Part A – Applicant

A.1 Main applicant

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Part B – Scientific proposal

B.1 Basic details

B.1.1 Title

Targeting the neurons that drive overconsumption in obesity

B.1.2 Abstract

The lateral hypothalamus (LH) is a brain region with a key role in regulating food intake. This function is served by specific populations of neurons within the LH that respond to food and that drive feeding when activated. However, it is completely unknown what the role of such specific LH neurons is in the actual development of obesity, a pressing medical and societal problem. Now, new technology allows us to (a) determine how these specific LH neurons alter their functionality during obesity development, and (b) precisely target the activity of these neurons to counteract the obesogenic process.

Just as in addiction, adaptative changes in the excitability and output of specific neurons likely drive uncontrolled consumption. We hypothesize that specific LH neurons become hyperactive upon exposure to an obesogenic diet, that these neurons become specifically responsive to palatable food when obese and that the alterations in these neurons drive overconsumption in obesity. We will therefore explore how the development of obesity impacts on specific LH neurons by determining 1) their responsiveness to palatable food using in vivo calcium imaging and 2) their neuronal output using patch clamp electrophysiology combined with optogenetics. Finally (3) we will determine whether chemogenetically manipulating their activity counteracts overconsumption and prevents obesity development. We will first focus on leptin receptor expressing neurons in the LH that project to the ventral tegmental area (VTA) using leptin receptor cre mice. We have preliminary data that chemogenetic activation of these neurons increases the motivation to obtain palatable food. Identifying the LH neurons that drive overconsumption when exposed to an obesogenic environment provides a mechanistic understanding of how neurons implicated in feeding contribute to obesity. Targeting specific neurons with drugs or chemogenetics is a novel therapeutic strategy that can be utilized for the treatment of many brain disorders.

B.1.3 Summary

The brain plays a key role in overeating and obesity. We will determine which neurons drive the overconsumption of rewarding highly caloric food in obesity. One key brain area of importance for feeding behavior is the lateral hypothalamus. Specific subpopulations of neurons in the lateral hypothalamus have been linked to feeding, but it remains unclear how the function of these neurons changes during the development of obesity, and to what extent these hypothalamic neuronal changes are instrumental in this process. We will apply novel technologies in mice to identify and target the neurons in the lateral hypothalamus that lead to obesity. We will begin by determining how the in vivo response to food and the electrical activity of specific neurons in the lateral hypothalamus alters during obesity. We will bring these neurons under the control of a drug, using a gene therapy approach named chemogenetics. We will explore whether chemogenetic manipulation of neurons in the lateral hypothalamus suppresses the development of obesity on a diet rich in fat and sugar. The same technology will then be used in mice that have already become obese to determine whether they start to eat less of high caloric food and lose weight. This fundamental research provides a framework to understand the specific role of neurons in overconsumption. Pharmaceutical industries could then target these neurons with a drug, but chemogenetics itself could also become a gene therapy approach in humans.

B.1.4 Keywords

Leptin, obesity, obesogenic diet, lateral hypothalamus, chemogenetics

B.2 Scientific proposal

B.2.1 Research topic

The aims of this proposal are (i) to identify how neurons in the lateral hypothalamus (LH) feeding circuit change their responsiveness when obesity develops and (ii) to target these LH neurons and counteract overeating and the development of obesity. Obesity represents a substantial global health challenge affecting >400 million people worldwide. Whatever the cause, obesity is the result of eating in excess of energy requirement and this is controlled by the brain. Diet-induced obesity triggers neurobiological adaptations that drive the intake of high-caloric foods(1). Just as in addiction, these adaptations include plastic changes in the output of specific neurons that then drive uncontrolled consumption (2,3). The theory behind this project is that specific LH neurons become hyperactive upon exposure to an obesogenic diet, that these neurons become specifically responsive to palatable food in obese individuals, and that the alterations in these neurons drive overconsumption in obesity. Our strategy will focus, in particular, on the role of the ***LH*** since A) this relatively understudied key nucleus integrates information about energy balance and nutritional status, which communicates to forebrain areas implicated in motivation and dietary behavioural control (4); B) several genetically identified populations of LH neurons respond to changes in energy balance, affect feeding and are connected to the reward system(5), although the identity of the LH neurons implicated in dietary control in the context of obesity is completely unknown; C) novel preliminary evidence from our lab suggests that leptin inhibits specific LH neurons that drive palatable food consumption (***Figure 1***). Obesity is associated with decreased leptin responsiveness (leptin resistance). Lack of leptin signaling is one mechanism via which these LH neurons might remain active driving the motivation to ingest palatable food. In this project we therefore first focus on leptin-receptor (lepR) expressing LH GABA neurons that project to the VTA (using LepR cre mice).

