mahler and Berridge, 2012, Anderson and Phelps, 2001); reward value computation (VTA, OFC, striatum) (Rangel and Hare, 2010, O’Doherty et al., 2004); interoceptive awareness of internal states, including hunger (anterior insula) (Craig, 2009); and valuation updating of internal states to invigorate state-dependent motivational drive (OFC, amygdala) (Rudebeck et al., 2013, Salzman and Fusi, 2010).

Taken together, these data indicate how ghrelin can act outside of the hypothalamic-mediated homeostatic regulation of food or energy intake, and in fact directly access central neural systems that process reward and motivation to drive desire or “wanting” behaviors.

Ghrelin and Stress

Environmental stressors recruit complex behavioral, neural and endocrine responses that are generally well conserved across species (Ulrich-Lai and Herman, 2009, Romero, 2004, Blanchard et al., 2001). Threats or stressful events activate brainstem nuclei responsible for alerting and orienting (e.g. locus coeruleus) and the sympathetic nervous system for the behavioral ‘fight or flight’ response (hypothalamus – pituitary – adrenal (HPA) axis), which elevates circulating cortisol (Charmandari et al., 2005) (Figure 1). Chronic stressors induce anxiety- and depression-like behaviors (Gregus et al., 2005), alongside long-term VTA and nucleus accumbens adaptations in rats (Fitzgerald et al., 1996, Ortiz et al., 1996, Nikulina et al., 2004). Deficits in fear learning also contribute to expressions of anxiety-like behaviors in rodents and anxiety disorders in humans (Britton et al., 2011, Lissek, 2012, Pattwell et al., 2012).

Ghrelin was intrinsically linked with cortisol and responses to stressors after its initial identification (Asakawa et al., 2001a). Early experiments demonstrated that intra-cerebroventricular ghrelin administration induced anxiogenesis on the elevated plus maze and elevated serum corticosterone levels in mice (Asakawa et al., 2001a), and increased fear responses and reduced exploratory responses in rats (Carlini et al., 2002). Chronic intracranial (Hansson et al., 2011) and direct ghrelin injection into the hippocampus, amygdala and dorsal raphe nucleus (Carlini et al., 2004) all induce anxiogenesis in rats. In humans, intravenous ghrelin also increases cortisol levels in overweight and lean individuals alike (Kluge et al., 2011, Lambert et al., 2011). The anxiogenic effects of ghrelin seem to act in part via the corticotropin releasing factor (CRF) pathway, as ghrelin-induced anxiogenesis is associated with increased CRF receptors in the amygdala (Hansson et al., 2011), and is blocked by the CRF receptor antagonist α -helical CRH9-41 (Asakawa et al., 2001a) in rodents.

Stressors increase ghrelin concentrations both in rodents and humans. Stressful stimuli (tail pinch and starvation stress) elevate stomach ghrelin gene expression in rats (Asakawa et al., 2001a). In lean and obese humans, plasma ghrelin concentrations are elevated under conditions of physiological stress (Geliebter et al., 2013, Schellekens et al., 2012, Gluck et al., 2014) and, interestingly, ghrelin concentrations rise in women who are even simply anticipating a stressor (Raspopow et al., 2014).

The intrinsic link between ghrelin and cortisol is demonstrated neatly by two studies. The first demonstrated that circulating ghrelin levels increase in response to psychological stress in humans, but only when cortisol levels rise (Rouach et al., 2007). Secondly, a recent elegant series of experiments in six healthy, lean males demonstrated that circulating ghrelin levels increase when endogenous plasma cortisol levels increase (in response to intravenous administration of adrenocorticotrophic hormone or hydrocortisone). However, stimulation of the HPA axis without concurrent synthesis of cortisol (i.e., adrenal cortisol synthesis antagonism by the steroid 11β -hydroxylase blocker metyrapone) does not induce an increase in ghrelin, suggesting a direct link between circulating ghrelin and circulating cortisol, rather than HPA axis activation itself (Azzam et al., 2017).

