



# Does the glucagon-like peptide-1 act through the vagal afferent neurons to modulate anxiety, food neophobia and food reward behaviour?

Marie Rouault

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- ☐ d'un autre établissement (étudiant arrivé en M2)

**Does the glucagon-like peptide-1 act through the  
vagal afferent neurons to modulate anxiety, food  
neophobia and food reward behaviour?**

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***Soutenu à Paris le 28 septembre 2015***

***Devant le jury composé de :***

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Maître de stage : Wolfgang Langhans, excusé,  
remplacé par Jean-Philippe Krieger

Enseignant référent : Nicolas Darcel

*Les analyses et les conclusions de ce travail d'étudiant n'engagent que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST*



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## List of abbreviations

CA: Closed Arm

Ctl: Control

CCK: Cholecystokinin

CPP: Conditioned Place Preference test

CZ: Central Zone

DPP-IV: Dipeptidyl-peptidase-IV

Ex-4: Exendin-4

Ex-9: Exendin-9

EPM: Elevated Plus Maze test

FI: Food intake

GFP: Green Fluorescent Protein

GLP-1: Glucagon-like peptide-1

GLP-1 R: Glucagon-like peptide-1 receptor

GLP-1 R kd: Knock-down of the glucagon-like peptide-1 receptor

HEK 293 cells: Human Embryonic Kidney 293 cells

HFD: High Fat Diet

IP: Intraperitoneal

kd: knock-down

LV-shCTL: lentiviral particles control

LV-shGLP-1R: lentiviral particles targeting GLP-1R

LV-shRNAi: lentivirus-mediated short hairpin RNA interference

min: minute

mRNA: messenger RNA

NAc: Nucleus Accumbens

ND: Nodose Ganglion

NT: Neurotransmitter

NTS: Nucleus tractus solitarius or Nucleus of the solitary tract

OA: Open Arm

OF: Open field test

PYY: Peptide YY

PPG: Preproglucagon

qPCR: Quantitative Polymerase Chain reaction

s: seconds

SDA: Subdiaphragmatic vagal deafferentation

SEM: Standard Error of the Mean

shRNA: short hairpin RNA interference

VAN: Vagal afferent neurons

VAN GLP-1 R kd: knock-down of the glucagon-like peptide-1 receptor in vagal afferent neurons

Veh: Vehicle

VTA: Ventral Tegmental Area

## Introduction

The development of obesity and type-2 diabetes has become a major health issue and represents an increasing burden on our societies and healthcare systems. The foremost explanation for these epidemics is the challenge exerted on the homeostatic regulation of energy balance by an obesogenic environment (Zheng and Berthoud, 2007; Zheng and Berthoud, 2008). Indeed, overeating beyond the metabolic needs is a crucial behaviour leading to obesity.

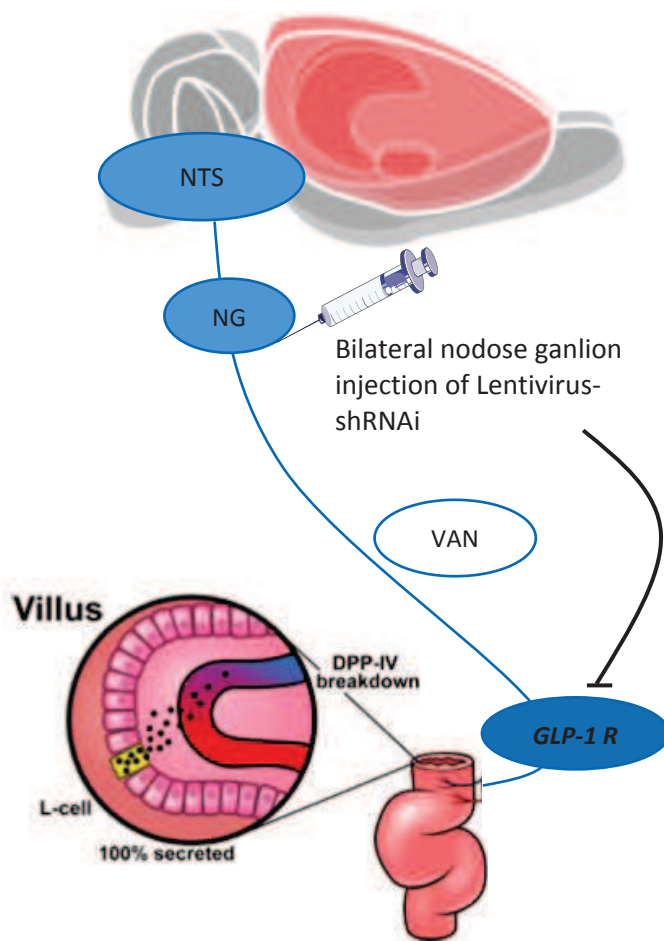
Obviously social and cultural factors also contribute to the epidemic, and it is now thought that some persons have a favourable nature and genetic predisposition to develop obesity in obesogenic environment (Berthoud and Morrison, 2008; Yeo and Heisler, 2012). A policy involving multiple strategies (health policy, lifestyle changes, physical activity, food diet habits, surgical and drug treatment) would enable to fight against these metabolic disorders. Thus understanding of physiological and neurological mechanisms related to obesity and T2D aim at finding potential target to develop new treatments.

Food intake is regulated by intermingled neuronal pathways that can be divided into those responsible for the regulation of metabolic processes and those responsible for cognitive and hedonic processes. Simplistically, the homeostatic control of food intake can be defined as the motivation to eat depending on the energy status of the body (Berthoud, 2002) while the hedonic control drives the motivation to consume rewarding food (Zheng and Berthoud, 2008).

Glucagon-like peptide-1 (GLP-1) is one of the satiety gut-derived hormones identified as a target to fight metabolic disorders. GLP-1 results from post-translational transformation of proglucagon. Peripheral GLP-1 is released by enteroendocrine L-cells from the intestinal tract in response to nutrients stimulation. GLP-1 is also secreted at the central level by a subset of neurons in the nucleus of the solitary tract (NTS).

GLP-1 was firstly described as an incretin hormone (Holst and Gromada, 2004). Indeed it is considered as the most stimulating agent enhancing the glucose-stimulated insulin secretion (Burcelin and Hosokawa, 2001), acting on the glucagon-like peptide-1 receptor (GLP-1R) of pancreatic  $\beta$ -cells via an endocrine mechanism (Doyle and Egan, 2007). A neuroincretin mechanism for the action of GLP-1 is also highly suspected (Nishizawa *et al.*, 1996; see Donath and Burcelin, 2013 for review, Krieger *et al.*, *Physiol Behav*, 2015). Thus pharmacological GLP-1R agonists such as exendin-4 (Ex-4) have been developed and are currently used in type 2 diabetes treatments. However GLP-1 effect on satiety and glycemia is not totally understood.

GLP-1 is rapidly degraded in the systemic circulation by the enzyme dipeptidyl-peptidase-4 (DPP-IV) (1-2 minute half-life) (Deacon *et al.*, 1995). This limits GLP-1's potential endocrine actions onto central GLP-1R and suggests the existence of a paracrine signalling complementary to the endocrine pathway (Deacon *et al.*, 1995).



**Figure 1:** VAN GLP-1R kd model. GLP-1R: Glucagon-Like Peptide-1 Receptor.

NTS: Nucleus Tractus Solitarius. NG: Nodose Ganglia. VAN: Vagal Afferent Neurons. DPP-IV: Dipeptidyl peptidase-4. L-cell: enteroendocrine L-cells.

GLP-1R are also expressed in the brain, notably in reward areas (VTA, NAcc ...). Recent studies explored the possibility that central GLP-1R activation can also modulate the reward value of food. Injection of GLP-1 in the NAcc was first shown to decrease food intake, involving for the first an area known for its role in reward processing in the control of food intake by GLP-1 (Dossat *et al.*, 2011). Centrally-administered Ex-4 (intra-VTA and intra-NAcc) decreased the reward value of food (Dickson *et al.*, 2012). Another study showed that blockade of GLP-1R by GLP-1R antagonist exendin-9 (Ex-9) in reward areas increased food intake (Alhadeff *et al.*, 2012) and palatability (Dossat *et al.*, 2013). These studies suggest a role for mesolimbic GLP-1R in controlling food intake by modulating the reward value of food. These studies, however, involve a pharmacological activation of central/mesolimbic GLP-1R and do not test whether GLP-1 secreted by the projections of NTS GLP-1 neurons play a physiological role in a similar process.

The vagus nerve innervates several organs located in the thoracic and abdominal cavities (Berthoud and Neuhuber, 2000), including the gut. Vagal afferent neurons end in the NTS, in the hindbrain. Thus it enables communication between the gut and the brain. It has been shown that vagal afferent neurons mediate gut peptides stimuli such as cholecystokinin (CCK) or GLP-1 and also mechanical stimuli such as gastric distension (see for review: de Lartigue, *Physiol Behav*, 2014).

Vagal afferent neurons (VAN) express the GLP-1R (Nakagawa *et al.*, 2004) and terminate in the lamina propria of the intestinal mucosa, thus being able to sense the release of GLP-1 by L-cells (Richards *et al.*, Diabetes, 2014) (**Appendix 1**). This implies that the vagus nerve may mediate a signal given by GLP-1 to the brain. Yet, the role of peripheral GLP-1 through the vagus nerve and the brain, on food intake control and glycemia, as well as its signalling pathway, is not fully understood (see Krieger *et al.*, *Physiol Behav*, 2015 for review). To test the role of VAN in mediating the peripheral GLP-1's effect on food intake and glycemia, our lab achieved a specific knock-down of the GLP-1R (GLP-1R kd) in the vagal afferent neurons (VAN GLP-1R kd, the abbreviation GLP-1R kd is used afterwards to design the group of rats with this model) in rats (**Fig.1**). A lentivirus vector carrying the short hairpin RNA sequence targeting the GLP-1R was injected in the left and right nodose ganglia (NG) of the vagus nerve. Thus, these rats have a markedly reduced expression of GLP-1R in the vagal afferent neurons (50 to 60% lower GLP-1R mRNA expression in the nodose ganglia). Several aspects of GLP-1R on the vagus nerve have already been explored confirming the hypothesis that GLP-1 activated GLP-1R on VAN to mediate a neural component reducing post-meal glycemia, acting on satiation by decreasing food intake and slowing down gastric emptying (Krieger *et al.*, Diabetes, 2015, accepted). Indeed, GLP-1R kd rats had bigger meals when fed *ad libitum* with normal chow food compared to control rats, but not higher daily food intake (FI) owing to a longer meal interval compensation. Moreover, another characteristic observed on GLP-1R kd rats was a blunted insulin peak after a meal.

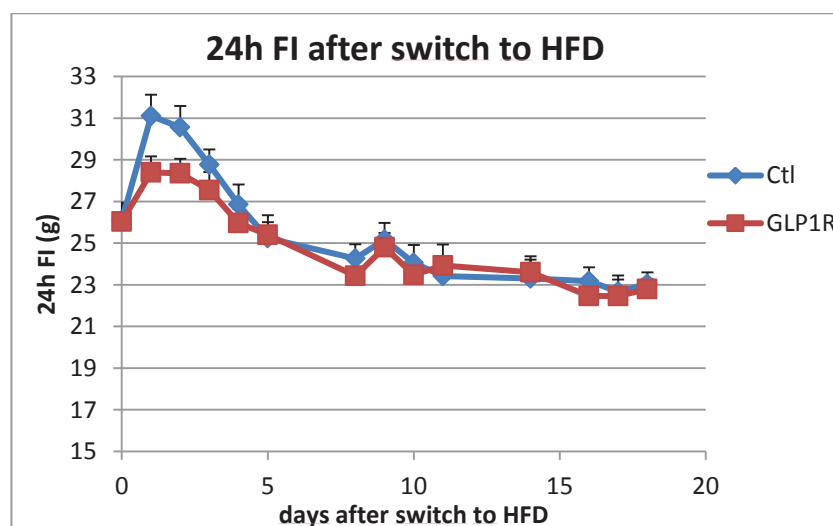


Besides, a preliminary observation of this model showed that GLP-1R kd rats had a lower food intake than control rats during the initial hyperphagic phase (0 to 5 days) when switched to a high fat diet (HFD) (60% from energy coming from fat) (**Fig. 2**, data from our laboratory not published yet). Then after five days, both GLP-1 R kd and control rats had the same food intake.

This observation led to the hypothesis that VAN GLP-1R differentially modulates food neophobia or the reward value of new palatable food. Moreover, based on other data from our lab showing a link between vagal afferents and innate anxiety (Klarer *et al.*, J. Neurosci., 2014), we also tested whether innate anxiety could be also modulated in the VAN GLP-1R kd model.

Our study explores for the first time the implication of peripheral GLP-1 on hedonic control through the vagus nerve. It aims at answering the following question: "does the glucagon-like peptide-1 receptor act through the vagal afferent neurons to modulate anxiety, food neophobia and food reward behaviour?"

To test this, we used the same model of Sprague-Dawley rats with a VAN GLP-1R kd as an efficient selective method to explore the role of vagal mediation of GLP-1 effects. We submitted rats to a series of behavioural tests assessing consumption of palatable food (sucrose and milk preference test) and non-palatable food (dextran preference test), innate anxiety (elevated plus maze and open field tests), food neophobia (food neophobia test) and food reward behaviour (conditioned place preference, incentive runway, taste reactivity).



**Figure 2:** Daily food intake of Ctl and GLP-1R kd rats after switch to HFD

While switched to HFD, GLP-1R kd rats displayed a lower food intake during the first 3 days following new exposure. After 3 days food consumption is same between control and GLP-1R kd rats.

## Materials and methods

### I. Virus Production (lentivirus-mediated short hairpin RNA interference LV-shRNAi).

Lentiviral particles were produced following a published protocol (Krieger *et al.*, Diabetes, 2015, accepted). A plasmid pLKO.1 (Sigma-Aldrich) expressed turbo Green Fluorescent Protein (GFP) and the U6 promoter driven shRNA sequence targeting the rat GLP-1R mRNA (for the lentiviral particles targeting GLP-1R (LV-shGLP-1R)) or a non-target shRNA sequence (for the lentiviral particles control (LV-shCTL)). We used the plasmids psPAX2 and pMD2G (Didier Trono, Addgene, #12259 and 12260) for the lentiviral construct. Briefly, lentiviral particles were transfected in Human Embryonic Kidney 293 cells (HEK 293 cells) with lipofectamine 2000 (4uL/mL of media, Life technologies) during 20 hours. The media containing the lentiviral particles was harvested 3 days after transfection (cells expressing GFP) and was concentrated using polyethylene glycol (PEG) 6000 (8% PEG 6000 and 0.3M NaCl in final concentration). Following a ten-fold serial dilution of the concentrated viral solution, and then infection of HEK 293T cells, the virus titre was measured by counting the number of cells expressing GFP. Unfortunately the desire concentration of  $10^{10}$  particles/mL was not reached. Our concentrated viral solution contained  $10^6$  particles/mL. Thus we used a previous production of the same lentivirus to knock down GLP-1 R in vagal afferent neurons.

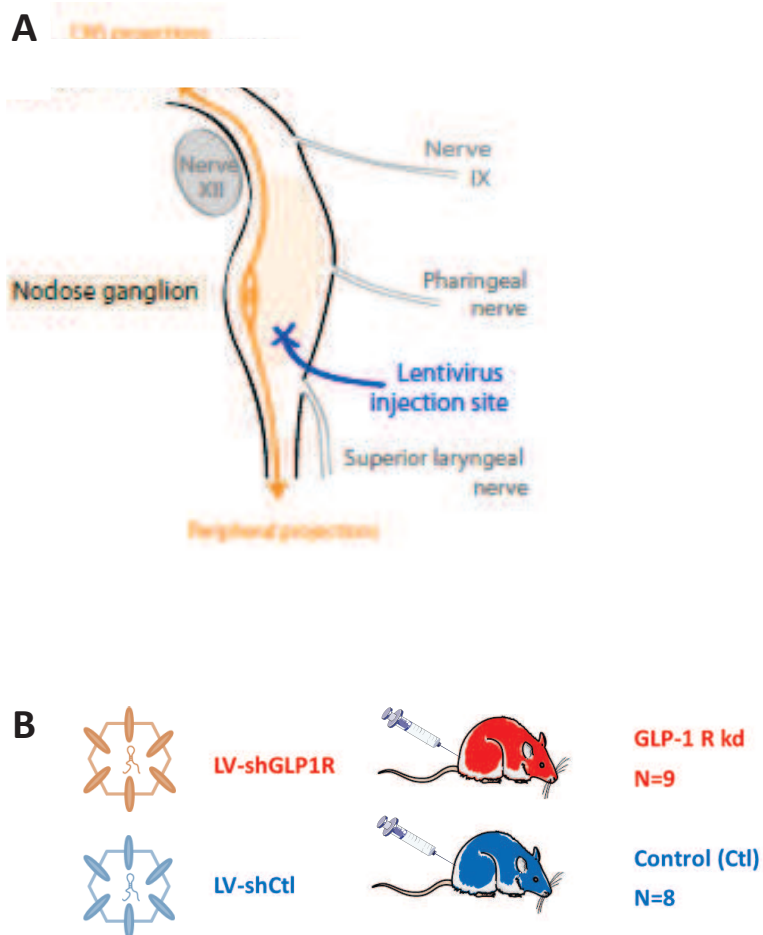
### II. Animals and housing

Seventeen adult male Sprague Dawley rats from ETH Schwerzenbach animal breeding facility were used in the study. Rats were five to seven weeks old (145-275 g body weight) upon reception and were housed in groups of three animals per cage (one cage had two animals) in acrylic cages with wood shaving floor (type IV, 595 × 380 × 200 mm) with 12/12 hour (h) light/dark cycle (dark onset from 08:00 to 20:00 h) at 21°C, 55-60% humidity. Unless specified otherwise, animals had *ad libitum* access to water and standard chow (Kliba 3436; Provimi Kliba Nafag, metabolisable energy: 3.13kcal/g). Before surgery, animals were acclimatized for one week and handled on a daily basis to habituate them to the experimenter. All experiments were approved by the Cantonal Veterinary Office of Zurich (license number 124/2014). Body weight was regularly measured along the study.

#### I. Surgery procedure

Two days before surgery, rats were fed with a special diet consisting of wet mash (unsweetened condensed milk mixed with powder chow, Kliba 3433; Provimi Kliba Nafag). The day before surgery, rats were fed with unsweetened condensed milk only. This diet was continued until three days after surgery. Then they were re-habituated to their standard chow with *ad-libitum* access.