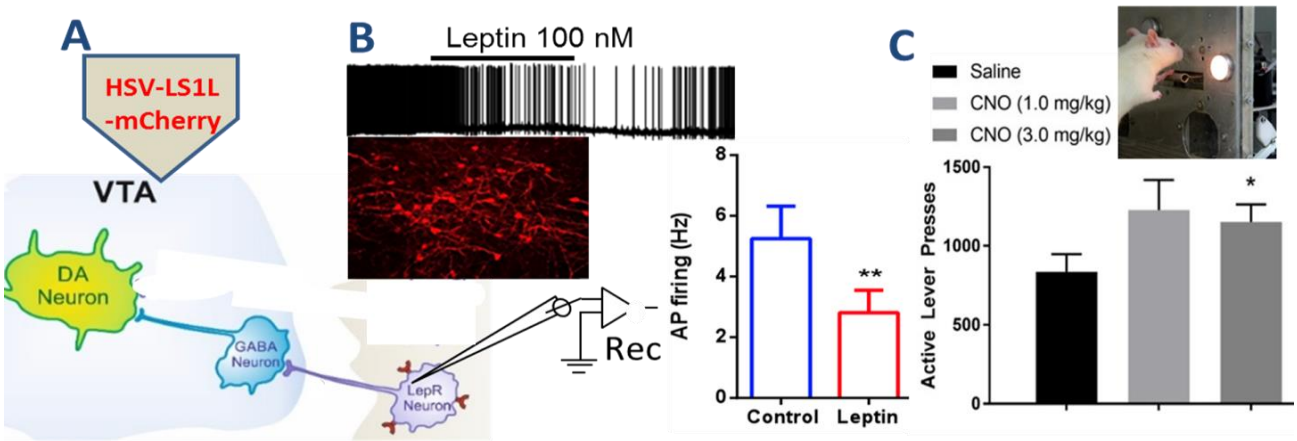

***Figure 1**: A:Lateral hypothalamic (LH) neurons of LepRcre mice expressing leptin receptor (LepR) projecting to the ventral tegmental area (VTA) stain red since they were infected with HSV-LS1L-mCherry injected into the VTA. B: recording from these neurons shows that leptin reduces action potential (AP) firing; C: Injection of adeno-associated viral vector (AAV) with a cre-dependent (DIO) receptor (hM3Dq: a mutated muscarinic acethylcholine receptor) Into the LH of LepR cre mice brings LH leptin receptor expressing neurons under chemogenetic control : CNO (clozapine-N-Oxide) activates leptin receptor expressing neurons and this increases lever pressing (motivation) to obtain a food reward.*

Lateral Hypothalamus (LH) neurons play a key role in obesity but are understudied Historically, the LH was designated a ''feeding center'' based on the finding that ablation produces hypophagia and starvation(6), whereas electrical stimulation elicits feeding and is rewarding (7,8). The LH is implicated in adaptive energy balance. This is the process by which the brain detects changes in energy status and directs appropriate feeding and energy expenditure behaviors to resolve the imbalance. The failure to sense and respond to increased adiposity and body weight with reducing (palatable) food consumption contributes to obesity.

LH neurons connect the sensing of positive energy balance with the initiation of behaviors that counteract it. The LH strongly projects to GABAergic neurons in the **ventral tegmental area** (VTA) that control VTA dopamine neurons that are implicated in motivation to obtain food and in food reward (12). Despite the identification of several populations of LH neurons with a role in feeding or energy balance, we do not know which LH neurons play a role in obesity and respond to delivery of palatable food (9-11). Nor has it been addressed whether plastic changes of LH neurons contribute to development of obesity and whether targeting them specifically with pharmacological tools is protective towards developing obesity on obesogenic diets.

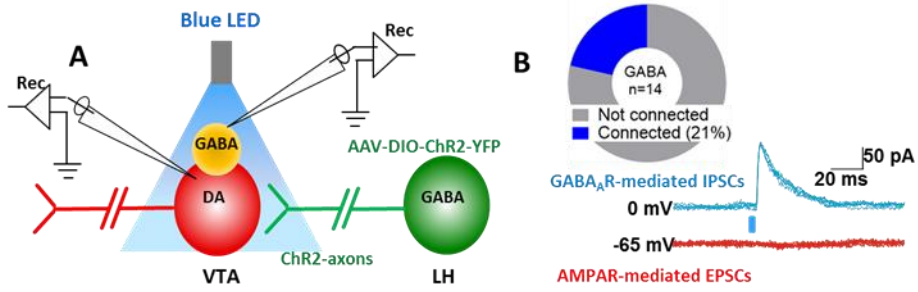

***Figure 2** A: setup for recording from either dopaminergic (DA) or GABAergic VTA neurons in LepR cre mice injected with AAV-DIO-ChR2-YFP into the LH such that blue laser light stimulates synaptic release from LH neurons projecting to VTA neurons; B: only in GABAergic VTA neurons IPSCs were induced by blue laser light, demonstrating that LepR LH neurons project directly to VTA GABA neurons*

