Psychoneuroendocrinology 100 (2019) 58–66

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Role of nesfatin-1 in anxiety, depression and the response to stress

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ARTICLE INFO

Keywords:
Animal model
Anorexia nervosa
Gut-brain axis
Obesity
Nucleobindin-2
Psychosomatic

ABSTRACT

Nesfatin-1 has been discovered a decade ago and since then drawn a lot of attention. The initially proposed anorexigenic effect was followed by the description of several other involvements such as a role in gastrointestinal motility, glucose homeostasis, cardiovascular functions and thermoregulation giving rise to a pleiotropic action of this peptide. The recent years witnessed mounting evidence on the involvement of nesfatin-1 in emotional processes as well. The present review will describe the peptide's relations to anxiety, depressiveness and stress in animal models and humans and also discuss existing gaps in knowledge in order to stimulate further research.

1. Introduction

Over the past years, nesfatin-1 received a lot of attention associated with the growing evidence for its role in various physiological processes. Discovered in 2006 in the rat hypothalamus (Oh-I et al., 2006), the 82-amino acid peptide is cleaved from its precursor protein nucleobindin 2 (NUCB2) before exerting pleiotropic effects. This is supported by the fact that apart from the hypothalamus, NUCB2 mRNA expression has been detected in various central and peripheral tissues including the hippocampus, prefrontal cortex, amygdala, brain stem, gastrointestinal tract, pancreatic islets or adipose tissue (Goebel-Stengel and Wang, 2013; Prinz and Stengel, 2016).

The first effect described for nesfatin-1 was its anorexigenic property. In animal studies, it was shown to suppress food intake after central and, much less robustly, after peripheral administration and subsequently also to reduce body weight (for review see: (Stengel et al., 2013b; Weibert and Stengel, 2017)). Furthermore, intracerebroventricular (icv) injection of nesfatin-1 resulted in a delay of gastric emptying (Stengel et al., 2009a) and a reduction of gastrointestinal motility (Atsuchi et al., 2010; Xu et al., 2015a), effects likely contributing to the reduction of appetite. Moreover, nesfatin-1 has been implicated in glucose homeostasis enhancing glucose-stimulated insulin secretion and sensitivity (Gonzalez et al., 2011; Li et al., 2013) as well as in lipid metabolism where nesfatin-1 administration decreased blood lipid concentrations and suppressed lipogenesis (Yin et al., 2015) contributing to the improvement of the metabolic profile. Other important effects described for nesfatin-1 include the increase of blood pressure (Mori et al., 2017; Osaki and Shimizu, 2014), stimulation of thermogenesis (Könczöl et al., 2012; Wernecke et al., 2014) and initiation of puberty (Garcia-Galiano et al., 2010), thus influencing reproductive functions as well.

The receptor mediating these pleiotropic effects has not been characterized yet. Nevertheless, converging evidence points towards a G protein-coupled receptor (Brailoiu et al., 2007), likely expressed in central (*e.g.* cortex, paraventricular nucleus (PVN) of the hypothalamus, dorsal motor nucleus of the vagus nerve) and peripheral tissues (*e.g.* pancreas, pituitary, stomach, small intestine, heart, skeletal muscle, visceral adipose tissue) as recently shown using autoradiography in rats (Prinz et al., 2016) corroborating the suspected broad range of nesfatin-1's effects.

However, subsequent studies showed that nesfatin-1 is not only restricted to metabolic functions. Over the years, the involvement of nesfatin-1 in the regulation of psychopathological conditions like depression and anxiety has been suggested (Emmerzaal and Kozicz, 2013). Differential findings have been described for women and men leading to the hypothesis of a sex-specific regulation of this peptide.

In this review we will outline the current knowledge on the role of NUCB2/nesfatin-1 in the regulation of emotional processes with an emphasis on the functional implications in depression, anxiety and the mediation of stress. In addition, we will acknowledge the sex-specific aspects in the modulation of these processes and discuss possible gaps in knowledge in order to stimulate future investigations. Since most

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https://doi.org/10.1016/j.psyneuen.2018.09.037
Received 20 July 2018; Received in revised form 2 September 2018; Accepted 26 September 2018
0306-4530/

commercially available antibodies targeting nesfatin-1 also recognize full length NUCB2 (Stengel et al., 2013b), we refer to these analytes as NUCB2/nesfatin-1.

2. Potential role in anxiety

Anxiety disorders, including generalized anxiety disorder, social anxiety disorder, specific phobias and panic disorder with or without agoraphobia are the most common psychiatric disorders worldwide (Kessler et al., 1994; Stein et al., 2017; Wittchen and Jacobi, 2005). In large epidemiological community surveys, the reported annual prevalence of anxiety disorders is up to 21% and the lifetime prevalence even up to 34% with women suffering approximately twice as likely than men (Bandelow and Michaelis, 2015). A recent systematic review and meta-regression indicated a global prevalence of 7% for anxiety disorders (Baxter et al., 2013). Due to these high prevalence rates, the associated reduction in quality of life of those affected as well as the high health care costs (Bandelow and Michaelis, 2015; Wittchen and Jacobi, 2005) it is necessary to characterize the pathomechanisms: psychosocial factors that maintain the symptoms but also possible biological contributors such as peptide hormones potentially involved in the development of these mental disorders.

Early after the identification of nesfatin-1, its implication in the modulation of different emotional states like anxiety has been examined. In particular, changes in behavior have been observed in animal studies after icv or peripheral injection of nesfatin-1 using standardized tests. Initially, innate and conditioned anxiety responses of rats were assessed in different behavioral tests after icv injection of 5 or 25 pmol of nesfatin-1 (Merali et al., 2008). In the elevated plus maze, a method to measure innate anxiety-like behavior (Rodgers and Dalvi, 1997), the higher dose resulted in a significant reduction of time spent in the open arms, the number of open arm entries and the number of unprotected head dips in comparison to the vehicle group reflective of a decreased explorative behavior (Merali et al., 2008). Moreover, both doses of nesfatin-1 resulted in a significantly prolonged latency to eat and a reduced consumption of palatable snacks when moved into a novel cage (Merali et al., 2008) as part of the novelty-induced hypophagia test (Merali et al., 2003). Although centrally injected nesfatin-1 was initially demonstrated to inhibit food intake (Oh-I et al., 2006), icv injected nesfatin-1 had no effect on snack consumption in the home cage (Merali et al., 2008), possibly pointing towards an indirect effect on food intake subsequent to the stimulation of anxiety. In order to assess conditioned fear responses the fear-potentiated startle test (Davis, 1990) was conducted, where noises (conditioned stimulus) and foot shocks (unconditioned stimulus) were combined and the startle amplitude after nesfatin-1 injection measured. Rats that received nesfatin-1 icv at a dose of 25 pmol displayed a significantly greater startle potentiation than controls (Merali et al., 2008). Lastly, rats treated with this dose showed a significantly elevated time engaged in freezing (Merali et al., 2008), a behavior displayed in response to conditioned fear (Davis, 1990). Based on this pioneer study, nesfatin-1 was implicated in the central mediation of anxiety-related behavior.