On surgery day, rats (190-320 g on surgery day) were pre-treated with a subcutaneous injection of borgeal



**Figure 3:** Details of the surgery procedure for lentivirus injection

**A** (from Krieger *et al.*, Diabetes, 2015, accepted, Fig.1A): detailed representation of the lentivirus injection containing LV-shCTL or LV-shGLP-1R in the nodose ganglion. **B:** Summary of the lentivirus injection

(5mg/kg, MSD) 1 to 2 hours before anaesthesia and atropine (50 ug/kg, Sintetica, Mendrisio) 15 to 30 min before anaesthesia. Rats were anesthetized by an intraperitoneal (IP) injection of ketamine (88 mg/kg, Ketalar, Kantonsapotheke Zürich) and xylazine (5 mg/kg, Rompun 2%, Kantonsapotheke Zürich). If required, additional ketamine was injected during surgery. Nodose ganglia were exposed. A custom-made glass capillary (50 µm tip) was used to inject 1.5 µL of the lentiviral solution (LV-shCTL or LV-shGLP-1R) into the left and right NG of each rat with a Picospritzer III injector (Parker Hannifin) (**Fig. 3A** from Krieger *et al.*, Diabetes, 2015, accepted, Fig.1A). Following the surgery, the incision was closed using sutures. Rats were post-treated immediately after the completion of the surgery with a subcutaneous injection of carprofen (5 mg/kg, Norocarp, Ufamed) for analgesia and with an intraperitoneal injection of 3 mL of warm Ringer's lactate solution for rehydration. To ensure expression of the lentiviral constructs and the subsequent GLP-1R knockdown, animals were allowed to recover for 18 days. The control (Ctl) group contained 8 animals and the GLP-1R kd group contained 9 animals (**Fig 3B**).

## II. Functional verification of GLP-1R kd by intraperitoneal injection of exendin-4

There is no fully admitted functional verification for a VAN GLP-1R kd. IP Ex-4 injection typically leads to a decrease in food intake, which depends on the dose (Mack *et al.*, 2006). Thus this test and assessment of GLP-1R kd was based on previous results (Krieger *et al.*, Diabetes, 2015, accepted): after IP Ex-4 injection related to vehicle (Veh), Ctl rats showed 30% food intake reduction, whereas GLP-1R kd rats showed a 10% reduction.

The GLP-1 R kd was verified by measuring food intake of rats after an intraperitoneal (IP) injection of the GLP-1 agonist Ex-4 0.3 ug/kg (Bachem, this dose already used by Krieger *et al.*, Diabetes, 2015, accepted, was sufficient to detect a difference in food consumed between control and GLP-1R kd rats). This functional verification was performed at the end, when the behaviour experiments and other food intake measures were finished, so as not to impair our behavioural tests by the stress due to IP injection. It also enabled to check if the knock-down (kd) was still efficient at the end of the study. Animals were trained on 3 days for restraining and IP injection prior to the experiment. Habituated to be single-caged, animals were food deprived when dark onset began for 3 h. Then they were injected with Ex4 or NaCl in a crossover design, with 2 days of wash out between the two injections, and had ad libitum access to their food cup filled with their usual powder chow (Kliba 3436; Provimi Kliba Nafag). Food intake was measured after 1 h, 2 h, 4 h and 24 h.



## I. Behaviour experiments

### 1) Experimental test order and condition

Behavioural tests started 18 days after surgery. During this period, we continued to handle rats on a daily basis to habituate them to the experimenter. All tests were performed during the active phase of the rats (dark phase from 08:00 to 20:00 h). For each behavioural test, cage order (when animal were group caged) or order of rats (when animals were single caged) and treatment groups were newly randomized.

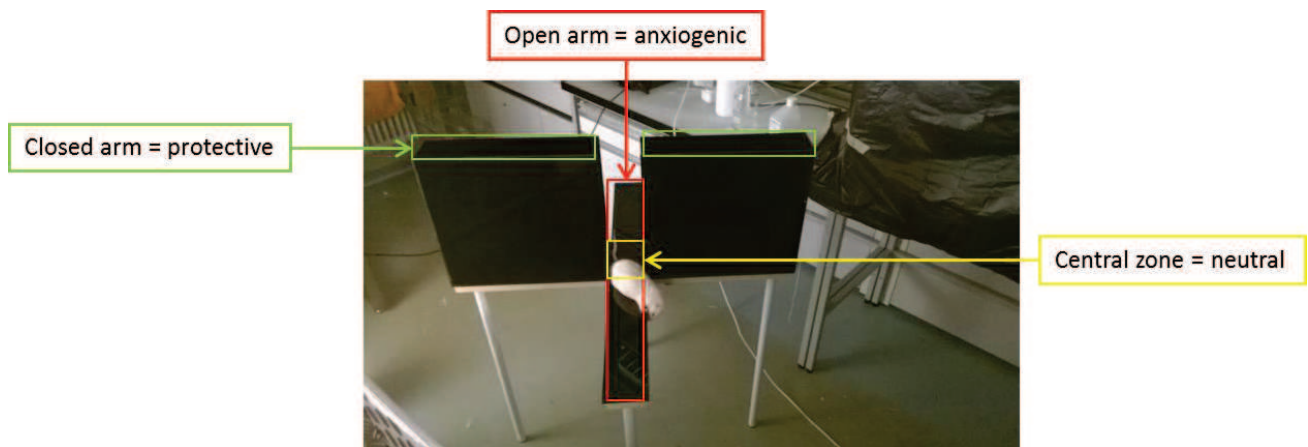
Behavioural experiments testing anxiety and food reward were performed first, before food intake measures and functional verification of the GLP-1R kd. The study was designed this way because we did not want to expose animals to drugs and injections and various novel foods before. Moreover, food intake measures require animals to be single-caged that can, when prolonged, be a stress for the animals and impact the outcome of our behavioural tests.

During the period of behavioural experiments testing anxiety and food reward, animals were housed in groups of three animals per cage (one cage had two animals) to avoid potent anxiogenic effects due to isolation and to keep social interaction (Zhang *et al.*, 2012; Klarer *et al.*, 2014). Between two behavioural tests, rats had a recovery period of 1 to 6 days. The order of the test performed was the following: (1) elevated plus maze test (EPM), (2) conditioned place preference test (CPP), (3) incentive runway test, (4) open field test (OF), (5) food neophobia test. Food intake records were performed afterwards. For each food intake record, rats were single caged. We reduced periods of single caging (maximum two weeks) and group caged the animals back whenever possible. During their single-cage period, rats had social time per initial group cage for several minutes to keep minimum social interactions. Before starting a new single cage period, rats were habituated for one day at least to the single cage. All measures were taken on a scale (PM 3000, Mettler Toledo) at the nearest 0.1 g. First exposure to the new food happened always 3 h after dark onset (11:00 h).

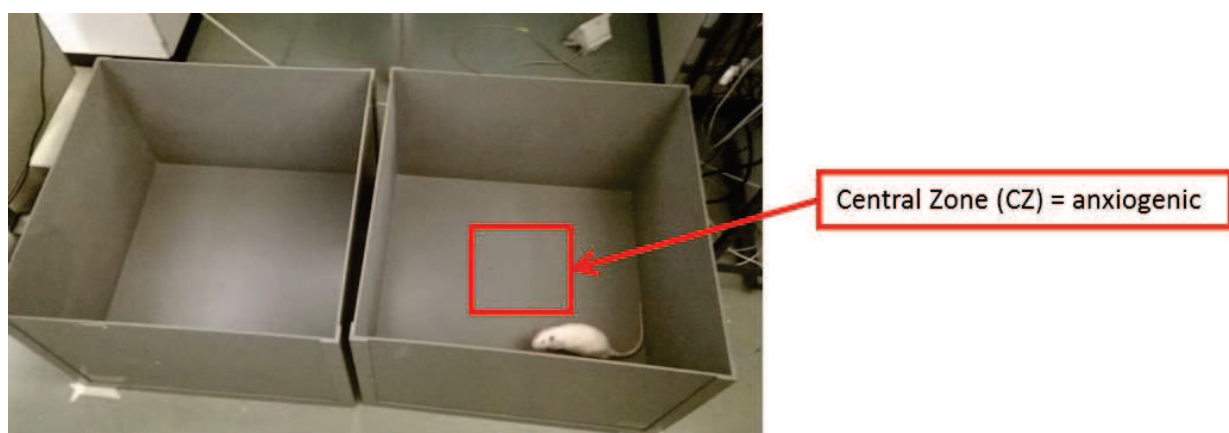
### 1) Test assessing anxiety

#### i. The elevated plus maze test

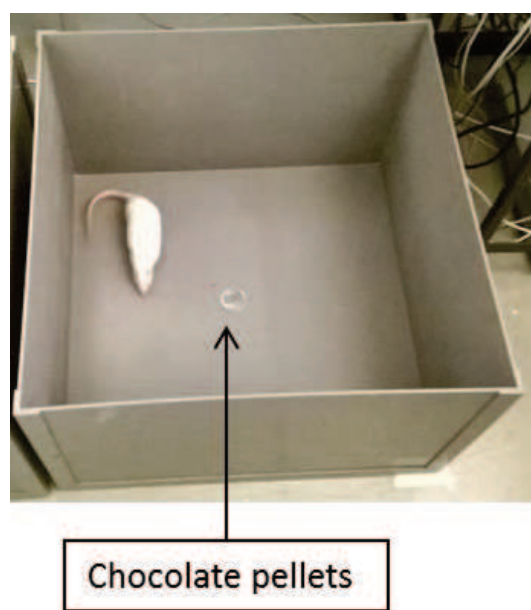
The elevated plus maze (EPM) test is a widely used method to assess innate anxiety-like behaviour in rodents (Walf and Frye, 2007). The test measures the animal's conflict between the natural exploratory behaviour in a new environment and their fear of unprotected and open space. Since the EPM is designed to test the innate component of anxiety, we conducted it first, when rats had not yet been exposed to any behavioural paradigm (Walf and Frye, 2007; Klarer *et al.*, 2014). The maze used was the same as the one described by Klarer *et al.*, 2014, and we followed her protocol. It consists in a black acryl glass, 85 cm elevated from the floor (**Fig. 4**). Four arms (10 cm wide, 45 cm long) equally spaced are attached to a central zone measuring 10x10 cm. The two opposing arms enclosed by a wall (40 cm high), except the part of the central zone, are the "closed arms". The two other arms, without walls but with a border of 1 mm,



**Figure 4:** The elevated plus maze apparatus



**Figure 5:** The open field apparatus



**Figure 6:** The food neophobia apparatus

are the "open arms". Innate anxiety is assessed by calculating the ratio of time spent on the open arms to the total time spent in the arms (open and closed arms) (Walf and Frye, 2007). Thus, an animal with a high level of innate anxiety is expected to spend less time in the open arms than an animal with a low level of innate anxiety (Belzung and Griebel, 2001; Cryan and Holmes, 2005). Rats were placed in the central zone (CZ), which is at the junction of the open arms (OA) and closed arms (CA). The rat was allowed to move in the maze for 5 minutes (min), and then it was brought back to its cage. After each rat, the maze was entirely cleaned with warm water to reduce the impact of olfactory cues on the next rat's behaviour. The experimenter was not present in the room. A camera above the maze recorded entries, durations and distance moved in each arm. Data were analysed by the video-tracking system Ethovision (Noldus IT) software. The total distance moved in the maze was also recorded to measure the locomotor activity during the test.

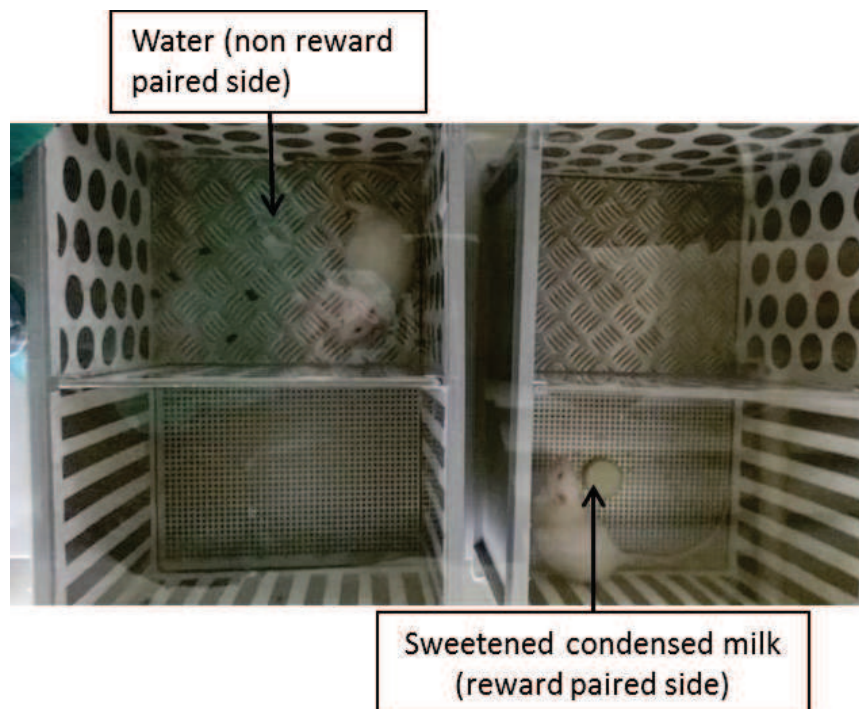
#### *ii. Open field test*

The open field test is also a widely used method to assess innate anxiety and locomotor activity in a novel environment (Belzung and Griebel, 2001; Cryan and Holmes, 2005). The protocol and material used are the same as the one described by Klarer *et al.*, 2014. The apparatus consisted of two square arena in grey Plexiglas (80x80 cm) surrounded by walls (50 cm high) (**Fig. 5**). Two different zones were defined in each arena: a central zone (25x25 cm) in the middle of the arena under a light of 30 lux and a peripheral zone including the rest of the arena where the luminosity was around 5 to 10 lux. Animals with a high level of innate anxiety are expected to avoid the "unprotected" central zone with strong luminosity, and spent more time along the wall in the shadow (Belzung and Griebel, 2001; Cryan and Holmes, 2005). The rat was put in one corner of the arena, always the same for all the rats, and was free to explore it for 30 min. After each animal trial, the arena was entirely cleaned with warm water. A camera above the open field recorded entries, durations and distance moved in each zone and that was analysed by the video-tracking system Ethovision software. The variables were measured during 30 min and analysed in 5 min time bins.

#### *i. Food neophobia test*

The food neophobia test of innate anxiety assesses the conflict between the natural trend of rat to avoid novel food and their hunger after a long period of fasting (File, 2001). The measure of the latency to start eating the novel food is taken to assess innate anxiety-like behaviour (Klarer *et al.*, 2014). Rats with a high level of anxiety towards food are expected to take longer to taste the novel food compared to less anxious rats (File, 2001). The apparatus consisted of a square arena in grey Plexiglas (80x80 cm) surrounded by walls (50 cm high) under a light of 30 lux in the centre (**Fig. 6**). To minimize stress reactions to food withdrawal, animals were trained to fast for 16 h a few days before the food neophobia test. The test was performed on three consecutive days. The first day was a habituation session where the animals were allowed to explore the arena for 30 min without food and without 16 h fasting. The actual food neophobia





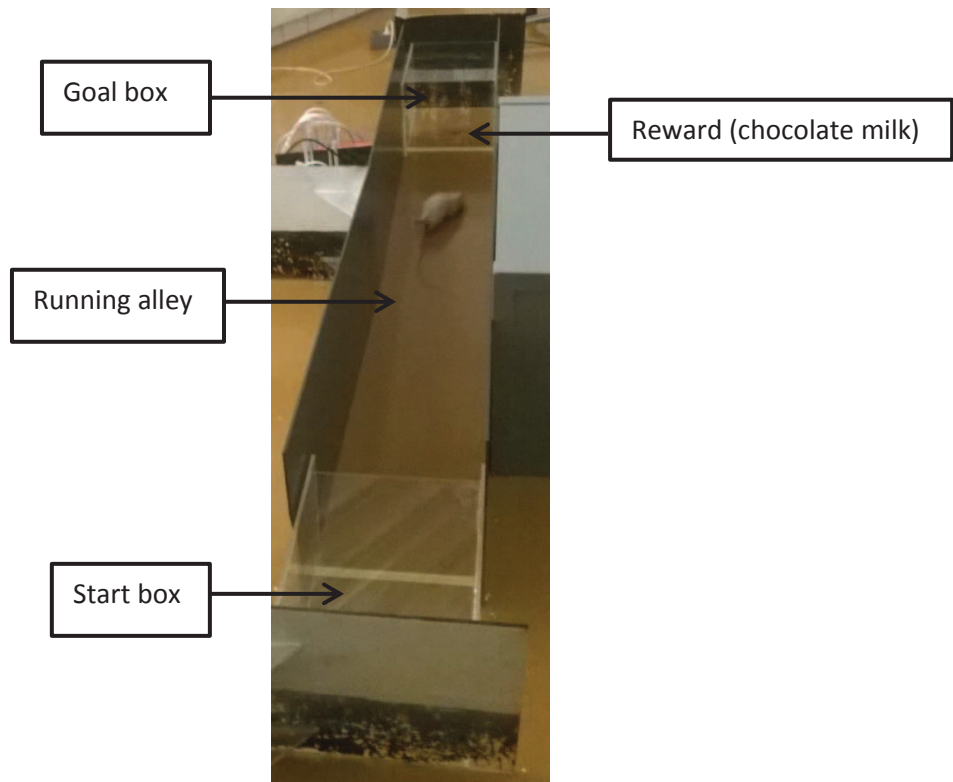
**Figure 7:** The conditioned place preference apparatus

test was performed on the next two days: the novel food (three chocolate pellets) was placed in a petri dish in the centre of the arena. There was one trial per animal on both days. Following 16 h of food deprivation, the rat was placed into the box and stayed for a maximum of 5 min while an experimenter recorded the latency to start eating. The experimenter was placed at 2 meters from the arena behind a door with a glass window, to minimize interactions with the animal. If the animal started eating and continued for 3 seconds, the trial was stopped and the animal was removed from the arena. This time was scored as the latency to eat. If a rat did not eat within 5 min, the trial was stopped and the latency to eat was scored as 300 seconds. The animal was then brought back to the animal room, but not with its cage mate to avoid social transmission of food preference (File, 2001) that could have altered the avoidance of the novel food for the following rats. Thus, it was put in a separate cage identical to its home cage, where it receives its usual chow immediately after the end of the trial. When its cage mate had also completed the trial, they were grouped cage again. A video camera above the runway recorded each animal trial for subsequent analysis.