Multiple subsets of LH neurons control feeding but their role in obesity is unknown

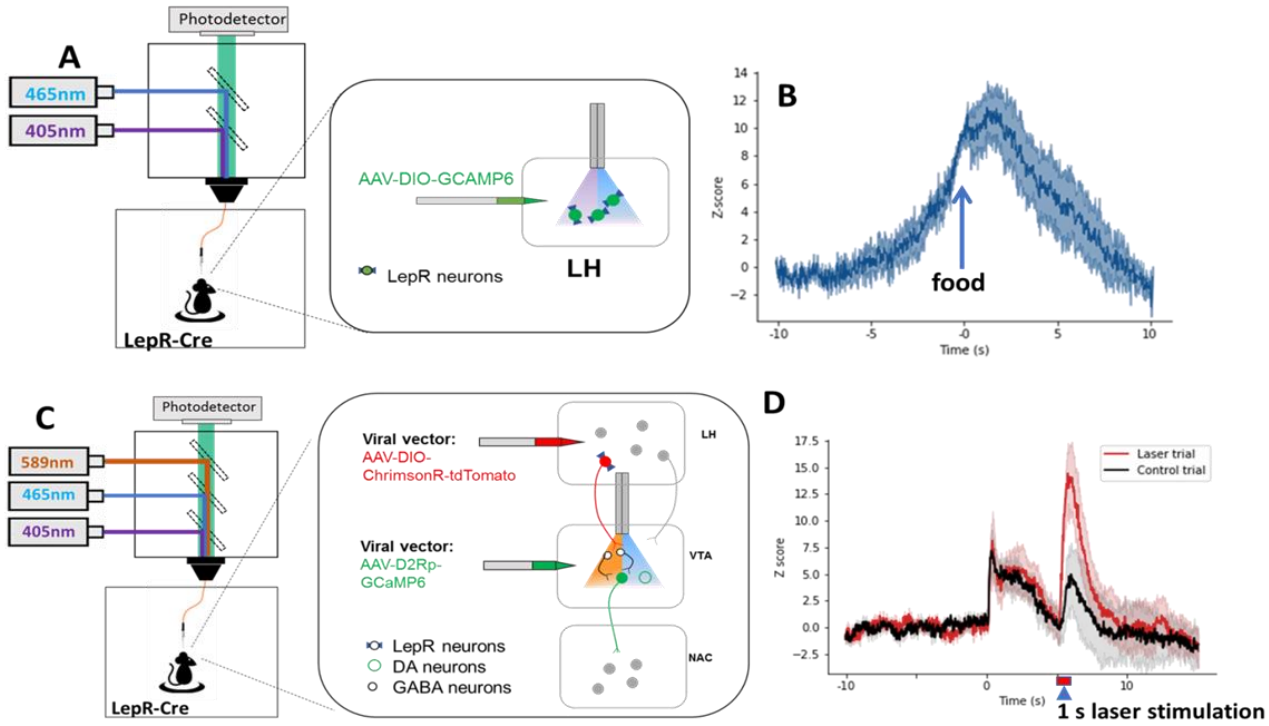

***Figure 3**: A. LepR-Cre mice were injected with AAV-DIO-GCAMP6 (cre-dependent fluorophore) in LH and a 400-micron optical fiber was placed just above the injection site. B. These mice were trained in a sucrose conditioning task and recorded using fiber photometry. Calcium-induced fluorescence was time-locked to sucrose delivery and was highest upon reward consumption. C. LepR-cre mice were injected with AAV-DIO-ChrimsonR-tdTomato (a redshifted optogenetic channel) in the LH and with AAV-D2Rp-GCaMP6 (the D2Rp refers to a dopamine receptor promoter driving fluorophore expression in dopamine neurons that express D2 receptor) in the VTA. An optic fiber was implanted above injection site in VTA for photometry D: Terminals of LepR LH neurons were stimulated in the VTA using a 589nm laser for 1 second (5mW, 40hz, 5ms pulses) during sucrose delivery and fluorescence signal time-locked to 5s tone (starting at t=0s) which predicted sucrose delivery at t= 5s.*