There is also contradictory evidence of the link between ghrelin and responses to stress. For example, ghrelin knockout mice also have reduced corticosterone following acute stress, suggesting that ghrelin mediates the response to stress at least in part via negative feedback (Spencer et al., 2012). Some studies have demonstrated that exogenous ghrelin is anxiolytic. Subcutaneous ghrelin administration in rats 45 minutes before stressors (i.e., elevated plus maze and forced swim test) results in anxiolysis, even though chronic stressors increase ghrelin levels (Lutter et al., 2008). These differences may be partly related to individual differences in animal state and trait anxiety levels. For example, stress-induced rises in ghrelin are somewhat blunted in high anxiety rats (Wistar Kyoto), compared to low anxiety control rats (Kristensson et al., 2006). This aspect suggests a potential protective mechanism, whereby if ghrelin reaches a certain high threshold, it may induce anxiolytic effects itself. In support of this theory, intravenous application of ghrelin, at a dose that induces a 2-3-fold increase in plasma ghrelin concentration, blunts the cardiovascular response to stress in overweight and lean humans (Lambert et al., 2011). Furthermore, individuals who do not have elevated baseline ghrelin levels tend to demonstrate emotional eating patterns, whereby stressful events or states of negative affect induce excessive food reward seeking and ingestive behavior (Raspow et al., 2010).

As excessive fear learning and generalization has been linked to the development of anxious behaviours (Britton et al., 2011, Lissek, 2012), ghrelin may mediate the development of anxious behaviors by directly altering fear learning. Rats with higher circulating acyl-ghrelin following brief fear conditioning have weaker long-term fear memories, mediated by ghrelin's actions on the amygdala (Harmatz et al., 2017).

Taken together, evidence suggests a mechanism whereby states of stress can increase ghrelin and cortisol, which intrinsically co-vary and increase in parallel. Ghrelin can also act centrally to induce motivated reward-seeking behavior. Additionally, or alternatively, ghrelin effects on reward-seeking behaviors may be peripherally mediated. The latter mechanism would be consistent with the knowledge that GHS-R1a is highly expressed in, and acts via, the vagus and solitary tract nucleus, and with evidence that peripheral ghrelin signal is necessary for ghrelin-induced control of food intake (for review, see: (Howick et al., 2017)) (Figure 1). This requirement suggests a novel link between stress and reward-seeking behaviors (for food or drugs of abuse) that might be mediated in part by the actions of ghrelin. Meanwhile speculative, higher baseline levels of ghrelin may also act in a protective manner to blunt the anxiogenic effects of stressors.

Ghrelin, Alcohol and Substance Use

Drugs of abuse, including alcohol, commonly increase dopamine transmission in the nucleus accumbens (Di Chiara and Imperato, 1988, Boileau et al., 2003), the expected site of their primary reinforcing effects (Everitt and Robbins, 2005). As discussed, there is extensive evidence for the modulatory role of ghrelin on the VTA – nucleus accumbens “reward pathway”, which naturally leads to questions around the role of ghrelin in alcohol and drug use. While there is a large body of evidence for the relationship between ghrelin and AUD, there is also growing evidence for interactions with stimulant drugs (for review, see: Zallar et al., 2017 and next section). Briefly, ghrelin administration in rodents increases nicotine-

induced striatal dopamine release (Palotai et al., 2013), motivation for heroin (Maric et al., 2012) and cocaine-induced locomotor hyperactivity (Wellman et al., 2005, Wellman et al., 2008, Jang et al., 2013). Interestingly, the enhancement of the rewarding effects of cocaine seems to be mediated by ghrelin's effects at the nucleus accumbens (Jang et al., 2013) and VTA (Schuette et al., 2013, Cepko et al., 2014). Methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) seem to increase circulating ghrelin (Crowley et al., 2005, Kobeissy et al., 2008). Finally, GHS-R1a antagonism in rodents reduces the motivating/rewarding effects (as indicated by conditioned place preference) of alcohol (Landgren et al., 2012, Jerlhag et al., 2009), nicotine (Jerlhag and Engel, 2011), morphine (Engel et al., 2015), cocaine (Wellman et al., 2005, Wellman et al., 2008) and amphetamine (Jerlhag et al., 2010).