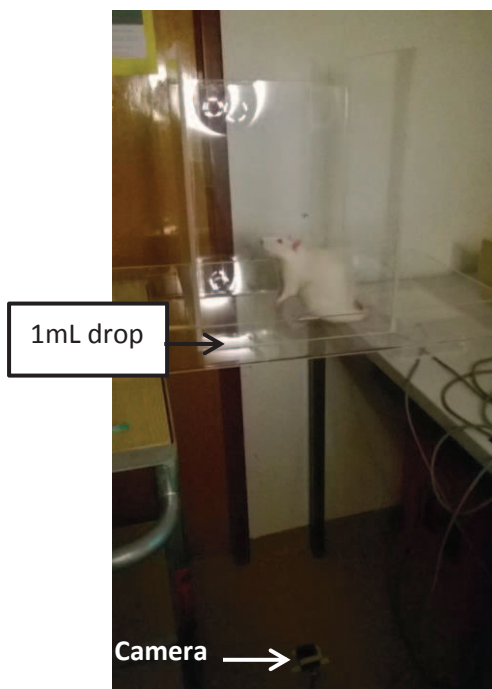
## 2) Test assessing the reward value of food

### ii. Conditioned place preference test

The conditioned place preference paradigm was primarily designed to study the rewarding and aversive effects of drugs (Prus *et al.*, 2009). It was also adapted to study the rewarding effect of food (Prus *et al.*, 2009, Dickson *et al.*, 2012). The animal is expected to spend more time in an environment previously associated with a food reward. The apparatus consisted of two connected chambers with distinct visual drawing on the walls and tactile protrusions on the floor, surrounded by a wall (25 cm length × 16 cm width × 16 cm height) and closed with a transparent Plexiglas roof (**Fig. 7**). One chamber had dots on the walls and square protrusion on its floor. The other chamber had line on the walls and a grid floor. Rats were previously habituated to the reward in their home cage (sweetened condensed milk, Migros, half diluted in water, 165 kcal/100g) during two daily sessions of 15 min. On the habituation session to the box, animal had access to both chambers for 20 min and time spent on both side was recorded. The least preferred chamber for each rat was paired with a reward (sweetened condensed milk, Migros, half diluted in water) and the preferred side was paired with a non-rewarding solution (water) in a so-called “biased approach”. Then, 8 conditioning sessions of 20 min each (2 sessions per day) followed, where the rat had access to one of the chamber. Rats were fasted 2 h previous to each conditioning session. Reward sessions and non-reward sessions alternated. The amount of reward and water consumed were weighed for each animal's trial, to ensure similar consumption of the reward at the end of the conditioning sessions. On the test day, animals had access to both chambers, without reward or water. Like on the conditioning sessions, rats were also 2 h fasted. The CPP test indicates how much rewarding is the palatable food depending on the time the rats spend in each chamber on the test day compared to the habituation session. All sessions (habituation, conditioning and test) were recorded by a camera above the CPP box. On habituation session and test session, the video-tracking system Ethovision software recorded entries, durations and distance moved in



**Figure 8:** The incentive runway apparatus



**Figure 9:** Taste reactivity apparatus



**Figure 10:** Camera picture of a rat licking the sweet vanilla flavoured water

each chamber. Rats were fasted 2 h prior to each conditioning and test sessions in order to standardize previous food intake.

### *iii. Incentive runway*

The incentive runway paradigm is a model used to evaluate the incentive salience or "wanting" (Ettenberg, 2009). During several test sessions, the speed to cross an alley and reach a positive incentive stimulus is recorded. Called "completion speed", it assesses the motivation to obtain a reward (Ettenberg, 2009; Shin *et al.*, 2011). The protocol used is based on the one describe by Shin *et al.*, 2011, and from our in-house setup. The apparatus consisted of a start box and a goal box joined by a 180 cm running alley (**Fig. 8**). As in the CCP test, rats were previously habituated to the reward in their home-cage (chocolate milk ChocoDrink M-Classic, Migros, 4g per rat, 61 kcal/100mL) during two daily sessions of 15 min. Then, rats were habituated to the start box and the running alley during one daily session of 5 min. Following 6 h food deprivation, rats were habituated to the goal box with the reward (4g) during two other daily sessions of 5 min. The runway behaviour was then assessed, performing one test per day over 5 days, after 4 h fasting. The rat was introduced into the start box and had to reach the goal box where the food reward were disposed (4g). The time started once his 4 limbs passed the line delimiting the exit of the goal box/start of the running alley. The time to reach the goal box (4 limbs crossed the goal box line) was measured and expressed as completion speed, as well as the time to start eating and the time to finish eating. A video camera above the runway recorded each animal trial for subsequent analysis.

### *i. Taste reactivity test (orofacial expressions and licking)*

We designed the taste reactivity test based on the research of Berridge (Berridge, 2000; Pecina and Berridge, 2005) and Gray and Cooper (Gray and Cooper, 1995) to assess the liking or hedonic appreciation of food of a new palatable solution of 2% sucrose with vanilla flavour. The apparatus consisted of an elevated transparent box (**Fig. 9**). Depending on the session, a 1mL drop of water or palatable solution was poured on the floor. Thus it was possible to observe the orofacial expression and body expression of the rat licking the solution (**Fig 10**). Rats were first habituated to the box during a 5 minute-session. The following day was the session with water. Expressions of the animal with a neutral stimulus were recorded. Rats were habituated during 7 min to the box, and then a 1mL drop of water was poured on the floor. The next day was the test day. Rats were habituated during 7 min to the box, and then a 1mL drop of palatable solution was put on the floor. The experimenter stayed in the room during all the session. All sessions were recorded by a camera for the subsequent analysis. Four variables were measured by the experimenter: number of bouts, number of total licks and number of licks per bout and number of paw licking. A bout was defined as an uninterrupted licking, i.e. pauses from more than 0.2 s, head up or turn, were considered sufficient to divide two bouts. Videos were analysed at 0.06 speed. For each subject's trial, analyses started at the first lick of the solution and continued for 1 min.

### 3) Food intake record

#### ii. *Baseline chow food intake, food intake after fasting and water intake*

- *Baseline chow food intake and after fasting*

To show that our GLP-1R kd rats replicated some of the aspects that have been previously seen (Krieger *et al.*, Diabetes, 2015, accepted), baseline chow food intake and food intake after fasting were measured. Daily baseline chow food intake (powder chow, Kliba 3433; Provimi Kliba Nafag) was measured every 24 h over 3 following days. Prior to start the baseline food intake, animals were given 3 days to adapt to the single cage and to be fed with the new powder chow food in a food cup. For food intake after 16 h fasting, food cup were returned back and food intake was weighed after 1 h, 2 h and 4 h.

- *Baseline water intake*

Following one day habituation to have access to two bottles in a single cage, baseline water intake was measure every 24 h during 2 days. We used two bottles to explain possible side preference.

#### iii. *Palatable food intake*

During all measurements of palatable food intake, rats had free access to water and chow food. When two bottles of different solutions were presented, they were switched every day to avoid possible side preferences (**Fig. 11**).



**Figure 11:** Apparatus for the solution measures

This apparatus was used for the baseline water intake, sucrose preference test, milk preference test, dextran preference test. Rats are single caged and have access to two bottles.

- *Sucrose preference test*

The sucrose preference test is usually used to measure anhedonia, in studies linked with depression (Harris *et al.*, 1997). We adapted it to use the intake of the palatable solution as an indication of liking, or at least to estimate the pleasure after a rewarding stimulus such as sucrose. Rats had free choice between one bottle of water and one bottle containing 2% sucrose solution (8 kcal/100 mL) for 2 days (**Fig. 11**). After presenting the new 2% sucrose solution, the intake of both bottles was measured at 1 h, 4 h, 24 h and 48 h.

After two washout days, we repeated the experience with a 4% sucrose solution (16 kcal/100 mL), measuring the intake of bottles after 1 h, 2 h, 4 h and 24 h exposure. The indication of liking is the percent of sucrose solution consumed over the total fluid intake.

- *Milk preference test*

Using a similar design as the sucrose preference test (**Fig. 11**), rats had free choice between one water bottle and one bottle of a mixture of raspberry flavoured sweet milk matching the nutritional values of the chocolate milk used in the running alley (50% of skimmed milk Léger, 40% of UHT Valflora Drink 1.5% fat milk, 10% of raspberry syrup Migros, energy density: 65 kcal/100 mL). Rats were exposed 4 h per day during 3 days. Measurements were taken at 30 min, 1 h, 2 h and 4 h after exposure.

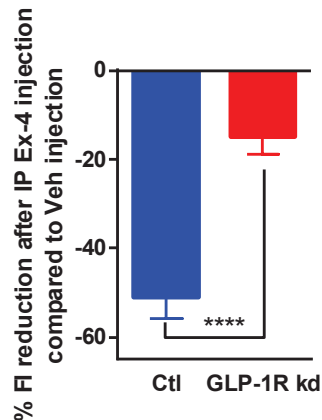
- iv. *Non-palatable food intake*

Using a similar design as the sucrose preference test (**Fig. 11**), rats had free choice access between one water bottle and one bottle of 2% Dextran solution (8 kcal/100 mL) (Dextran from *Leuconostoc mesenteroides*, average molecular weight 64 000-76 000 mol/g, Sigma-Aldrich). Long glucose polymers such as Dextran have a reduced sweet taste, and this Dextran solution does not taste sweet (at least for humans) and brings calories. During one day, measures were taken after 1 h, 2 h, 4 h and 24 h after exposure. The Dextran solution was design to control for caloric density and brought similar energy as the 2% sucrose solution.

## II. Statistical Analysis

Data were checked for normality. When data were normally distributed, the presence of an outlier within our data was checked using the Graphpad outlier test. If an outlier was detected, it was excluded for the rest of the analysis. Statistical significance was analysed using Student t-test, one-way or two-way ANOVA. If data were not normally distributed, they were transformed or non-parametric tests were performed. Only food neophobia data were not normally distributed and they were log-transformed (ln) for normality. Data are presented as mean  $\pm$  Standard Error of the Mean (SEM). Statistical significance was set at  $p$ -value $<0.05$ . All statistical analysis and graphics were performed with GraphPad Prism (version 6.05 for Windows).

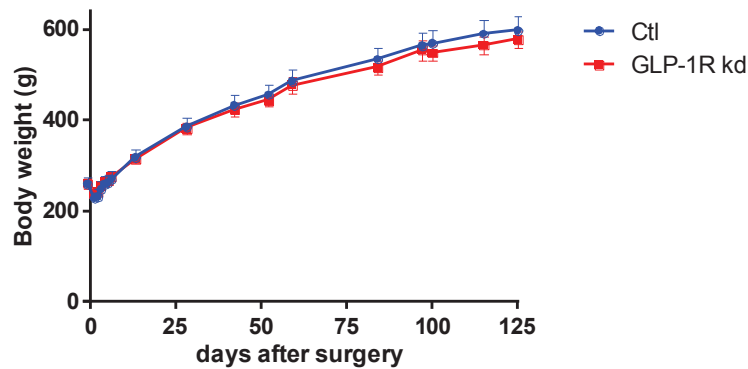
In graphics, all values are mean  $\pm$  SEM. In graphics, the following legend is used for significant difference: \* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ ; \*\*\*\* $p<0.0001$ .



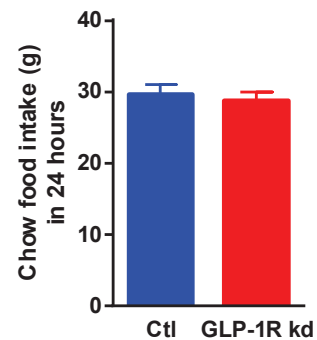
**Figure 12:** Functional verification of the VAN GLP-1R by IP Ex-4 injection

Percent of reduction in food intake 1 h after intraperitoneal Ex-4 injection (0.3µg/kg) compared to vehicle injection of Ctl and GLP-1R kd rats. Ctl rats displayed a 50% reduction in response to Ex-4 relative to the baseline in response to vehicle significantly different from GLP-1R kd with 15% food intake reduction. Ctl: -50.97 ± 4.823, n=8; GLP-1R kd: -14.91 ± 3.892, n=8; t-test, p<0.0001. Values are means ± SEM

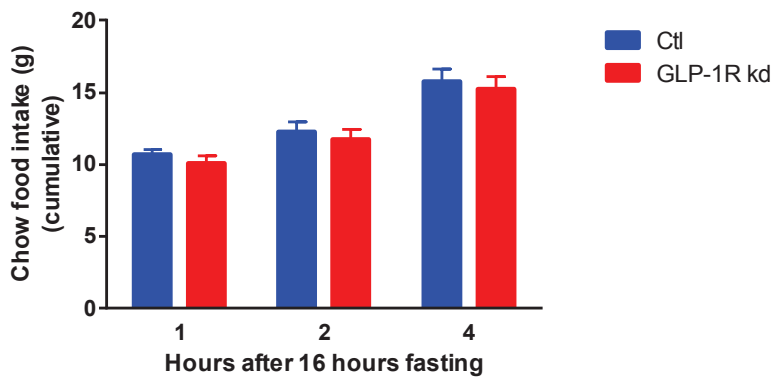
**A Body Weight (relative to surgery day)**



**B Baseline 24H chow food intake average upon 3 days**



**C Baseline food intake after 16H fasting**



**Figure 13:** Body weight, daily food intake of chow food

VAN GLP-1 R kd did not affect body weight, daily food intake of chow food. **A:** Body weight (day 0 indicates surgery day). No significant differences were detected between Ctl and GLP-1R kd rats. n(Ctl)=8, n(GLP-1R kd)=9. **B:** Baseline 24h chow food intake. Ctl: 29.68 ± 1.420, n=8; GLP-1R kd: 28.80 ± 1.132, n=9; t-test; ns. **C:** Chow food intake following 16h fasting (2 × 3 (treatment × time) parametric ANOVA, ns). All values are means ± SEM



## Results

### I. Verification of the VAN GLP-1R kd

#### 1) VAN GLP-1R kd did not reduce their food intake after functional verification by intraperitoneal exendin-4 injection

Following an IP injection of the GLP-1R agonist Ex-4, food intake 1 h after Ex-4 injection relative to food intake after vehicle injection was significantly reduced in Ctl rats compared to GLP-1R kd rats (Ctl:  $-50.97 \pm 4.823$ ,  $n=8$ ; GLP-1R kd:  $-14.91 \pm 3.892$ ,  $n=8$ ; t-test,  $p<0.0001$ ) (**Fig. 12**). The effect was still noticeable 2 h and 4 h after Ex-4 injection, and the difference between Ctl and GLP-1R kd remained significant (t-test,  $p<0.001$ ). These results were consistent with the results of Krieger *et al.*, Diabetes, 2015, accepted (**Appendix 2**).

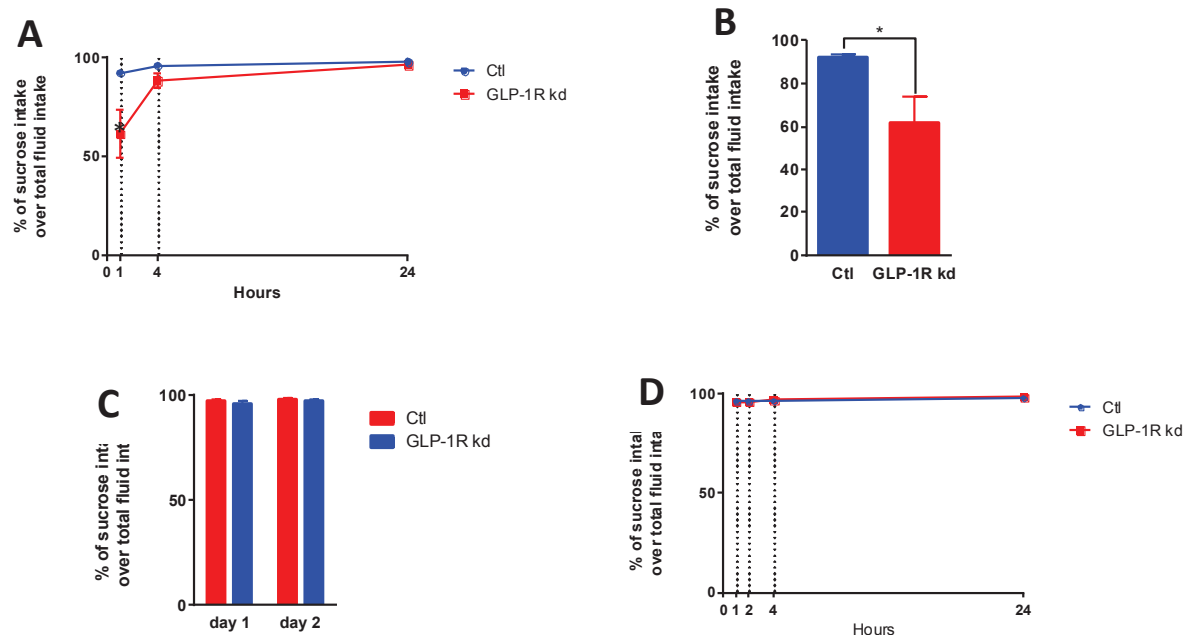
The purpose of this newly-established functional test was also to decide which individual animal could be included in the study. Based on previous experiments, the inclusion criteria for the VAN GLP-1R kd rats was a maximum decrease of 30% in 1 h food intake. At 1 h time point, within our GLP-1R kd rats, individuals had maximum 28% and minimum 3% food intake reduction in response to Ex-4 injection relative to vehicle injection. Thus, all animals were included in the study.

#### 2) VAN GLP-1 R kd did not affect body weight and daily food intake of chow food

As previously observed (Krieger *et al.*, Diabetes, 2015, accepted), GLP-1R kd and Ctl rats showed no difference in body weight along the study (**Fig 13A**).

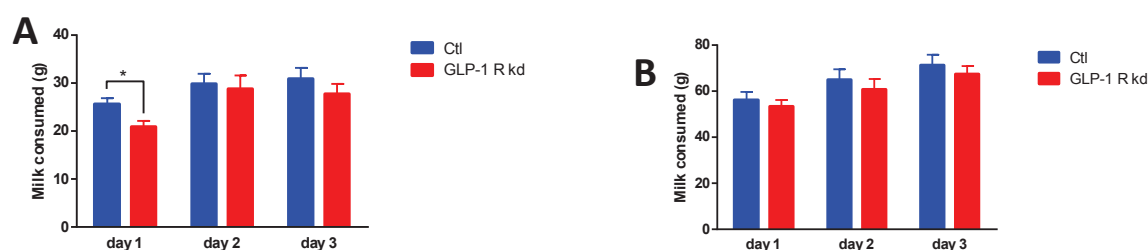
As expected (Krieger *et al.*, Diabetes, 2015, accepted), no difference in 24 h chow food intake was noticed between GLP-1R kd and Ctl rats (**Fig 13B**). While baseline chow food intake was measured, both groups ate 30g/day (average upon 3 days) (Ctl:  $29.68 \pm 1.420$ ,  $n=8$ ; GLP-1R kd:  $28.80 \pm 1.132$ ,  $n=9$ ; t-test; ns). Besides, following 16 h of fasting, no significant difference in chow food intake was seen between groups at any time point ( $2 \times 3$  (treatment  $\times$  time) parametric ANOVA, ns) (**Fig. 13C**).





