Aus der Medizinischen Klinik mit Schwerpunkt Psychosomatik der
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DISSERTATION

Nesfatin-1$_{30\text{-}59}$ und Adipositas: Beeinflussung von
Angstverhalten und Depressivität

Nesfatin-1$_{30\text{-}59}$ and obesity: influence on anxiety and
depressiveness

zur Erlangung des akademischen Grades
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Table of abbreviations

AgRP……………………..………………………….…………… agouti-related peptide

ARC………………………………………………….…………arcuate nucleus

BMI……………………………………………………………body mass index

CART…………………………………………… cocaine- and amphetamine-regulated transcript

CRF………………………………………………......…corticotropin-releasing factor

DA……………………………………………………...……. dopamine

DIO…………………………………………...…….... diet-induced obesity

DL Box……………………………...………………………dark-light box

ERK…………………………………...……. extracellular signal-regulated kinase

EZM……………………………………………………elevated zero maze

HFD………………………...……………………………………high fat diet

HPA…………………………………………...hypothalamus-pituitary-adrenal

IBI……………………………...……………...………. inter-bout interval

ICV……………………………………...…………………intracerebroventricular

IMI…………………………………………………...……inter-meal interval

IP……………………………………………….…… intraperitoneal

MDD……………………………………...…………….... major depressive disorder

NIH……………………………………………… novelty-induced hypophagia

NPY……………………………………………………. neuropeptide Y

NUCB2………………………………………………………nucleobindin-2

NW…………………………………………..………………… normal weight

POMC…………………………………………….. pro-opiomelanocortin

PVN……………………………...…………...……. paraventricular nucleus

SC………………………………………………….....subcutaneous

SPT…………………………………………………...sucrose preference test

VTA……………………………………………….……… ventral tegmental area

Abstracts

English

Background and aim:

Obesity is a worldwide health issue with numerous subsequent or related adverse health effects such as cardiovascular diseases or mental health disorders. Studies have indicated that the food regulatory peptide nesfatin-1 not only has anorexigenic properties but also might induce anxious or depressive behavior. Since obesity is associated with significant neuroendocrine alterations, the present investigation aimed to explore, whether the anxiogenic/anhedonic effect of nesfatin-1 can be observed under normal weight as well as obese conditions.

Methods:

For this study, we used adolescent male normal weight (NW) and diet-induced obese (DIO) Sprague-Dawley rats. All animals were cannulated intracerebroventricularly for central peptide injections. To assess depressive-like behavior/anhedonia, we performed the sucrose-preference test (SPT), which was used for dose finding (0.1, 0.3 and 0.9 nmol nesfatin-$1_{30\text{-}59}$/rat), and the novelty-induced hypophagia test (NIH). In a second set of experiments, the protocols of the SPT and NIH test using an automated food intake monitoring system (BioDaq system) were investigated in greater detail. To test for anxiety-like behavior we performed the open field test (OFT), the elevated-zero maze (EZM) and the dark-light box test (DL Box).

Results:

The dose finding SPT experiment indicated the dose of 0.3 nmol nesfatin-$1_{30\text{-}59}$ as the effective dose, where NW animals displayed a significantly reduced sucrose/water ratio after 0.3 nmol nesfatin-$1_{30\text{-}59}$ injected ICV (-33%, $p < 0.05$, n=11) compared to their respective vehicle group (injected with 5 µL of sterile H_2O, n=11). In the NIH test, NW animals reacted with a significant reduction in cookie intake (-62%, $p < 0.05$, n=8) compared to controls (n=8). In the OFT and EZM Test, NW test animals visited/entered the exposed areas significantly less often (OFT: -45%, $p < 0.01$, n=12, EZM: -39%, $p < 0.01$, n=9) compared to their controls (OFT: n=11, EZM: n=8). In all the above-mentioned experiments 0.3 nmol nesfatin-$1_{30\text{-}59}$ did not significantly affect anxious or anhedonic behavior in DIO rats ($p > 0.05$).

The second set of experiments generated food intake microstructure data such as meal size (g/meal), meal duration (sec/meal), bout size (g/bout), bout duration (sec/bout), or number of bouts during training and test periods of the SPT (n=8) and NIH (n=8) test.

Conclusion:

Our investigations further support the possible anxiogenic and anhedonic effects of centrally injected nesfatin-1$_{30-59}$ in NW male rats. However, the dose of 0.3 nmol nesfatin-1$_{30-59}$ was not capable of inducing behavioral alterations in DIO animals. Further, using the automated intake system BioDaq allows detailed food or liquid intake monitoring without disturbing the rats' behavior.

German

Hintergrund und Zielsetzung:

Adipositas ist eine weltweite Gesundheitsproblematik mit zahlreichen (Folge-)Erkrankungen wie kardiovaskulären Krankheitsbildern oder psychischen Erkrankungen. Studien deuten darauf hin, dass das nahrungsregulatorische Peptid Nesfatin-1 nicht nur anorexigene Eigenschaften aufweist, sondern auch Angst und depressives Verhalten hervorrufen kann. Nachdem Adipositas mit bedeutenden neuroendokrinen Veränderungen assoziiert ist, war das Ziel der Studie herauszufinden, ob der anxiogene/anhedonische Effekt von Nesfatin-1 sowohl unter normalgewichtigen als auch unter adipösen Bedingungen beobachtet werden kann.

Methodik:

Für diese Studie haben wir adoleszente männliche normalgewichtige (NW) und nahrungsinduziert adipöse (DIO) Sprague-Dawley Ratten verwendet. Alle Tiere wurden zur Applikation des Peptids mit einer intrazerebroventrikulären (IZV) Kanüle versehen. Um depressives Verhalten/Anhedonie zu untersuchen, haben wir den „sucrose-preference test" (SPT), welcher auch zur Dosisfindung (0,1, 0,3 und 0,9 nmol nesfatin-1$_{30-59}$/Ratte) verwendet wurde, und den „novelty-induced hypophagia test" (NIH) durchgeführt. In einem zweiten Experimentteil wurden die Protokolle des SPT und NIH-Tests unter Verwendung des automatisierten Nahrungsaufnahmesystem (BioDaq-System) detaillierter beschrieben. Zur Testung von Angstverhalten haben wir den „open-field test" (OFT), den „elevated-zero-maze" (EZM) und den „dark-light box test" (DL Box) durchgeführt.

Ergebnisse:

Im Dosisfindungsexperiment SPT ergab sich eine Dosis von 0,3 nmol Nesfatin-1$_{30-59}$, da NW Ratten bei dieser Dosis eine signifikant reduzierte Zuckerlösung/Wasser-Aufnahme (-33%, $p < 0,05$, n= 11) im Vergleich zur Kontrollgruppe (n=11) zeigten. Beim NIH-Versuch reagierten NW Ratten mit einer signifikanten Reduktion der verzehrten Keksmenge (-62%, $p < 0,05$, n=8) im Vergleich zu den Kontrolltieren (n=8). Im OFT und EZM Test betraten/besuchten NW Tiere die exponierten Zonen signifikant weniger häufig (OFT: -45%, $p <0,01$, n=12, EZM: -39%, $p < 0,01$, n=9) verglichen zur Kontrollgruppe (OFT: n=11, EZM: n = 8). In keinem der beschriebenen Experimente führte 0,3 nmol Nesfatin-1$_{30-59}$ zu einer Beeinflussung von Angstverhalten und Anhedonie bei DIO-Ratten ($p > 0,05$).

In der zweiten Experimentreihe konnten Nahrungsaufnahmemikrostrukturdaten wie die Mahlzeitengröße (g/Mahlzeit), die Mahlzeitendauer (Sek/Mahlzeit), die Dauer eines Bouts (Sek/Bout), oder die Anzahl der Bouts während der Trainings- und Testperiode des NIH (n=8) und SPT (n=8) generiert werden.

Schlussfolgerung:

Unsere Untersuchungen untermauern den möglichen anxiogenen und anhedonischen Einfluss von zentral injiziertem Nesfatin-1$_{30-59}$ bei NW männlichen Ratten. Jedoch konnte die Dosis 0,3 nmol Nesfatin-1$_{30-59}$ keine Verhaltensänderungen bei DIO Tieren hervorrufen. Des Weiteren ermöglicht die Verwendung des BioDaq Systems eine detaillierte Flüssigkeits- und Nahrungsaufnahmeermittlung, ohne dabei das Verhalten der Ratten zu stören.

1. Introduction

Nesfatin-1 was initially described in rats by Oh-I and colleagues as a peptide with anorexigenic properties (1). The precursor protein nucleobindin-2 (NUCB-2) is cleaved into nesfatin-1, nesfatin-2, and nesfatin-3 (1). However, the food intake regulatory effect was only shown for nesfatin-1 (1). The midsegment nesfatin-1$_{30-59}$ was found to be the active core of the peptide (2). Nesfatin-1 is expressed centrally most notably in food regulatory nuclei of the hypothalamus – namely the paraventricular nucleus (PVN) and the arcuate nucleus (ARC). Peripherally, its major expression site are the gastric X/A-like cells (3), where nesfatin-1 is co-expressed with the orexigenic hormone ghrelin. It was further described in the adipose tissue (4), the intestine and in the endocrine pancreas tissue (5) . Nesfatin-1 is capable of crossing the blood-brain barrier (6), further accounting for its involvement in the bidirectional communication pathway of the gut-brain axis. The pioneer study on nesfatin-1 indicated that central (ICV) injection of nesfatin-1 in rats reduces food intake dose-dependently – an effect that could be blocked by injection of an anti-nesfatin-1 antibody (1). Chronic ICV injection of the anorexigenic peptide nesfatin-1 led to weight loss in rats (1), accounting for its association with long-term body weight changes.

Obesity, defined as a BMI $\geq$ 30 kg/m^2 (7), is accompanied by profound endocrine alterations, in particular in regards to orexigenic and anorexigenic peptidergic gut brain signaling (for review see (8)). For instance, the orexigenic peptide ghrelin is decreased under obese conditions, while the anorexigenic factor cholecystokinin (CCK) was shown to exert reduced satiety effects under obese conditions in mice (9). NUCB2/nesfatin-1 levels and secretion were increased in a high fat diet (HFD) mouse model, while food deprivation led to reverse effects (4). The correlation of NUCB2/nesfatin-1 and obesity was further characterized by a study in humans showing that the number of nesfatin-1 immunoreactive cells in the gastric oxyntic mucosa increased with the degree of obesity (10). This finding was underlined by a study conducted in obese subjects, stating the same correlation of fat percentage and circulating nesfatin-1 levels (11). These changes might influence feeding behavior, since a key study conducted in diet-induced obese (DIO) and normal weight (NW) rats indicated that ICV administered nesfatin-1$_{30-59}$ differentially affects food intake microstructure: In NW rats meal size decreased after ICV nesfatin-1$_{30-59}$ injection, accounting for a satiation effect, while in DIO rats food intake was decreased by a reduction in meal frequency, accounting for a satiety effect of ICV nesfatin-1$_{30-59}$ under obese conditions (12).

Obesity however not only goes along with hormonal changes but is also associated with symptoms of <u>depressiveness and anxiety</u> (13). Interestingly, a study conducted by Hofmann and collaborators showed a strong positive correlation between anxiety scores and NUCB2/nesfatin-1 plasma levels in obese women ($r = 0.54$; $p < 0.001$) (14). In an anxiety-like gastric hypersensitivity model, induced by sequential stress, hippocampal and plasma NUCB2/nesfatin-1 levels were increased (15), giving further rise to the involvement of NUCB2/nesfatin-1 in not only weight regulation, but also anxiety behavior. Merali further investigated this association by injecting nesfatin-1 ICV in NW rats and found that nesfatin-1 had a dose-dependent effect on anxiety parameters and fear-related behavior (16), indicating an anxiogenic effect of the peptide.

In order to assess anxiety and depressive-like behavior in animals, a broad battery of <u>behavioral experimental tests</u> has been implemented. For anxiety assessment, the open-field test (OFT), the elevated plus maze and its further development to the elevated zero maze (EZM) or the dark light box (DL Box) test have been developed. For measuring depression-like behavior, tests like the novelty-induced hypophagia (NIH) test, the forced swim test, the tail suspension test or the sucrose-preference test (SPT) are well established tools. Hereby, surrogate parameters such as a reduced intake of a palatable snack or drink may account for anhedonia, a key symptom in depression. Reduced explorative behavior in a novel environment as well as an increase in avoidance of open spaces along with reduced locomotion in the anxiety tests indicate anxious behavior.