In line with these findings, a subsequent study demonstrated a stimulated anxiety-like behavior in rats after repeated intraperitoneal (ip) injections of nesfatin-1 over a period of three weeks (Ge et al., 2015a). In the open field test, rats treated with nesfatin-1 spent significantly less time in the center representing the vulnerable zone of the test, and displayed significantly decreased frequencies of grooming and rearing as well as reduced moving distances than the control group (Ge et al., 2015a). Furthermore, a significantly reduced frequency of entering all three arms of the Y maze, an overall reduced moving distance and a decreased preference index of the unexplored arm were observed after nesfatin-1 administration (Ge et al., 2015a), supporting the findings of the open field test and the assumption of an exploration-suppressive action by peripherally administered nesfatin-1 and therefore, an anxiety-enhancing effect when faced with a novel environment.

In a recent rat study, the influence of sequentially applied stress early in life on anxiety-like behavior in adulthood was investigated (Jing et al., 2017). Young animals underwent maternal separation (postnatal day 2-21) and were periodically treated with acute gastric irritation (day 10-16) followed by daily 90-min restraint stress over a period of seven days (Jing et al., 2017). Since NUCB2 mRNA expression in the gastric oxyntic mucosa was reported to be 10-fold higher than in the brain (Stengel et al., 2009b), alterations of NUCB2/nesfatin-1 were assessed – besides in hippocampus and plasma – also in the gastric fundus. Following the sequential stress, rats displayed increased anxiety-like behavior as indicated by less time spent in and fewer entries into the open arms of the elevated plus maze (Jing et al., 2017). Similarly, animals showed significantly less attendance to stay in the central area as well as significantly lower moving distance and slower movement in the open field test compared to non-stressed controls (Jing et al., 2017). Since this was accompanied by higher NUCB2/nesfatin-1 peptide levels in hippocampus, plasma and stomach (Jing et al., 2017), endogenous nesfatin-1 upregulated under conditions of stress may play a role in the development of anxiety.

Subsequently, the potential association of nesfatin-1 and anxiety was also examined in human studies. A study in male normal-weight patients diagnosed with generalized anxiety disorder (GAD) reported 45% lower NUCB2/nesfatin-1 plasma levels in these patients compared to age- and sex-matched healthy controls (Gunay et al., 2012). Another study reported an increase of NUCB2/nesfatin-1 plasma levels in a mixed-sex, normal weight population with panic disorder compared to a healthy control group (Bez et al., 2010). Furthermore, a positive correlation between NUCB2/nesfatin-1 and the severity of the panic disorder ($r = 0.467$, $p = 0.011$) was found (Bez et al., 2010). Also in male and female patients with an obsessive compulsive disorder significantly higher NUCB2/nesfatin-1 plasma levels were found compared to healthy controls (Bez et al., 2012). However, no association was observed between NUCB2/nesfatin-1 and the severity of obsessions and compulsions (Bez et al., 2012). It is important to note that these studies did not assess possible sex differences in the regulation of NUCB2/nesfatin-1 that may contribute to the partly discrepant findings described above.

Psychiatric diseases like anxiety and depression are often comorbid disorders in obesity. In particular, obesity is reported to be associated with anxiety (Gariepy et al., 2010). Moreover, NUCB2/nesfatin-1 was reported to be positively associated with body mass index and fat mass in humans (Ramanjaneya et al., 2010; Tan et al., 2011). Therefore, our group primarily investigated the association between nesfatin-1 and anxiety in female obese patients (Hofmann et al., 2013) in order to shed more light on nesfatin-1 as a potential link between food intake/nutrition and the emotional state. Plasma NUCB2/nesfatin-1 concentrations were assessed along with the severity of anxiety symptoms using the generalized anxiety disorder scale (GAD-7, 7-item scale, scores range from 0 to 21), a self-report questionnaire for the diagnosis of generalized anxiety disorder but also a sensitive screening tool for social anxiety, panic and posttraumatic stress disorders (Spitzer et al., 2006). According to the GAD-7 scores, the study population was divided in females with low anxiety (mean ± SD, 5.0 ± 2.7) and high anxiety (14.2 ± 3.3, $p < 0.001$) (Hofmann et al., 2013). We observed that women with high anxiety scores had significantly higher NUCB2/nesfatin-1 plasma levels (+33%) compared to females with low anxiety (Hofmann et al., 2013). Moreover, we detected a positive correlation between NUCB2/nesfatin-1 levels and GAD-7 scores ($r = 0.680$, $p < 0.001$) (Hofmann et al., 2013) suggesting a role for nesfatin-1 in the mediation of anxiety in humans as well. This finding was corroborated in patients with anorexia nervosa, a disease also frequently associated with anxiety (Kaye et al., 2004), with 65% higher NUCB2/nesfatin-1 plasma levels in patients reporting high anxiety (GAD-7 score: 16.0 ± 3.0) compared to those with lower anxiety levels (7.5 ± 3.3, $p < 0.001$) resulting in a positive association between anxiety scores and NUCB2/nesfatin-1 ($r = 0.32$, $p = 0.04$) (Hofmann et al., 2015a). Since the study investigating male patients described an association opposite to the one observed in females (Gunay et al., 2012), a sex-specific regulation of NUCB2/nesfatin-1 has been hypothesized.