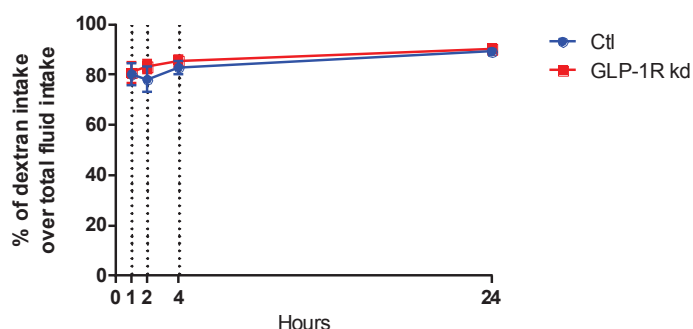
**Figure 14:** 2% sucrose consumption upon 2 days and 4% sucrose consumption upon 1 day of Ctl and GLP-1R kd

GLP-1R kd reduced their consumption of 2% sucrose solution over total fluid consumption 1 h after exposure on day 1. **A:** 2% sucrose solution intake over total fluid consumption (cumulative measures) on day 1 of exposure.  $3 \times 2$  (time  $\times$  treatment) ANOVA give a significant treatment difference ( $F(1,14)=5.085$ ,  $p<0.05$ ). **B:** Subsequent comparison confirmed a significant difference between Ctl and GLP-1R kd on day 1 after 1 h exposure to 2% sucrose solution over total fluid consumption (Ctl:  $92.38 \pm 1.165$ ,  $n=7$ ; GLP-1R kd:  $61.63 \pm 11.99$ ,  $n=9$ ; Unpaired t-test with Welch's correction;  $p=0.03<0.05$ ; F test to compare variances:  $F=136$ ,  $Df=8$ ,  $Dfd=6$ ;  $p<0.0001$ ). **C:** Day 1 and day 2 2% sucrose solution intake over total fluid consumption. **D:** 24H 4% sucrose solution intake over total fluid consumption ( $4 \times 2$  (time  $\times$  treatment) ANOVA, treatment ns).



**Figure 15:** Raspberry flavoured sweet milk consumption upon 3 days (milk is removed after 4H of exposure) of Ctl and GLP-1R kd

GLP-1R kd reduced their milk consumption 1 h after exposure on day 1. **A:** 1 h milk consumption upon 3 days. 1h Day1; Ctl:  $25.57 \pm 1.212$ ,  $n=7$ ; GLP-1R kd:  $20.82 \pm 1.307$ ,  $n=9$ ; t-test,  $p=0.02$ . T-test differences between groups were not significant the other days at 1H. **B:** 4h milk consumption upon 3 days. T-test differences between groups were not significant.



**Figure 16:** 2% dextran consumption upon 1 day of Ctl and GLP-1R kd

GLP-1R kd did not affect consumption of non-palatable solution: 2% dextran solution intake over total fluid consumption during the first 24 h of exposure.  $4 \times 2$  (time  $\times$  treatment) ANOVA, treatment was not significant.

## II. VAN GLP-1 R kd showed short-term changes in palatable food intake

### 1) Short-term changes in palatable food intake during first exposure in home cage

We conducted two tests, the sucrose preference test and the flavour sweet milk preference test, in home-cage. We measured the consumption when animals had access to a more rewarding food.

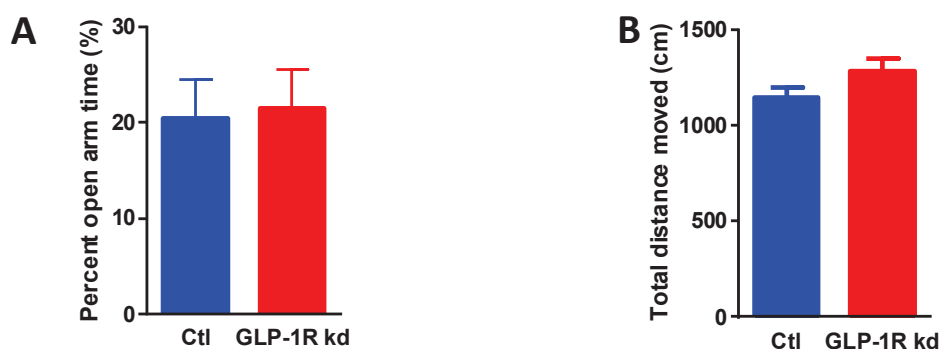
In the 2% sucrose preference test, 2% sucrose consumption over the total fluid intake was significantly decreased 1 h after first exposure in the GLP-1R kd group compared to the Ctl group (Ctl:  $92.38 \pm 1.165$ ,  $n=7$ ; GLP-1R kd  $61.63 \pm 11.99$ ,  $n=9$ ; t-test,  $p<0.05$ ) (**Fig. 14B**). After 4 h of exposure, GLP-1R kd rats reached the sucrose consumption of Ctl and there were no difference between groups anymore. Upon 2 days of continuous exposure to the sucrose solution, the consumption of 2% sucrose solution over total fluid intake was similar in both groups ( $2 \times 2$  (treatment  $\times$  time) ANOVA, ns) (**Fig. 14C**). We reproduced this design by increasing the sucrose concentration to 4%. No difference in 4% sucrose consumption was observed at any time point ( $2 \times 4$  (treatment  $\times$  time) RM ANOVA, ns), (**Fig. 14D**).

To consolidate this observation of lower food intake at first exposure, we used a solution of raspberry flavoured sweet milk (to which rats had never been exposed) and repeated a similar design, to the exception that the solution was withdrawn after 4 h of exposure. Again, 1 h after the first exposure, GLP-1R kd rats consumed significantly less than the Ctl (Ctl:  $25.57 \pm 1.212$ ,  $n=7$ ; GLP-1R kd:  $20.82 \pm 1.307$ ,  $n=9$ ; t-test,  $p<0.05$ ) (**Fig. 15A**). After 2 h of exposure, milk consumption was similar between the two groups. Upon the two following days, no difference was noticed in milk consumption between groups (**Fig 15B**).

### 2) First exposure in home cage to a new non-palatable food did not induce change at short-term

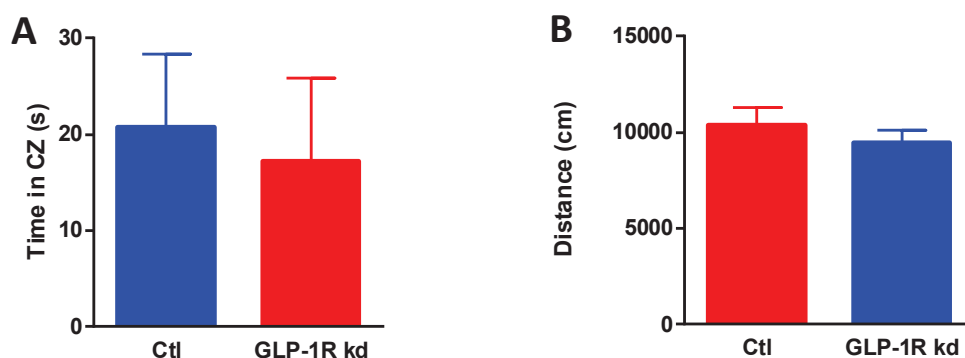
The previous changes in food intake were seen with sweet and/or fatty solutions, in other words what is considered as "palatable" food or rewarding food. We aimed to see if this short-term reduced consumption of a novel food was specific to palatable food, or if the same results can be obtained with a "non-palatable solution". To investigate this, we used a design similar to the sucrose preference test, using the polysaccharide dextran that probably does not taste sweet for rats owing to its high molecular weight and that we considered as non-palatable. We measured no significant difference between groups in Dextran consumption over the total fluid consumption at any time point ( $2 \times 4$  (treatment  $\times$  time) ANOVA, treatment ns) (**Fig. 16**), strengthening that this short term reduction of novel food consumption was specific to palatable food.

In brief, the phenotype observed in VAN GLP-1 R kd is a short-term reduction in consumption of a new palatable food, which is consistent with the first observation of a lower consumption of HFD. We hypothesized that this phenotype could be the result of any of the 3 following factors (or a combination of them): a/ an increased innate anxiety, b/ a specific food neophobia or c/ a decrease in the reward value of food. We consequently specifically tested whether VAN GLP-1R kd rats differ from control rats in any of these parameters.



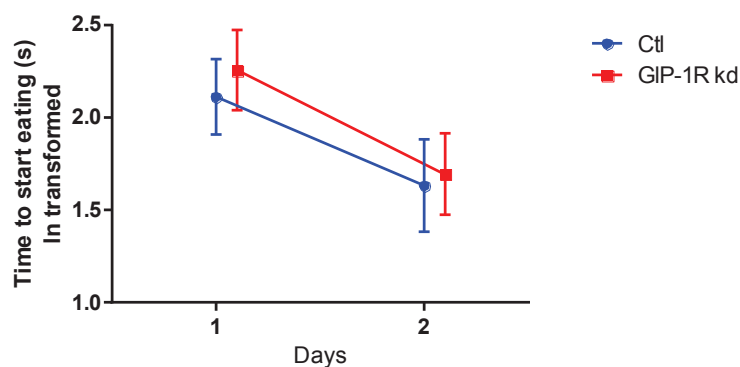
**Figure 17:** In the EPM, percent time spent in open arm and total distance moved of Ctl and GLP-1R kd

GLP-1R kd did not significantly affect measures of innate anxiety and locomotor activity in the elevated plus maze test. **A:** Percent of time spent in open arms to the total time spent in the arms (open and closed arms). Ctl:  $20.47 \pm 3.962$ ,  $n=8$ , GLP-1R kd:  $21.55 \pm 3.970$ ,  $n=9$ ; t-test, ns. **B:** Total distanced moved in the EPM. Ctl:  $1147 \pm 57.15$ ,  $n=8$ ; GLP-1R kd:  $1286 \pm 67.66$ ,  $n=9$ ; t-test, ns.



**Figure 18:** In the OF, time spent in CZ and total distance moved of Ctl and GLP-1R kd

GLP-1R kd did not significantly affect measures of innate anxiety and locomotor activity in the open field test. **A:** Time spent in the central zone of the open field during 30 min of test. Ctl:  $20.78 \pm 7.591$ ,  $n=8$ , GLP-1R kd:  $17.22 \pm 8.607$ ,  $n=9$ ; t-test, ns. **B:** Total distance moved in the arena during 30 min of test. Ctl:  $10389 \pm 918.8$ ,  $n=8$ ; GLP-1R kd:  $9429 \pm 677.4$ ,  $n=9$ ; t-test, ns.



**Figure 19:** Latency to eat on day 1 and day 2 of Ctl and GLP-1R kd rats in the food neophobia test.

Latency to eat on day 1 and day 2 was not significantly different between Ctl and GLP-1R kd rats. The criteria of exclusion « did not to eat on both days » resulted in excluding two rats in both groups. Time to start eating was ln transformed for normality.  $2 \times 2$  (treatment  $\times$  time) ANOVA, interaction and treatment ns, time  $p=0.01$  means that latency to eat on day 2 is significantly reduced.

### III. Assessment of innate anxiety, food neophobia and reward value of food

#### 1) VAN GLP-1R kd did not significantly affect measures of innate anxiety in the elevated plus maze and open field tests

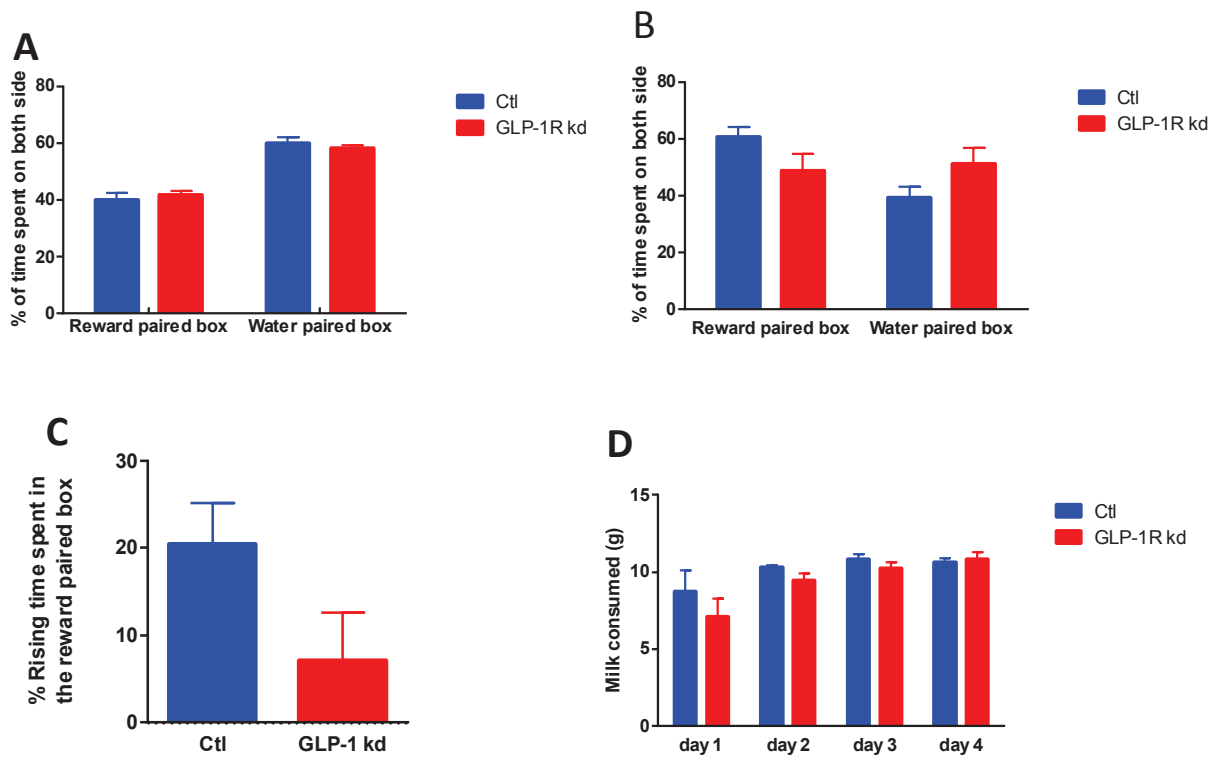
Measures of innate anxiety were evaluated first in the elevated plus maze and later in the open field.

In the EPM, the ratio of time spent in open arms to the total time spent in the arms (open and closed arms) was not affected in GLP-1R kd (Ctl:  $20.47 \pm 3.962$ ,  $n=8$ , GLP-1R kd:  $21.55 \pm 3.970$ ,  $n=9$ ; t-test, ns) (**Fig. 17A**) (**Appendix 3**), as well as the percentage of distance moved in the open arms (Ctl:  $1147 \pm 57.15$ ,  $n=8$ ; GLP-1R kd:  $1286 \pm 67.66$ ,  $n=9$ , t-test, ns) (**Fig. 17B**). These results indicated that GLP-1R kd did not affect measures of innate anxiety.

VAN GLP-1 R kd did not affect time spent in central zone (CZ), that is the anxiogenic part of the open field (Ctl:  $20.78 \pm 7.591$ ,  $n=8$ , GLP-1R kd:  $17.22 \pm 8.607$ ,  $n=9$ ; t-test, ns) (**Fig. 18A**), as well as the total distance moved. Overall, locomotor activity was not changed in VAN GLP-1R kd rats (Ctl:  $10389 \pm 918.8$ ,  $n=8$ ; GLP-1R kd:  $9429 \pm 677.4$ ,  $n=9$ ; t-test, ns) (**Fig. 18B**). Moreover, looking at the total distance moved by 5 min time bins, both GLP-1R kd and Ctl groups showed a similar decrease, that is the normal trend of the exploratory behaviour of rats in a new environment (**Appendix 4**). The results of the open field test are consistent with the results of the EPM in term of anxiety and locomotor activity.

#### 2) VAN GLP-1R kd did not significantly affect measures of fear towards novel food in the food neophobia test

The food neophobia test assessed the fear to eat a novel food by measuring the latency to eat upon two following days. The latency to eat the first day was similar in both group ( $p>0.1$ ), and followed the same reduction the second day ( $2 \times 2$  (treatment  $\times$  time) ANOVA, interaction and treatment ns) (**Fig. 19**), indicating that GLP-1 R kd and Ctl rats had the same level of food neophobia.



**Figure 20:** In the CPP, percent of time spent on both side on habituation session and test day, percent of rising time on test day relative to habituation session, milk consumed, of Ctl and GLP-1R kd

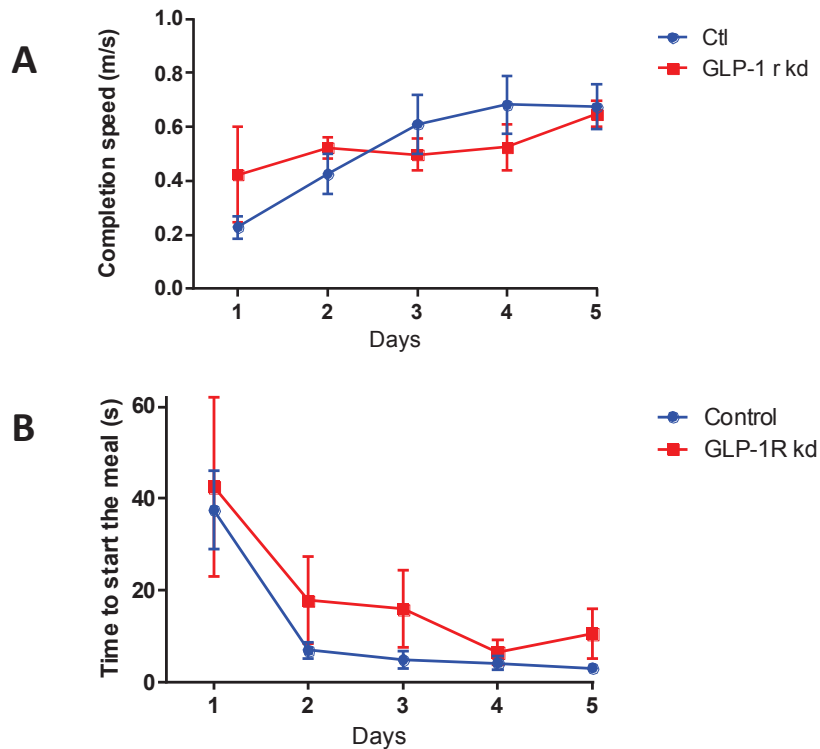
GLP-1R kd tended to show a deficit in palatable food-induced side preference (CPP paradigm) that the control group did not displayed. **A:** CPP habituation session, time spent on both side during the 20 min of the session. **B:** CPP test day, time spent on both side during the 20 min of the session. **C:** CPP Habituation session day vs test day: Rising time spent in the reward paired box in Ctl and GLP-1R kd rats. Ctl:  $20.42 \pm 4.723$ ,  $n=8$ ; GLP-1R kd:  $7.034 \pm 5.583$ ,  $n=9$ , t-test,  $p=0.09$ , ns. **D:** CPP Milk consumed on the 4 days of conditioning sessions

### 3) VAN GLP-1R kd failed to show a palatable food-induced side preference in a CPP paradigm

To test whether VAN GLP-1 R kd modulated the reward value of palatable food, we compared GLP-1R kd rats and Ctl rats in the conditioned place preference paradigm. The time spent in the chamber previously paired with a food reward (here sweet condensed milk, whereas the other chamber was paired with water), after several conditioning sessions, was taken as a measure of how rewarding the rat finds the food.