Opto- and chemogenetic stimulation of different (9,12,13) LH neurons induces feeding in satiated mice. E.g. acute chemogenetic activation of vesicular GABA transporter (**VGAT**)-expressing LH neurons increases food intake (13), but it is not known whether long term inhibition of these neurons protects from diet-induced obesity. Many LH neurons project to the VTA and these neurons are good candidates to drive the motivation to obtain reward from palatable food (14-16). Using in vivo calcium imaging, it was recently shown that subsets of LH VGAT neurons respond to delivery of food in food-restricted mice (13,17). Leptin targets subsets of VGAT LH neurons (18) that impact on the motivation for food and on sensitivity to food cues. We found LH LepR neurons to connect directly onto VTA GABA neurons (***Figure 2***). The dieting associated decrease in leptin explains why dieting increases food reward behavior and it increases the sensitivity to food cues in humans and rodents (19-23). Leptin-receptor expressing (LepR) LH neurons projecting to the VTA mediate at least part of the effect of leptin on food reward (18,24,25). We therefore first focus on the role of these LH LepR neurons in obesity. We already found that LH LepR neuronal activity is increased during the consumption of sucrose, and that optogenetic activation of nerve terminals from these neurons in the VTA potently stimulates dopamine neuronal activity during food reward consumption (***Figure 3***).

Development of obesity is associated with plasticity in LH neurocircuitry We found that exposure to a free choice high fat (lard) and high (20%) sucrose solution (HFHS) diet increased motivation to work for a sucrose pellet (1) suggesting that obesogenic diets alter the neurocircuitry underlying reward-seeking behavior for palatable food. Limited evidence indeed shows that these circuits can exhibit plasticity related to energy balance (e.g. potentiation of glutamatergic inputs to LH neurons after fasting) (2). Development of diet-induced obesity is also associated with plastic changes in the electrophysiological effects of leptin on LH neurons that project to the VTA (26). However, there remains a gap in knowledge whether LH neurons that control feeding, e.g. those that express leptin receptor, show such plastic changes upon exposure to obesogenic diets and how this impacts on obesity. We found that leptin's efficacy to suppress food intake was inversely related to the development of obesity upon exposure to HFHS diet (27), supporting the importance of leptin-sensitive neuronal circuit activity in obesity development.

Taken together it is clear that subpopulations of LH neurons such as LepR expressing LH neurons, affect feeding and food reward. However, there is almost no information regarding which of these neurons change their properties in a way that contributes to obesity development. Despite the fact that short term chemogenetic activation of LH neurons stimulates food intake, it is unknown whether long term inhibition of these neurons counteracts obesity. ***It is essential to address these gaps in knowledge because this will make possible the identification and validation of discrete cell groups within the LH that provide a potential target for therapeutic intervention.***

Thus, to progress towards a better understanding of the role of specific LH neurons in dietary control and obesity we will first record from LepR LH neurons using in vivo calcium imaging to determine how these neurons respond to palatable food in obese mice (**Aim 1**). We will also expose mice to an obesogenic diet and determine the adaptations in neuronal output (changes in excitability and in synaptic output) of these LH neurons (**Aim 2**). Finally, we will demonstrate that targeting these LH neurons with chemogenetics can successfully control behaviors important for dietary control and counteract development of obesity (**Aim 3**). By using state of the art technologies (chemogenetics, optogenetics, and patch clamp electrophysiology and the combined use of genetically engineered viruses) that my lab masters, it is only now that we can identify and target the neurons underlying development and maintenance of obesity.

B.2.2 Approach

Project 1: Determine the response of LH neurons to palatable versus bland food upon development of obesity (Aim 1, months1-18) Mice (LepRcre) will be injected with a (non-toxic) virus (HSV-LS1L-GCAMP6s) into the VTA. This will express the calcium indicator GCaMP6 in a cre-dependent manner in those (LH) neurons that (a) have a leptin receptor, and (b) project to the VTA (***Figure 1B*** shows these neurons). By placing an optic fiber implanted specifically above the LH, we will be able to follow the activity of LH_{LepR}→VTA neurons in real time, while the animals are exposed to different (palatable) foods. Mice will be denied access to food for 6h and thus motivated to obtain food and trained (with optic fibers attached) on a food delivery task in which after a 3 sec tone either a grain-based, a sucrose or a fatty pellet will be delivered in a balanced order in a 30 min session (50 trials). 3 weeks after surgery the mice will be connected for fiber photometry. A calcium fluorescent signal will be time locked to tone, food delivery and nose poke into the receptacle delivering food. These mice will then be fed a HFHS diet for 8 weeks which induces obesity. Then we will again expose them to the food delivery task and record neuronal activity as described above. In case fiber photometry is not sensitive enough to detect neuronal activity during the food delivery task, we will implant grin lenses and attached a miniscope camera on the skull (as done before (13)). These experiments will test the hypothesis that LH_{LepR}→VTA neurons increase their responsiveness to palatable food compared to bland food upon development of obesity, by determining the ratio of the calcium-induced fluorescence of palatable to bland food before and after exposure to HFHS diet.