It is important to conduct these behavioral investigations, since large epidemiological studies showed a life-time prevalence of 33.7% for anxiety disorders (17) and more than 264 million people worldwide are affected with depression (18). Besides, obesity has already been seen as a global epidemic in the year 2000 by the WHO (19), giving rise to the term "globesity". Since obesity poses a risk factor for a wide variety of illnesses such as cardiovascular diseases, type two diabetes, sleep-breathing abnormalities and many more, obesity is a severe medical problem (20). In contrast to its topicality, the underlying factors and neuroendocrine alterations are not yet fully understood. Therefore, this dissertation aims to investigate the effects of the anorexigenic neuropeptide nesfatin-1$_{30\text{-}59}$ on anxiety and depression-like behavior in normal weight and obese rats using several behavioral experiments. Additionally, the combination of two of these tests – namely the NIH and the SPT – was methodologically further described in a second set of experiments, since these two tests are performed using a special automated food intake monitoring system.

2. Materials and methods

2.1 Laboratory animals

For all our experiments, male adolescent Sprague-Dawley rats with a starting weight of 200-250 g were utilized. Animals were kept under standard temperature (21-23 °C) and humidity (45-65%) conditions. The animal rooms were provided with a standardized illumination cycle, with the light turned on at 6 am and turned off at 6 pm. When the rats arrived at the animal facility, they were kept in groups of 4 animals per cage for acclimatization. After ICV surgery, animals were kept in single cages in close contact to each other (cages stand close together, transparent PVC cages) to keep the housing conditions as natural as possible. All cages were equipped with bedding and enrichment which was changed on a weekly basis. The laboratory animals were handled regularly including cautious immobilization of the animals for one minute in order to get the animals used to the injection procedure. This was done to reduce any additional restraint stress during injections prior to the experiments, that could influence the behavior of the animal during the test. The rats were checked on a daily schedule to ensure the well-being of the (operated) animals and check their daily body weight and food intake (see next sub-chapter). Documentation of possible unusual behavior, appearance or weight loss was documented in scoresheets on a daily basis. Except during the experiments, tap water and food access was provided *ad libitum* at any time.

2.2 Diets and food intake

2.2.1 Standard diet vs. high fat diet

For our experimental setting, normal weight animals received a standard rodent diet, while DIO rats were fed a high fat diet. Both diets were standardized in regards of composition. The standard diet contained 3.9 kcal/g, with 70% carbohydrates, 20% proteins and 10% fat. The high fat diet was composed of 4.7 kcal/g, with 45% fat, 35% carbohydrates and 20% proteins. The composition of the standard diet was chosen to mimic a natural food intake. The amount of 45% fat was chosen to provide sufficient weight gain for the DIO model, but not to induce anhedonia by raising the fat content too high. In the second publication only normal weight animals receiving standard chow were used.

2.2.2 Diet-induced obesity (DIO) model

The diet-induced obesity model is a well-established tool for obesity studies in rats and mice (21). Our DIO protocol was as follows: After an acclimatization period of one week after arrival to the animal facility, rats received the high fat diet as described above. The special diet and tap water in a bottle were provided from the top of the cage and were accessible by the animals at any time. Body weight, food intake and well-being of the animals was assessed and documented at around the same time every day. After the 10-week period of high-fat diet feeding, the 50% of the animals who gained the most weight were chosen for the experiments, while the other 50% of the rats, who did not develop diet-induced obesity (22), were excluded from the study.

2.2.3 Food intake assessment: manual vs. automated food intake monitoring system (BioDaq)

<u>Manual food intake assessment</u>

Manual food intake assessment was performed by daily weighing the food on top of the cage and calculating the daily food intake by subtracting the current food weight from the food weight of the day before. In order to keep the intervals equal, manual food intake assessment was done at approximately the same time every day. Manual food intake assessment was the common method used for rats undergoing the OFT, EZM and DL Box test.

<u>Automated food intake monitoring</u>

For the SPT and the NIH test, an automated food intake monitoring system (BioDaq, Research Diets Inc.) was used.

Experimental setup of the BioDaq system:

In this setting, rats are kept in single PVC cages with access to one food and one water hopper each, with no food or water offered from the top of the cage. The hoppers holding the food containers and water bottles are placed on microbalances, which are weighing the hoppers every second. The hoppers can be manually opened and closed by gates. Gates are usually open to give animals access to the hoppers and can be closed for maintenance or experimental settings. The microbalances are connected to a software system (BioDaq Data viewer) on a laboratory computer, where the recorded data can be extracted.

Assessment of food intake microstructure data:

This system is not only capable of assessing overall values of total food intake but also records food intake microstructure data. Hereby, the system distinguishes between changes in weight and stable weight of the hoppers, which are interpreted as food or liquid consumption of the animal in case of weight alterations and no consumption for steady weights. The timeframe between two meals is called an inter-meal interval (IMI). Meals, meaning the period where animals consume food and liquids, can be further broken down to bouts and inter-bout intervals (IBI). Bouts are the smallest food intake microstructure unit and can further be characterized by the parameters "start time", "duration" and "consumed amount". The total food or liquid consumption during a meal must be at least 0.02 g. As soon as two bouts are 5 seconds or more apart from each other, this time between the two bouts is called an inter-bout interval (IBI). An inter-meal interval (IMI) is defined as two bouts being more than five minutes apart from each other (Figure 1) (23).

Animals need to be habituated to the system, to learn how to eat from the hoppers, which approximately takes one week. During adaptation of the animals to the system, it might be necessary to provide additional food from top of the cage.
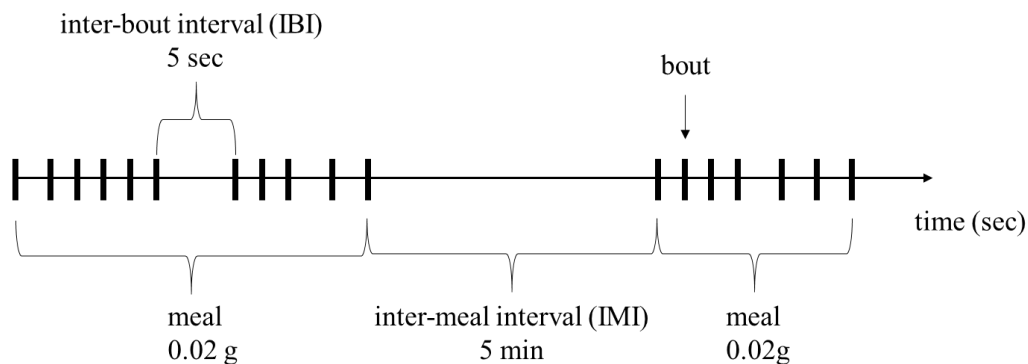
Figure 1: Food intake microstructure assessment using the BioDaq system. The values given are the minimum values for the respective microstructure parameter. **Abbreviations:** sec – seconds; min – minutes; g – grams Source: S.G. Kühne

Daily system and animal maintenance:

The BioDaq system needs daily maintenance. This includes cleaning of the microbalances, checking of possible errors that occurs during the recording period (e.g. caused by spillage on the balances, refilling of food and water), documentation of the body weight, appearance and behavior of the rat as a surrogate parameter of the well-being of the rat and inspection of the rats' ICV cannula. Further, bedding and enrichment needs to be changed on a weekly basis as well as calibration of all balances.

2.3 Intracerebroventricular cannulation

2.3.1 Materials for ICV surgery

For ICV cannulation, the following surgical instruments are needed: an electric shaver, sterile gloves, surgical masks, one scalpel with blade, two anatomic pincers, one scraper, one scissor, one needle holder, one spreader, one screwdriver and one micromotors skull drill. Further, suture material (nylon 3-0), ICV cannulas and cannula caps (1 per rat), miniature skull stainless-steel screws (4 per rat), dental cement, liquid activator for dental cement, small gauze sponges, cotton sticks, ethanol 75% and ddH_2O (to rinse screws and caps), a scale, medication (anesthesia, antidot, pain killers, antibiosis, ointment), pipettes and pipette tips, an infrared lamp, small syringes (1 ml) and cannulas, a sharps container and small falcon tubes are necessary for the procedure. To handle the stereotactic apparatus a light source, a warm pad, a sterile cover, stereotactic instruments, a binocular, and Paxions and Watsons stereotaxic atlas (24) are needed.

2.3.2 Medications for ICV cannulation

For anesthesia, a mixture of ¾ ketamine (Ketanest™, Curamed, Karlsruhe, Germany; 100 mg/ml) and ¼ xylazine (Rompun™, 2%, Bayer, Leverkusen, Germany; xylavet 20 mg/ml) was administered intraperitoneally (IP). A volume of 0.1 ml/100g body weight was administered for anesthetizing the animals. Watery povidone-iodine solution (Braunol) was used for surgical site disinfection. For pain control, animals received buprenorphine (Temgesic®, Individor UK Limited, 0.1 ml/ rat; 0.03 mg/kg body weight; 1:3 dilution in sterile 0.9% saline) subcutaneously (SC) after surgery and twice daily for the first three postoperative days. Further, bupivacaine 1% gel was applied locally on the top of the rat's head. To prevent infection, an oral antibiosis (enrofloxacin; Baytril™ 2.5% ad us. vet., 0.1 ml/L, 10 mg/kg) was added to the drinking water for 5 days. Dexpanthenol 5% (Bepanthen®, Bayer Vital GmbH) ointment was used to cover and protect the rat's eyes during surgical procedure.

2.3.3 Surgical procedure

The aim of this procedure is the placement and fixation of an ICV cannula to the right lateral ventricle of the rat's brain.

Before surgery, the rat was anesthetized as described above, the eyes were covered with ointment for protection and the fur on top of the rat's head was shaved off. The animal was then fixated in the stereotaxic apparatus. The right position of the head was checked by a small

reflexive eye twitch caused by the vagal reflex. Then, bupivacaine gel as a local anesthetic and povidone-iodine for surgical site disinfection were applied with small gauze sponges. A small vertical incision was made with a scalpel and the underlying membranes were rubbed off with a scraper. After that, the wound was kept open with a spreader and the bregma (meeting point of the cranial sutures) was located. From there, the location of the cannula -0.8 mm dorsal, -1.5 mm lateral and -3.5 mm ventral was identified, and a small hole was drilled in the skull at this location. Then, four miniature skull screws (Plastics One Inc., Roanoke, VA, USA) were drilled in the skull for fixation and the guide cannula (22-gauge, Plastics One Inc., Roanoke, VA, USA) was cautiously inserted to the right lateral ventricle. The skull was dried off with cotton sticks, followed by the application of dental cement (Stoelting Co., Wood Dale, IL, USA) and a liquid activator until all screws were covered and only the upper plastic part of the guide cannula was visible. After the beginning of hardening, the material was modulated to the head and the spreader was removed. The animal was carefully released from the stereotaxic apparatus, a dummy cannula was placed into the guide cannula, the surrounding skin/fur and eyes were cleaned, and buprenorphine (SC) was administered.

2.3.4 Verification of cannula placement

The insertion of the ICV cannula follows stereotactic coordinates; however, anatomical reality might vary. Therefore, after all experiments are done, the correct placement of the ICV cannula needs to be verified. Therefore, all animals were finally anesthetized (IP) and injected with toluidine blue 0.1% (10 µL, ICV). After waiting for one minute to let the fluid spread throughout the ventricle, the brain was extracted from the animal. Finally, the brain was cut horizontally at the visible puncture site of the cannula. A blue coloration of the ventricle verified the correct placement of the cannula.

2.4 Peptide

2.4.1 Storage and aliquotation

The peptide nesfatin-$1_{30\text{-}59}$ (mouse, rat) was obtained as powder from Bachem AG, Weil am Rhein, Germany. It was diluted in ddH_2O (500 µg/200 µl) and aliquoted to 10 µl solution each. The powder and the aliquots were stored at -80 °C. For temporary storage, e.g. for aliquotation or experiments, the peptide was kept on ice. For dose establishment, the three nesfatin-$1_{30\text{-}59}$ doses, that were based on our previously obtained nesfatin-$1_{30\text{-}59}$ food intake data (12) were used (0.1, 0.3 and 0.9 nmol). Later, only the effective dose of 0.3 nmol nesfatin-$1_{30\text{-}59}$/rat was utilized.

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2.4.2 Injection of the peptide

Each ICV injection contained 5 µl. The vehicle group always received 5 µl ddH$_2$O/rat, while the intervention group received a dose of 0.1, 0.3 or 0.9 nmol nesfatin-1$_{30-59}$ in 5 µl ddH$_2$O/rat. Injection of the peptide always occurred 30 minutes before testing. This timeframe is based on data, assessed in a previous study of our workgroup, where activity of the peptide was observed 30 minutes after ICV injection (25). All experiments were conducted at the onset of the dark phase (6:30 pm), since the anorexigenic effects of the peptide have been shown during this photoperiod (25).

