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Obesity and Neural Networks Involved in Olfaction - Food Intake Interactions: Role of the Hypothalamus in Odor Processing

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THÈSE DE DOCTORAT

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« J'ai appris que la voie du progrès n'était ni rapide ni facile »

Marie Curie

R É S U M É

Obésité et réseaux neuronaux impliqués dans les interactions olfaction – prise alimentaire : rôle de l'hypothalamus dans le traitement des odeurs.

Le poids corporel de la plupart des animaux est stable dans le temps, suggérant qu'un système physiologique complexe équilibre l'apport alimentaire et la dépense énergétique sur le long terme. Le système de contrôle homéostatique le plus étudié est le noyau arqué (ARC) de l'hypothalamus. Dans cette structure, les neurones AgRP contrôlent la prise alimentaire. En réponse au jeûne, l'hormone de faim, la ghréline, active les neurones AgRP, ce qui entraîne une augmentation de la prise alimentaire. Après ingestion, d'autres hormones réduisent la prise alimentaire en diminuant l'activité des neurones AgRP.

Des études récentes ont montré que ces mêmes hormones ciblent également le bulbe olfactif (BO), suggérant que l'état métabolique peut directement modifier la sensibilité du système olfactif. La faim augmente la capacité de détection olfactive, tandis que la satiété réduit cette capacité. Ces études suggèrent qu'une relation bidirectionnelle établie entre le système olfactif et l'hypothalamus est essentielle pour le maintien de l'homéostasie métabolique.

Dans ce contexte, étant donné que les neurones AgRP favorisent la prise alimentaire et les comportements de recherche de nourriture, mon projet de thèse vise à déterminer comment, outre l'impact des hormones, l'activité des neurones AgRP peut moduler le traitement olfactif au sein du BO (en particulier au niveau de la couche de cellules granulaires (CG)) et influencer les capacités olfactives chez la souris. Deux modèles murins ont été utilisés pour examiner cette question.

J'ai tout d'abord étudié les souris AgRP-DTR qui ont subi une ablation périnatale des neurones AgRP par injection de toxine 1 semaine après la naissance. Les souris AgRP-ablated et les souris témoins ont été soumises à plusieurs tests comportementaux olfactifs (test d'habituation-déshabituation olfactif, ...). L'activité calcique des CGs en réponse aux odeurs a été enregistrée in vivo sur ces animaux en utilisant la photométrie fibrée.

Mes résultats montrent que le jeûne est associé à une augmentation des capacités olfactives chez les souris témoins. De plus, les souris témoins présentent, à jeun, une modification de l'activité des CGs en réponse à différents stimuli olfactifs par rapport aux animaux témoins rassasiés. En revanche, les souris AgRP-ablated ne montrent aucun changement significatif ni de leurs capacités olfactives (constamment élevées), ni de l'activité des CGs en réponse à de nouveaux stimuli olfactifs, qu'elles soient nourries ou à jeun. Il semble qu'en absence des neurones AgRP, l'activité du système olfactif n'est plus corrélée aux besoins nutritionnels du corps. Cependant, l'activité des CGs est modulée chez les souris AgRP-ablated, nourries et à jeun, en réponse à la présentation de l'odeur Chow.

Dans un deuxième temps, des approches chémo génétiques (expression virale de DREADDs) ont été appliquées sur des souris AgRP-cre rassasiées (ad libitum) pour activer artificiellement et transitoirement les neurones AgRP tout en enregistrant in vivo l'activité des CGs en réponse à la présentation d'odeurs. En plus de favoriser la prise alimentaire, l'activation des neurones AgRP augmente les capacités olfactives. Les données d'imagerie calcique in vivo révèlent une activité réduite des CGs en réponse à différents stimuli olfactifs suite à l'activation des neurones AgRP.

Enfin, l'obésité entraîne une résistance à la ghréline en réduisant la réactivité des neurones AgRP à la ghréline plasmatique. Par conséquent, un modèle murin d'obésité induite par l'alimentation a été utilisé pour étudier l'impact de l'activité altérée des neurones AgRP sur les performances olfactives. Nos données suggèrent que l'obésité entraîne des modifications des capacités olfactives chez la souris. Les enregistrements dans le BO de souris obèses ne montrent aucun changement significatif dans l'activité des CGs dans différents états métaboliques.

En conclusion, mes résultats suggèrent que l'activation des neurones AgRP induite par la faim influence potentiellement le traitement granulaire du message olfactif dans le BO, permettant des meilleures performances olfactives.

Mots clés : hypothalamus, neurones AgRP, prise alimentaire, bulbe olfactif, comportement, obésité.