Project 2: Determination of changes in neuronal output of LH_{LepR}→VTA neurons after development of obesity (Aim 2, months 12-36). ***Does diet-induced obesity induce plastic changes in the excitability of candidate LH neurons?*** We will determine whether the neuronal output of LH_{LepR}→VTA neurons is altered upon the development of obesity. To visualize these LH_{lepR}→VTA neurons we will inject HSV-LS1L-GFP into the VTA of LepR-cre mice. This will lead to the cre-dependent expression of GFP in LepR-containing input neurons in the VTA. After a recovery period, we will divide the animals into two groups, one with access to chow (Chow diet), and one to HFHS diet for 8 weeks, during which HFHS animals gain excessive weight and become obese. Next we will prepare acute LH brain slices and perform patch-clamp recordings from neurons in the LH that exhibit green (i.e. projecting to the VTA and possessing LepRs) fluorescence. Subsequently we will assess whether the excitability or synaptic output of these neurons are altered as a function of obesity.

Assessing neuronal excitability: To assess membrane properties we will perform whole-cell recordings in current-clamp mode from LH_{lepR}→VTA neurons. Apart from resting membrane potential and I/V relationship we will assess active membrane properties such as the threshold for action potential (AP) firing, AP latency and AP frequency. By doing so, we will assess whether LH_{lepR}→VTA neurons are more excitable (i.e. more prone to fire APs after current injections) after exposure to an HFHS diet compared to chow diet. Moreover, we will assess whether this is due to alterations in the membrane resistance (calculable from the IV plot) and/or primarily due to synaptic influences (performing the IV plots both in the absence, and the presence of blockers for AMPA and GABA-A receptors).

This work will clarify whether these neurons show plastic changes upon the development of obesity which may compromise dietary control. The results will guide how to manipulate neurons (inhibit or stimulate) with chemogenetics (see aim 3) in order to test whether altered activity of these neurons contributes to development of obesity.

Determining synaptic output of LH_{lepR}→VTA neurons in obesity development: Our preliminary results show that LH_{lepR} neurons that project to the VTA are predominantly GABAergic and preferentially innervate GABA neurons in the VTA (***Figure 2***). We will assess whether a HFHS diet induces neuroadaptations directly at the nerve terminal output level to enhance GABAergic neurotransmission from LH_{lepR} neurons onto VTA_{GABA} neurons. To this end we will cross LepR-Cre mice with GAD67-GFP (CB6-Tg(Gad1-EGFP)G42Zjh/J)mice (28,29) and stereotactically inject them with a viral vector into the LH to cre-dependently express channelrhodopsin-2 (AAV-DIO-hsyn-ChR2-mCherry). Since the Cre recombinase is only expressed in LepR expressing neurons, the channelrhodopsin (ChR2) and mCherry cDNA will only be expressed in LH_{lepR} neurons. As before, animals are allowed to recover and are then placed on either the Chow or HFHS diet for 8 weeks. Subsequently, brain slices are prepared and patch-clamp recordings are now performed in the VTA. VTA_{GABA} neurons (green fluorescence) will be voltage-clamped at +20 mV in the presence of synaptic blockers for AMPA and NMDA receptors, to optimally record GABA-A receptor-mediated transmission. To specifically investigate GABA transmission on VTA_{GABA} neurons originating from LH_{lepR} neurons, we will deliver short (1-10 ms) light pulses (470 nm) onto the slice to stimulate ChR2 on LH_{lepR} nerve terminals whilst recording. The relative strength of the resultant time-locked GABAergic transmission will then be assessed.

To determine whether the probability of GABA release has been enhanced in HFHS diet animals, we will repeatedly deliver two light pulses in quick succession of each other (50 ms apart). By assessing the size of the response to the 2^{nd} pulse relative to the response to the 1^{st} pulse we will calculate the paired-pulse ratio (PPR), which is inversely related to release probability. Then we will assess whether HFHS increases the impact of each

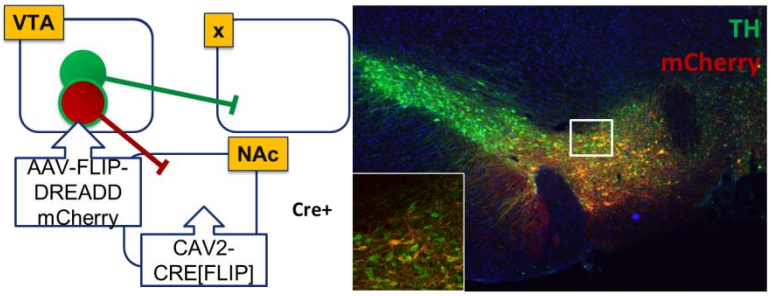

***Figure 4**: All dopamine (DA) neurons in ventral tegmental area (VTA) expres cre (in TH tyrosine hydroxylse) cre animals). Those VTA DA neurons that project to the accumbens (NAc) also express a cre-dependent flipase. A flipase-dependent DREADD injected into the VTA expresses DREADD (fused to mCherry) only in VTA DA neurons projecting to the NAc*