Subsequently, we conducted a study with male and female obese inpatients hospitalized due to obesity and its somatic as well as mental comorbidities (Hofmann et al., 2015b). The female population displayed significantly higher NUCB2/nesfatin-1 plasma levels (+25%) and mean anxiety scores (+54%) compared to males (Hofmann et al., 2015b), possibly pointing towards a higher mental burden and fearful behavior in the female study population. Similar to our previous studies, both groups were then divided in subgroups with high and low anxiety scores (men: 10.0 ± 3.9 *vs.* 1.9 ± 1.7, $p < 0.001$; women: 13.9 ± 3.4 *vs.* 4.6 ± 2.5, $p < 0.001$) (Hofmann et al., 2015b). Females displaying high anxiety scores had significantly elevated NUCB2/nesfatin-1 levels (+51%) in comparison to women with low anxiety. In contrast, male patients with higher anxiety scores demonstrated an inverse alteration with significantly decreased NUCB2/nesfatin-1 levels (-17%) compared to men with low anxiety scores. These findings resulted in a positive association of anxiety and circulating NUCB2/nesfatin-1 levels in women ($r = 0.54$, $p < 0.001$) and a negative correlation in men ($r = -0.32$, $p = 0.04$) (Hofmann et al., 2015b). At least under conditions of obesity these results support the assumption of a sex-specific regulation of NUCB2/nesfatin-1. Whether this is associated with a NUCB2/nesfatin-1-induced stimulation of anxiety in women and a differential effect in men remains to be further investigated.

One possible explanation for the sex-specific regulation of NUCB2/nesfatin-1 could result from the already proposed implication of nesfatin-1 in reproductive functions and the association with sex hormones. There is mounting evidence suggesting that nesfatin-1 exerts a stimulatory effect on the hypothalamus-pituitary-gonadal axis at different levels and is implicated in pubertal maturation (Garcia-Galiano and Tena-Sempere, 2013). Conditions leading to weight loss and a negative energy balance like fasting or chronic undernutrition downregulated NUCB2 mRNA expression and inhibition of NUCB2/nesfatin-1 mRNA expression resulted in a delay of pubertal onset in rats (Garcia-Galiano et al., 2010). Moreover, significantly higher NUCB2/nesfatin-1 levels were found in girls with premature thelarche compared to prepubertal controls (Çatli et al., 2015) suggesting a role of nesfatin-1 in the initiation of puberty. NUCB2/nesfatin-1 is expressed in the hypothalamus, adipose tissue and gonads, giving rise to a peptidergic link between energy homeostasis and reproduction (Garcia-Galiano and Tena-Sempere, 2013). Indeed, reproductive hormones as testosterone and estradiol were reported to stimulate NUCB2 mRNA expression in the hypothalamus and pituitary *in vitro* (Chung et al., 2015; Hatef and Unniappan, 2017; Seon et al., 2017). Furthermore, estrogen was reported to attenuate fat accumulation, prevent insulin resistance and glucose intolerance indicating a protective effect on the development of obesity (Lizcano and Guzman, 2014). Whether the anorexigenic action of nesfatin-1 could be enhanced by estrogen in order to prevent metabolic dysfunctions will have to be further investigated. Interestingly, NUCB2/nesfatin-1 levels were decreased in patients with polycystic ovary syndrome (Alp et al., 2015; Deniz et al., 2012) possibly contributing to the weight gain observed in these patients. Whether the relationship between NUCB2/nesfatin-1 and sex hormones plays a pivotal role in the modulation of emotional responses warrants further investigation. For this purpose, the development of anxiety during menstrual cycle with simultaneous assessment of sex steroids and circulating NUCB2/nesfatin-1 would be interesting.

While the studies discussed above described the association between NUCB2/nesfatin-1 and anxiety at a single time point, studies with longitudinal design are indispensable in order to investigate causal interrelations. Therefore, in our subsequent study, we examined the association of NUCB2/nesfatin-1 with psychometrically measured anxiety and their alterations in response to inpatient treatment in an obese mixed-sex population (Hofmann et al., 2017). In light of our previous findings we hypothesized a decrease of NUCB2/nesfatin-1 plasma levels in females and an increase in males associated with a clinical improvement of anxiety. A clinically meaningful improvement was defined as a reduction of GAD-7 scores by at least 5 points (Hofmann et al., 2017) based on a definition of mild, moderate and severe anxiety disorders reflected by GAD-7 scores of 5, 10 and 15, respectively (Spitzer et al., 2006). As expected, in women we observed a positive correlation between NUCB2/nesfatin-1 plasma levels and anxiety scores at the beginning ($r = 0.411$, $p = 0.006$) and following clinical improvement of anxiety during the treatment ($r = 0.301$, $p = 0.047$) (Hofmann et al., 2017). In the male study population only a trend towards a negative correlation between NUCB2/nesfatin-1 levels and anxiety scores was observed ($r = -0.381$, $p = 0.059$) at the beginning of the treatment. However, after clinically improved anxiety a significant negative correlation was detectable ($r = -0.469$, $p = 0.018$) (Hofmann et al., 2017). This inverse association between NUCB2/nesfatin-1 and anxiety in males and females was consistent with previous findings. Surprisingly, neither in women nor in men who showed significantly improved anxiety scores during inpatient treatment, significant changes in NUCB2/nesfatin-1 plasma levels were seen (Hofmann et al., 2017). The trend of the alterations was as suggested with a decrease in NUCB2/nesfatin-1 plasma levels in women (-23%) and an increase in men (+12%), however, this did not reach statistical significance (Hofmann et al., 2017). Moreover, the alteration of NUCB2/nesfatin-1 levels was not associated with the development of the anxiety symptoms (women: $p = 0.587$; men: $p = 0.373$). Therefore, alterations of NUCB2/nesfatin-1 were not predicted by the course of anxiety during treatment (Hofmann et al., 2017). The absence of changes might be due to the relatively short observation period (~ 2 weeks), and longer intervals might be necessary in order to induce significant changes in NUCB2/nesfatin-1. Moreover, the improvement of anxiety – although clinically meaningful – defined by a reduction of at least 5 points might not be sufficient to have an impact on NUCB2/nesfatin-1. Furthermore, the levels reported at the beginning of the study with a mean GAD-7 score of 8.2 ± 6.0 (range 0-20) in the whole study population may have been too low in order to observe significant changes of anxiety-regulating mediators. Lastly, NUCB2/nesfatin-1 could be rather involved in the acute regulation of anxiety as in rats acute but not chronic stress induced an elevation of NUCB2/nesfatin-1 plasma levels and hypothalamic NUCB2 mRNA expression (Xu et al., 2015b). This hypothesis remains to be further investigated. In conclusion, the study design should be enhanced with a longer observation time as well as with a larger study sample and matched healthy controls. Furthermore, patients with diagnosis of an anxiety disorder and more severe anxiety symptoms should be enrolled in future studies. In addition, it should be analyzed whether circulating NUCB2/nesfatin-1 is derived from central (brain) or peripheral (stomach) production sites.