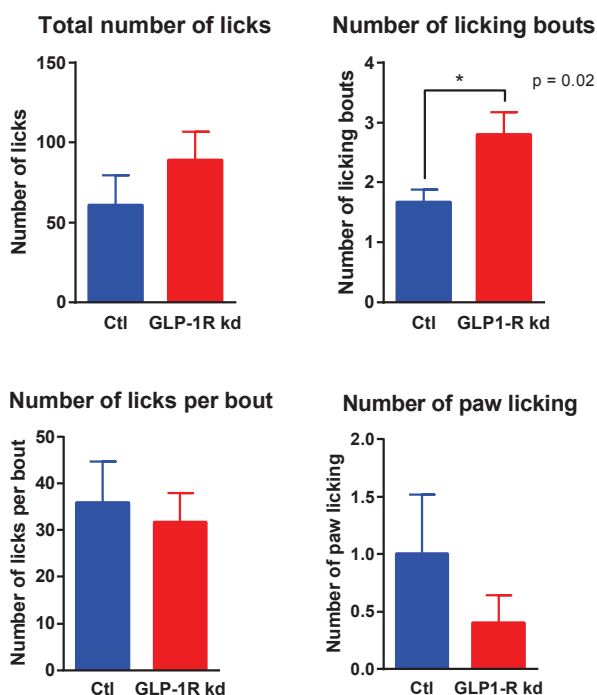
On habituation session, time spent in both chamber was recorded and the time spent in the dot and in the stripe chambers were similar in both groups (**Fig 20A**). However, some rats showed a clear preference for one of the side. Thus, we went for a biased approach: the side the less preferred was paired with a reward and the side the most preferred was paired with water.

On the test day, Ctl rats increased of more than 20% ( $20.42 \pm 4.723$ , one sample t-test difference from 0,  $p=0.003<0.01$ ) their time spent in the reward paired chamber whereas GLP-1R kd rats time spent in the reward paired chamber was not significantly increased from 0 ( $7.034\% \pm 5.583$ , one sample t-test difference from 0,  $p=0.24$  ns). The difference between both groups only tended to be significant (Ctl:  $20.42 \pm 4.723$ ,  $n=8$ ; GLP-1R kd:  $7.034 \pm 5.583$ ,  $n=9$ ; t-test,  $p=0.9$ ) (**Fig. 20C**). The milk consumption in the two last conditioning sessions indicated that there was no difference in hunger (**Fig. 20D**). Furthermore the latency to eat during the last reward session was less than 10 seconds and similar in both group. These two points indicated that both groups were conditioned the same way. The general locomotor activity was not changed in both groups, consistent with the results of the EPM and the open field tests. Following the results of the CPP, we performed experiments testing specific components of food reward, the “wanting” and the “liking”.



**Figure 21:** In the incentive runway, completion speed and time to start the meal of Ctl and GLP-1R kd

GLP-1R kd showed identical behaviour in an incentive runway paradigm. **A:** Completion speed to reach the goal box.  $2 \times 5$  (treatment  $\times$  time) ANOVA, interaction and treatment ns, time  $F(4,60) = 4.505$ ,  $p=0.003$ . Subsequent t-test analysis comparing groups per day did not give any significance difference. **B:** Time to start the meal in the incentive runway paradigm.  $2 \times 5$  (treatment  $\times$  time) ANOVA, interaction and treatment ns, time  $F(4, 60) = 12.07$   $p<0.0001$ . Subsequent t-test analysis between groups per day did not give any significance difference.



**Figure 22:** In the taste reactivity, total number of licks, number of licking bouts, number of licks per bout and number of paw licking of Ctl and GLP-1R kd

Taste reactivity test did not give interpretable results in term of liking. Number of licking bouts was significant between Ctl and GLP-1R kd rats: Ctl:  $1.667 \pm 0.2108$ ,  $n=6$ ; GLP-1R kd:  $2.800 \pm 0.3742$ ,  $n=5$ ; t-test,  $p=0.02$ . The t-test analysis of the other variables (total number of licks, number of licks per bout, number of paw licking) were not significantly different.

#### 4) VAN GLP-1R kd showed identical behaviour in an incentive runway paradigm

To investigate the "wanting" component of food reward (or motivation to obtain food), we measured the completion speed (based on the time to reach the goal box) and the time to start the meal in the incentive runway paradigm. Both GLP-1R kd and Ctl groups showed the same completion speed that increased similarly between day 1 and 3 until reaching a plateau (**Fig. 21A**). The latter information indicated that they learnt very quickly where the food reward was. Furthermore, time to start the meal was not significantly different between both groups, and decreased the same way on day 2 (**Fig 21B**). These results indicated that both groups had a similar motivation for food reward. Data of food reward consumption are shown in **Appendix 5**.

#### 5) VAN GLP-1R kd did not show clear difference enabling interpretation in the taste reactivity test

To investigate the "liking" component of food reward, we used the taste reactivity test. Total number of licks, number of licks per bout and number of paw licking was not significantly affected in both groups. GLP-1R kd rats showed a higher number of licking bouts than the control (Ctl:  $1.667 \pm 0.2108$ , n=6; GLP-1R kd:  $2.800 \pm 0.3742$ , n=5, t-test, p=0.02), and Ctl rats tended to show more paw licking than GLP-1R kd (Ctl  $1.000 \pm 0.5164$ , n=6; GLP-1R kd:  $0.4000 \pm 0.2449$ , n=5; t-test, ns) (**Fig 22**). These results did not enable us to conclude on liking aspect of food reward.



## Discussion

### I. Relevance of the VAN GLP-1R kd model

#### 1) The importance of the vagus nerve as a mediator of intestinal GLP-1

A large number of studies supported the hypothesis that VAN mediates gut signal from the intestine to the brain, such as GLP-1 (see for review: Krieger *et al.*, Physiology & Behavior, 2015; Ronveaux *et al.*, 2015): these studies mostly used lesions of the vagus nerve (such as SDA, the most selective method to date to disconnect vagal afferents below the diaphragm (Norgren et Smith, 1994)) to suggest that VAN were involved in mediating GLP-1's effect on food intake to the brain (Abbott *et al.*, 2005; Kanoski *et al.*, 2011; Labouesse *et al.*, 2012; Plamboeck *et al.*, 2013). Results of Krieger *et al.*, Diabetes, 2015, accepted, showed using VAN GLP-1R kd model that VAN GLP-1R mediates the neuroincretin and satiation effects of intestinal GLP-1.

#### 2) The choice of the lentivirus strategy for a VAN GLP-1R kd

##### i. Physiological relevance of a VAN GLP-1R kd model

The bilateral injection into the nodose ganglia of a lentivirus expressing a shRNA enabled to target specifically the GLP-1R in the VAN. Contrary to previous VAN lesion-based approaches, this approach does not damage the vagus nerve and is GLP-1R specific (Krieger *et al.*, Diabetes, 2015, accepted, see **Appendix 6** for further tests confirming the efficiency and tissue-specificity of a VAN GLP-1R kd). So far, injection in the NG were followed by non-survival experiment to measure electrophysiology activity (Calik *et al.*, 2014), and a recent study used unilateral nodose ganglion injection of an AAV to perform optogenetics activation of VAN (Chang *et al.*, 2015). By our method, shRNA binds to GLP-1R mRNA and prevents GLP-1R synthesis. As a result, the amount of GLP-1R present at the surface of VAN should be reduced, without affecting its expression in other organs. We could specifically examine the role of GLP-1R in the VAN in the mediation of a signal triggered by its ligand, gut GLP-1. Furthermore, even if several areas expressed GLP-1R in the brain, it is unlikely that a high concentration of gut GLP-1 reaches central GLP-1R via the systemic circulation because of its rapid degradation in the blood (Deacon *et al.*, 1995) and the brain blood barrier that prevents GLP-1 to enter in brain areas. It is possible, however, that physiological concentrations of circulating GLP-1 activate GLP-1R in brain areas that are devoid of a brain blood barrier, such as the area postrema (Punjabi *et al.*, 2014). Although several studies using pharmacological doses of GLP-1 or its agonist Ex-4 reported modulation of food reward behaviour (Dossat *et al.*, 2011; Dickson *et al.*, 2012; Alhadeff *et al.*, 2012; Dossat *et al.*, 2013; Richard *et al.*, 2015), the mechanisms by which endogenous GLP-1 act might be different. Our goal was not to investigate the mechanisms of action of the long-lasting GLP-1R agonists but to unravel a possible impact of VAN GLP-1R activation of the reward value of the ingested food.

## ii. *The lentivirus strategy*

To transfer genetic material in an animal model, the injection of a viral vector is an efficient and simple method. Another transfection method, electroporation of a plasmid, has been successfully used to transfect siRNA into the NG of rats (Heldsinger *et al.*, 2012), but the long-term efficiency of such a method, as well the extent to which the nerve may be damaged, remain questionable. Several types of viruses are possible vectors: adenoviruses, adeno-associated viruses (AAV) and lentiviruses. AAV and lentiviruses are preferred to adenoviruses because the latter lead to a high inflammatory response. AAV have the advantage to be easily spread owing to their small size (<20 nm) whereas lentivirus particles are larger (50 to 100 nm). Yet our laboratory tested different AAV serotypes, seeing only limited infection of the NG, while in-house produced lentiviruses showed a promising infection potential. Moreover, technically, the lentivirus production was faster and its purification and concentration were easier than with AAV, allowing an on-demand production. Lentiviruses are known to have a long period of incubation because they integrate the genome of their host cell after a reverse transcription from RNA to DNA (retroviruses family). Thus, their expression is more stable compared to AAV which rarely integrates. This characteristic was an advantage for a long-term study (several months) like ours. For all these reasons, we used a lentiviral vector rather than AAV.

## iii. *Cre-lox approach*

Another approach than the lentivirus strategy could have been used, the *cre-lox* method which has the advantage to selectively activate or inactivate genes in a tissue-specific manner and to have a theoretical total knock out. For VAN, the main obstacle remains to find a promoter for the *cre-recombinase* expression that is truly specific to this cell type. So far, *Nav1.8* (de Lartigue *et al.*, Mol Metab., 2014) and *Phox2b* (Sisley *et al.*, 2014; Liu *et al.*, 2014) were used for this purpose but the expression of these two genes is not limited to VAN (Scott *et al.*, 2011; Stirling *et al.*, 2005).

## 3) Limits of the VAN GLP-1R kd

The main limit is that the reduction of GLP-1R expression in the VAN is partial. All neurons may not have been infected by the lentivirus and infected neurons will residually express GLP-1R. Consequently, it is possible that remaining GLP-1R expression may mask some of the physiological effects of VAN GLP-1R that we try to highlight with our method.

Functional aspects of the VAN GLP-1R kd were noticeable until 14 weeks after the lentivirus injection. In Krieger *et al.*, Diabetes, 2015, accepted, this functional aspect was observed beyond 12 weeks. It is, however, possible that knocking down VAN GLP-1R for this period engenders compensatory changes.

Another limit of the kd approach is the relative difficulty to verify the kd. In previous experiments, the kd was verified by measuring the expression of GLP-1R mRNA in the nodose ganglia, i.e. in the end of the experiments. To avoid waiting for the end of all experiments to collect the nodose, we established a new

functional test to verify the GLP-1R kd. This functional test, however, could have interfered with our behavioural experiments as they required an IP injection of Ex-4 and single-caging.

On the whole, our method resulted in a specific and permanent reduction of GLP-1R in the VAN. Even if the knock down was not total, it enabled to test endogenous peripheral GLP-1 effect on the VAN, employing a physiological approach.

## II. Further interpretation of the results

### 1) Body weight and daily food intake of chow food and functional verification

The fact that VAN GLP-1 R kd did not affect body weight and daily food intake confirms previous data showing that total food intake and energy balance were not affected in this model (Krieger *et al.*, Diabetes, 2015, accepted). Other satiation factors (gastric distension, PYY, CCK...) remained unaffected by the GLP-1R kd and may be consequently sufficient to maintain the overall energy balance.

A discrepancy was noticed with the results of Krieger *et al.*, Diabetes, 2015, accepted. In this study, food intake after 16 h fasting was increased 1 h after a prolonged period of fasting in GLP-1R rats, whereas it was not affected in our study.

The functional verification confirmed that the kd was efficient in all rats. Thus, all animals could be included in the study. Food intake after Ex-4 injection was not reduced at the same level in all GLP-1R kd rats. This can be due to the variability between rats in response to the drug, the access of Ex-4 to other GLP-1R in the periphery or brain, or the residual expression of GLP-1R in VAN. There are also other satiety signals that modulate food intake and balance the effect of Ex-4.

### 2) Short-term changes in palatable food intake

Both GLP-1R kd and Ctl rats showed a clear preference for the palatable solution compared to the water, when freely accessible. However, when exposed to a new palatable solution, GLP-1R kd rats decreased their consumption compared to the Ctl during the first hour of exposure, and after consumption of GLP-1R kd and Ctl looked identical. Daily consumption of palatable food intake was not affected, consistent with the absence of change in energy homeostasis in GLP-1R kd (Krieger *et al.*, Diabetes, 2015, accepted). As the palatable food used was liquid, it may be possible that GLP-1R kd were not able to drink as much as the controls. Yet, they drank the same quantity of water in the baseline water consumption (**Appendix 7**) and during these two tests with palatable solution, daily total fluid intake (palatable solution and water) was identical in both groups. Also the intensity of the food reward was maybe not high enough to feel it rewarding. A 2% sucrose solution was considered as palatable since rats taste is strongly developed. The milk solution contained more sugar (8%) and fat (1.6%) that is a much rewarding combination. Besides when giving a solution of higher sucrose concentration (4%), no difference was observed between both

groups. Our explanation is that the 4% sucrose solution did not represent a new food as it was not a new taste but only a solution of higher intensity of the sucrose taste. On the whole the lower consumption of a new palatable food considered as rewarding was similar to the previous observation on HFD, considering as rewarding (Krieger *et al.*, in preparation). With a non-palatable solution, GLP-1R kd rats did not display a lower consumption, suggesting that this decrease in consumption was specific to a palatable solution. Sweetness of carbohydrates decreases with polymer length (low dextrose equivalent DE) and dextran has a very high chain length, which allowed us to think that dextran does not taste sweet particularly sweet for rats.

The phenotype observed in VAN GLP-1 R kd is a reduction in consumption of a new palatable food at short term. Then we investigated if this observation resulted from a/innate anxiety, b/fear toward a novel food, or c/decrease in the reward value of food.

### 3) Anxiety test: innate anxiety and food neophobia

Innate anxiety and food neophobia are plausible explanation why an animal would eat less than expected during first exposure. Moreover, a study has shown that disconnection of the vagal afferent neurons below the diaphragm (subdiaphragmatic vagal deafferentation SDA) resulted in a reduction of innate anxiety in the EPM, OF, and food neophobia tests (Klarer *et al.*, 2014). No difference in innate anxiety and food neophobia were observed in both Ctl and GLP-1R kd rats in these three tests. It is important to note, however, that for technical reasons, the food neophobia test was done after animals were trained to behaviour experiments and novel food, which could reduce a natural food neophobic behaviour. Indeed, a food neophagia/neophobia test was first done after the EPM, so that the animal had not been exposed to many novel foods. This test, however, did not give interpretable results since a high proportion of both controls and GLP-1R kd rats did not interact with the food. Plausible explanations are that the arena might have been too anxiogenic or that the rats were not sufficiently trained and consequently stressed.

### 4) Reward value of food

#### i. CPP

GLP-1R kd rats showed a trend to spend less time in the reward paired box. At first sight, as satiety peptide, GLP-1 would be expected to decrease the reward value of food. Thus rats feeling less or delayed satiation (GLP-1R kd phenotype typically shows bigger meal), should expect more food. Moreover, this behaviour should be reinforced by the rewarding value of sweet milk. Together with the observation on HFD and the lower palatable food intake at first exposure, the CPP suggested the hypothesis that GLP-1R kd rats had a reduction of hedonic appreciation of food. In other words VAN GLP-1R signalling led to an increase of hedonic appreciation of palatable food. A further step led to think that VAN GLP-1R signalling is linked with brain reward areas.

Yet, several other suggestions may explain this trend:

1/ *GLP-1R kd are less hungry than the controls.* They, however, ate the same during the conditioning sessions, assuming that both groups have the same hunger.

2/ *GLP-1R kd are not conditioned the same.* On the last reward sessions, all rats went directly eating the reward, highlighting that they were conditioned the same way. Additionally, they did not consume the water during all the conditioning sessions. A deficit of learning or association cues may be an explanation, but the fact that they were conditioned the same is not in favour of this hypothesis. Besides, learning aspect is low for this test.

3/ *GLP-1R kd have a different locomotor behaviour.* No difference in total locomotor activity in the chamber on habituation sessions was measured. Indeed, if they would have moved more, they would have been more occupied to explore (and not to eat or to look for food), explaining changes in time spend in reward paired box on the test day. Besides, changes in exploratory behaviour could be excluded because no differences in locomotor activity were seen in the EPM and the OF tests.

4/ *The effort is too high for VAN GLP-1R kd rats.* This test does not require a high effort to obtain the reward, so that an excessive task is likely not at stake. Moreover, no differences were observed in the incentive runway, going in that sense.

To conclude, we can interpret the behaviour of the GLP-1R kd rats as an altered feeling of reward following the reduction of GLP-1R expression in the VAN. GLP-1R kd rats may need a higher amount of palatable food to feel the reward at the same level of the Ctl. While this amount is enough for Ctl rats to feel the reward value of food, VAN GLP-1R kd rats have something to overcome.

## *ii. Incentive runway*

In the incentive runway, rats showed the same willingness to run for food, an indicator of wanting. This does not entirely exclude the possibility of a change in wanting. Indeed, animals had performed several behavioural experiments and had been trained to find palatable food in a new environment. Thus, our animals reached a plateau of completion speed after 3 sessions. Compared to the incentive runway results of Shin *et al.*, 2011, their rats needed at least 6 sessions to reach a plateau. Consequently, it is plausible that the fast learning curve of our rats has made our tests not sensitive enough to detect a potential effect of the GLP-1R kd. Another limit of this test is that the effort to reach the food (running a 1.8 meters corridor) may not be enough discriminant. As improvement, some obstacles could be added in the corridor to make it less easy. Another disadvantage is the design of this test which can make measures difficult to take accurately. The operant responding, which also measures the incentive motivational value of food reward, gives more accurate measures. It could have been done to complement the incentive runway test. Unfortunately, we did not have this apparatus adapted to rats in our laboratory.

### iii. Taste reactivity

The taste reactivity did not enable us to clearly assess liking component of reward value of food. Several reasons can explain it. The main bias was that the quantity ingested was not properly controlled, compared to Berridge who controlled the exact amount delivered in the rat's mouth thanks to a cannula. Moreover as it was the last test, rats were maybe habituated to sweet taste and in spite of the vanilla flavour, this was not "new" for them. In addition, the number of licks is not a true measure of liking but more a measure of how fast and how much rats eat (lickometers, which count the number of licks, are usually used to measure food consumption). As stated by Berridge, 2000, tongue protrusions (extensions of the tongue outside the mouth to lick the lips) and paw licking are the true measures of liking. Yet in our video, distinguishing tongue protrusion from licking was difficult). The number of paw licking may tend to be higher in the Ctl group and may reflect more a hedonic control of food intake, compared to the number of licks that may more show the ingestion and thus the homeostatic control of food intake.