Ghrelin, Alcohol and Alcohol Use Disorder

To date, considerable evidence has started to elucidate the complex interactions between ghrelin, reward, craving and stress-reactivity systems in the context of alcohol use and AUD. In rodents, ghrelin administration, both systemically and directly into the VTA, increase alcohol intake in drug naïve and alcohol-habituated models (Jerlhag et al., 2009, Davis et al., 2012, Cepko et al., 2014, Jerlhag et al., 2011b). In contrast, GHS-R1a antagonism reduces alcohol intake, self-administration, preference, alcohol-induced locomotion and conditioned place preference (the latter indicating a reduction in the rewarding properties of alcohol) in wildtype, Long-Evans and alcohol-preferring rats, wild type C57BL/6J mice and alcohol-habituated female prairie voles (Bahi et al., 2013, Gomez and Ryabinin, 2014, Landgren et al., 2012, Davis et al., 2012, Suchankova et al., 2013, Stevenson et al., 2015, Stevenson et al., 2016, Suchankova et al., 2016, Jerlhag et al., 2009). Alcohol-induced increases in nucleus accumbal dopamine release is not observed in ghrelin knockout (Jerlhag et al., 2011a) and GHS-R1a knockout mice (Jerlhag et al., 2009). As mentioned earlier, GHS-R1a expression is high in EW cells, which have been implicated in rodents characterized by high alcohol intake (Bachtell et al., 2002, Weitemier et al., 2001, Fonareva et al., 2009). The reduction in alcohol preference and intake caused by ghrelin receptor antagonism in mice may be mediated by EW rather than VTA (Kaur and Ryabinin, 2010).

Chronic alcohol exposure in high alcohol-preferring rodents also modulates reward and motivational systems, increasing GHS-R gene expression in the VTA and other cortical and subcortical regions involved with reward processing (Landgren et al., 2011). Higher fasting ghrelin concentrations in healthy individuals predict more intense subjective rewarding effects of alcohol (Ralevski et al., 2017), pointing to a role for ghrelin in the acute hedonic aspects of alcohol use in healthy individuals too. These observations suggest a critical role for ghrelin in mediating the link between alcohol and central mesolimbic dopamine pathway integrity, as well as the accompanying subjective rewarding effects of alcohol.

There is now a growing body of evidence implicating disturbances in the ghrelin system in AUD. Acute alcohol administration results in a reduction in blood ghrelin concentrations or a blunting in fasting-induced ghrelin increase in AUD (Calissendorff et al., 2006, Calissendorff et al., 2005, Zimmermann et al., 2007, Leggio et al., 2013, Ralevski et al., 2017), although these studies have been limited by the use of water or saline as a control

condition, rather than an isocaloric non-alcoholic control (see also Commentary by: (Cummings et al., 2007)). Actively drinking AUD individuals seem to have lower circulating ghrelin, but abstinence is associated with increasingly higher circulating ghrelin compared to healthy controls (Kraus et al., 2005, Akkisi Kumsar and Dilbaz, 2015, Wurst et al., 2007, Leggio et al., 2012, Kim et al., 2005, Ralevski et al., 2017). Ghrelin concentrations correlate positively with alcohol craving (Addolorato et al., 2006, Koopmann et al., 2012, Leggio et al., 2012); baseline ghrelin levels are higher in AUD individuals who subsequently relapse, but lower in those able to maintain abstinence (Leggio et al., 2012). Furthermore, higher baseline fasting levels of ghrelin predict more reward sensitivity and impulsivity (Ralevski et al., 2018), traits associated with compulsive alcohol use (Belin et al., 2008, Everitt and Robbins, 2005). As mentioned, higher baseline ghrelin is also associated with more subjective rewarding effects of alcohol in healthy social drinkers (Ralevski et al., 2017). Together, this research implicates high circulating ghrelin levels as a risk factor for craving-induced relapse in AUD.

These studies are limited by the investigation of associations between endogenous ghrelin concentrations and self-reported behaviors (some of them reported retrospectively, therefore subject to recall bias) like drinking, craving and subjective effects of alcohol. More recent work, however, supports a direct and causal link between ghrelin signaling and alcohol craving and consumption. In a double-blind, placebo-controlled human pharmacological manipulation study of ghrelin in non-obese AUD individuals, individuals received either intravenous acyl-ghrelin administration or placebo, and were then exposed to alcohol and juice cues in a naturalistic bar-like laboratory setting (Leggio et al., 2014). Intravenous ghrelin, as compared to placebo, significantly increased alcohol cue-induced craving. Furthermore, post-infusion blood ghrelin levels were positively correlated with cue-induced increases in alcohol craving (Leggio et al., 2014). Interestingly, intravenous ghrelin increased alcohol craving, without increasing either the urge to drink juice or food craving (Leggio et al., 2014), suggesting a direct and specific link between ghrelin and alcohol craving, at least in non-obese human subjects with AUD. A more recent double-blind, placebo-controlled within-subject counterbalanced human laboratory study further corroborates the link between ghrelin and alcohol-seeking behaviors. This study demonstrated for the first time that intravenous ghrelin administration, as compared to placebo, increases intravenous alcohol self-administration in a progressive ratio schedule in actively drinking AUD individuals (Farokhnia et al., 2017). Participants also started self-administering alcohol significantly sooner under intravenous ghrelin versus placebo. Interestingly, higher breath alcohol concentration was associated with higher alcohol stimulation, pleasure ratings, intoxication ratings and food craving under intravenous ghrelin, but not placebo administration (Farokhnia et al., 2017). Furthermore, distinct brain regions involved in motivation, reward and stress regulation were engaged by intravenous ghrelin, compared to placebo, in anticipation of alcohol cues (amygdala activation) versus food cues (nucleus accumbens activation and medial OFC deactivation) (Farokhnia et al., 2017).