3. Potential role in depression

Since there is a high comorbidity between anxiety and depressive disorders (Thibaut, 2017), it might be speculated that NUCB2/nesfatin-1 plays a role in the development of depressive symptoms as well. In rats, both single and repeated (daily over 3 weeks) ip administration of nesfatin-1 dose-dependently increased immobility in the forced swim test (Ge et al., 2015b), pointing towards an increased despair-like behavior. In the open-field test, total moving distance, the frequencies of rearing and grooming as well as the time spent in the center were significantly decreased after repeated ip administration of nesfatin-1 compared to the control group (Ge et al., 2015b), reflecting depressive-like behavior. Moreover, plasma interleukin 6 (IL-6) and C-reactive protein (CRP) levels were dose-dependently increased after administration of nesfatin-1 for 3 weeks (Ge et al., 2015b), giving rise to IL-6 and CRP contributing to nesfatin-1's-induced depression-like behavior, a hypothesis in line with the assumption that activated immune-inflammatory signaling cascades are involved in the pathogenesis of depression (Valkanova et al., 2013).

Depressive states are often accompanied by sleep disorders which may be accompanied by abnormal rapid eye movement (REM) sleep (Steiger and Kimura, 2010). Since NUCB2/nesfatin-1 and melanin-concentrating hormone (MCH) were found to be highly co-expressed in the tuberal hypothalamic area (Fort et al., 2008) with MCH showing orexigenic (Qu et al., 1996) as well as sleep-promoting (Verret et al., 2003) properties, the role of the anorexigenic NUCB2/nesfatin-1 in sleep regulation received attention. In male rats, NUCB2 mRNA and NUCB2/nesfatin-1 protein expression were significantly down-regulated in the zona incerta and dorsolateral hypothalamus in response to REM sleep deprivation (Vas et al., 2013). It is to note that these regions display large amounts of NUCB2/nesfatin-1 positive neurons (Brailoiu et al., 2007; Goebel-Stengel et al., 2011) and are involved in the control of food intake, sleep-wake regulation and also in the development of depressive symptoms (Vas et al., 2013). Interestingly, REM sleep rebound resulted in an activation of NUCB2/nesfatin-1 positive cells in the same brain areas, especially those co-localized with MCH (Vas et al., 2013). When nesfatin-1 (25 pmol) was injected icv at the beginning of the light phase, significantly elevated sleep fragmentation and decreased time spent in REM sleep as well as in the intermediate stage of sleep were observed (Vas et al., 2013), indicating sleep-modulating properties of NUCB2/nesfatin-1. Whether nesfatin-1 and MCH play a counter-balancing role in sleep regulation will have to be further investigated.

However, another study reported a slight increase in REM sleep following icv nesfatin-1 administration of a high dose (50 pmol) or a very high dose (250 pmol) 1 h before the onset of the dark phase (Jego et al., 2012). Conversely, REM sleep was significantly impaired in response to nesfatin-1 antiserum or NUCB2 antisense administration (Jego et al., 2012). These discrepant results may be due to the different doses used or relate to the different time points of administration. Whether NUCB2/nesfatin-1 displays a circadian rhythm should be investigated in follow-up studies.

More recently, a mouse study investigated the changes of NUCB2/nesfatin-1 in a gastric cancer model comorbid with depression under different stress conditions (Zhang et al., 2018). Female mice were either exposed to 5 weeks of chronic unpredicted mild stress (CUMS) including periodic fasting or normal feeding without stress exposure. As a reliable and validated tool to induce depressiveness in mice, CUMS is characterized by sequential exposure to various mild stressors over a period of several weeks, *e.g.* 24 h fasting or 24 h water withdrawal (Willner, 1997). In both, the control and the CUMS group, gastric cancer was induced after 5 weeks by subcutaneous administration of gastric carcinoma cells. No differences were detected between groups in the sucrose preference test, but significantly reduced moving distance and time spent in the center of the open field test were observed in the CUMS group compared to gastric cancer mice without stress exposure (Zhang et al., 2018). In addition, the immobility in the forced swim test was significantly elevated in the stress group (Zhang et al., 2018), pointing towards a CUMS-induced depressive-like behavior including decreased explorative activity and enhanced despair. With regards to NUCB2/nesfatin-1 levels, female mice with gastric cancer and CUMS displayed significantly higher plasma, hippocampal and midbrain concentrations in comparison to the group without stress exposure and healthy control animals (Zhang et al., 2018), giving rise to the assumption that NUCB2/nesfatin-1 increased under these combined stressful conditions (cancer and CUMS) is involved in the development of the depression-like phenotype in female mice. Further studies might focus on a single stressor in order to better/easier relate cause and effect.