To conclude, when GLP-1R of the vagal afferent neurons undergo a partial knock-down, the lower consumption of new palatable food is not due to a higher innate anxiety or food neophobia. The phenotype observed is a decrease of the hedonic appreciation of new palatable food, as concluded from the different palatable food intake and the CPP test. So far, trying to investigate the reward component of reward, we are not yet in a position to define which component is the most likely to explain this phenotype.

## III. Going back to the literature to link GLP-1R kd with a reduced hedonic appreciation of food

The reduction of hedonic appreciation of new palatable food when GLP-1R expression is reduced in VAN of rats seems contradictory. As gut-GLP-1 is a satiety signal, we would expect that it decreases food reward. Therefore when transmission of GLP-1 signal to the brain is prevented, *a priori*, reward value of food would not be decreased. Furthermore, GLP-1R kd showed bigger meal on chow food, compensated by longer meal interval so that there is no change in daily food intake (Krieger *et al.*, Diabetes, 2015, accepted). These results led us to consider a change at the central level and the existence of a compensatory mechanism. We examined in the literature what was known from GLP-1 and GLP-1R at the brain level.

### 1) From gut-GLP-1 to the NTS

The pathway by which peripheral GLP-1 through VAN GLP-1R acts on the brain to modulate food reward still needs to be fully elucidated. Vagal afferent neurons end in the NTS (hindbrain) (Grill and Hayes, 2012) and anatomically, VAN have direct connection (Trapp and Cork, 2015) to preproglucagon (PPG) expressing neurons of the NTS (Hisadome *et al.*, 2010). PPG neurons are known to be activated by CCK, leptin, and gastric distension (Hisadome *et al.*, 2010; Hisadome *et al.*, 2011). Then, although not directly reported, VAN may mediate a signal to PPG neurons (Hisadome *et al.*, 2010).

## 2) From the NTS to the Ventral Tegmental Area (VTA) and the Nucleus Accumbens (NAc)

GLP-1 is produced at the brain level in the NTS (Jin *et al.*, 1988). PPG neurons are known to release GLP-1 in the brain (Larsen *et al.*, 1997). It is hypothesized that PPG neurons activated after food intake release GLP-1 (Trapp and Cork, 2015). The areas, where PPG neurons project, have been studied by LLewellyn-Smith *et al.*, 2011. Among the areas the most innervated by PPG neurons, there were the dorsomedial hypothalamic nuclei, thalamic paraventricular nucleus, paraventricular nucleus of the hypothalamus (LLewellyn-Smith *et al.*, 2011). These findings are consistent with another study using IP ex-4 and extensive FOS expression (Gu *et al.*, 2013). These areas located in the hypothalamus are involved in the control of energy homeostasis (LLewellyn-Smith *et al.*, 2011; Larsen *et al.*, 1997; Hayes *et al.*, 2009). Recently, it has been discovered that PPG neurons of the NTS projected directly to the VTA and the NAc (Alhadeff *et al.*, 2011) (**Appendix 8** shows location and projections on the rat brain). These areas from the forebrain are involved in the mesolimbic reward system. The areas, where PPG neurons project, match the areas where GLP-1R is expressed, notably the VTA and the NAc (Merchenthaler *et al.*, 1999). Thus if a signal is released by PPG neurons, it may arrive to the VTA and NAc. The link between PPG neurons and these two areas implied in the reward system started to be studied only for the first time in 2011 (Dossat *et al.*, 2011).

## 3) Overview of the neurobiological mechanisms involved in food reward

Recently the neurobiological networks related to food reward have been reviewed (Egecioglu *et al.*, 2011). Incentive salience theory of reward distinguishes three components: liking, wanting and learning. This theory can be applicable to food (Berridge and Robinson, 1998):

- Liking refers to the hedonic aspect of food, immediate experience or anticipation of pleasure
- Wanting reflects the incentive motivation to obtain food also called reward seeking.
- Learning is related to associative prediction and knowledge acquisition (Berridge and Robinson, 1998).

These three components involved different neurobiological mechanisms.

### i. Dopamine and food reward

The mesolimbic dopamine system is the well-known mechanism of reward. VTA and NAc belong to the mesolimbic system. Dopamine is the main neurotransmitter (NT) involved in the neurobiological process of **incentive motivation** (Berridge, 2007). The main mechanism is the release of dopamine by the VTA to the NAc (Wise and Bozart, 1987) (see **Appendix 8** for the dopaminergic projections of the VTA in green arrow). The neural network related to liking does not seem to be directly linked with the mesolimbic dopamine system (Berridge, 2007). Nonetheless this system is involved in the reinforcement of consumption of hedonic food (Berridge, 2007). Mice unable to feel sweet taste showed a preference for sucrose and activation of their mesolimbic reward system (de Araujo *et al.*, 2008). This study proposed that the taste was not necessary to feel food reward.



## *ii. Opioid and food reward*

The neurobiological process of the “liking” component of food reward implicates the neural network of taste feeling (Grill and Norgren, 1978; Pecina and Berridge, 2000). The NAc is one of the areas involved, with the brainstem, the pons, the ventral pallidum, amygdala and prefrontal cortex (Grill and Norgren, 1978; Pecina and Berridge, 2000). The principal NT here is the mu-opioid (Berridge and Robinson, 2003).

## *iii. Cannabinoid and orexin and food reward*

There are suspicions that two other NT, cannabinoid (Kirkham and Williams, 2001) and orexin (Harris *et al.*, 2005), could play a role in food reward, as they interact with the dopaminergic and opioid network.

## 4) Central GLP-1 implication in food reward

Several studies provide proofs of the implication of central GLP-1 and GLP-1R and food reward in studies on rodent or humans. Blockade of NAc GLP-1R with Ex-9 on rats led to an overconsumption of food (Dossat *et al.*, 2011). GLP-1 injection in the NAc increased c-fos activation (Dossat *et al.*, 2011). Together these findings suggested that central GLP-1 might reduce food intake (Dossat *et al.*, 2011). GLP-1R activation by pharmacological doses of Ex-4 in the VTA and NAc has been shown to reduce the intake of palatable foods (Alhadeff *et al.*, 2011; Dickson *et al.*, 2012). GLP-1R stimulation in the NAc and VTA by a central Ex-4 injection on rats led to a reduction of food reward behaviour (decrease in motivation to work for food in the operant responding paradigm and lack of induced preference side in the CCP paradigm) (Dickson *et al.*, 2012). GLP-1R of the NAc is suggested to modulate appreciation of palatable food: when NAc GLP-1 receptors are blocked by Ex-9, rats consumed more of palatable food (Dossat *et al.*, 2013). A reduction of the food reward behaviour was also observed following injection of GLP-1 or its agonist EX-4 in the NTS (Richard *et al.*, 2015). They also showed that the mesolimbic system was involved as these injections were correlated with a reduction of dopamine gene expression. A link between central GLP-1R and the mesolimbic system is not only suspected in rodents but also on humans. In obese subject Ex-9 injected, the cerebral areas related to food reward increased their activity in presence of a visual stimulus (food picture) (Van Bloemendaal *et al.*, 2014). Recently, it has been shown that an artificial activation of GLP-1 neurons by a DREADD system seems to decrease high fat food intake (Wang *et al.*, 2015). The activation resulted in a lower secretion of dopamine in the VTA. However the technique employed in this study might have activated the whole NTS and not specifically PPG neurons.

Together, these findings suggest a potential implication of central-GLP-1R signalling in the modulation of palatable food intake and reward value of food (food motivated behaviour and hedonic value of food) (Skibicka, 2013). As it is highly suspected that peripheral GLP-1 might act on PPG neurons via VAN (Trapp and Cork, 2015), and that PPG neurons which project to the reward areas VTA and NAc, our studies give indications for an implication of peripheral GLP-1 in food reward.



#### IV. Hypothesis explaining a decrease in hedonic appreciation of palatable food at short term

##### 1) Three plausible hypothesis

We defined three hypotheses for the decrease in hedonic appreciation of new palatable food.

1/ In VAN GLP-1R kd rats, GLP-1 can bind on residual receptors. VAN GLP-1 receptors are only partially activated, and only partially recruit central pathways via the NTS. As a result the amount of signal transmitted to the brain may be reduced. So the transmission of feeling information may be slower, brain areas may be activated later, and GLP-1R kd rats need more time to associate the pleasure felt with the palatable food. Therefore the decrease in food intake occurs only at the beginning of the first exposure and after a few hours of exposure consumption is the same in the two groups.

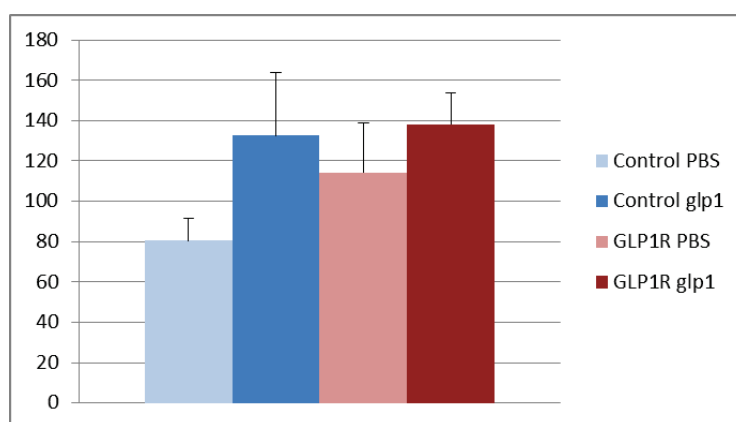
2/ A central compensatory mechanism can account for the chronic loss of VAN GLP-1R and explained the changes in hedonic appreciation of palatable food. The system the most likely to be involved is the dopaminergic system as it is linked to reward processes.

3/ The last hypothesis that cannot be excluded is a change in liking appreciation of food, which is linked to the opioid system, meaning that something could be changed in the u-opioid release or in the expression or sensitivity of opioid receptors.

##### 2) Two preliminary observation in favour of the hypothesis of a compensatory mechanism at the central level

Two preliminary observations obtained on a previous batch of VAN GLP-1R kd rats (participating in the publication of Krieger *et al.*, Diabetes, 2015, accepted) directed us towards a compensatory mechanism at the central level. The first observation is an **increase in basal c-fos neuronal activation in the NTS of GLP-1R kd rats** (data non published, **Fig. 23**). The NTS neurons of Ctl rats following a vehicle injection showed low basal activation, and, as expected, they were more activated after GLP-1 injection. Surprisingly, NTS neurons of GLP-1R kd rats showed an elevated c-fos activation (same level as the Ctl GLP-1 injected), whether they were injected with a vehicle or GLP-1. This suggested that the NTS can react to the VAN GLP-1R kd in increasing the basal neuronal activation. This observation, if confirmed, raises the questions: by which mechanisms is there a permanent basal activation and what are the activated neurons? The second observation concerned the proglucagon expression in the NTS of Ctl and GLP-1R kd rats on chow and HFD. On chow, proglucagon expression was at the same level in Ctl and GLP-1R kd. While switched to HFD, proglucagon expression decreased in Ctl rats whereas it stayed at the same level as on chow in GLP-1R kd rats. In other words, when exposed to HFD, VAN GLP-1R kd rescues the diet-induced reduction in NTS *ppg* expression.

Knowing this, it is plausible that the chronic reduction of GLP-1R expression in the VAN induces basal GLP-1 NTS neuronal activation that accounts for **the decreased in hedonic appreciation of palatable food at first exposure**. Even if the NTS received fewer signals from GLP-1, some neurons that should not be activated are activated. Based on these two observations and the publications quoted above, notably that the increase of activation of PPG neurons seems to be linked with a decreased intake of palatable (Wang *et al.*, 2015), it may be possible that a compensation at the central level, i.e. a permanent basal GLP-1 NTS neuronal activation, engaged the dopaminergic system (we can also not exclude a change in the opioid receptor system) and led to a decrease in hedonic appreciation of a new palatable food at short term.



**Figure 23:** Number of c-fos activated neurons in the NTS of Ctl and GLP-1R kd after GLP-1 or vehicle (PBS) injection

Increase in basal c-fos neuronal activation in the NTS of GLP-1R kd (called GLP-1R on the graph) rats (data from our laboratory non published). The NTS neurons of Ctl rats following a vehicle injection were low activated, and they were more activated after GLP-1 injection as expected. Surprisingly, NTS neurons of GLP-1R kd rats showed an elevated c-fos activation (same level as the Ctl GLP-1 injected), whether they were injected with a vehicle or GLP-1.

#### V. Further research to test our hypothesis

In this part we present how we plan to test the different hypotheses in the next few months. We also propose other experiments that could be done if we would have the technical tools. In the end, we expose which experiments could have been done along our project to implement our analysis.

## 1) Reflexion about the next step of the project

### *i. Double immunostaining of the NTS*

The increase basal c-fos activation of NTS neurons in GLP-1R kd rats, raise the question of the phenotypes of these neurons. Are they PPG neurons expressing GLP-1? We plan to perform double immunostaining of our c-fos activated NTS neurons to check if they are also PPG neurons.

### *ii. Quantitative Polymerase Chain reaction (qPCR) of the NTS*

Another experiment to investigate the hypothesis of the compensatory mechanism of an upregulation of GLP-1 in the NTS is a qPCR to measure the level of GLP-1 expression in the NTS.

### *iii. Investigating change in neurotransmitter levels in the brain*

- In vivo voltammetry was considerate but could not be performed*

*In vivo* voltammetry provides a measure of dopamine signal in real time. It is consequently particularly suited to track potential changes in the dopaminergic system. It was, however, not a reasonable approach for this project since this technique is currently not fully established in our laboratory. A joint group of research is currently developing this technique, yet it is not possible to perform it on non-anaesthetized animals. Legally, the technique implies another surgery requiring another license at the Kanton Veterinary office, implying to wait a few months. Voltammetry is especially used for phasic dopamine release which is the very immediate response to a pleasure stimulus, as for a novel palatable food.

- Terminal experiment after exposure to a novel palatable food*

The decrease in hedonic appreciation in VAN GLP-1R rats always occurred with a new palatable food during the beginning of the first exposure. In order to investigate the hypothesis of a potential change in dopamine or opioid levels in the brain at short-term, in our terminal experiment rats were sacrificed 10 min after the first exposure to a new food reward (peanut butter) for half of the rats, or their usual chow food for the other half, and fresh brain were collected.

- Three possible approaches: c-fos, qPCR, High Performance Liquid Chromatography (HPLC)*

By analysing their brains, we wish to explain the hypothesis of the slower kinetic of a signal to the brain, a change in the reward system (dopaminergic system) or a change in the liking pathway (opioid system, cannabinoid). Three approaches are possible: c-fos, qPCR and HPLC. After selecting the areas involved in the dopaminergic and/or opioid system, c-fos enables to see neuronal activation. Yet c-fos is not a specific technique: it describes which neurons are activated, but does not inform on the phenotype of these neurons, or on potential neuronal inhibitions. Furthermore, it requires the brain to be perfused (fixed by a solution) and limits the techniques that could be performed afterwards. With the qPCR, we can punch the area expressing dopamine and/or opioid receptors and measure gene expression. Its limitation is that it will

inform us about expression and not the activity. HPLC directly measures a targeted compound. It enables to quantify neurotransmitters released in selected areas.

To allow more direct measurements of brain functional markers, we intend to measure the neurotransmitter level in selected brain areas (VTA and NAc) with HPLC, by collaborating to the department of Psychiatry and Psychotherapy of the University Hospital Carl Gustav Carus in Dresden, Germany. In correlating neurotransmitter concentration in several brain areas after a palatable (peanut butter) or a low-palatable (chow) meal with the differences in behaviour previously measured, we hope to test our hypotheses and concentrate research toward one or several of them.

## 2) Clues for further research

As the VAN GLP-1R knock-down was only partial, another genetic manipulation would be needed to knock-out entirely the GLP-1R in the vagus nerve. The *cre-lox* approach could be used, to our knowledge, no specific promoter for the *cre-recombinase* is known for the VAN. Paired with the knock-down approach, it would allow understanding further our hypothesis and the mechanisms underlying by GLP-1R in the VAN.

Other tests could have been performed. To check if the CPP could be explained by deficit in conditioning experience, a contextual fear conditioning test could be performed. This test would have investigated if GLP-1R kd rats show a similar deficit in aversive learning. To have a more accurate measure of the amount of solution consumed, the lickometer test could give interesting measure, but we did not have the apparatus. A test enabling to have accurately the quantity ingested as a continuous function of time, at the nearest minute, seems an adapted experiment to better understand our phenotype. In the taste reactivity, the liking did not appear different. Yet this test is designed to observe the very immediate response to a pleasure stimulus. This window of time may be too short to detect a change in behaviour as differences appeared in the food consumption during the first hour. Thus focusing on a one hour period of time to analyse the food consumption and behaviour seem more relevant.

The first observation of the lower HFD (60% energy coming from fat, and a few coming from carbohydrates) consumption was seen upon 3 days, whereas in our food intake records (the energy coming mostly from sugar), the consumption was decreased during the first hour. We focus on the fact that the food was palatable or not, yet we could look closer at the nutrient composition and the ratio between fat, protein and carbohydrate.

## VI. Reflexion about the relevance and scope of our project for society and scientific implications of the research

In our project, we investigated the link between the satiety factor GLP-1 and its repercussion in behaviour, underpinning an effect at the central level regarding the reward value of food. We are the first to explore

further the connection of VAG GLP-1R in the central nervous system for the hedonic control on food intake. The effects of gut-GLP-1 on food intake, glycemia and gastric emptying through the GLP-1R of vagal afferent neurons have recently been emphasized by Krieger *et al.*, Diabetes, 2015, accepted. As vagal afferent neurons were involved, it suggested an implication of GLP-1R on the brain for energy homeostatic control.

The first interest of our research lies in the progress of the scientific knowledge.

In the field of GLP-1, its interest is that it supports the idea that gut GLP-1 mediates a signal to the brain via GLP-1R of vagal afferent neurons and that this signal seems to have implication in term of food reward behaviour. As GLP-1R agonists are used in the treatment of type 1 and 2 diabetes and obesity, it appears important to better understand the mechanisms related to the GLP-1 system. Here, we focused on the vagus nerve which is not yet specifically targeted by drug treatments. Therefore, the vagus nerve could appear as a potential target for future therapy. Our research highlights that a satiety signal may be linked with the hedonic control, whereas it was firstly thought to be link with the homeostatic control. The latter point is relevant concerning obesity. When calorie intake is beyond the needs of the organism and not balanced by energy expenses, it will lead to a positive energy balance and weight gain. Obviously, causes of this imbalance between energy consumed and expended are numerous and multifactorial. The monogenic cause exists but remains rare. More often it is a combination of a favourable genetic field involving multigene factors in the so-called "obesogenic" environment (an environment driving the motivation to eat, where food rich in calories is plethora available, and a sedentary lifestyle), social aspect (culture, education, habits), economic aspect, and emotion (Berthoud and Morrison, 2008). In other words, this metabolic disorder is thought to be the result of a negative modulation of homeostatic regulatory processes induced by an increasing pressure of hedonic, cognitive and emotional processes in an obesogenic environment (Zheng and Berthoud, Physiology, 2008). Obesity is dramatically rising up not only in western countries but also in developing countries. Nonetheless, looking at the current food availability in some part of the world, it may be surprising that the rate of obesity is not higher, meaning that physiological processes maintain the balance energy consumption and expenses. The cultural aspect of eating behaviour plays certainly a major role in the rising rate of obesity in some region, whereas some place seems to be protected.