single vesicle of GABA released at these specific synapses (which in part depends on the density of postsynaptic GABA-A receptors, as well as on vesicular GABA content). To this end we will replace the calcium in the extracellular medium with strontium, which (when combined with optogenetic stimulation) induces input-specific single-vesicle-like responses whose amplitudes can be quantified to determine the postsynaptic response of a VTA_{GABA} neuron to individual GABA vesicles released from LH_{lepR} neurons (35).

Overall we predict that LH_{LepR} neurons that project to the VTA will exhibit a higher excitability and drive larger GABAergic responses (reduced PPR and/or enhanced amplitude of single-vesicle-like responses) in mice that have become obese on the HFHS diet.

Project 3: Determination of the extent to which chemogenetic stimulation of specific LH neurons counteracts diet-induced obesity (Aim 3, months 30-48). Leptin receptor expressing neurons in the LH will be targeted with chemogenetics to determine whether they can be utilized to counteract obesity.

3.1 Impact of long-term chemogenetic stimulation of target neurons on body weight gain. We will inject a viral vector that delivers a flipase-dependent DREADD (we generated both AAV-fl-hM3Dq or AAV-fl-hM4Di) into the LH of LepRcre mice. A canine adenovirus (CAVFlxFlp: a replication-defective, CAV-2 vector harbouring the CMV promoter that leads to Cre-inducible (DIO) Flipase expression) will be injected in to the VTA. CAV2 infects neurons at their projection terminals and delivers cDNA retrogradely to the nucleus (15,30). When injected in LH projection sites, this cre dependent flipase will be expressed in LH neurons projecting to that site. Only in cre expressing cells, flipase will become active and subsequently express the (flipase-dependent) DREADD ***(Figure 4)***. As controls we will express fluorescent protein (no receptor) in the targeted cells and treat these non-DREADD expressing mice with designer drug. Thus, in simple terms, we can exert chemogenetic control of a specific projection site (eg to VTA) of the LepR-expressing cells in the LH.

Three weeks after viral injections, these mice will be exposed to the HFHS diet and will receive designer drug or vehicle via drinking water and food intake and body weight gain will be monitored for 8 weeks and upon sacrifice quantify the amount of adipose tissue. Since stimulation of hM3Dq (the DREADD we used in figure 1) expressed in LepR+ LH neurons increased motivation to obtain food, we will express hM4Di, the inhibitory DREADD in these neurons to address whether this suppresses development of diet-induced obesity. We expect that inhibition of these neurons during exposure to the HFHS diet reduces (palatable) food consumption and makes mice less obese.

3.2 Therapeutic targeting of LH neurons to treat diet-induced obesity Similar as in 3.1, but now 3 weeks after viral injections we will expose mice to the HFHS diet. After 8 weeks of exposure to this diet, we will continue this diet and start treatment by giving designer drug or vehicle. We will determine whether therapeutic targeting of these neurons results in decreasing obesity as compared to controls by measuring food intake and body weight daily and upon sacrifice quantify the amount of adipose tissue. We predict that suppressing the activity of these neurons will reduce (palatable) food intake and make the mice less obese. These experiments address the potential of targeting specific LH neurons to treat diet-induced obesity.

B.2.3 Justification

The work will be performed in 45 months by a post-doc with experience in electrophysiology and fiber photometry that we will hire for this project. All equipment and expertise is available and up and running in my department, such as reflected in results in figures 2 and 3. Running costs (material costs) associated with the project concern optic fibers and connectors, antibodies for immunohistochemistry, purchasing viral vectors, mice (breeding, genotyping and housing) which adds up to approximately 1k per month. Allowances for animal experimentation (3 k), publication costs (open access) (5k)and congress attendance (4K) add up to 12 k in 4 years.