Together, these two human laboratory studies (Leggio et al., 2014, Farokhnia et al., 2017) represent the first human evidence of a causal link between pharmacologically manipulated ghrelin signaling and alcohol craving, alcohol self-administration and brain activity in

current AUD individuals. This relationship between ghrelin, craving, alcohol intake, the subjective rewarding effects of alcohol and subsequent relapse together suggest a mechanism whereby ghrelin facilitates the maladaptive alcohol-seeking behaviors that persist in addictive disorders. These findings, in turn, suggest that GHS-R1a blockade may represent a novel pharmacological treatment for AUD patients. Consistent with previous work indicating that GHS-R1a antagonists (e.g., JMV2959, DLys3-GHRP-6) reduce alcohol-seeking behaviors in pre-clinical models (Bahi et al., 2013, Gomez and Ryabinin, 2014, Landgren et al., 2012, Davis et al., 2012, Suchankova et al., 2013, Stevenson et al., 2015, Stevenson et al., 2016, Suchankova et al., 2016, Jerlhag et al., 2009), a recent human study provides preliminary evidence in the same direction. Specifically, a Phase 1b human laboratory study has recently reported the safety of the co-administration of alcohol with a novel ghrelin receptor inverse agonist, PF-5190457, in heavy drinking humans (Lee et al., 2018). This preliminary study showed that PF-5190457 reduced cue-induced craving for alcohol and food in a natural bar-like laboratory setting (Lee et al., 2018).

Ghrelin, Alcohol and Stress

It is established that in individuals with AUD and substance use disorders, stressors or negative affect can facilitate drug seeking and drug taking behaviors (Baker et al., 2004, Koob, 2003). Indeed, elevated physiological stress responses, or aversive, stress-like states are observed during acute and longer-term withdrawal (Rasmussen et al., 2000, Valdez et al., 2002, Koob and Le Moal, 2005). Cortisol is heightened in actively drinking AUD humans (Stalder et al., 2010, Sinha et al., 2009, Lovallo et al., 2000, Fox et al., 2007), and while evidence linking response to stressors and ghrelin is inconsistent, the relationship between increased cortisol and increased ghrelin is clear (although in healthy controls). As ghrelin can drive reward-seeking behaviors, induce alcohol craving and self-administration, and is associated with higher risk for relapse in AUD, it is possible that states of stress can increase vulnerability to states of craving in AUD via central actions of ghrelin.

This hypothesis needs to be further developed and supported by future studies examining both cortisol and ghrelin in AUD, at resting states and in response to acute stressors. Another important inquiry will be to parse out stress-induced relapse and craving-induced relapse. Since lower ghrelin levels in actively drinking AUD individuals might reduce the protective effects of ghrelin against stress-induced reward-seeking, it is possible that individuals more sensitive to environmental cues and craving will be at higher risk for relapse when they have higher baseline ghrelin levels, whereas those who have difficulty dealing with life stressors and experience stress-induced relapse will be protected by higher baseline ghrelin.