Earlier, the relationship between NUCB2/nesfatin-1 and depression has been investigated in humans. In a mixed-sex study population comprised of normal weight patients with major depressive disorder twofold higher mean NUCB2/nesfatin-1 plasma levels were found in patients diagnosed with major depression compared to healthy controls (Ari et al., 2011). Furthermore, a positive correlation between NUCB2/nesfatin-1 plasma levels and scores of the Hamilton Depression Rating Scale (HAM-D), a diagnostic tool to assess the severity of depressive disorders (Hamilton, 1960), were described in both, patients ($r = 0.393$, $p = 0.035$) and controls ($r = 0.429$, $p = 0.023$) (Ari et al., 2011), suggesting that NUCB2/nesfatin-1 plays a role in the development and/or maintenance of depressive symptoms. Interestingly, no statistically significant difference between women and men was detected concerning the mean NUCB2/nesfatin-1 plasma levels within both groups (Ari et al., 2011). More recently, in a mixed-sex study population diagnosed with either moderate or severe major depression, NUCB2/nesfatin-1 plasma levels were significantly higher in all depressive patients compared to healthy volunteers (Algul and Ozcelik, 2018). In addition, patients with severe major depression had significantly higher NUCB2/nesfatin-1 levels compared to depressive patients with moderate symptoms (Algul and Ozcelik, 2018). Another study confirmed the association between NUCB2/nesfatin-1 and the severity of depression, showing a significant positive correlation between plasma NUCB2/nesfatin-1 levels and HAM-D scores ($r = 0.651$, $p < 0.001$) (Xiao et al., 2018). In addition, a significant increase of NUCB2/nesfatin-1 levels in patients diagnosed with mild, moderate and severe depression was observed from group to group and compared to healthy controls (Xiao et al., 2018), demonstrating a strong association of NUCB2/nesfatin-1 with the severity of mood disorders. With an odds ratio of 1.3, higher plasma NUCB2/nesfatin-1 might be a potential risk factor for severe depression (Xiao et al., 2018). However, longitudinal studies in depressive patients are needed in order to examine whether increased NUCB2/nesfatin-1 levels are cause or effect of mood disorders. Furthermore, plasma NUCB2/nesfatin-1 levels were significantly higher in females than in males (Xiao et al., 2018), unfortunately, this was not examined with regards to the different manifestation of depressive symptoms.

Supporting the hypothesis of NUCB2/nesfatin-1 acting in a sex-dependent manner also under conditions of depression, intriguing results were reported in a study where NUCB2 mRNA expression of drug-free depressed suicide victims was analyzed in the brain (Bloem et al., 2012). The study focused on the Edinger-Westphal nucleus (EW) of the midbrain (Bloem et al., 2012), a brain region with high expression of NUCB2/nesfatin-1 (Brailoiu et al., 2007) and involved in the regulation of stress-related mood disorders (Kozicz et al., 2011). While NUCB2 mRNA expression in the EW was 1.8-fold higher in male suicide victims compared to the control group without any diagnosed psychiatric or neurodegenerative disorder, in female suicide victims an inverse regulation was observed with 2.7-fold lower NUCB2 mRNA levels compared to non-depressed individuals (Bloem et al., 2012). Since women are twice as likely affected from depressive disorders (Kuehner, 2003), the observed sex-specific associations with NUCB2/nesfatin-1 might contribute to a better understanding of the different pathobiology of depression in men and women. Due to the fact that Ari et al. did not detect differences between plasma NUCB2/nesfatin-1 levels of depressed men and women (Ari et al., 2011), it remains to be established whether differential alterations in the brain relate to peripheral changes.

In light of these findings, we also aimed to investigate the relationship between NUCB2/nesfatin-1 and depressive symptoms in patients showing a broad range of body weight as obesity is often associated with comorbid depression (Luppino et al., 2010). First, we assessed depressive symptoms in an obese female study population (Hofmann et al., 2013) as NUCB2/nesfatin-1 was implicated in regulating both, the nutritional and emotional state. Using the patient health questionnaire (PHQ-9), a widely used self-report screening instrument for the diagnosis of major depression and evaluation of the severity of depressive symptoms (Spitzer et al., 1999), we examined the association between depressive symptoms and plasma NUCB2/nesfatin-1 levels. After dividing the study population into patients with low and high anxiety we observed that patients with high anxiety also displayed higher levels of depression (+98%) compared to females with low anxiety (Hofmann et al., 2013). Furthermore, we found a positive correlation between circulating NUCB2/nesfatin-1 levels and total PHQ-9 scores ($r = 0.45$, $p < 0.001$) (Hofmann et al., 2013). Interestingly, in one of our subsequent studies investigating females diagnosed with anorexia nervosa, a disease also often associated with depression (Godart et al., 2006), the PHQ-9 sum scores were significantly elevated (+83%) in the high anxiety group as well (Hofmann et al., 2015a), however, no correlation was observed with NUCB2/nesfatin-1 plasma levels. This might be explained by the rather small sample sizes and – more importantly – overall very low NUCB2/nesfatin-1 plasma levels in the anorexic female population (Hofmann et al., 2015a).

In a subsequent study we followed up on the sex-dependent regulation of NUCB2/nesfatin-1 under depressive conditions suggested by Bloem and colleagues (Bloem et al., 2012). Obese females displayed significantly higher mean scores for depressiveness (+32%) in comparison to males and moreover, a positive correlation between NUCB2/nesfatin-1 plasma levels and mean PHQ-9 scores ($r = 0.35$, $p < 0.01$) was detected in women, while no correlation was seen in men (Hofmann et al., 2015b). Whether NUCB2/nesfatin-1 is involved in the development and/or maintenance of depressive symptoms in men will have to be further investigated.

Lastly, we investigated, whether the severity of depressive-like behavior would change NUCB2/nesfatin-1 levels in an obese mixed-sex population in association with the improvement of anxiety symptoms during inpatient treatment (Hofmann et al., 2017). In women who displayed an improvement of anxiety scores, the grade of depressiveness depicted by PHQ-9 scores also significantly improved (-43%) (Hofmann et al., 2017). Compared to females, we did not observe a significant change of PHQ-9 scores in the male study population along with a change of reported anxiety (Hofmann et al., 2017). No significant alterations of circulating NUCB2/nesfatin-1 were observed in association with altered depressiveness (Hofmann et al., 2017). Future studies should focus on patients with major depression undergoing a regimen specifically intended to treat depression (with or without the use of antidepressants) and may have to follow up patients over a longer period of time (> 2 weeks) as changes of NUCB2/nesfatin-1 may require more time. However, it should be kept in mind that brain alterations of NUCB2/nesfatin-1 might be more important for the development of depressiveness and may be missed by the measurement of plasma NUCB2/nesfatin-1. Besides that, future studies should shed light on potential interrelations between NUCB2/nesfatin-1 and other peptides that have been also implicated in depressive-like behavior. Recent studies reported, that decreased levels of brain-derived neurotrophic factor (BDNF) (Aas et al., 2018; Nunes et al., 2018), oxytocin (Frasch et al., 1995) and neuropeptide Y (NPY) (Ozsoy et al., 2016) are associated with depressive disorders in humans. Moreover, increased pituitary adenylate cyclase-activating polypeptide (PACAP) (Seiglie et al., 2015) and neurokinin (NK) receptor 2 signaling (Bardelli et al., 2013) seem to maintain the depressive phenotype. Lastly, the orexigenic peptide ghrelin, derived from the same gastric cell as NUCB2/nesfatin-1 (Stengel et al., 2009b, a), might also play a role in the development of depression, although findings are inconsistent in human studies (Barim et al., 2009; Ozsoy et al., 2014). Therefore, the development of mood disorders should be rather viewed in light of complex alterations of these peptides. Future studies are warranted investigating the interactions between NUCB2/nesfatin-1 and these different peptides.