For injections, the animal was light hand-fixated by one researcher. Due to the regular handling of the animals as described above, this stimulus should not add any additional stress to the rat. Then, the dummy cannula was removed from the guide cannula and the peptide was slowly (over 15 seconds) injected to the lateral ventricle. Therefore, the peptide was filled into a Hamilton syringe. A PE-50 catheter (Intramedic Polyethylene Tubing, Clay Adams, Parsippany, NJ, USA) connected the syringe to a 28-gauge cannula (Plastics One Inc., Roanoke, VA, USA), that was inserted to the guide cannula in the rat. After injection, the cannula was left in the guide cannula for another minute to let the fluid drain off. Then, the injection cannula was carefully removed, and the dummy cannula was inserted back to the guide cannula.

2.5 Behavioral tests

The tests to assess anxiety, namely the OFT, EZM and DL Box test, were conducted at the Animal Outcome Core facility of the Charité Campus Mitte, while the BioDaq system, needed for conduction of the NIH and SPT test, was located at the animal facility of the Charité Campus Virchow. All animal experiments were approved by the LaGeSo, Berlin, Germany.
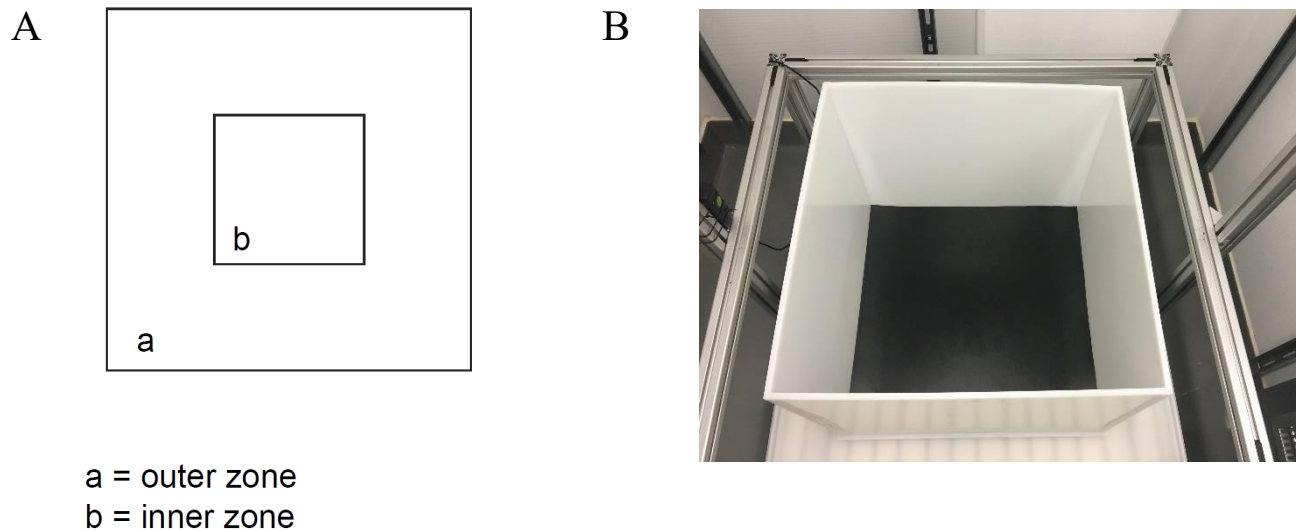
2.5.1 Open field test

Experimental setup

The open field test is performed in a 50 x 50 cm white PVC box with a black mat on the ground. A camera, which is connected to a computer outside the experimental room, is placed above the box and records a video of the animal during the test. The computer is equipped with the special software viewer (Biobserve GmbH, Bonn, Germany), recording various behavioral data. This software divides the box into an outer zone (area **a** in Figure 2) and an inner zone, also called center zone (area **b** in Figure 2). For the experiment, the rat is placed in the center of the experimental box and the researcher leaves the room to not disturb or influence the animal's behavior. The experimental room has a standard temperature of 20-24 °C and a

standard humidity of 45-65% in order to keep environmental influence factors between the test settings as equal as possible. After 5 minutes of testing, the software stops recording and the animal is removed from the open field test. Before all experiments, the box needs to be cleaned with 70% ethanol. Between two animals, the box is cleaned with 5% ethanol.

a = outer zone
b = inner zone

Figure 2: experimental setup of the open-field test. **A.** schematic setup of the OFT. **B.** test apparatus OFT; Source of A: Kühne SG, Schalla MA et. al., *Nutrients* 2018. Source of B: picture by S. G. Kühne

Purpose of the test

The OFT is a well-established behavioral test (26) that assesses anxiety behavior in animals. For this test laboratory animals are exposed to a novel environment. A test animal, that has a higher level of anxiety will have a decrease in locomotion compared to a normal animal. However, it is not only locomotion, but also the amount of time, that the animal spends in the central (inner) zone, that can indicate anxiety. An animal, who is more explorative and less anxious will spend more time and make more entries in the central zone, where the animal feels more exposed and vulnerable, whereas an animal, who is more anxious will spend more time in the corners and the outer areas of the arena. Therefore, the parameters average velocity (cm/s), entries in center zone (number), and duration in center zone (s) are parameters for anxiety assessment in this test.

2.5.2 Elevated-zero-maze test

Experimental setup

The EZM is a zero shaped elevated platform with two open and two closed areas (Figure 3). The EZM apparatus is, as the OFT test, located in a separate room with standard environmental

conditions and shielded from noise and disturbances. The EZM platform is filmed with a camera located above the experimental stetting and is connected to a laboratory computer,
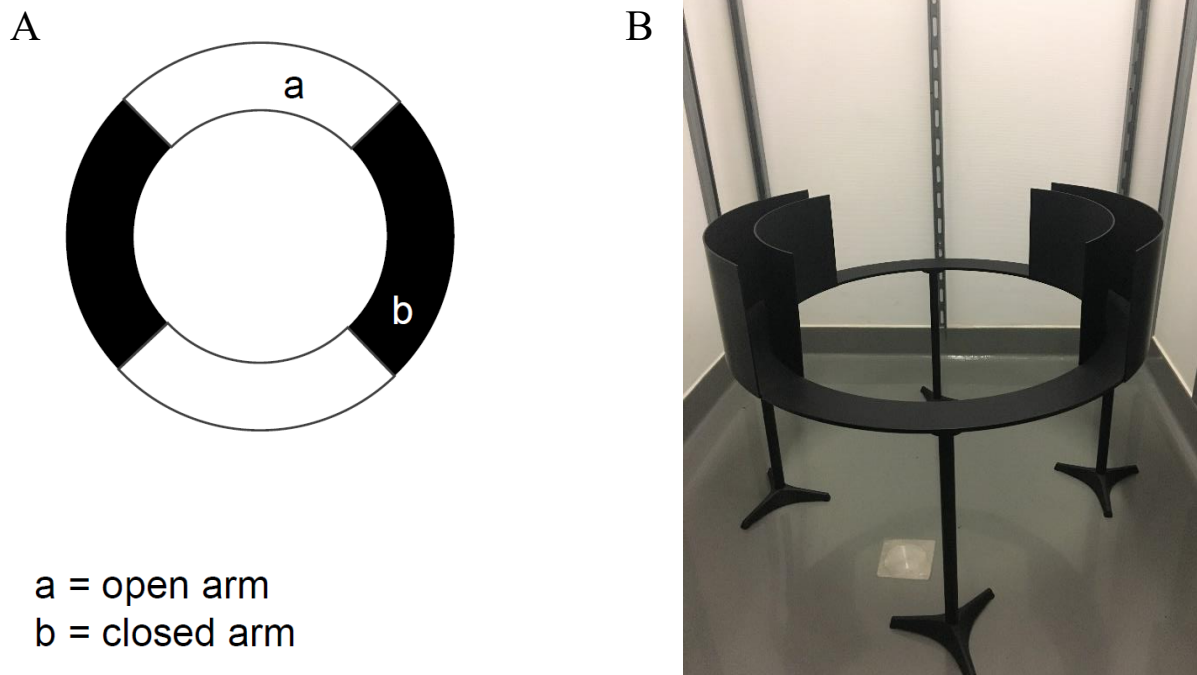
Figure 3: experimental setup of the elevated-zero maze **A.** schematic setup of the EZM. **B.** test apparatus EZM;
Source of A: Kühne SG, Schalla MA et. al., *Nutrients* 2018. Source of B: picture by S. G. Kühne

located outside the test room. The software Viewer (Biobserve GmbH, Bonn, Germany) records a video of the animal and tracks behavioral data. For the test, the animals are all placed in the open arm facing the same direction towards a closed arm. The test duration is also 5 minutes. Cleaning of the test apparatus also is conducted with 70% ethanol before all experiments and with 5% ethanol in between the tests.

Purpose of the test

The EZM test also follows the goal to assess anxiety parameters of an animal. Hereby, an indicator of anxiety is an animal that hides in the closed arms, spending more time there, where it feels safer and more shielded than in the open arms. Also, these animals show less locomotor activity and avoid the open and exposed areas. In contrast, a less anxious rat explores the open arms of the maze, indicated by a larger number of visits of the open arms and by more time spent in the open arms. Also, the activity, indicated by the overall track length during the test period, is higher in less anxious test animals.

2.5.3   Dark-Light Box test

<u>Experimental setup</u>

The DL Box test is performed within the TSE Multi Conditioning System (MCS), a multi-purpose modular test platform that is sound attenuating. The animals are tested in a 50 cm x 50 cm arena that is divided into two equal chambers, one dark and one light. The two chambers a are connected through a small door, where the animal can cross to the other compartment. The left chamber is illuminated with approximately 100 lux, while the right chamber (dark chamber) has no illumination (Figure 4). The DL Box is connected to a laboratory computer equipped
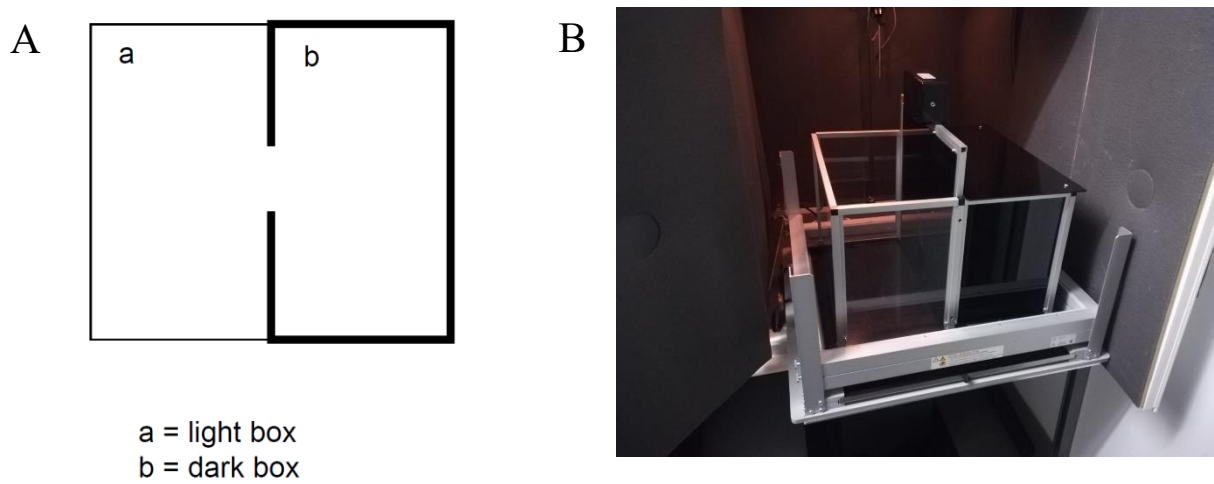
Figure 4: experimental setup of the dark-light box; **A.** schematic setup of the DL Box. **B.** test apparatus DL Box; Source of A: Kühne SG, Schalla MA et. al., *Nutrients* 2018. Source of B: picture by S. G. Kühne

with the TSE software system, that tracks the animal's movements using high-resolution infrared sensors. Also, a video of the animal is recorded. All animals are placed in the light compartment at the beginning of the experiment and allowed to explore the entire area for 10 minutes. As in the OFT and EZM test, the experiment takes place in a noise shielded environment with standard conditions (temperature 20-24 °C, humidity 45-65 %, researcher leaves the room during the experimental period). In line with the OFT and EZM test, the DL Box is cleaned with 70% ethanol before all experiments and with 5% ethanol between to test animals.

<u>Purpose of the test</u>

The DL box test is a behavioral test that assesses the anxiety level of an animal. It is a sensitive tool also applied in pharmacological research of anxiolytics such as benzodiazepines (27). Hereby, the test targets the instinct of a rat to hide in the dark and the contradictory desire to explore the light compartment. The parameters for measuring anxiety are the duration of time

spent in the dark chamber, number of visits in the bright chamber and the latency. Latency is defined as the time from the start of the experiment until the animal first enters the dark compartment. An animal with anxiety-like behavior will spend more time in the dark chamber, with less visits to the light chamber and a shorter latency to the dark chamber. Consequently, a curious/less anxious animal has a greater latency to enter the dark chamber, more time spent in the light chamber and more visits to the light chamber.