Conclusions

Since its discovery, ghrelin has been implicated in diverse functional roles in the CNS, with central and peripheral interactions with other hormones governing complex behavioral responses to external cues and internal states. By acting at the VTA – nucleus accumbens ‘reward link’, ghrelin can induce both general and directed motivation for specific or preferred rewards. Stress-induced increases in cortisol seem to cause an increase of ghrelin in the periphery, suggesting a pathway by which stressful life events trigger motivation for

rewards, whether they are natural food rewards or drugs of abuse. However, some work also suggests that higher baseline levels of ghrelin might be protective against angiogenesis (Mani and Zigman, 2017). In healthy individuals, acute alcohol administration seems to reduce ghrelin and actively drinking AUD individuals have lower circulating ghrelin, which increases during abstinence and is associated with cue-induced craving and subsequent relapse. Therefore, the role of ghrelin in cue-induced craving and relapse is becoming clear, but its role in stress-induced relapse requires further delineation. Furthermore, future studies should also address the role of the EW nucleus as a mediator of the effects of ghrelin on both stress reactivity and alcohol intake.

Identifying novel targets that may lead to the development of new treatments for AUD is a priority (Litten et al., 2018). The ghrelin system represents a promising target, yet a lot of work is still needed. It is also conceivable that, even if future studies confirm the hypothesis that targeting the ghrelin system will lead to novel effective treatments, these will be limited to specific sub-populations. For example, recent animal and human work indicates that gastric by-pass for obesity may lead to *de novo* excessive alcohol drinking (for review, see: (Blackburn et al., 2017)). Rodent work also suggests that GHS-R1a blockade reduces excessive alcohol use in rats but increases alcohol intake after gastric by-pass (for review, see: (Blackburn et al., 2017)). However, this work is preliminary. At present, the characteristics that may help predict effective ghrelin-modulating personalized approaches remain unknown.

In summary, the ghrelin system may represent a novel target for personalized therapeutics, although additional work is needed to shed light on the role of ghrelin in AUD. While this is a growing area of study, there remain several unanswered questions alluded to in this critical review. Inconsistencies across studies are likely due to differences in the study populations (healthy human, AUD individual, rodent, specific rodent strain); differences in peripheral cortisol levels; whether they examine chronic or acute alcohol use or abstinence; and whether they examine endogenous or administered ghrelin, and acylated ghrelin or UAG. Future work should aim to delineate the interactions between ghrelin, stress responses and craving, for both a clearer understanding of the pathophysiology of neuropsychiatric disorders and for the development of more comprehensive therapeutic models.

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Figure 1. Central and peripheral actions of ghrelin: Simplified schematic of the complex interacting peripheral and central actions of ghrelin and cortisol

Ghrelin is synthesized primarily by endocrine cells in the stomach where it is acylated by the ghrelin O-acyl transferase (GOAT) enzyme. Acyl-ghrelin is released into the peripheral blood and crosses the blood-brain-barrier to reach the growth-hormone secretagogue receptor (GHS-R) on the hypothalamus (Hyp) and centrally-projecting Edinger-Westphal (EW) nucleus. In the hypothalamic arcuate nucleus (AN), acyl-ghrelin from periphery, and possibly from ghrelin-producing neurons, induces the release of neuropeptide Y (NPY), an orexigenic neuropeptide, stimulating appetite and ingestion.

Acyl-ghrelin also acts at the hypothalamic paraventricular nucleus (PVN), where it stimulates NPY release. NPY release in turn inhibits GABA release, thereby stimulating corticotropin releasing factor (CRF) release, leading to adrenocorticotrophic hormone (ACTH) release from the anterior pituitary (AP) to the adrenal cortex (AC). This cascade through the hypothalamic-pituitary-adrenal (HPA) axis causes the release of cortisol, which is also triggered by stress. Increased cortisol in the periphery causes increased peripheral acyl-ghrelin, suggesting a mechanism whereby states of stress can increase ghrelin and cortisol, which intrinsically covary and increase in parallel.

Acyl-ghrelin also activates ventral tegmental area (VTA) neurons by binding to GHS-R's and causing the release of dopamine in the nucleus accumbens (NAc). This mesolimbic dopamine projections result in increased reward processing, motivated behaviors and cravings, including craving for alcohol. Peripheral ghrelin levels are reduced during acute alcohol intake in healthy volunteers and in actively drinking individuals with chronic alcohol use disorder (AUD). On the other hand, peripheral ghrelin levels are increased during abstinence in individuals with chronic AUD.

Peripherally, GHS-R are also synthesized and expressed in cells of the nodose ganglion (ND) with stomach-projected vagal afferent fibers. Peripheral acyl-ghrelin also acts via its GHS-R at the vagus nerve. The nucleus tractus solitarius (NTS), located at the medulla oblongata, receives information via the vagal afferent pathway and signals to the AN to induce appetite and ingestion.