4. Potential role in stress mediation

Mood and anxiety disorders are often characterized by a high vulnerability for stress and therefore, altered stress responses often further enhance clinical symptoms. Therefore, it is not surprising that NUCB2/nesfatin-1 has been investigated also in light of different stressors.

Early on, our group described a wide distribution of NUCB2/nesfatin-1 in several rat brain regions including autonomic nuclei (Goebel et al., 2009a), often involved in the response to stress. Subsequently, we demonstrated that an exposure to restraint stress for 30 min resulted in a robust activation of c-Fos immunoreactive (ir) neurons in different brain nuclei of male rats. Double immunohistochemical staining for c-Fos and nesfatin-1 indicated that the majority of activated (c-Fos positive) neurons was NUCB2/nesfatin-1 ir, namely 95% in the supraoptic nucleus (SON), 90% in the ventrolateral medulla (VLM), 80% in the locus coerulerus (LC), 57% in the rostral raphe pallidus (rRPa) 48% in the caudal nucleus of the solitary tract (NTS) and only 10-20% in the PVN except for the anterior parvocellular part (48%) (Goebel et al., 2009b). This activation of NUCB2/nesfatin-1 ir neurons in hypothalamic and hindbrain stress-related regions after an acute psychological stressor implies a possible role of NUCB2/nesfatin-1 in the response to acute stress, at least at the level of the brain.

Similarly, another study reported an activation of NUCB2/nesfatin-1 ir cells in the PVN, SON, LC, dorsal raphe nucleus (DR) and NTS following acute restraint stress in rats (Yoshida et al., 2010). Interestingly, plasma NUCB2/nesfatin-1 levels were not altered by acute restraint stress with a duration of 15 min (Yoshida et al., 2010), giving rise to a central alteration of NUCB2/nesfatin-1. Whether this also relates to a purely central mode of action under these conditions will have to be further investigated.

Moreover, icv administered nesfatin-1 induced c-Fos protein expression in the above-mentioned nuclei including corticotropin-releasing factor (CRF) ir neurons in the PVN, noradrenaline positive neurons in the NTS and LC and serotonin positive neurons in the DR (Yoshida et al., 2010), all involved in the response to stress. As all these regions also express NUCB2/nesfatin-1 (Foo et al., 2008), nesfatin-1 might (also) act in a paracrine manner. In isolated CRF ir neurons of the PVN *in vitro*, nesfatin-1 administration resulted in an increased Ca^{2+} concentration, indicating a direct stimulating effect of nesfatin-1 on these cells (Yoshida et al., 2010). In line with this finding, plasma ACTH and corticosterone levels were significantly increased following icv injection of nesfatin-1 in comparison to control animals (Yoshida et al., 2010). Furthermore, hypothalamic CRF mRNA expression and serum corticosterone were significantly elevated following single and repeated ip nesfatin-1 administration (Ge et al., 2015b). These results indicate that nesfatin-1 affects both central as well as peripheral stress signaling *via* the hypothalamus-pituitary-adrenal (HPA) axis. In humans, studies investigating the effect of nesfatin-1 on the HPA axis are lacking so far. However, NUCB2/nesfatin-1 levels were positively associated with heart rate (Celik et al., 2013) and blood pressure (Kovalyova et al., 2017; Zhao et al., 2015). Similarly, the stress response mediated *via* the HPA axis could also alter other parameters like energy expenditure or glucose supply by altered nesfatin-1 signaling, hypotheses to be further investigated.

In another rat model of restraint stress, NUCB2 mRNA expression as well as NUCB2/nesfatin-1 immunoreactivity were elevated in the parvocellular part of the hypothalamic PVN and caudal VLM following single, long-duration (4 h) restraint stress compared to non-stressed rats (Könczöl et al., 2010). Furthermore, both ACTH and corticosterone plasma levels were significantly elevated after icv administration of nesfatin-1 without restraint stress compared to the vehicle group (Könczöl et al., 2010). In addition to the increased NUCB2 mRNA expression in response to restraint stress, the peptide's mRNA expression in the parvocellular PVN was also significantly enhanced after bilateral adrenalectomy (Könczöl et al., 2010), indicating that endogenous NUCB2/nesfatin-1 is controlled by adrenal steroids in a negative feedback mechanism.

Not only restraint stress was demonstrated to activate NUCB2/nesfatin-1 ir neurons in the brain. Our group used a well-established physical stressor, abdominal surgery, in order to assess NUCB2/nesfatin-1 immunoreactivity in the rat brain (Stengel et al., 2010), since an elevated neuronal activation in stress-related brain regions, *e.g.* SON, PVN and LC, after abdominal surgery was reported before (Bonaz et al., 1994). Compared to anesthesia alone, the expression of c-Fos in SON, PVN, LC, rRPA, NTS, VLM and EW neurons was significantly increased after abdominal surgery (Stengel et al., 2010). Double c-Fos + NUCB2/nesfatin-1 labeling indicated that the majority of the c-Fos ir cells was also NUCB2/nesfatin-1 ir, namely 99% in the SON, 91% in the LC, 82% in the rRPa, 74% in the EW and VLM as well as 9-71% in the PVN (dependent on the subregion) (Stengel et al., 2010). In line with our previous finding that nesfatin-1 delays gastric emptying (Stengel et al., 2009a), the upregulation of brain nesfatin-1 may be involved in the development of postoperative gastric ileus.