2.5.4   Novelty-induced hypophagia test

Habituation and training period

The NIH test was conducted using the BioDaq system (BioDAQ, Research Diets Inc., Jules Lane, New Brunswick, NJ, USA) as described above (section 6.2.3). Prior to experimental testing, the rats needed to be surgically equipped with an ICV cannula, recover from the intervention, and adapted to eating and drinking from the hoppers of the automated food intake monitoring system. Then, a NIH training period was started.
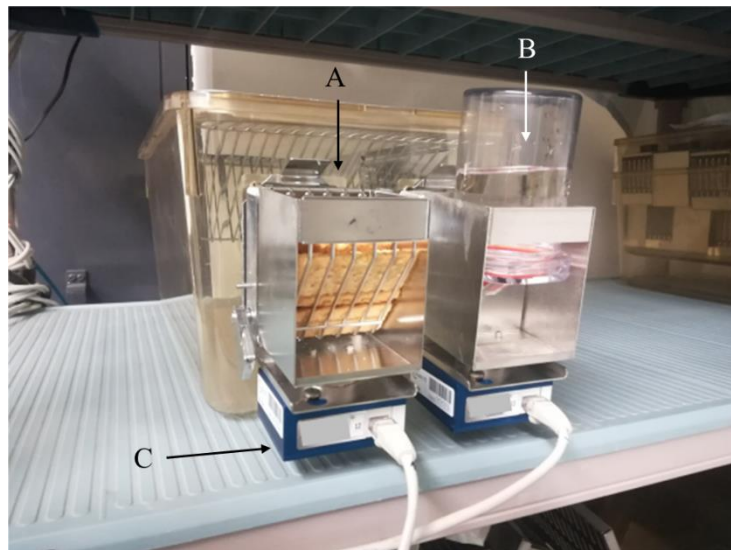
Figure 5: experimental setup of the novelty-induced hypophagia test. **A.** cookie hopper **B.** tap water **C.** microbalance. Source: adapted from Schalla MA, Kühne SG, et. al., *J. Vis. Exp.* 2020

Hereby, at the onset of the dark phase, the food hoppers were replaced by cookies (HoneyMaid™ Graham Cracker Crumbs, Nabisco, East Hanover, NJ, USA). After that, the animals had access to the cookies as a palatable snack for 30 minutes to familiarize the animals with it (Figure 5). Then, the cookie hoppers were removed, and their normal chow was provided again. Water was always accessible *ad libitum* during the procedure. After the 30-minute training period, the cookie and water intake were documented. All interventions during the training, such as opening/closing gates, replacing chow or documentation were done using red light, which is not visible to rats, in order to not disturb the animals during the dark phase. This training procedure was repeated for 5 consecutive days, so that a stable cookie intake was reached.

NIH experimental setting

On day 6, the NIH experiment was conducted as an adapted and modified version of the NIH test of Merali and colleagues (16). Hereby, unfasted animals were disconnected from the BioDaq system for injections but stayed in their home cages. Then, novel cages without bedding and enrichment were connected to the BioDaq system. The animals were injected 30 minutes before the experiment. For the experimental testing, the rats were placed into the novel and empty cages and water and cookies gates, with the familiar palatable snack, were opened (Figure 5). After 30 minutes of NIH testing, water gates remained open, cookie gates were closed, and the palatable snack was replaced by regular chow. The cookie intake and further food intake microstructure data were all recorded by the BioDaq system.

Purpose of the test

The NIH test has a focus on anhedonia and anxiety assessment. The novel cage, where the rats are placed in during the experiment, induces novelty stress, which is anxiogenic. The amount of the consumed snack during the test is an indicator of anhedonic behavior as well as a reduced latency to first bout (in seconds). Latency to first bout means the time period, starting at the beginning of the experiments, when the cookie gates are opened until the time point, when the rat first touches the cookie.

2.5.5 Sucrose Preference test

Habituation and training period

In line with the NIH test setting, the animals also need to be surgically equipped with an ICV cannula, recover from the intervention and adapt to eating and drinking from the BioDaq system (BioDAQ, Research Diets Inc., Jules Lane, New Brunswick, NJ, USA). Then, a training period starts, where the chow hopper is replaced by a second water hopper. The second water hopper is filled with 1%-sucrose solution, while the other one remains filled with tap water (Figure 6). In this setting, chow is provided from top of the cage and food intake is therefore assessed manually. Every 24 hours, the position of
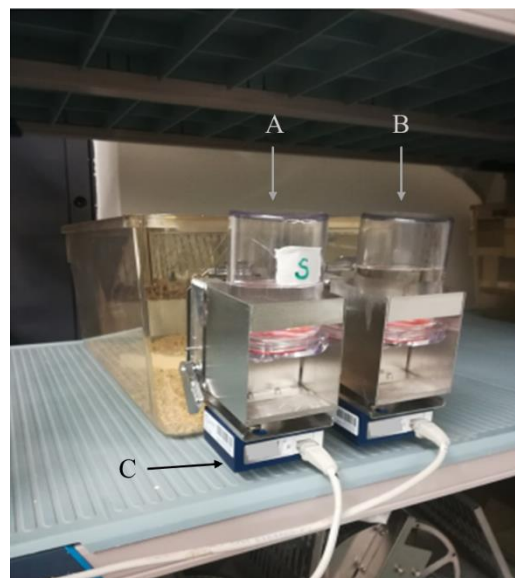
Figure 6: experimental setup of the sucrose-preference test; **A.** sucrose solution **B.** tap water **C.** microbalance
Source: adapted from Schalla MA, Kühne SG, et. al., *J. Vis. Exp.* 2020

the tap water and the sucrose solution are exchanged, to avoid habituation to the position to the preferred bottle. The training period must last at least two consecutive days. Then, the sucrose solution gets removed for 24 hours prior to the test. A successful training period is marked by a sucrose to water ratio close to 1.

<u>SPT experimental setting</u>

On the test day, the animals are injected at the onset of the dark phase (6 pm). After 30 minutes, the experiment begins and animals are offered tap water and the sucrose solution from the hoppers, as well as food from the top of the cage. After one hour of test period, the sucrose gates are closed, and the animals are offered chow and tap water as usual. It is important, to document the position of the sucrose and the tap water bottle, as well as which animal received which injection to the BioDaq system for later data analysis.

<u>Purpose of the test</u>

The aim of the SPT test is to assess anhedonia in rats. The sucrose solution offered during the training period and on the test day displays a preferable drink to the rats. This can be observed by the sucrose/water ratio of almost 1 during the training period. If the injected peptide induces an anhedonic effect, these rats consume significantly less sucrose solution than the controls.

2.6 Statistics

2.6.1 Statistical analysis

Statistical analysis was performed with SigmaStat 3.1 (Systat Software, San Jose, CA, USA). Hereby, we first checked whether our data were normally distributed or not, using the Kolmogorov-Smirnov test. If normal distribution was assured, two groups were compared using *t*-test. The Mann-Whitney-U test was applied for non-normally distributed data. In case of multi group comparison, one-way ANOVA followed by Turkey *post hoc* test was utilized for normally distributed data or in case of non-normal distribution followed by Dunn's multiple comparison test. The significance level was set at $p < 0.05$ and data are all shown as mean ± sem.

2.6.2 Data and animal exclusion criteria

Animal data had to be excluded in case any disturbances happed during the tests. This includes for example sudden noises or if the recording did not work. Further, if animals showed any odd

behavior before the test or had a significant weight loss/reduced food intake, animals were excluded from the behavioral tests. Also, if the injection prior to the test did not drain off or cannula placement was proven wrong after finalization (n=1), data were excluded from the statistics.

3. Results

3.1 Publication 1: Nesfatin-1 injected intracerebroventricularly increases anxiety, depression-like behavior, and anhedonia in normal weight rats

3.1.1 Influence of nesfatin-1$_{30-59}$ on anhedonic/depressive behavior under normal weight and DIO conditions
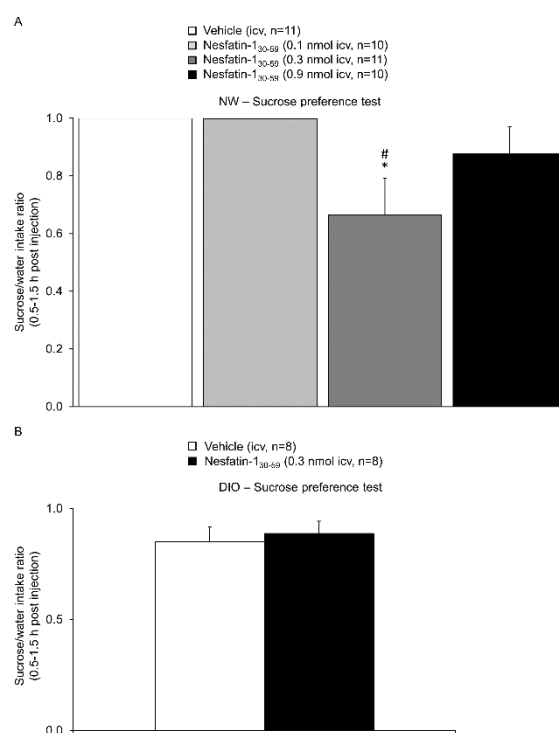
Both experiments, conducted to assess anhedonic/depressive-like behavior, showed that nesfatin-1$_{30-59}$ injected ICV significantly induced anhedonia under NW conditions but not in obese rats.

In the **SPT** test, the comparison between 0.1 (n=10), 0.3 (n=11), 0.9 (n=10) nmol nesfatin-1$_{30-59}$ ICV and the vehicle group receiving 5 µl ddH$_2$O ICV (n=11) in NW male rats showed a significant reduction in the sucrose/water ratio in the 0.3 nmol group (0.66 ± 0.13) compared to the control group (1.00 ± 0.00) and the dose 0.1 nmol nesfatin-1$_{30-59}$ (1.00 ± 0.00, p = 0.3; Figure 7). Nesfatin-1$_{30-59}$ injected at a dose of 0.9 nmol induced a sucrose/water ratio of 0.88 ± 0.09; however,

Figure 7: SPT under normal weight and obese conditions; **A.** normal weight rats **B.** obese rats. Data expressed as mean ± SEM. * p < 0.05 vs. vehicle # p < 0.05 vs. nesfatin-1$_{(30-59)}$ 0.1 nmol.
Source: Kühne SG, Schalla MA et. al., *Nutrients* 2018

this did not reach significance (p = 0.30). Based on this dose establishment, all further experiments used the dose of 0.3 nmol nesfatin-1$_{30-59}$ as the effective dose.

Injection of 0.3 nmol nesfatin-1$_{30-59}$ (ICV) in DIO rats and assessment of the sucrose/water intake ratio from 30 to 90 minutes post injection had no significant effect in the intervention group (n=8) compared to controls (n=8, p = 0.69; Figure 7).

In the **NIH** test, normal weight rats injected ICV with 0.3 nmol nesfatin-1_{30-59} (n=8) consumed significantly less cookies (1.09 ± 0.32 kcal) during the test than their respective controls (4.36 ± 1.31 kcal, p=0.04; n=8, Figure 8), accounting for an anhedonic effect of the peptide. However, nesfatin-1_{30-59} did not have a significant effect on the parameter "latency to first bout (s)" (207.0 ± 58.4 vs. 241.3 ± 48.8 s, p = 0.68).