To summarize, we used a new method of a bilateral injection in the nodose ganglion of a vector to target specifically the expression of one gene (GLP-1R) in one type of cells (nodose ganglion neurons). The previous research of Krieger *et al.*, Diabetes, 2015, accepted, as well as this project, pointed out that it allowed investigating in-depth biological mechanisms in which the vagus nerve plays a role. Consequently, this technique itself is of outstanding interested and could be performed in other research areas to investigate the role of vagus nerve in a wide spectrum of other biological processes.

## Conclusion about a six-month master thesis

This project was the opportunity to work in a research laboratory on a subject related to the physiology of nutrition and behaviour. It enabled me to acquire new scientific knowledge about GLP-1, the vagus nerve, the neurobiological networks related to food reward and different behavioural tests. The broad range of the research topics in the group allowed me having discussions with the other students and enriched my knowledge in the area of energy homeostasis and metabolism. I also learnt and get acquainted with *in vivo* and *in vitro* technics. During these six months, I gained experience in several animal behaviour tests and molecular and cellular biology technics, as well as animal handling, monitoring and post-surgery care.

Working in a research laboratory was also an occasion to develop professional skills that complete my educational background. I had to carefully think to the planning of the series of experiments that I manage, and also to carry out the project in time. I had to show a strong sense of independence owing to the nature of the behavioural experiments. Even if I principally worked in autonomy, the regular communication with my supervisor Jean-Philippe Krieger was essential to successfully lead this project and make progress of the thought. Nevertheless I also worked in team for several experiments or to help other people in the laboratory. I strengthen my adaptability in term of schedule, which was necessary when experiments had to be conducted upon seven or eight following days, or when we had to reproduce an experiment that did not work technically. Flexibility was also needed when we adjusted our hypothesis. This master thesis also reinforced my analytical skills to answer to this scientific problem. I exercised the rigour of the scientific reasoning.

However, it is difficult to put an end in this scientific research project as it calls other questions. Furthermore a few tests were frustrating because clear measures giving interpretable results were not easy to take. Especially in term of behaviour, the animals can show various reactions.

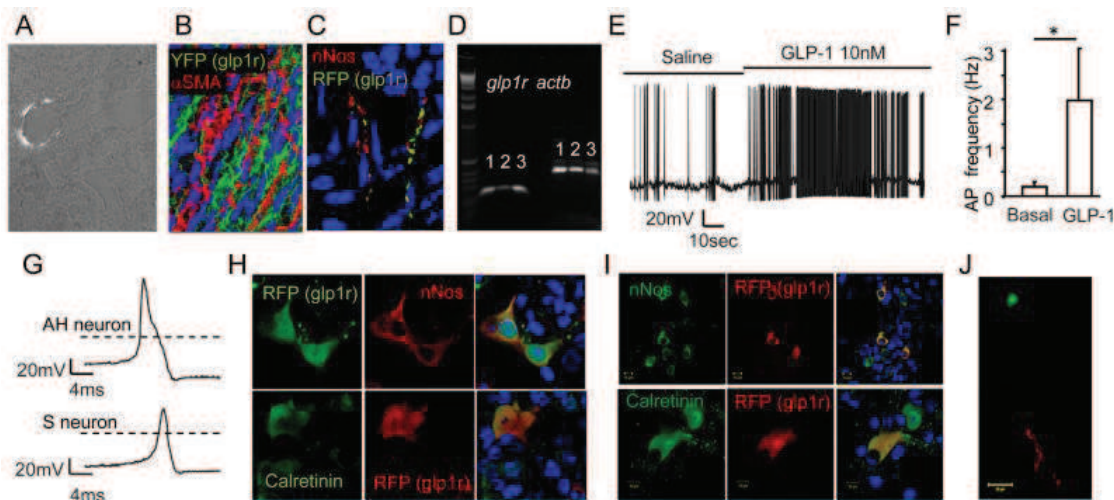
This experience was also very enriching from a cultural point of view. With people mainly coming from various countries, I spoke English on a daily basis and learnt new vocabulary related to the topic. Once again it led me to integrate myself in an international work environment and in a foreign country, experience that is always stimulant.



## Appendixes

### Appendix 1 : « GLP-1R in enteric neurons » (Fig.5 from Richards *et al.*, 2014)

Figure 5J shows an enteroendocrine L-cell in proximity to a GLP-1R fluorescent fiber.



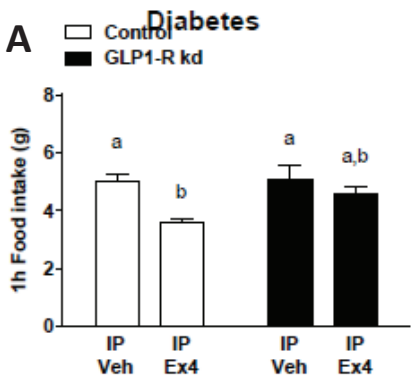
**“Fig 5: Glp1r in enteric neurons**

A. Fixed tissue section of liver from a *glp1r-cre/ROSA26-tdRFP* mouse, showing *glp1r*-fluorescence in structures surrounding a portal vein. B,C. Antral area of the stomach from a *glp1r-cre/ROSA26-tdRFP/ROSA26-YFP* mouse, immunostained in B for YFP (green) and  $\alpha$ SMA (red) and in C for RFP (green) and nNOS (red). D. Nested PCR amplification of *glp1r* and  $\beta$ -actin, performed on 3 hand-picked *glp1r*-fluorescent enteric ganglia from *glp1r-cre/ROSA26-tdRFP* mice. E. Representative recording of a partially dissociated small intestinal *glp1r*-fluorescent enteric neuron in primary culture, monitored in whole cell current clamp, following addition of GLP-1 (10 nM). F. Mean action potential (AP) frequency of 7 cells under basal conditions and following GLP-1 application. \*  $p < 0.05$  by Wilcoxon signed rank test. G. Enteric neurons, recorded as in E, were divided into AH-type (with characteristic hump on the action potential downstroke and after-hyperpolarisation) and S-type. The dashed line represents the 0 mV line. Small intestinal (H) and colonic (I) tissue sections from a *glp1r-cre/ROSA26-tdRFP* mouse co-stained for RFP together with nNOS or calretinin, as indicated. As direct RFP fluorescence is lost on fixation, a green secondary antibody was used to visualise RFP in the upper panel of H. J. Intestinal tissue section from a *glp1r-cre/ROSA26-tdRFP/GLU-Venus* mouse showing proximity of an L-cell (green) and a red *glp1r*-fluorescent fibre.”

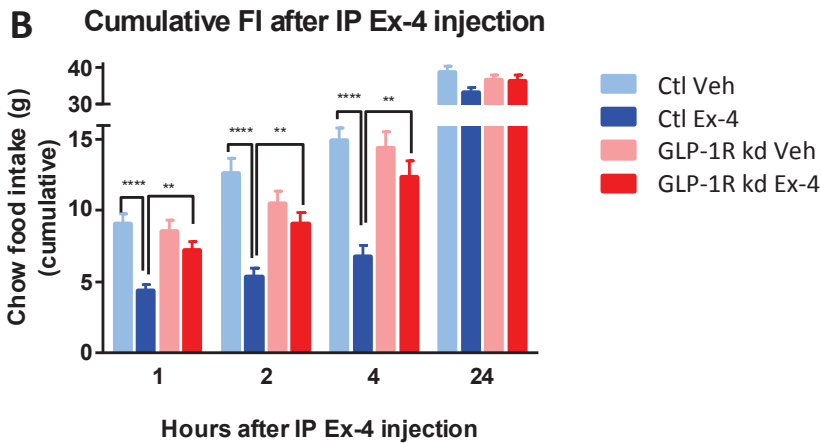
From Richards, P., Parker, H. E., Adriaenssens, A. E., Hodgson, J. M., Cork, S. C., Trapp, S., Gribble, FM., Reimann, F. (2014). Identification and characterisation of glucagon-like peptide-1 receptor expressing cells using a new transgenic mouse model. *Diabetes*, 63(4), 1224–1233. <http://doi.org/10.2337/db13-1440>



**Appendix 2:** Complements to the results of the functional verification of the VAN GLP-1R kd by IP Ex-4 injection

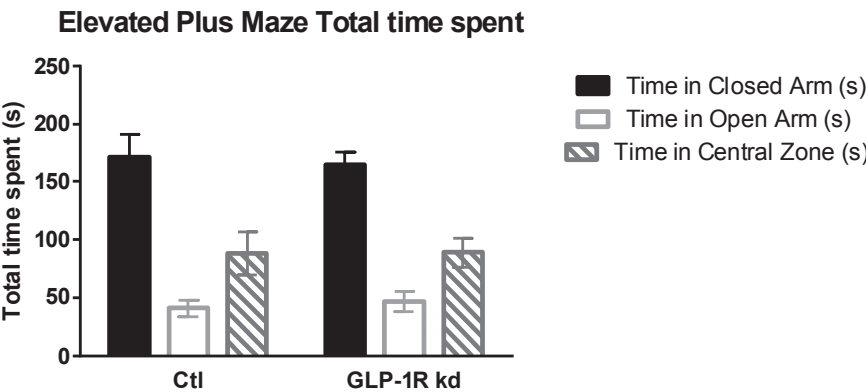


**A:** “Figure 4B: VAN GLP-1R kd attenuated the effects of IP Ex-4, 1 h food intake after (B) IP Ex-4 (0.3 μg/kg; n=12/11). Different letters indicate a significant difference between two groups after post-hoc Bonferroni-corrected comparison ( $P<0.05$ ).” of Krieger *et al.*, Diabetes, 2015, accepted shows the food intake of Ctl of GLP-1R kd rats 1h after IP Ex-4 or Veh injection. These results corroborate our results from our functional verification.



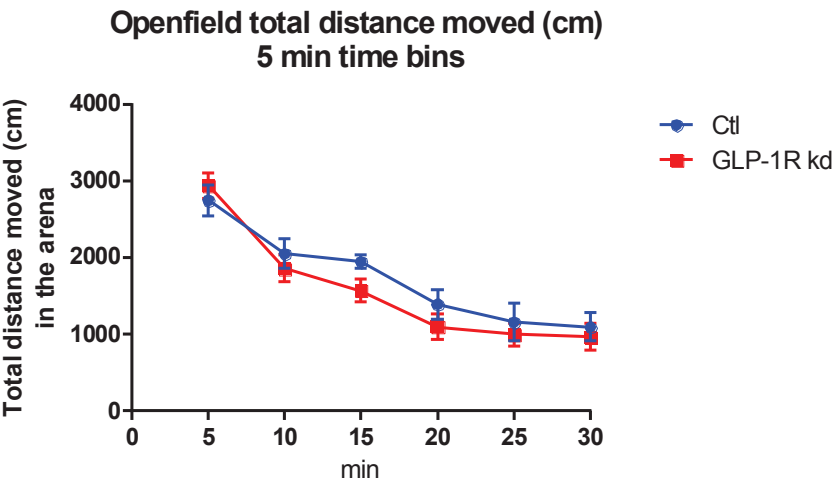
**B:** Cumulative chow food intake 1h, 2h, 4h, 24h after IP Ex-4 injection or vehicle injection in Ctl and GLP-1R kd rats. Stars indicate a significant difference between two groups after t-test comparison (\* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ ; \*\*\*\* $p<0.0001$ ). All values are mean  $\pm$  SEM.

**Appendix 3:** In the elevated plus maze test, total time spent in the different zones in Ctl and GLP-1R kd



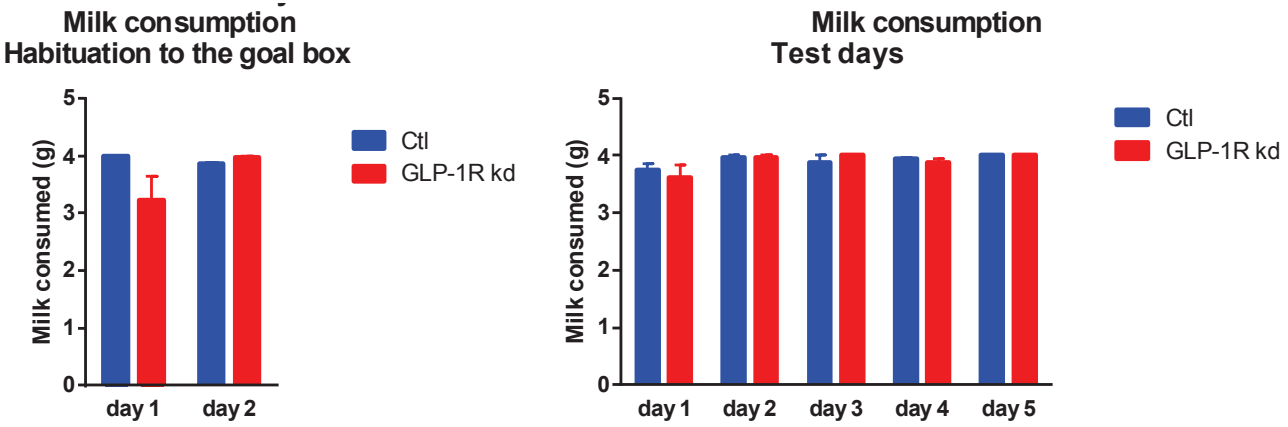
All values are mean ± SEM.

**Appendix 4:** In the open field test, total distance moved in the arena as a function of 5 min time bins in Ctl and GLP-1R kd



All values are mean  $\pm$  SEM.

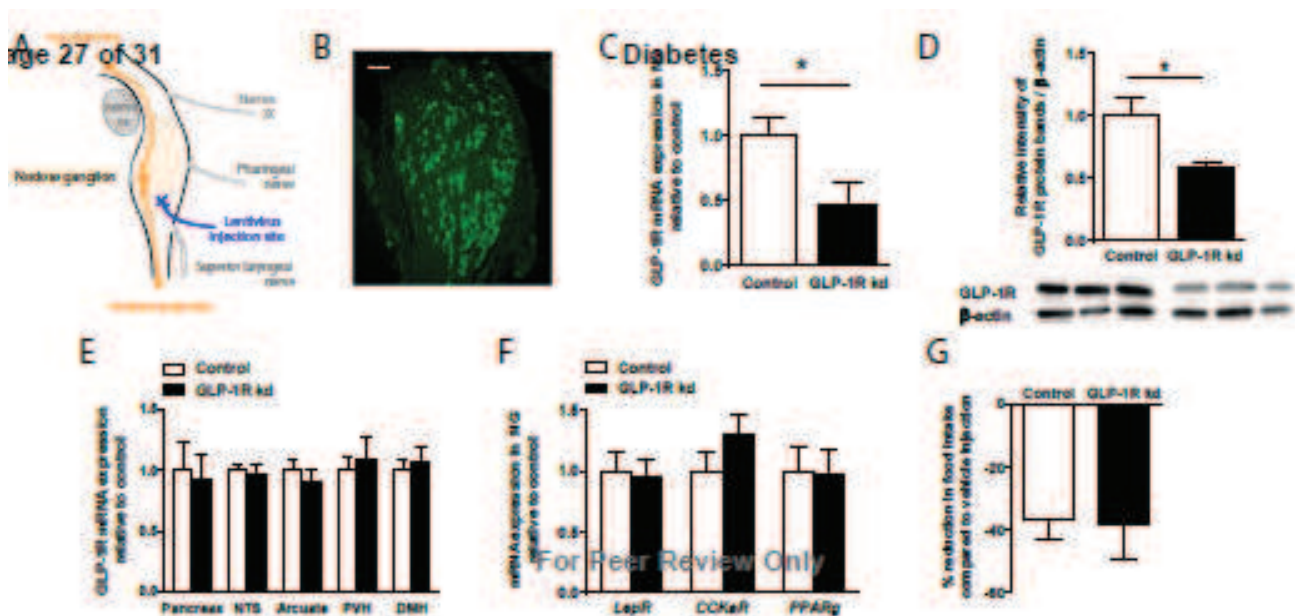
**Appendix 5:** Milk consumption during the habituation to the goal box and test days of the incentive runway test in Ctl and GLP-1R kd



All values are mean  $\pm$  SEM.

**Appendix 6:** Further tests confirming the efficiency and tissue-specificity of a VAN GLP-1R kd (Fig.1 from Krieger *et al.*, Diabetes, 2015)

Figure 1 from Krieger *et al.*, Diabetes, 2015 detailed further tests confirming reduction of GLP-1R expression in the nodose ganglia. GFP expression in infected nodose ganglion (B) confirmed that the nodose ganglion was effectively infected by the lentivirus. Relative expression of GLP-1R mRNA (C) by qPCR of the nodose ganglion confirmed that GLP-1R expression was significantly reduced in the NG. Relative expression of GLP-1R protein in NG (D) by Western Blot confirmed that the lentivirus injection led to significantly reduce GLP-1R available in the vagus nerve. Relative expression of GLP-1R mRNA (E) by qPCR of other organs where GLP-1R is expressed (pancreas, the brain areas DMH, Arc and PVH), confirm that the reduction of the GLP-1R expression was specific to the vagus nerve and did not affect other organs.

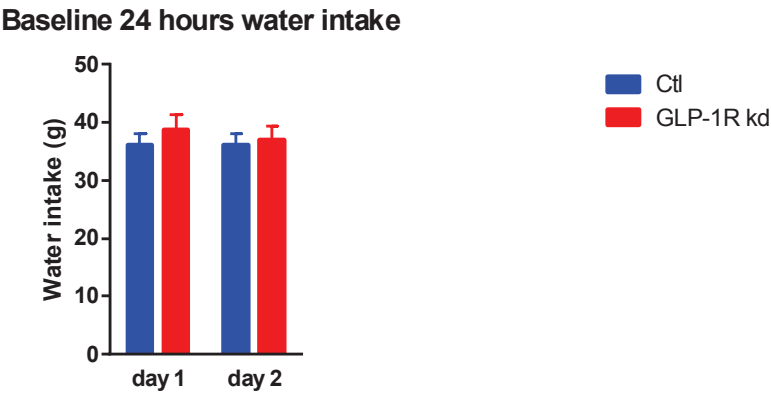


“Figure 1: VAN LV-shGLP-1R injection leads to a significant and specific reduction in GLP-1R expression in the nodose ganglia

(A) Schematic representation of lentiviral injection site in a rat nodose ganglion. (B) Visualization of GFP expression in the nodose ganglion of a LV-shGLP-1R injected rat, representative picture. Scale bar, 50 μm. (C) Relative expression of GLP-1R mRNA in the nodose ganglia of control and GLP-1R kd rats (n=10/8). (D) Relative expression of the GLP-1R protein levels in the nodose ganglia of control and GLP-1R kd rats (n=5/5) as measured by the relative intensity of the GLP-1R detection band normalized by the intensity of the β-actin band, with representative examples. (E) Relative expression of GLP-1R mRNA in the pancreas (n=6/7), NTS (n=7/7), Arc(n=7/7), PVH (n=7/6) and DMH (n=7/7) of control and GLP-1R kd rats. (F) Relative expression of LepR (n=6/7), CCKaR (n=6/7) and PPARγ (n=6/7) mRNA in the nodose ganglia of control and GLP-1R kd rats. (G) Percentage of decrease in 1 h food intake after IP injection CCK (4 μg/kg) relative to vehicle injection (n=6/6) of control and GLP-1R kd rats. \* indicates a significant difference between the control and GLP-1R kd groups (P<0.05).”