Additionally, central NUCB2/nesfatin-1 ir cells were activated under conditions of inflammation in response to an ip injection of lipopolysaccharide (LPS) (Bonnet et al., 2009). LPS significantly increased the number of c-Fos + NUCB2/nesfatin-1 ir neurons in the PVN, SON, arcuate nucleus (Arc) and NTS compared to control rats (Bonnet et al., 2009), suggesting a role for NUCB2/nesfatin-1 in mediating peripheral inflammatory signals and potentially induce behavioral responses. We extended these finding by showing that ip injected LPS significantly increased peripheral NUCB2/nesfatin-1 plasma levels in male rats by 43%, 78% and 62% compared to control animals at 2, 5 and 7 h after the injection, respectively, while levels returned to baseline at 24 h (Stengel et al., 2011). Since gastric corpus NUCB2 mRNA expression was increased by 109% and gastric NUCB2/nesfatin-1 protein content was elevated by 62% after LPS administration compared to controls, peripheral (stomach) NUCB2/nesfatin-1 very likely contributes to the increase in circulating NUCB2/nesfatin-1 (Stengel et al., 2011).

Lastly, we examined, whether a continued metabolic stress is able to affect NUCB2/nesfatin-1 ir cells in the brain and used activity-based anorexia (ABA), a well-established animal model for anorexia nervosa combining food restriction and voluntary activity in a running wheel applied for a period of 21 days (Scharner et al., 2017). The ABA group was compared to a group of *ad libitum* fed rats (AL), to rats with access to a running wheel and to food (activity, AC) and to rats with restricted feeding (RF). In the ABA group, we observed a significant increase in the number of NUCB2/nesfatin-1 ir neurons in the PVN, Arc, dorsomedial hypothalamic nucleus (DMH), LC and NTS in comparison to AL and AC groups, but not to the RF group (Scharner et al., 2017). Moreover, significantly more c-Fos + NUCB2/nesfatin-1 ir were detected in the SON of ABA rats compared to the other three groups and in the PVN, Arc, DMH, DR and the rRP compared with the AL and AC group (Scharner et al., 2017), suggesting that metabolic status/feeding regimen has the greatest impact on the activation of brain NUCB2/nesfatin-1. The activation of NUCB2/nesfatin-1 ir cells in stress-related brain regions in response to ABA leads to the speculation of an involvement of nesfatin-1 in the modulation of food intake and/or emotional processes under conditions of anorexia nervosa, a hypothesis that warrants further investigation.

Interestingly, another study suggested that NUCB2/nesfatin-1 might be involved in acute stress responses but not in the mediation of chronic stress. Hypothalamic NUCB2 mRNA expression and NUCB2/nesfatin-1 plasma levels were elevated following acute water avoidance stress (WAS) (Xu et al., 2015b). In contrast, under conditions of chronic stress applying a CUMS regimen for 3 weeks neither hypothalamic NUCB2 mRNA expression nor circulating NUCB2/nesfatin-1 concentrations were affected (Xu et al., 2015b). Time course experiments will help to unravel whether an adaptation/desensitization occurs under conditions of continued stress exposure.

In human studies, the extent of individually perceived stress was examined in relation to NUCB2/nesfatin-1. Our research group focused on the association of NUCB2/nesfatin-1 and different emotional states in patients displaying a broad range of body weight often diagnosed with eating disorders, since impaired eating behavior often coincides with emotional disturbances. In a study population comprised of obese females we detected significantly higher stress levels (+60%) as assessed by the perceived stress questionnaire (PSQ-20) (Fliege et al., 2005) in women reporting high anxiety compared to obese females with low anxiety scores (Hofmann et al., 2013). In addition, NUCB2/nesfatin-1 plasma levels were positively correlated with PSQ-20 sum scores ($r = 0.57$, $p < 0.001$) (Hofmann et al., 2013), leading to the speculation of nesfatin-1 being involved in the mediation of stress in humans as well. It is to note that the scores of the PSQ-20 subscales “worries”, “tension” and “demands” were positively associated with NUCB2/nesfatin-1 ($p < 0.001$), while the “joy” scores showed an inverse correlation with NUCB2/nesfatin-1 ($p = 0.015$) (Hofmann et al., 2013). Also women with anorexia nervosa and a high grade of anxiety displayed significantly higher PSQ-20 levels (+51%) than females with low anxiety (Hofmann et al., 2015a). However, these scores did not correlate with circulating NUCB2/nesfatin-1 levels (Hofmann et al., 2015a) possibly due to the overall low NUCB2/nesfatin-1 concentrations in patients with anorexia nervosa.

Subsequently, we assessed the grade of perceived stress as well as peripheral NUCB2/nesfatin-1 concentrations in obese males and females, expecting NUCB2/nesfatin-1 to be differentially implicated in stress mediation dependent on sex (Hofmann et al., 2015b). In female patients we observed significantly higher levels of stress (+26%) than in males, and while NUCB2/nesfatin-1 plasma levels were positively correlated with PSQ-20 scores in women ($r = 0.39$, $p < 0.001$), in men no correlation was observed (Hofmann et al., 2015b). Lastly, when assessed in a longitudinal fashion, female obese inpatients that reported an improvement of their anxiety scores also improved with regards to their perceived stress levels (-26%), while in men no significant changes in perceived stress were observed (Hofmann et al., 2017). No association between changes in perceived stress and alterations of plasma NUCB2/nesfatin-1 has been detected either (Hofmann et al., 2017). Taken together, these findings along with the described differential association of NUCB2/nesfatin-1 with anxiety in this study population (Hofmann et al., 2015b) emphasize the predominant implication of NUCB2/nesfatin-1 in the mediation of anxiety in a sex-dependent manner.