The ICV injection of 0.3 nmol nesfatin-1_{30-59} in DIO animals had no significant effect on food intake as well as the latency to first bout (s) (p > 0.05) compared to their obese controls (n=5/group, Figure 8).
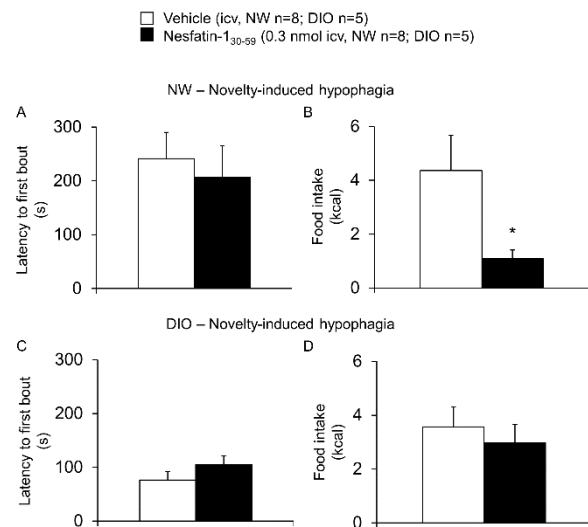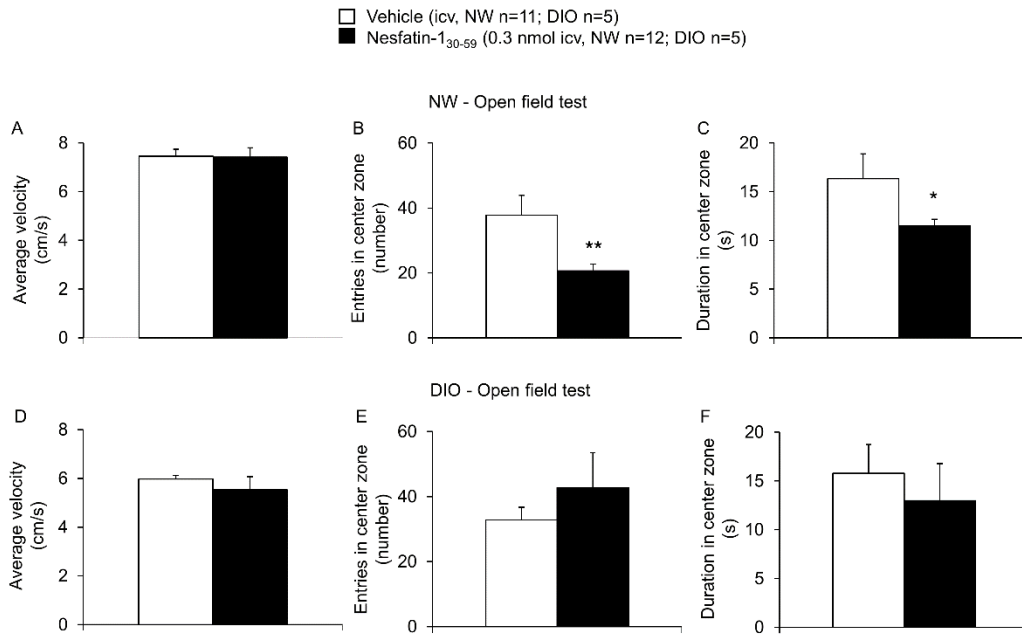

Figure 8: NIH under normal weight and DIO conditions **A.** normal weight rats **B.** obese rats. Data expressed as mean ± SEM. * p < 0.05 vs. vehicle. Source: Kühne SG, Schalla MA et. al., *Nutrients* 2018

*3.1.2   Influence of nesfatin-1_{30-59} on anxiety under normal weight and DIO conditions*

In the following, the results of the three tests used for anxiety assessment – namely the OFT, EZM and DL box test – are displayed.

In the **OFT** test, an ICV injection of nesfatin-1_{30-59} (0.3 nmol) in normal weight rats (n=12) induced a significant reduction (p = 0.008) in the number of entries in the center zone of the maze (20.6 ± 2.0) compared to vehicle injected animals (37.8 ± 6.1), accounting for an anxiogenic effect of the peptide (Figure 9). This observation is underlined by the fact that also the duration in the center zone, measured in seconds, was significantly reduced (p = 0.04) after ICV nesfatin-1_{30-59} injection (11.5 ± 0.7 s), when compared to the normal weight control rats (16.3 ± 2.6 s). However, average velocity (cm/s) did not differ (p = 0.47) between the two normal weight groups (7.4 ± 0.4 vs. 7.4 ± 0.5 cm/s). When performing the OFT on DIO rats, none of the assessed parameters (average velocity (cm/s), entries in enter zone (number), duration in center zone (s) was influenced by the peptide (p > 0.05; Figure 9).

Figure 9: OFT under normal weight and obese conditions **A.** normal weight rats **B.** obese rats. Data expressed as mean ± SEM. * $p < 0.05$ vs. vehicle ** $p < 0.01$ vs. vehicle. Source: Kühne SG, Schalla MA et. al., *Nutrients* 2018

In the **EZM** test, central injection of 0.3 nmol nesfatin-1_{30-59} induced a significant ($p = 0.002$) reduction in the number of visits of the open arms in normal weight rats (19.8 ± 1.8, n=9), when comparing them to vehicle treated animals (32.5 ± 2.8, n=8, Figure 10). The parameters time in open arms (92.7 ± 11.9 vs. 128.9 ± 15.6 s, $p = 0.05$) as well as the overall track length (25.6 ± 1.4 vs. 27.9 ± 1.1 m, $p = 0.14$) were not significantly altered after ICV injection of nesfatin-1_{30-59} in normal weight laboratory animals. Performing the EZM test under obese conditions
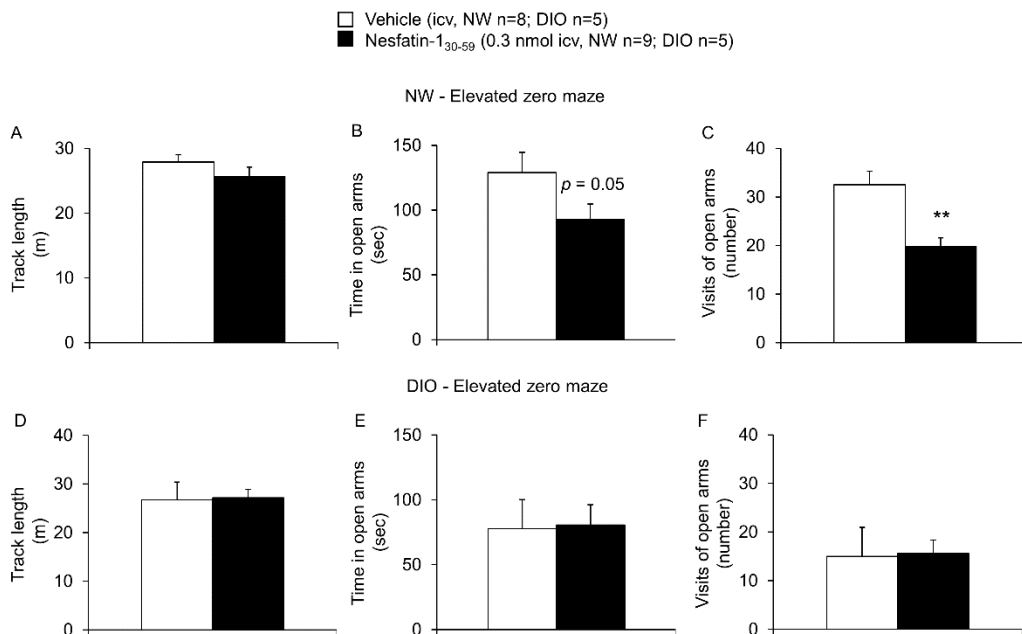
Figure 10: EZM under normal weight and obese conditions **A.** normal weight rats **B.** obese rats. Data expressed as mean ± SEM. ** $p < 0.01$ vs. vehicle. Source: Kühne SG, Schalla MA et. al., *Nutrients* 2018

(n=5/group), 0.3 nmol of central nesfatin-1_{30-59} did not alter behavior when comparing nesfatin-1_{30-59} and vehicle rats (Figure 10).

In the **DL Box** test none of the assessed parameters – namely time im dark (381.9 ± 12.2 vs. 413.0 ± 15.9 s, $p = 0.10$), latency (21.5 ± 3.2 vs. 32.6 ± 5.6 s, $p = 0.08$), visits of bright side (number, 12.0 ± 0.9 vs. 11.5 ± 0.6, $p = 0.33$) and time in bright (281.1 ± 12.2 vs. 187.0 ± 15.9 s, $p = 0.10$) – was significantly affected ($p > 0.05$) by ICV nesfatin-1_{30-59} injection in normal weight animals (Figure 11). Also under DIO conditions, no significant behavioral alterations could be oberserved after ICV nesfatin-1_{30-59} injection in the DL Box test (Figure 11).
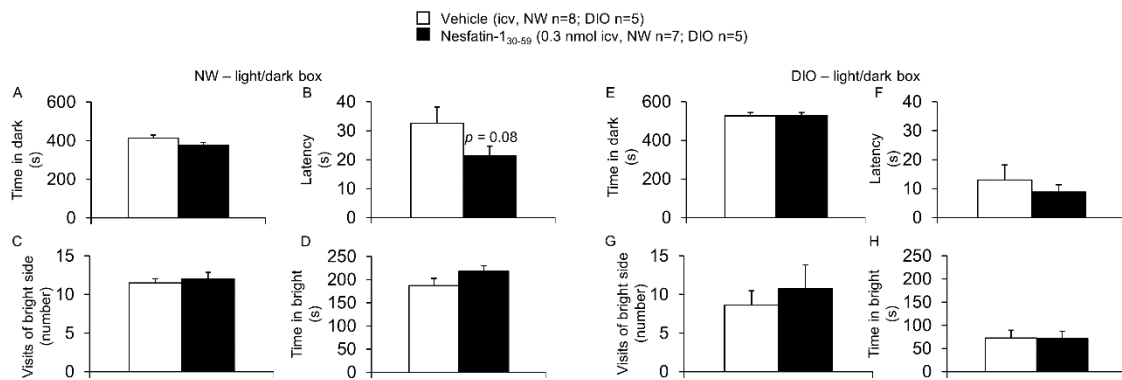
Figure 11: DL Box under normal weight and obese conditions **A.** normal weight rats **B.** obese rats. Data expressed as mean $\pm$ SEM. $p > 0.05$. Source: Kühne SG, Schalla MA et. al., *Nutrients* 2018
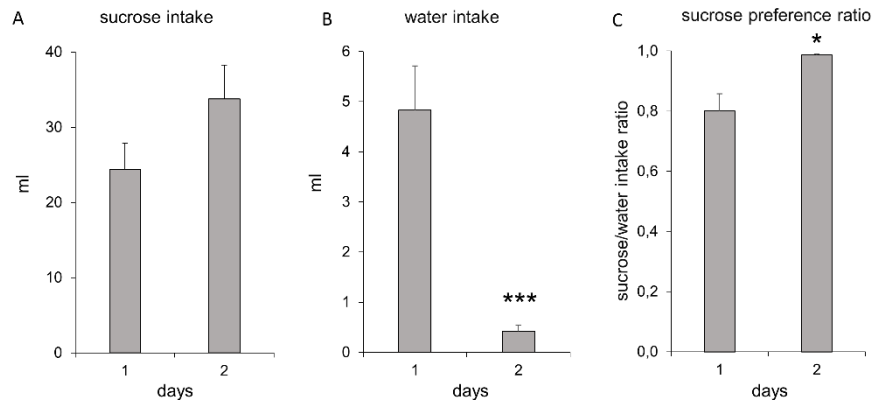
3.2 Publication 2: Sucrose preference and novelty-induced hypophagia tests in rats using an automated food intake monitoring system

3.2.1 Representative results of the SPT

At the end of the SPT training period the sucrose preference ratio is almost 1.0 (0.99 ± 0.004, $p < 0.05$ vs. day 1, Figure 12). Over the training period, the animals tend to consume more sucrose solution from day to day (day 1: 24.40 ml $\pm$ 3.48 ml, day 2: 33.77 ml $\pm$ 4.49 ml, $p = 0.17$; Figure 12)

Figure 12: SPT training period. **A.** sucrose solution consumption on day 1 and 2 in ml. **B.** water consumption on day 1 and 2 in ml. **C.** sucrose/water intake ratio on day 1 and 2. Data expressed as mean $\pm$ sem; n=8; * $p < 0.05$, *** $p < 0.001$. Source: Schalla MA, Kühne SG, et. al., *J. Vis. Exp*. 2020

and water intake decreases (day 1: 4.83 $\pm$0.89 ml, day 2: 0.42 ml $\pm$ 0.13 ml, $p < 0.001$; Figure 12). All data were obtained using the BioDaq data analysis software.

3.2.2 Representative results of the NIH test

During the NIH training period and on the test day, the automated food intake monitoring system is not only capable of assessing food and water intake values, but also records microstructure data, as shown in the following. During the 5-day training period, the meal size gradually increases (day 1: 0.48 ± 0.14 g, day 2: 1.05 ± 0.32 g, day 3: 1.48 ± 0.56 g, day 4: 1.1 ± 0.39 g, day 5: 1.91 ± 0.68 g, Figure 13), while parameters such as meal duration, time spent in meals or time spent in bouts do not change significantly over the days ($p > 0.05$, n=8; Figure 13).