B.2.4. Embedding

This project finds its origin in 1) EU projects Full4Health, Neurofast and Nudge-IT in which I applied optogenetics, in vivo electrophysiology and calcium imaging to study brain circuits controlling feeding in collaboration with (amongst others) Dr. G. Stuber (Chapel Hill, United States) and Prof. dr. G.Leng (University of Edinburgh) 2) a NWO TOP project "Shining light on loss of control over substance and food intake" on the interface of overconsumption and addiction in which I was PI, 3) an STW funded project on neural mechanisms underlying leptin resistance in obesity in which I collaborated with Prof dr. S la Fleur (AMC) and 4) an ALW project titled "Unraveling the neural circuits that drive food choices" which involved my colleague Dr Frank Meye (VENI and ERC laureate) as electrophysiologist. I regularly meet and discuss projects with these scientists and will also discuss this project. I share facilities and expertise with my colleague Frank Meye with whom I closely collaborate. The techniques (optogenetics, chemogenetics, fiber photometry) that were implemented during these projects, their combination with patch-clamp electrophysiology and the strong background in

leptin resistance and development of obesity provide the excellent background for the current project. I am an ideal mentor for the post-doc that will work on this project since I have more than 15 years experience in working with viral vectors and models studying feeding behaviour, and the last 7 years supervised projects using slice electrophysiology, optogenetics and fiber photometry. The gaps in knowledge we address in this project pave the way towards novel therapeutic strategies to treat obesity. Which LH neurons are implicated in development of obesity and whether long term inhibition of these neurons protects from diet-induced obesity is one such gap on knowledge. How LH leptin receptor expressing neurons that project to the VTA change upon obesity development and whether targeting them is a therapeutic strategy to counteract obesity is not known. This proposal builds upon work of many others in my field and aims to utilize current knowledge to impact on treatment in the future. It is my ambition to contribute to designing novel therapies, such as chemogenetics, and bring them to the clinic.
The Department of Translational Neuroscience is embedded within the Brain Center Rudolf Magnus at the University Medical Center Utrecht. My research benefits from the complementary expertise of my colleagues, such as Jeroen Pasterkamp and Elly Hol. I collaborate with Prof. S.L. Dickson (Department of Neuroscience and Physiology, Sahlgrenska Academy, Gothenburg), Prof. G Leng (Dept of Experimental Physiology, Centre for Integrative Physiology, Edinburgh), Prof C. Dieguez (Department of Physiology, CIMUS, University of Santiago de Compostela-Instituto), and others and together we form a network of scientists focusing on neural circuits underlying feeding. I am also board member of the SSIB (society for the study of ingestive behaviour) and well connected to other scientists in this field. We collaborate with clinicians from who we get advise on directions of research that are important for society and clinic that ensure that our work impacts to improve treatment of eating disorders in the future: Prof A. van Elburg (Rintveld eating disorder clinic, Altrecht), Prof. J. Hebebrand (Child and Adolescent Psychiatry, Rheinische Kliniken Essen, University Duisburg-Essen), Professor B. Herpertz-Dahlmann (Klinik für Psychiatrie, Psychosomatik und Psychotherapie des Kindes- und Jugendalters, Aachen). I also hold a position as scientific advisor at Rintveld eating disorder clinic in Zeist, a Top-GGZ accredited clinic, via which I translate and communicate fundamental research to practice. I recently became guest professor at Sahlgrenska Academy at the University of Gothenburg to introduce viral vector technologies, chemogenetics and fiber photometery there. I collaborate with Dr English, University of North Carolina, Chapel Hill (Bryan Roth lab) on development of AAV vectors expressing novel DREADDS and with Dr Jin, School of Medicine at Mount Sinai, New York who will provide novel DREADD ligands. The proposed project is thus very well embedded nationally as well as internationally.

B.2.5 Risk assessment

This ambitious project should be achievable in its entirety. We have all methods, techniques and infrastructure already in place. Besides LepR cre mice, we also have VGATcre mice that can be used as backup strategy. In combination with CavFlxFlp we can target LH VGAT neurons projecting to the VTA or target LH LepR neurons projecting to other sites. All projects can run independently, although the earlier work will inform later work. Project 1 and 2 will inform on Project 3 but it is not dependent on it since LH leptin receptor expressing neurons are strong candidate neurons for exploring chemogenetic treatment. If in project 2 for some reason viral tracing technology does not work for the targeted neuron, we will use conventional tracers. If necessary we will use minimal promoters to target specific populations of neurons. Relevant to the LH we have successfully targeted melanin-concentrating hormone neurons with AAV vectors that my lab generated. In case long term treatment with CNO results in receptor down-regulation, we also have versions of DREADDs (designer receptors) in which the C-terminal tail of the receptor has been deleted (in collaboration with J.Wess(31). These receptors (such as Gq-Biased DREADD) lack the sequences necessary for binding arrestin and can therefore not be down-regulated. I am aware that CNO can be backmetabolized into clozapine which may act as ligand and induce side effects (36). Therefore non-transgenic littermates will be injected with viral vectors expressing cre-dependent DREADDs and treated with CNO as we have done previously (33,34). As alternatives for DREADD agonists, besides CNO we use compound 21 and I will obtain novel agonists as soon as they become available. I have published eight papers now in which we used chemogenetics in rodent models (e.g. 30,32,33,34).