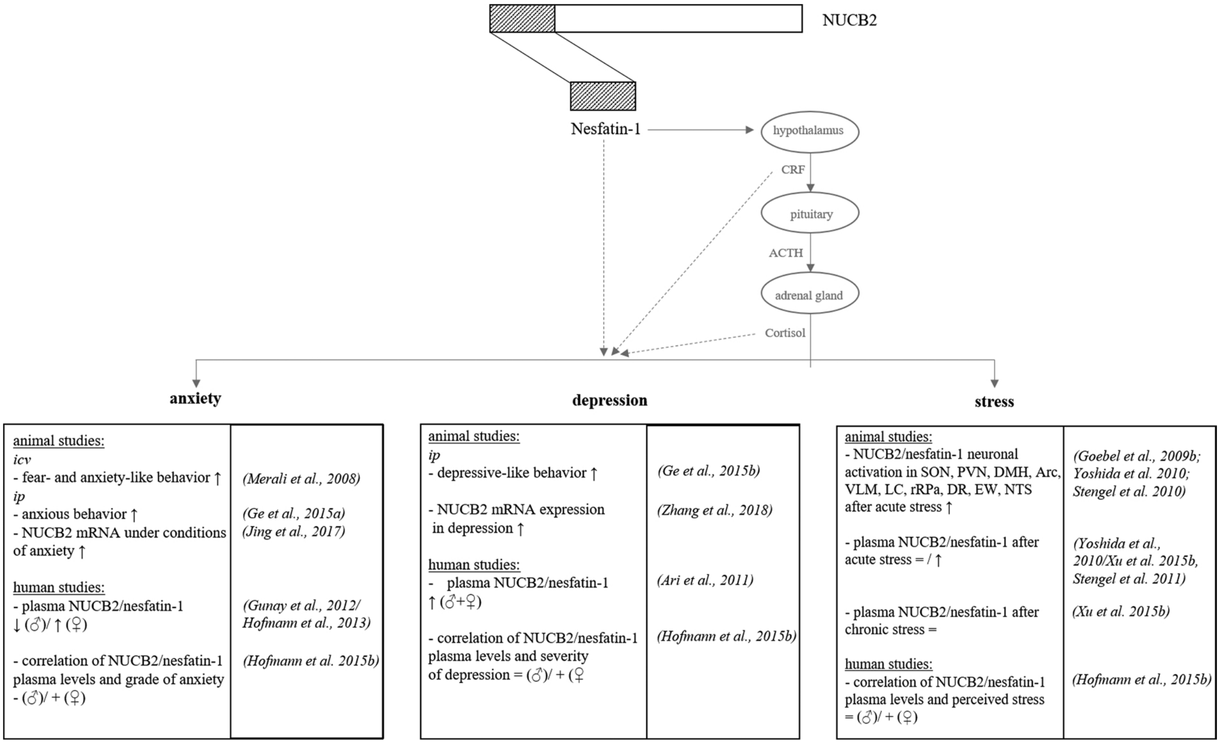
It is important to note that so far investigations are lacking that assess the effects of acute stress on NUCB2/nesfatin-1 signaling in humans. These studies should be performed in healthy subjects but also in subjects that already report an increased burden of perceived stress in order to investigate possible adaptive changes of NUCB2/nesfatin-1 signaling.

5. Summary

In recent years, nesfatin-1 received much attention as a peptide hormone considered to play a role at the interface between metabolic circuitries and emotional processes. Converging evidence points towards anxiogenic, depression- and stress-promoting effects of nesfatin-1 in animal and human studies (Fig. 1). However, the mechanism underlying these actions is often unclear so far and should be further investigated. New approaches such as optogenetics will help to unravel these pathways, first in animal models. Since the receptor mediating nesfatin-1’s actions still remains unknown, its discovery will represent a big leap forward in the understanding of nesfatin-1’s (patho)physiology. The sex-specific aspects require further investigation with systematically conducted studies and control of influencing factors like sex steroids or menstrual cycle. Longitudinal studies enrolling patients with one psychiatric diagnosis (*e.g.* anxiety disorder, major depression) are needed in order to reduce confounding factors and further investigate the possible role of nesfatin-1 in the development and/or maintenance of emotional processes. Lastly, longitudinal studies are also required in order to investigate whether the alterations of NUCB2/nesfatin-1 are response to emotional dysfunctions or trigger these psychopathological states.

Conflicts of interest

The authors have no conflicts of interest.



anxiety

animal studies:	
icv	
- fear- and anxiety-like behavior ↑	*(Merali et al., 2008)*
ip	
- anxious behavior ↑	*(Ge et al., 2015a)*
- NUCB2 mRNA under conditions of anxiety ↑	*(Jing et al., 2017)*
human studies:	
- plasma NUCB2/nesfatin-1 ↓ (♂)/ ↑ (♀)	*(Gunay et al., 2012/ Hofmann et al., 2013)*
- correlation of NUCB2/nesfatin-1 plasma levels and grade of anxiety - (♂)/ + (♀)	*(Hofmann et al. 2015b)*

depression

animal studies:	
ip	
- depressive-like behavior ↑	*(Ge et al., 2015b)*
- NUCB2 mRNA expression in depression ↑	*(Zhang et al., 2018)*
human studies:	
- plasma NUCB2/nesfatin-1 ↑ (♂+♀)	*(Ari et al., 2011)*
- correlation of NUCB2/nesfatin-1 plasma levels and severity of depression = (♂)/ + (♀	*(Hofmann et al., 2015b)*

stress

animal studies:	
- NUCB2/nesfatin-1 neuronal activation in SON, PVN, DMH, Arc, VLM, LC, rRPa, DR, EW, NTS after acute stress ↑	*(Goebel et al., 2009b; Yoshida et al. 2010; Stengel et al. 2010)*
- plasma NUCB2/nesfatin-1 after acute stress = / ↑	*(Yoshida et al., 2010/Xu et al. 2015b, Stengel et al. 2011)*
- plasma NUCB2/nesfatin-1 after chronic stress =	*(Xu et al. 2015b)*
human studies:	
- correlation of NUCB2/nesfatin-1 plasma levels and perceived stress = (♂)/ + (♀)	*(Hofmann et al., 2015b)*

Fig. 1. Implications of NUCB2/nesfatin-1 in the mediation of anxiety, depression and stress and its potential action *via* activation of the hypothalamic-pituitary-adrenal axis. The dotted line depicts an unknown (direct) mode of action. Abbreviations: ↑, stimulation/increase; ↓, inhibition/decrease; ♂, men; ♀, women; -, negative; +, positive; =, no effect; ACTH, adrenocorticotropic hormone; ARC, arcuate nucleus; CRF, corticotropin-releasing factor; DMH, dorsomedial hypothalamic nucleus; DR, dorsal raphe nucleus; EW, Edinger-Westphal nucleus; icv, intracerebroventricular; ip, intraperitoneal, LC, locus coeruleus; NTS, nucleus of the solitary tract; NUCB2, nucleobindin-2; PVN, paraventricular nucleus; rRPa, rostral raphe pallidus; SON, supraoptic nucleus; VLM, ventrolateral medulla.

Acknowledgments

This work was supported by funding of the German Research Foundation (STE 1765/3-2) and Charité University Funding (UFF 89/441-176, AS).

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