During the NIH test, the following parameters were obtained (Figure 14):

- meal size, meaning how many grams of cookies were consumed in one meal (0.98 ± 0.34 g/meal),

- meal duration, meaning the length of a meal in seconds (998.29 ± 192.16 sec/meal),

- time spent in meals, meaning the cumulated duration of meals over the test period measured in seconds (998.29 ± 192.16 sec),

- latency to first bout, meaning the time between the start of the test ("open all gates") to the first bout (241.25 ± 45.96 sec),
- bout size, meaning the amount of consumed cookies in grams consumed during one bout (0.21 ± 0.04 g/bout),
- bout duration, meaning length of one bout in seconds (70.70 ± 16.56 sec/bout),
- time spent in bouts, meaning the cumulated duration of bouts over the test period measured in seconds (439.75 ± 147.67 sec),
- number of bouts, meaning the number of bouts over the test period (5.63 ± 0.74)
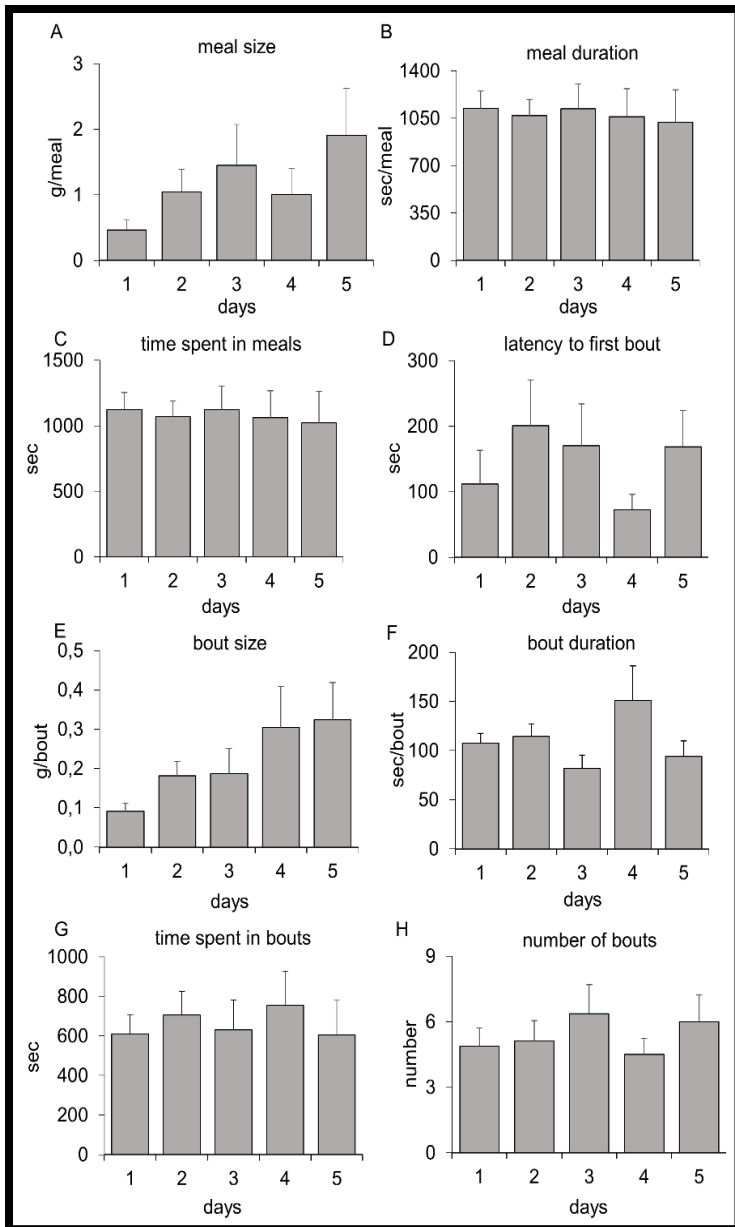
Figure 13: food intake microstructure assessed by automated intake monitoring system during 5-day NIH training period. **A.** meal size (g/meal) **B.** meal duration (sec/meal) **C.** time spent in meals (sec) **D.** latency to first bout (sec) **E.** bout size (g/bout) **F.** bout duration (sec/bout) **G.** time spent in bouts (sec) **H.** number of bouts (number). Data expressed as mean ± SEM. n = 8. Source: Schalla MA, Kühne SG, et. al., *J. Vis. Exp*. 2020
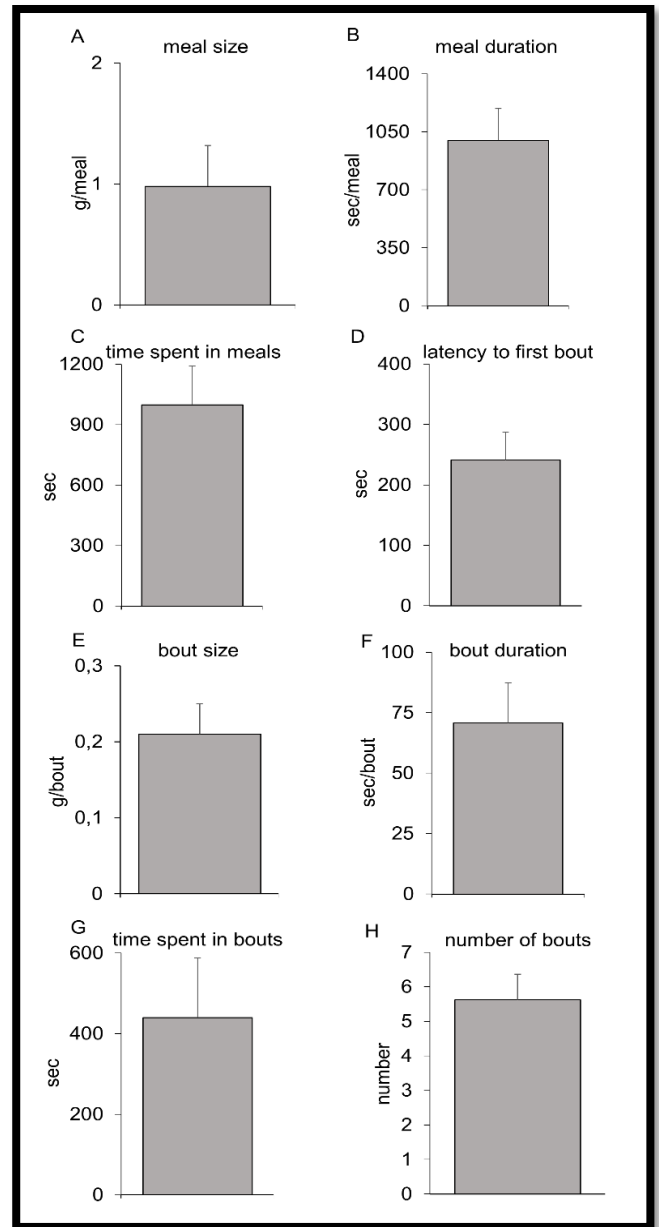
Figure 14: Intake microstructure assessed by BioDaq system during NIH test day. **A.** meal size (g/meal) **B.** meal duration (sec/meal) **C.** time spent in meals (sec) **D.** latency to first bout (sec) **E.** bout size (g/bout) **F.** bout duration (sec/bout) **G.** time spent in bouts (sec) **H.** number of bouts (number). Data expressed as mean ± SEM. n = 8. Source: Schalla MA, Kühne SG, et. al., *J. Vis. Exp*. 2020

4. Discussion

4.1 Key findings of the study

This dissertation showed that injection of 0.3 nmol of the active midsegment nesfatin-1_{30-59} injected ICV in rats induces anxiety and depression-like behavior in NW animals. However, no behavioral alterations could be observed when injecting the same dose of the peptide ICV in obese rats. These data were generated using several behavioral tests, of which two were conducted with the automated food intake monitoring system. This system is capable of providing additional food intake microstructure data in an automated manner, leaving the animals undisturbed during the behavioral tests, which would not have been possible with manual intake assessments.

4.2 Possible (patho)mechanism of the anxiogenic/anhedonic effect of nesfatin-1_{30-59} under normal weight and under obese conditions

Besides its key feature of having an anorexigenic effect and thereby also playing a role in long-term body weight regulation (1), nesfatin-1 has been shown to have pleiotropic effects as being involved in glucose metabolism (28) but also in thermoregulation, heart rate variation, gastric emptying or reproductive functions (29). This work further supports the idea that nesfatin-1 plays a role in the mediation of anxiety and depression-like behavior – an effect not observed under DIO conditions (30).

The possible (patho)physiological mechanism behind the injection of a satiety peptide and behavioral alteration is not yet fully discovered. However, the cross-link between stress and food intake can be assumed in the hypothalamus – the center of food intake regulation and the begin of the stress axis. In the hypothalamus, nesfatin-1 has been shown to be expressed in the arcuate nucleus (ARC), where the orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP) are co-expressed with the anorexigenic peptides pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) (31). The food intake-suppressing peptides POMC and CART subsequently activate the paraventricular nucleus (PVN), where among others the anorexigenic substance corticotropin-releasing factor (CRF) is secreted (31). This peptide not only plays a role in appetite control but is also involved in the hypothalamus-pituitary-adrenal (HPA) axis (32). ICV injected nesfatin-1_{30-59} has been shown to activate CRF-positive neurons (25) and thereby possibly upregulates the HPA axis leading to an increase in peripheral cortisol release and consequently to more anxious behavior. Noteworthy, 13% of the nesfatin-1 positive neurons in the PVN co-express CRF (33), giving

further support to the idea, that anxiety mediation of nesfatin-1 is related to CRF signaling. The anxiogenic effect that our study observed in normal weight animals after ICV nesfatin-1$_{30-59}$ injection was further supported by our follow up study (34). Injecting a nesfatin-1 antibody in normal weight rats and thereby blocking endogenous nesfatin-1 resulted in reduced anxiety behavior (34). This result further substantiates our findings by validating a physiological anxiogenic nesfatin-1 effect and excludes that our results are exclusively a pharmacological effect of the peptide.

The neuroendocrine status and in consequence the central and peripheral stress and food intake regulatory systems are altered under obese conditions (8). This is a multifactorial occurrence, wherein – besides inflammation processes or genetics – the dysregulation of the HPA axis plays a crucial role (35). It has been observed that obesity is associated with hyperactivity of the HPA axis, resulting in permanently increased cortisol production and release (35, 36), promoting obesity development due to its hypothalamic stimulatory effect on orexigenic peptides (37). Long-term cortisol elevation is not only linked to adipogenesis but also to depression, as seen in patients suffering from Cushing Syndrome (hypercortisolism), where 50-80% of these patients develop major depressive disorder (MDD) (38). In our study the hyperactivity of the HPA axis in obese animals might have led to such high cortisol levels that the additional impulse of the ICV injected nesfatin-1 did not make a difference (30). Therefore, a dose finding/escalation study in DIO rats needs to be conducted in the future to clarify the appropriate – likely higher – dose under obese conditions. Also, whether elevated peripheral nesfatin-1 levels contribute to the HPA hyperactivity by crossing the blood-brain barrier (6), and additionally activating central nesfatin-1 positive neurons, needs to be further investigated. The idea appears plausible since the increased amount of peripheral visceral adipose tissue in obesity expresses NUCB2/nesfatin-1 (4) and could therefore be causative for the increase in plasma nesfatin-1 (4). However, studies on nesfatin-1 levels under obese conditions are inconsistent – some studies also report reduced nesfatin-1 levels in obesity (39), contributing to the obese status by its reduced inhibitory satiety signal. Further studies need to clarify this and need to further focus on the identification of the nesfatin-1 receptor to substantiate the above-described concept.

Additionally, obesity is linked to loss of appropriate nutrient sensing (40) and hormonal desensitization. One of the best-known examples therefore is the leptin resistance occurring under obese conditions, where the hypothalamus does not respond any longer to leptin signals (41). Neuroimaging studies have found that also further brain areas are functionally altered

under obese conditions (35) and thus can be less sensitive to satiety signals (35, 42). Therefore, a possible explanation why nesfatin-1 did not show a behavioral effect in DIO rats might be, that the brain areas – especially the hypothalamus, but also other in areas involved in food intake regulation – might be desensitized to the anorexigenic signal of nesfatin-1 (30).

However, the model of the mediation of nesfatin-1 via CRF downstream signaling has been challenged by more recent findings of our work group. Our study assessed – among other parameters like phoenixin – cortisol and NUCB2/nesfatin-1 levels in normal weight rats (n=7/group) undergoing restraint stress at different timepoints between 0 min – 240 min. (43). Cortisol levels, a parameter of the successful activation of the HPA axis, rose over the time of the restraint stress (43). However, the rise in NUCB2/nesfatin-1 levels was not parallel to the cortisol curve but remained relatively constant until it rose significantly at 240 min (43). This divergent rise of cortisol and NUCB2/nesfatin-1 questions a direct downstream mechanism of nesfatin-1 *via* the HPA axis.