B.2.6 Scientific and/or societal impact

At present, obesity represents a substantial global health concern with more than 400 million obese individuals worldwide. Moreover, the co-morbidities arising as a consequence obesity rank obesity within the World Health Organisation’s top 10 causes of death. The brain represents the master coordinator of appetite and body weight, and within the brain the lateral hypothalamus (LH) is a critical hub in regulating energy balance. A clear understanding of the precise mechanism how the LH is involved will provide the insight that is necessary to develop novel treatment strategies for obesity. However, discoveries in this direction have been hindered by a lack of precise tools. Taking advantage of the latest technology, here we aim to discover the role that specific

LH neurons play in development of obesity. We will unravel how molecularly defined LH neurons play a role in energy balance and feeding, whether they play a role in becoming obese and whether they can be targeted to treat obesity. We aim to precisely determine which LH neurons show plastic changes that underlie counter regulatory physiological processes that make it hard to resist overconsumption of palatable and energy-dense diets when obese. More knowledge about how neurons adapt to obesogenic diets helps to increase public awareness that the brain changes in obesity similar as in addiction. I consider it critical that my research is disseminated to the public to increase understanding about why controlling body weight is difficult and potential strategies for changing behaviours and improving metabolic health. I seek to disseminate my own results as well as to distill the lay meaning from other scientific advances using multiple media. As I do regularly, I will continue to disseminate how the fundamental research we do impacts on improving health of patients with eating disorders and obesity via social media and by speaking in national congresses of caretakers and other stakeholders.

Targeting specific LH neurons with drugs would be a next step towards a pharmacotherapy for obesity. Since chemogenetics can successfully control behaviors important for dietary control and counteract development of obesity, chemogenetics itself could be developed as gene therapy. The strategies that we use and further develop in this project are state-of-the-art and applicable to a variety of brain disorders in which altered neuronal activity of specific neurons contribute to the disease process. One of my long term ambitions is to develop chemogenetics further towards application in humans. This is a showcase project with which I hope to demonstrate that targeting specific neurons with chemogenetics is a straightforward approach to achieve therapeutic efficacy. Related to this, I am planning to start a company around the treatment of eating disorders with the serial entrepreneur Gerard Platenburg.

We will use novel viral vectors, part of which have been developed in our lab (such as flipase-dependent DREADDs, ***Figure 4***). I have shared these tools with other as they can also be applied to other fields of neuroscience.

B.2.7 Literature/references

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Part C – Additional information

C.1 Research areas

Neurosciences (80%)

Animal behaviour (20%)

C.2 Additional applicant information

C.2.1 Main applicant

Name:	Roger Adan
Affiliation:	Dept Translational Neuroscience, Brain division, UMCU, UU
Position:	professor
Paid position:	yes
Full time or part time:	full time (1.0fte)
End date of contract:	N/A

C.3 Other grant applications

I have not submitted project proposals that overlap with the current proposal.

C.4 Data management plan

1. Will you collect or generate data that is suitable for reuse?
   No, the data generated are very specific and result in the description of specific properties of neurons in feeding and obesity. The data will be stored at servers of UMCU, each night these data are backed up
   During the project the generated data (raw and analyzed data files) will be saved on servers of the UMC Utrecht. Preservation of the data is ensured by a daily back-up system. We will share our results in peer-reviewed articles in high quality scientific journals and present them at (inter-)national conferences. Furthermore, we will draft a full data management plan for long-term storage together with our in-house data management team (Hans Troost and Leo Swart).
2. Where will the data be stored during the research?
   During the project the generated data (raw and analyzed data files) will be saved on servers of the UMC Utrecht. Preservation of the data is ensured by a daily back-up system.
3. After the project has been completed, how will the data be stored for the long-term and made available for use by third parties? To whom will the data be accessible?
   The data will be stored on hard disks and available to the PI of the project and available upon request
4. Which facilities (ICT, glove box, refrigerator, etc.) do you expect you need for the storage of data during and after the research? Are these available?
   We are currently implementing a server that allows to manage the amount of data generated from fiber photometry that can easily absorb 3 Terrabyts of data per year

C.5 Public summary

De neuronen die leiden tot te veel eten in obesitas

Van de specifieke populaties neuronen die eetgedrag stimuleren, weten we niet welke een rol spelen bij overconsumptie bij obesitas. In dit project richten wij ons op neuronen in de laterale hypothalamus, een hersengebied betrokken bij het aansturen van eten. We bestuderen hoe deze neuronen veranderen bij obesitas en of manipulatie van deze neuronen er voor kan zorgen dat je niet meer dik wordt als je wordt blootgesteld aan een omgeving met vet- en suikerrijk voedsel. Dit inzicht is nodig voor de ontwikkeling van nieuwe strategieën om obesitas te behandelen.