**Appendix 7:** Baseline 24 hours water intake upon 2 days of Ctl and GLP-1R rats

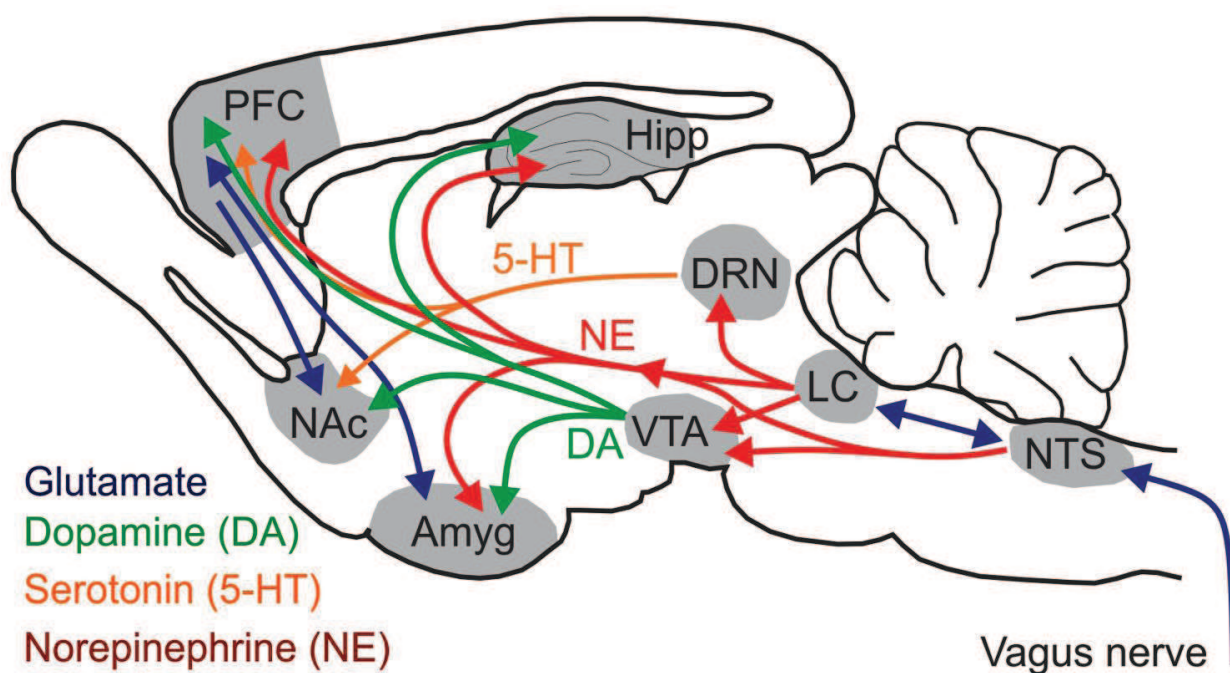
2×2 (treatment × time) RM ANOVA, interaction, treatment and time ns



All values are mean ± SEM.

**Appendix 8 :** Schematic overview of projection from the NTS showing dopaminergic projections (Childs *et al.*, 2015)

The vagus nerve ends in the NTS which projects to the VTA. The VTA have dopaminergic projection (shown in green) to the NAc but also other brain areas.



**“Figure 5:** Simplified schematic of the brainstem innervation by the vagus nerve and second or higher order projections. Through its indirect modulation of monoamine nuclei in the brainstem, including the locus coeruleus (LC) and the raphe nuclei (DRN), the vagus nerve can modulate important components of the extinction circuitry, which include the prefrontal cortex (PFC), the amygdala (Amyg), the hippocampus (Hipp) and the nucleus accumbens (NAc).”

From Childs, J.E., Alvarez-Dieppa, A.C., McIntyre, C.K., Kroener, S. Vagus Nerve Stimulation as a Tool to Induce Plasticity in Pathways Relevant for Extinction Learning. *J. Vis. Exp.* (102), e53032, doi:10.3791/53032 (2015).

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## French summary / Résumé français

Le glucagon-like peptide-1 (GLP-1) est une hormone connue pour son effet incrétine et anorexigénique. Elle est produite par les cellules endocrines L suite à une stimulation par des nutriments. Le nerf vague innerve de nombreux organes abdominaux dont l'intestin et exprime des récepteurs à GLP-1 (GLP-1R). Le nerf vague transporte des informations de l'intestin au cerveau, tel que les signaux de satiété venant de la cholécystokinine et du peptide YY. Il est également soupçonné que le nerf vague soit impliqué dans la transmission au cerveau de signaux provenant de GLP-1. Cependant, la relation entre GLP-1 intestinal, nerf vague et système nerveux central reste méconnue. En raison de son action sur les GLP-1R des cellules  $\beta$  du pancréas sur l'augmentation d'insuline, des agonistes du GLP-1R sont utilisés dans les traitements du diabète de type 2 et de l'obésité. C'est pourquoi il apparaît important de mieux comprendre les mécanismes d'action sous-jacents à GLP-1R.

Récemment, une étude de notre laboratoire utilisant un modèle de rat dont l'expression des GLP-1R dans les afférences vagales était diminuée (GLP-1R kd) a montré que le GLP-1 jouait un rôle dans la prise alimentaire, la glycémie, la satiété et la vidange gastrique via les GLP-1R des afférences vagales. Par ailleurs, lorsque ces rats GLP-1R kd passent à un régime riche en lipide (60% de l'énergie provenant de lipides), ces rats GLP-1R kd consomment moins que le groupe contrôle durant les trois à quatre premiers jours suivant le début d'exposition. Après, leur consommation est identique.

Cette observation apparaît paradoxale. Connaissant le rôle de GLP-1 en tant qu'hormone de satiété, il serait attendu qu'elle diminue le plaisir associé à la nourriture. Or ces rats qui doivent recevoir moins de signaux de satiété ou avec un retard consomment moins d'une nourriture riche en lipide, considérée comme palatable. À priori cette diminution de consommation d'un aliment est intrigante et questionne, d'autant plus que son caractère palatable devrait renforcer sa consommation. Dans notre étude, nous avons testé les hypothèses d'une implication de GLP-1R kd dans A/ l'anxiété innée, B/ la neophobie alimentaire, C/ la diminution du plaisir alimentaire.

Pour cela, nous avons utilisé un modèle de rats mâles Sprague Dawley dont l'expression du GLP-1R dans les afférences vagales est diminuée (GLP-1R kd). Un vecteur lentivirus porteur d'une séquence de petit ARN interférant en épingle à cheveux (shRNAi) est injecté dans les deux ganglions inférieurs du nerf vague des rats. Ce shRNAi interfère avec l'ARNm de GLP-1R, empêchant son expression. L'efficacité et l'intégrité du modèle GLP-1R a été vérifiée par un test fonctionnel. Suivant une injection intrapéritonéale d'un agoniste de GLP-1R, exendine-4 (Ex-4), les rats contrôles ont diminué leur prise alimentaire d'avantage que les rats GLP-1R kd. Ce test a été utilisé pour inclure ou exclure des rats dont le knock-down n'aurait pas fonctionné.

Pour explorer nos différentes hypothèses, nos rats ont suivi une série de tests comportementaux évaluant ces différentes hypothèses : mesure de la prise alimentaire d'aliments palatables (sucrose preference test,



milk preference test) et non-palatable (dextran preference test), elevated plus maze test, open field test, food neophobia test, conditioned place preference (CPP) test, incentive runway test, taste reactivity test.

Nous avons mesuré chez les rats GLP-1R kd comparé aux contrôles une réduction significative de la consommation d'une nouvelle solution palatable (solution à 2% de concentration en saccharose et solution de lait sucré à la framboise) durant la première heure d'exposition. La consommation des deux groupes était similaire par la suite. Ces résultats concordent avec l'observation préliminaire sur un régime riche en gras. Pour vérifier que cette réduction était spécifique à un aliment palatable, nous avons reproduit ce même test avec une solution non palatable (solution à 2% de concentration en dextran, un long polymère de glucose qui n'a pas un goût sucré). Les deux groupes de rats n'ont pas présenté de différence de consommation de ce nouvel aliment non palatable. Nous en avons conclu que les GLP-1R kd avaient une réduction de la consommation d'une nouvelle nourriture palatable au tout début de l'exposition. Aucune différence en terme d'anxiété innée n'a été mesurée entre les deux groupes dans les tests du elevated plus maze et de l'open field. De même la peur vis à vis d'une nouvelle nourriture n'était pas affectée dans le food neophobia test. Dans le CPP, le groupe de rats GLP-1R kd n'a pas montré une préférence pour un environnement associé à un plaisir alimentaire. Cela nous a mené à tester deux composants du plaisir alimentaire. Ni la motivation à vouloir de la nourriture ('wanting') dans l'incentive runway test, ni la réponse immédiate à un nouveau stimulus alimentaire ('liking') ne semblent affecté chez les rats GLP-1R kd. Néanmoins, ces deux dernières expériences ne permettent pas de réfuter une absence de 'wanting' ou de 'liking'. En résumé, l'ensemble de ces résultats nous indiquent que le GLP-1R kd dans les afférences vagues entraîne une faible diminution de l'appréciation du plaisir qui se manifeste au début de l'exposition à un nouvel aliment palatable.

Les afférences vagues relient l'intestin aux neurones exprimant le preproglucagon (PPG). Ces neurones situés dans le noyau du tractus solitaire (NTS) projettent vers des aires cérébrales impliquées dans la régulation homéostatique de la prise alimentaire (hypothalamus), et vers des aires du système de la récompense : l'aire tegmental ventral (VTA) et le noyau accumbens (NAc). Dans une expérience précédente sur le modèle GLP-1R kd, nous avons observé par activation c-fos que les neurones du NTS avaient un niveau d'activation basal plus haut chez les rats GLP-1R kd que chez les rats contrôles. Cela nous a amené à faire l'hypothèse que le GLP-1R kd dans les afférences vagues induit la mise en place d'un mécanisme compensatoire au niveau central qui entraîne cette faible diminution du plaisir ressenti suite à l'exposition à un nouvel aliment palatable qui se manifeste au début. Nous orientons maintenant nos recherches vers l'étude de changement dans les mécanismes cérébraux impliqués dans le plaisir associé à la prise alimentaire. Nous avons l'intention de mesurer les niveaux de dopamine dans les aires cérébrales du système de la récompense VTA et NAc, ainsi que les niveaux d'opioïde. Nous souhaitons également élucider la nature des neurones activés dans le NTS par c-fos, qui amène par ailleurs la question du mécanisme par lequel ces neurones sont d'avantage activés.

Un des intérêts de notre recherche réside dans la technique d'injection d'un vecteur viral dans le ganglion inférieur du nerf vague. Cette technique pourrait avoir des applications pour d'autres recherches s'intéressant au rôle du nerf vague par une manipulation génétique.

Nos recherches supportent l'hypothèse que les afférences vagales transportent un signal enclenché par GLP-1 intestinal jusqu'au cerveau. Ce signal semble diminuer faiblement le plaisir associé à un aliment palatable. Cela suggère une implication du GLP-1 intestinal ou GLP-1R dans le contrôle hédonique de la prise alimentaire, alors qu'il joue aussi un rôle dans la régulation homéostatique de la prise alimentaire.

Une poursuite de cette étude pourrait permettre de donner des indications pour mieux comprendre le dérèglement entre les contrôles homéostatique et hédonique de la prise alimentaire, qui sont soupçonnés d'être parmi les causes de l'obésité. L'épidémie d'obésité progresse considérablement et devient un problème de santé publique majeur. Une politique impliquant des stratégies à plusieurs niveaux (politique de santé publique, promotion d'un modes de vie sain, alimentation saine et activité physique, traitement médicamenteux) semble nécessaire afin d'enrayer l'épidémie. Une meilleure compréhension des mécanismes nerveux régulant la prise alimentaire pourrait permettre de découvrir de potentielles cibles pour le traitement de l'obésité et d'autres maladies métaboliques.

## English summary


Glucagon-Like peptide-1 (GLP-1) is an incretin and anorexigenic hormone released by the gut in response to nutrient stimulation. The vagus nerve innervates the intestine and expresses GLP-1 receptors (GLP-1R). It is suspected to mediate gut-GLP-1 effects to the brain. Yet this aspect has not been fully explored. As GLP-1R agonists are used in type-2 diabetes and obesity treatments, it is relevant to better understand the mechanisms related to GLP-1R.

Here, we knocked down the expression of GLP-1R in vagal afferent neurons (GLP-1R kd) in male Sprague Dawley rats to investigate its consequences on innate anxiety, food neophobia and food reward behaviour. The expression of GLP-1R in VAN was specifically downregulated by the injection in the nodose ganglia (NG) of a lentivirus carrying a RNA interference silencing sequence. We found that GLP-1R kd rats displayed a specific short-term reduction in consumption of a novel palatable food. We did not observe this reduction with a non-palatable food. Innate anxiety in the elevated plus maze and open field tests was not affected by the GLP-1R kd, as well as fear to eat a novel food in the food neophobia test. In the conditioned place preference paradigm, GLP-1R kd rats showed a deficit in palatable food-induced side preference. Investigating further food reward components, the motivation to obtain food in the incentive runway paradigm and the liking in the taste reactivity test were not altered in GLP-1R kd rats. Together these findings suggest that VAN GLP-1R kd rats have a reduction in the short-term hedonic appreciation of a new palatable food.

VAN have connection to preproglucagon (PPG) neurons in the nucleus of the tractus solitarius (NTS). These PPG neurons project not only to brain areas implicated in the homeostatic control of food intake the brain, but also to the reward areas ventral tegmental area (VTA) and nucleus accumbens (NAc). Previously to this study, we observed that GLP-1R kd rats had an increase in basal c-fos neuronal activation in the NTS. Together, it leads us to hypothesis that VAN GLP-1R kd induces to a compensatory mechanism at the central level that accounts for the short-term reduction in hedonic appreciation of food reward. We are now going to investigate which these c-fos activated neurons are, as well as the implication of our VAN GLP-1R kd model at the brain level focusing on potential changes in dopamine in the VTA and NAc and opioid level to explore possible change in the reward value of food.

Our research contributes to support the hypothesis that gut-GLP-1 mediates a signal to the brain via VAN GLP-1R. This signal seems to have implication in term of food reward behaviour. A further study may also provide clue to better understand the dysregulation of homeostatic versus hedonic control of food intake that appears as one of the reason for the dramatic rise of obesity epidemic.



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Titre français : Le glucagon-like peptide-1 joue-t-il un rôle via les afférences vagales dans la modulation de l'anxiété, la néophobie alimentaire et le comportement de la récompense alimentaire ? Titre anglais : Does the glucagon-like peptide-1 act through the vagal afferent neurons to modulate anxiety, food neophobia and food reward behaviour?		
<p>Résumé : Le glucagon-like peptide-1 (GLP-1) est une hormone incrétine et anorexigénique sécrétée par l'intestin. Des agonistes du récepteur à GLP-1 (GLP-1R) sont utilisés dans les traitements du diabète de type 2 et de l'obésité. Les afférences vagales (AV) expriment le GLP-1R et transmettent des effets de GLP-1 sur la satiété et la glycémie. Ici, nous avons étudié l'hypothèse d'une implication du GLP-1R des AV sur le comportement de la récompense alimentaire.</p> <p>Sur un modèle de rats, nous avons réduit spécifiquement l'expression du GLP-1R des AV (GLP-1R kd) en injectant un lentivirus porteur d'une séquence shARN dans les ganglions inférieurs du nerf vague. Nous avons observé que les GLP-1R kd réduisaient spécifiquement la consommation d'une nouvelle nourriture palatable en début d'exposition. Ce comportement n'était pas dû à un changement d'anxiété innée (tests du <i>elevated plus maze</i> et de l'<i>open field</i>) ni de néophobie alimentaire. Dans le test du <i>conditioned place preference</i>, les GLP-1R kd n'ont pas montré de préférence pour un environnement associé à un plaisir alimentaire. En résumé, ces résultats suggèrent que le GLP-1R kd entraîne une réduction de l'appréciation du plaisir à manger un nouvel aliment palatable à court-terme. Connaissant le rôle de GLP-1R des AV sur la satiété, nous avons fait l'hypothèse que ce comportement s'expliquait par un mécanisme compensatoire cérébral suite au GLP-1R kd. Nous cherchons maintenant à l'élucider.</p> <p>Brièvement, nos recherches supportent l'hypothèse que les AV transmettent un signal GLP-1 au cerveau impliqué dans le plaisir alimentaire.</p>		
<p>Abstract: Glucagon-like peptide-1 (GLP-1) is an incretin and anorexigenic hormone released by the gut. GLP-1 receptor (GLP-1R) agonists are currently used in type-2 diabetes and obesity treatments. The vagal afferent neurons (VAN) express GLP-1R and have been implicated in the mediation of gut-GLP-1 effects on satiety and glycemia. Here, we study the hypothesis that vagal GLP-1R also modulates food reward behaviour.</p> <p>We used a rat model with a specific knockdown of GLP-1R expression in VAN (GLP-1R kd) by injecting a lentivirus carrying a shRNA sequence in the nodose ganglia. We found that GLP-1R kd rats displayed a specific short-term reduction in consumption of a novel palatable food, that was not supported by changes in innate anxiety (<i>elevated plus maze</i> and <i>open field</i> tests) or food neophobia. Moreover, in a <i>conditioned place preference</i> paradigm, GLP-1R kd showed a deficit in palatable food-induced side preference. Together these findings suggested that GLP-1R kd rats had a reduction in short-term hedonic appreciation of a novel palatable food. Based on the classical role of VAN GLP-1R in satiety, we hypothesized that GLP-1R kd induced compensatory mechanism at the central level that accounted for the short-term reduction in hedonic appreciation of food reward. Our research will further focus on elucidating these compensatory mechanisms.</p> <p>Overall, our research contributes to support the hypothesis that gut-GLP-1 mediates a signal to the brain via VAN GLP-1R that is involved in some aspects of the food reward behaviour.</p>		
Mots-clés : afférences vagales, glucagon-like peptide-1, injection bilatéral dans les ganglions inférieurs du nerf vague, plaisir alimentaire, tests comportementaux		
Key Words: behavioural tests, bilateral nodose ganglia injection, food reward, glucagon-like peptide-1, vagal afferent neurons		

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