Another follow-up study conducted by our group showed, that the co-injection of a nesfatin-1 antibody/vehicle resulted in a significantly increased number of entries in the open arms of the elevated-zero maze (EZM) compared to control-antibody/vehicle injected in normal weight rats (n=11/group) indicating an involvement of nesfatin-1 in the mediation of anxiety (34). The application of a nesfatin-1 antibody was not capable of preventing CRF-induced anxiety (34). Therefore, further peptides and pathways – e.g., the melanocortin – 4 receptor system (1, 44) or the m-TOR pathway (45, 46) – might be involved in the downstream mediation of nesfatin-1's anxiogenic effect and should further be explored.

When establishing a dose for the experiments, we did not observe a linear increase in effect as it was seen previously in the anorexigenic effect of nesfatin-1 (1), but a rather U-shaped dose effect (30) with the middle dose 0.3 nmol being the most effective dose in normal weight animals. This might possibly be explained with an oversaturation of the nesfatin-1 receptor with the highest dose or antagonistic effects starting to appear (30). It might also be possible that a desensitization might occur or that different nesfatin-1 receptors with opposing effects depending on the nesfatin-1 levels exist. A further idea is that the mediation of nesfatin-1-induced anxiety is mediated by another receptor, just like the effects of nesfatin-1 on glucose metabolism requires the ghrelin receptor (47). Thereby, nesfatin-1 levels exceeding the amount of available co-receptor do not have any further effect. Further research might use our doses (0.1, 0.3 and 0.9 nmol) in a knockout rodent model to investigate the effects of nesfatin-1 while knocking out/blocking possible target co-receptors and to evaluate whether nesfatin-1 actually

has a specific receptor or whether its effects are mediated trough other receptors/target molecules using an existing pathway.

4.3 Behavioral tests

Despite the fact, that using an animal model only allows indirect anxiety/depression assessment such as by approach and avoidance parameters in the behavioral tests, the performed experiments are a valuable method in behavioral science and pharmacological studies. Our finding that 0.3 nmol nesfatin-1_{30-59} induces anxiety/depression-like behavior was replicable in different test settings indicated by a significantly reduced sucrose/water ratio (SPT), a significantly reduced palatable snack intake (NIH), a significantly reduced number of entries and duration in the center zone (OFT) or a significantly reduced number of visits in open arms (EZM) in normal weight rats. Further beneficial is that the NIH and SPT test were conducted using the automated food intake monitoring system which avoided disturbance of the animals by manual weight assessment. Also, additional food intake microstructure values, broken down to the smallest unit of food intake – one bout – was possible.

Among others, we used the EZM test for anxiety assessment. The EZM is a further development of the elevated plus maze. The elevated plus maze is, as the name indicates, plus shaped with two open and two closed arms. The advantage of the EZM over the plus maze is that there is no center area, which can neither be calculated as open or closed and is therefore excluded from the results. A study comparing the two test settings found that they are comparable (48). However, the EZM has a higher detection sensitivity when comparing to a control group because the EZM has higher baseline levels (48). We therefore used the EZM for our experiments.

The NIH test is a behavioral test with high predictive validity and reliability for anxiety and depressive-like behavior assessment (49). Due to its repeatability the test allows a cross-over design and enables reduction of needed animals. Also, no food-deprivation prior to testing is necessary. An immunohistochemistry study showed a feeding/fasting-dependent nesfatin-1 c-Fos immunoreactivity in the PVN, being increased after refeeding (33) and decreased following fasting (1). Therefore, we chose a hyponeophagia model that does not require rodent fasting prior to the investigation. The novel cage during the test induces a novelty stress (49) to both control and nesfatin-1_{30-59} injected animals, so that a possible stress-induced anorexia (50) that might be mediated by elevated CRF levels (51) due to the novelty-stress only decreases the baseline values of total snack consumption but does not influence the nesfatin-1 effect. The

novelty-stress compared to other stress-inducing methods allows a very precise evaluation of the nesfatin-1 effect since it does not add any confounding stimuli such as inducing stress by pain stimuli (49). Additional stress due to manual food assessment was avoided using the automated system to leave the animals as undisturbed as possible.

The NIH test is also used for pharmacologic evaluation of anxiolytic substances or antidepressants (49). Therefore, the NIH test is capable of assessing anxiety as well as anhedonia (49). Investigating an anxiogenic/anxiolytic effect, the parameter "latency to first bout" plays a crucial role since it demonstrates how long the animal in the novel environment takes to overcome the conflict to approach the familiar palatable snack (16, 52). The parameter food intake in contrast is suited to assess anhedonia, where a reduced amount of totally consumed snack points toward anhedonic behavior (30). In our data, we saw a non-significant trend in the parameter "latency to first bout" but a significant reduction in the total snack intake during testing in normal weight nesfatin-1_{30-59}-injected rats compared to the control group. Therefore, the NIH test in our setting displayed the anhedonic effect of centrally injected nesfatin-1_{30-59}. Using a behavioral test based on reduction of food intake while injecting an anorexigenic peptide might raise the question whether the observed effect might be a food regulatory effect. However, one of our previous analysis showed that the anorexigenic effect of the peptide occurs between the fourth and the eights hour after injection (12), while our experiments here were performed 30 minutes post injection, where we observed the highest behavioral effect. Also, another rodent study that conducted the SPT further supports the temporal discrepancy between the food intake and the behavioral effect of the peptide (53).

A disadvantage seen in the NIH test is that it requires single housing for the training period as well as for the test (49). However, in our study single housing was required anyways due to ICV cannulation and the use of the automated system. To minimize this disadvantage transparent PVC cages were used, where rats have eye contact to each other.

Our second paper focusing on the methodical aspects of our experiments presented representative food intake microstructure data of the training and test period (54). Over the five training days before the experiment the meal size and the bout size increased steadily (54). This shows that the rodents get more and more accustomed to the palatable snack, implying a successful training period. Interestingly, the parameters meal duration, time spent in meals and time spent in bouts remained relatively stable during the 5-day training period. A possible explanation for this phenomenon is the circadian feeding behavior of rats (55). The training period in our experiments was conducted during the first 30 min of the dark phase (30). Strubbe

and colleagues described that rats have two peaks in nocturnal food intake with the first occurring during the first three hours of the night and a second one in the last three hours of the dark phase (55). Based on this finding, our training period lies in the first circadian food intake peak. Therefore, a relatively stable meal duration, time spent in meals and time spent in bouts might be due to the focus on food intake during this time. Additionally, since the animals were as undisturbed as possible due to the automated food and liquid monitoring, these parameters might display the natural feeding behavior of a rat in the beginning of the dark phase.

Interestingly, we did not see the expected continuous fall in the latency to first bout over the training period. This might be due to the technical issues. When starting the NIH training, the recording needs to be started on the computer and then all gates need to be opened manually. This process needs to be done quickly to avoid a discrepancy between the first and last opened gates, however the opening process also needs to be done carefully to avoid spillage and in consequence microbalance errors. Since the parameter latency to first bout is extremely sensitive by counting the seconds, the manual opening process might confound this parameter. To avoid this, a further development of the automated system would be necessary. Hereby, when giving the instruction "open all gates", all gates would open automatically at the same time.

The SPT is a commonly used test for anhedonia assessment (56). The 1% sucrose solution offered to the rats poses a reward system stimulus (56). The consumption of the palatable sucrose solution leads to an activation of dopamine (DA) neurons in the ventral tegmental area (VTA), the location of reward processing (57). During the training period of the SPT, where rats are not exposed to any additional external stimulus, the sucrose preference ratio as seen in our second publication, shifts to almost exclusive sucrose consumption – a sign of successful implementation of the test (54). In anhedonia; however, animals lose the ability to experience pleasure due to this reward-related deficit occurring in anhedonic, depressive conditions (58). When injecting the active center part of nesfatin-1 ICV, the sucrose/preference ratio decreased significantly compared to control rats (30), indicating an anhedonia-inducing effect of central nesfatin-1. NUCB2/nesfatin-1 mRNA was shown to be expressed in the reward-related brain areas such as the VTA and that DA neurons in the VTA express NUCB2/nesfatin-1 (53). The study further indicated that ICV injected nesfatin-1 reduced the reward value of sucrose (53) further substantiating our findings. Moreover, an *ex vivo* experiment showed that nesfatin-1 hyperpolarized DA neurons by inducing an outward directed potassium current, implying a direct influence of nesfatin-1 on DA neurons (53). These investigations give a

neuropsychopharmacological background to our finding and thereby corroborate the validity and accuracy of our data.

Interestingly, we did not find an anhedonia-inducing effect of nesfatin-1 in obese rats (30). A study conducted in mice indicated that acute high-fat diet (HFD) activates c-Fos expression in the VTA (59). Consequently, long-term HFD feeding as conducted in our rats, might lead to a permanent mesolimbic reward system activation. During the SPT training period, the sucrose solution further contributes to the hyperactivated mesolimbic system signaling. Consequently, that might lead to a desensitization to the ICV nesfatin-1 stimulus, so that the increased activity in the VTA overrules the anhedonic nesfatin-1 stimulus. This hypothesis should be validated in a further study, possibly by immunohistochemical investigations after ICV nesfatin-1 injection in DIO rats. Also, not only the parameters sucrose and water intake for calculating the sucrose-preference ratio, but also further food/liquid intake microstructure parameters as presented in our second paper should be recorded to enlarge the knowledge on a possible (patho)physiological link between the hypothalamic food intake regulation in obesity and reward-related deficits like anhedonia.

4.4 Clinical applications and further research

Based on the findings of this dissertation future studies need to focus on the psycho-neuroendocrine dysregulation under obese conditions. A follow-up study of this presented research might test whether nesfatin-1 has an anxiogenic/anhedonic effect in DIO rats when injected in higher doses. Furthermore, other anhedonia assessing behavioral tests such as the social interaction test (56) should be used to explore whether nesfatin-1 might not only induce anhedonic feeding behavior but also social anhedonia, which is a phenomenon often seen in depressed patients. Another anhedonia test called the female urine sniffing test (56) is a further valuable tool to be assessed in DIO rats after nesfatin-1 injection, since nesfatin-1 was also shown to have an effect on reproduction. Also, the combination of the hedonic taste reactivity test (56) in combination with the automated system (54) would greatly contribute to the existing knowledge on anhedonia and obesity research, since in this setting the licking reactions to sweet taste could be monitored with the automated microstructure assessment.

Furthermore, future research should replicate our experiments (30) using female rats, since a human study has indicated a sex-specific regulation of NUCB2/nesfatin-1 (14). Hereby, obese women showed a positive correlation between anxiety scores and NUCB2/nesfatin-1 levels ($r = 0.54$; $p < 0.001$), while in obese men, this correlation was found to be inverse (14). A majority

of investigations in the field of endocrine research is conducted in males since females do not have as stable baseline endocrine values as males due to cyclic hormonal changes. Therefore, when investigating the anhedonic/anxiogenic effect of nesfatin-1$_{30-59}$ in female rodents, prepubertal rats might be used to avoid confounding hormonal changes.

Since nesfatin-1 is only known since 2006 and new peptides are discovered constantly by bioinformatics – like phoenixin in 2013 (60), further research should focus on blocking – for example by using an antibody or a knockout animal model – one of the novel satiety/food regulatory peptides to further elucidate the mediation pathway of nesfatin-1. Also, it is conceivable that other (newly discovered) food intake regulatory peptides also have an impact on behavioral alterations under obese conditions. Therefore, the behavioral tests conducted in this investigation (30) like the SPT or NIH in combination with the automated system (54) should be applied to DIO rats exposed to another food-regulatory substance.

Moreover, we discussed, that the HFD might interfere with the reward system in rats. Therefore, using an obesity model not based on a HFD, such as leptin- or leptin-receptor deficient rodent models, no reward system stimulus would interfere because a normal caloric chow would be used.

Since anxiety disorders and depression are often-seen comorbidities in obesity, nesfatin-1 poses a future-directing peptide with its food regulatory as well as anxiogenic/anhedonic properties. Regarding to the high prevalence of obesity worldwide, the need of further understanding of the psychoneuroendocrine pathophysiology is urgent. The search for anti-obesity drugs has not been successful yet, since most drugs were proven ineffective or had enormous side effects and were consequently withdrawn from the market. For now, the only effective treatment for morbid obesity remains bariatric surgery. However, before therapy options can be developed, the underlying neuroendocrine mechanisms need to be understood, to which this dissertation contributes to.

5. Conclusion/Summary

In conclusion, our first study demonstrated that nesfatin-1$_{30-59}$ injected ICV in NW rats induced anxiety behavior indicated by a significantly reduced number of entries and duration in the center zone of the OFT or by significantly reduced visits of the open arms in the EZM. The exact (patho)physiological mechanism behind this phenomenon is still to be unraveled; however, a possible link between nesfatin-1 signaling and CRF – a peptide involved in appetite regulation as well as in stress/anxiety mediation *via* the HPA axis – was discussed. Furthermore,

ICV injected nesfatin-1$_{30\text{-}59}$ induced anhedonic behavior in NW animals indicated by a significant decrease in cookie intake during the NIH and a significantly decreased sucrose preference ratio in the SPT compared to controls. It was discussed that the nesfatin-1$_{30\text{-}59}$ injection possibly interacts with DA neurons in the VTA, reducing the reward value of sucrose. In DIO animals no nesfatin-1$_{30\text{-}59}$-induced anxious behavior was observed, possibly due to the pathological neuroendocrine dysregulations in obesity such as hyperactivation of the HPA axis, alterations in nesfatin-1 levels or desensitization processes in the yet unknown nesfatin-1 receptor. The lacking anhedonic nesfatin-1$_{30\text{-}59}$ effect might result from the HFD constantly activating the mesolimbic reward system and thereby overruling the nesfatin-1$_{30\text{-}59}$ effect. All the discussed possible (patho)mechanisms need to be further explored by additional basic research and then applied to the human. In this further research process, the methods described in our second paper combining the SPT and NIH test with the automated food intake monitoring system are a valuable tool for detailed and reliable food/liquid intake assessment.

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7. Statutory declaration and declaration of contribution

"I, Stephanie Gladys Kühne, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic "Nesfatin-1$_{30-59}$ und Adipositas: Beeinflussung von Angstverhalten und Depressivität / Nesfatin-1$_{30-59}$ and obesity: influence on anxiety and depressiveness", independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.
The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date: _____

Stephanie Gladys Kühne

Detailed Declaration of Contribution

Stephanie Gladys Kühne contributed to the following publication summarized in this thesis:

Publication 1:

Kühne S.G.*, Schalla M.A.*, Friedrich T., Kobelt P., Goebel-Stengel M., Long M., Rivalan M., Winter Y., Rose M., Stengel A. Nesfatin-1$_{30-59}$ Injected Intracerebroventricularly Increases Anxiety, Depression-Like Behavior, and Anhedonia in normal weight rats. *Nutrients* vol. 10,12 1889. 3 Dec. 2018, doi:10.3390/nu10121889

Contribution in particular:
- Performance of behavioral experiments (SPT, NIH, OFT, EZM, DL Box)
- Assistance to intracerebroventricular cannulation (surgery)
- Daily maintenance of the automated food intake monitoring system (BioDaq)
- Daily assessment of animal well-being (body weight, food intake)
- Data analysis
- Writing of the first draft and revision of the manuscript
- Creation of all figures in the manuscript

Publication 2:

Schalla, M.A.*, **Kühne, S.G.***, Friedrich, T., Hanel V., Kobelt, P., Goebel-Stengel M., Rose, M., Stengel, A. Sucrose Preference and Novelty Induced Hypophagia Tests in Rats using an Automated Food Intake Monitoring System. J. Vis. Exp. (), e60953, doi:10.3791/60953 (2020).

Contribution in particular:
- Conduction of animal experiments
- Daily maintenance of the automated food intake monitoring system (BioDaq)
- Daily assessment of animal well-being (body weight, food intake)
- Contribution to data analysis (representative results of NIH and SPT test)
- Contribution to writing of the manuscript (critical revision and adjustments of the first draft of the manuscript, submission of the manuscript, initial creation of figure 1-5)

* shared first authorship

8. Print copies of the selected publications

8.1 Publication 1: Nesfatin-1$_{30-59}$ Injected Intracerebroventricularly Increases Anxiety, Depression-Like Behavior, and Anhedonia in normal weight rats

https://doi.org/10.3390/nu10121889

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI
Selected Categories: **"NUTRITION and DIETETICS"** Selected Category
Scheme: WoS
Gesamtanzahl: 81 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	Annual Review of Nutrition	5,528	8.886	0.005230
2	PROGRESS IN LIPID RESEARCH	5,302	8.435	0.006750
3	Advances in Nutrition	3,937	6.853	0.012870
4	AMERICAN JOURNAL OF CLINICAL NUTRITION	58,213	6.549	0.055760
5	CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION	10,197	6.015	0.011670
6	NUTRITION REVIEWS	7,526	5.788	0.010600
7	International Journal of Behavioral Nutrition and Physical Activity	8,371	5.548	0.019780
8	CLINICAL NUTRITION	10,558	5.496	0.016870
9	PROCEEDINGS OF THE NUTRITION SOCIETY	5,238	5.347	0.006230
10	INTERNATIONAL JOURNAL OF OBESITY	22,185	5.151	0.032040
11	FOOD CHEMISTRY	90,665	4.946	0.101120
12	NUTRITION RESEARCH REVIEWS	2,164	4.586	0.001840
13	CURRENT OPINION IN CLINICAL NUTRITION AND METABOLIC CARE	4,842	4.534	0.007130
14	EUROPEAN JOURNAL OF NUTRITION	5,669	4.423	0.011650
15	JOURNAL OF NUTRITIONAL BIOCHEMISTRY	9,815	4.414	0.014150
16	JOURNAL OF NUTRITION	38,804	4.398	0.029930
17	JOURNAL OF PARENTERAL AND ENTERAL NUTRITION	5,287	4.249	0.007990
18	Nutrients	12,031	4.196	0.032520
19	Obesity	17,578	4.042	0.037840
20	Journal of the Academy of Nutrition and Dietetics	3,687	4.021	0.014370
21	INTERNATIONAL JOURNAL OF EATING DISORDERS	8,732	3.897	0.010160
22	NUTRITION	10,167	3.734	0.013010
23	BRITISH JOURNAL OF NUTRITION	26,011	3.657	0.035400
24	Nutrition Journal	4,484	3.568	0.009540

Selected JCR Year: 2017; Selected Categories: "NUTRITION and DIETETICS"

8.2 Publication 2: Sucrose Preference and Novelty-Induced Hypophagia Tests in Rats using an Automated Food Intake Monitoring System

9. Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

10. List of publications

ORIGINAL ARTICLES

December 2018

Title: Nesfatin-1$_{30\text{-}59}$ Injected Intracerebroventricularly Increases Anxiety, Depression-Like Behavior, and Anhedonia in normal weight rats
Authors: **Stephanie Gladys Kühne***, Martha Anna Schalla*, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Matthias Rose, Andreas Stengel
Journal: Nutrients 2018 Dec 3;10(12):1889. doi: 10.3390/nu10121889. PMID: 30513901
Impact factor: 4.2 (2017)

July 2019

Title: Intracerebroventricular Injection of Phoenixin Alters Feeding Behavior and Activates nesfatin-1 Immunoreactive Neurons in Rats
Authors: Tiemo Friedrich, Martha Anna Schalla, Sophie Scharner, **Stephanie Gladys Kühne**, Miriam Goebel-Stengel, Peter Kobelt, Matthias Rose, Andreas Stengel
Journal: Brain Res. 2019 Jul 15;1715:188-195. doi: 10.1016/j.brainres.2019.03.034. Epub 2019 Mar 28. PMID: 30930149

May 2020

Title: Sucrose Preference and Novelty-Induced Hypophagia Tests in Rats using an Automated Food Intake Monitoring System
Authors: Martha Anna Schalla*, **Stephanie Gladys Kühne***, Tiemo Friedrich, Vivien Hanel, Peter Kobelt, Miriam Goebel-Stengel, Matthias Rose, Andreas Stengel
Journal: J Vis Exp. 2020 May 8;(159). doi: 10.3791/60953. PMID: 32449711

August 2020

Title: Central blockage of nesfatin-1 has anxiolytic effects but does not prevent corticotropin-releasing factor-induced anxiety in male rats
Authors: Martha Anna Schalla, **Stephanie Gladys Kühne**, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Masatomo Mori, Matthias Rose, Andreas Stengel

Journal: Biochem Biophys Res Commun. 2020 Aug 27;529(3):773-777. doi: 10.1016/j.bbrc.2020.05.163. Epub 2020 Jul 19. PMID: 32736706

October 2020

Title: Restraint stress affects circulating NUCB2/nesfatin-1 and phoenixin levels in male rats.

Authors: Martha Anna Schalla, Miriam Goebel-Stengel, Tiemo Friedrich, **Stephanie Gladys Kühne**, Peter Kobelt, Matthias Rose, Andreas Stengel

Journal: Psychoneuroendocrinology. 2020 Oct 7;122:104906. doi: 10.1016/j.psyneuen.2020.104906. PMID: 33059202

Impact factor: 4.7 (2019)

REVIEW ARTICLES

September 2018

Title: Placental miR-340 expression: a key to gestational programming of activity-based anorexia?

Authors: **Stephanie Gladys Kühne**, Andreas Stengel

Journal: Non-coding RNA Investig 2018;2:53. doi: 10.21037/ncri.2018.09.01

September 2019

Title: Veränderungen der Darm-Gehirn-Achse beim Reizdarmsyndrom – Ein Update

Authors: **Stephanie Gladys Kühne**, Andreas Stengel

Journal: Thieme Erfahrungsheilkunde. vol.: 68, issue 05, doi: 10.1055/a-1008-7780

October 2019

Title: Alterations of peptidergic gut-brain signaling under conditions of obesity

Authors: **Stephanie Gladys Kühne**, Andreas Stengel

Journal: J Physiol Pharmacol. 2019 Oct;70(5). doi: 10.26402/jpp.2019.5.01. Epub 2019 Dec 26. PMID: 31889037

August 2018

<u>Title</u>: Nesfatin-1 (30-59) injected intracerebroventricularly increases anxiety, depressiveness and anhedonia in normal weight but not in diet-induced obese rats

<u>Authors</u>: Martha Anna Schalla, **Stephanie Gladys Kühne**, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Matthias Rose, Andreas Stengel

<u>Journal</u>: Neurogastroenterology and Motility 2018, Vol. 30, Suppl. 1, Special Issue: SI, Meeting Abstract 051

March 2019

<u>Title</u>: Nesfatin-1 30-59 increases anxiety, depressive behavior and anhedonia in normal weight but not in diet-induced obese rats

<u>Authors</u>: **Stephanie Gladys Kühne**, Martha Anna Schalla, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Matthias Rose, Andreas Stengel

<u>Journal</u>: Annual Meeting of the German Society for Neurogastroenterology and Motility, 2019; Berlin Germany

<u>Title</u>: Central blockage of nesfatin-1 has anxiolytic effects but does not prevent corticotropin-releasing factor-induced anxiety or depressiveness in male rats

<u>Authors</u>: **Stephanie Gladys Kühne**, Martha Anna Schalla, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Matthias Rose, Andreas Stengel

<u>Journal</u>: Annual Meeting of the German Society for Neurogastroenterology and Motility, 2019; Freising Germany

<u>Title</u>: Nesfatin-1 30-59 injected intracerebroventricularly increases anxiety, depression-like behavior, and anhedonia in normal weight rats

<u>Authors</u>: **Stephanie Gladys Kühne**, Martha Anna Schalla, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Matthias Rose, Andreas Stengel

Journal: 2nd Adipocyte Brain Crosstalk Symposium 2019; Lübeck, Germany

Title: Nesfatin-1 30-59 steigert Ängstlichkeit, depressives Verhalten und Anhedonie bei normalgewichtigen, jedoch nicht bei adipösen Ratten

Authors: **Stephanie Gladys Kühne**, Martha Anna Schalla, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Matthias Rose, Andreas Stengel

Journal: Deutscher Kongress für Psychosomatische Medizin und Psychotherapie. 27. Jahrestagung der Deutschen Gesellschaft für psychosomatische Medizin und Ärztliche Psychotherapie (DGPM). 70. Arbeitstagung des Deutschen Kollegiums für psychosomatische Medizin (DKPM).

August 2019

Title: Central blockage of nesfatin-1 has anxiolytic effects but does not prevent corticotropin-releasing factor-induced anxiety or depressiveness in male rats

Authors: **Stephanie Gladys Kühne**, Martha Anna Schalla, Tiemo Friedrich, Peter Kobelt, Miriam Goebel-Stengel, Melissa Long, Marion Rivalan, York Winter, Matthias Rose, Andreas Stengel

Journal: Abstracts. Neurogastroenterology & Motility, 31: e13671. doi:10.1111/nmo.13671

February 2020

Title: Immobilization Stress modifies activity and immunoreactivity of Phoenixin-expressing brain regions in rats

Authors: Tiemo Friedrich, Martha Anna Schalla, **Stephanie Gladys Kühne**, Reinhard Lommel, Miriam Goebel-Stengel, Matthias Rose, Andreas Stengel

Journal: Zeitschrift für Psychosomatische Medizin und Psychotherapie 2019, Vol. 66, Issue 1

* = shared first authorship

11. Acknowledgements