S U M M A R Y

Obesity and Neural Networks Involved in Olfaction–Food Intake Interactions: Role of the Hypothalamus in Odor Processing.

Body weight of most animals is remarkably stable over time, suggesting that a complex physiological system balances food intake and energy expenditure over the long term. The most studied homeostatic control system is the arcuate nucleus (ARC) of the hypothalamus. By integrating internal (hormones) as well as external (sensory cues) feeding signals, the ARC regulates homeostasis and controls eating behavior.

Among the well-characterized ARC neurons, AgRP neurons govern a critical aspect of survival: the drive to eat. In response to food deprivation, the hunger hormone ghrelin activates AgRP neurons resulting in an enhanced appetite and an increased food intake. Upon eating, other hormones (such as leptin) suppress appetite and reduce food intake by deactivating AgRP neurons.

Recent studies showed that these same hormones also target the olfactory bulb (OB), suggesting that the metabolic status of the body can directly change the sensitivity of the olfactory system. Hunger increases the ability to detect odors, whereas satiety reduces this ability. Most of these studies linked smell to metabolism while suggesting that a bidirectional relationship established between the olfactory and the hypothalamic systems is key for the maintenance of metabolic homeostasis.

In this context, besides the undeniable impact that peripheral signals have on olfaction, and since AgRP neurons promote not only food consumption but also behaviors that lead to food discovery, my thesis project aims to determine how hypothalamic AgRP neuron activity modulates odor processing within the OB (in particular at the level of granule cell (GC) layer) and impacts olfactory capacities in mice. Two mice models were used to examine this question.

First, AgRP-DTR mice underwent perinatal ablation of AgRP neurons by toxin injection in the first week after birth; AgRP-ablated and AgRP-control mice were subjected to multiple olfactory behavioral tests (olfactory habituation dishabituation test, aversive odor test, and buried food test) and in vivo calcium recording of GC activity (using fiber photometry).

My results show that fasting is associated with increased olfactory capacities and enhanced food-seeking behavior in AgRP-control mice. Moreover, in AgRP-control mice, GC activity is modulated between the fed and the fasted state in response to different odor stimuli. On the other hand, AgRP-ablated mice show no significant changes neither in their olfactory capacities (constantly upregulated) nor in GC activity in response to novel odor cues whether fed or fasted. It seems that in the absence of AgRP neurons (AgRP-ablated mice), the activity of the olfactory system does not match the nutritional needs of the body. However, GC activity is modulated in fed vs. fasted AgRP-ablated mice in response to chow odor presentation.

Second, chemogenetic approaches (using virally mediated expression of DREADDs) were applied on sated (ad libitum) AgRP-cre mice to artificially and transiently activate AgRP neurons while recording in vivo GC activity in response to odor presentation. Besides promoting food intake, activating AgRP neurons in sated mice was found to increase olfactory capacities and enhance food-seeking behaviors. In vivo calcium imaging experiments showed reduced GC activity in the OB of AgRP-activated mice in response to different odor stimuli.

Finally, obesity is associated with impaired metabolism and altered olfactory capacities. In the ARC, diet-induced obesity (DIO) causes ghrelin resistance by reducing AgRP neuron responsiveness to plasma ghrelin. Hence, a DIO mouse model was used to study the impact of altered AgRP neuron activity on olfactory performance. Our data suggest that DIO leads to changed olfactory capacities in mice. Recordings of GCs in the OB of obese mice show no significant changes in GC activity under different metabolic states.

Together, my results suggest that hunger-induced AgRP neuron activation potentially influences the granular processing of the olfactory message within the OB leading to an enhanced olfactory performance.

Keywords: hypothalamus, AgRP neurons, food intake, olfactory bulb, behaviors, obesity.

RÉSUMÉ SUBSTANTIEL

L'homéostasie énergétique résulte d'un équilibre dynamique entre l'apport et la dépense énergétique. La stabilité à long terme du poids corporel reflète cet état d'équilibre, ce qui suggère qu'un système de contrôle physiologique complexe est impliqué dans la régulation homéostatique de l'équilibre énergétique au fil du temps. La perturbation de cette régulation est à l'origine de conditions physiopathologiques potentiellement mortelles, dont l'anorexie mentale à un extrême et le syndrome métabolique à l'autre. Pour maintenir l'homéostasie énergétique, l'hypothalamus reçoit en permanence des signaux neuronaux et hormonaux relayant des informations sur l'état nutritionnel de l'organisme. Les nutriments, métabolites et hormones en circulation, libérés par des organes périphériques agissent comme des signaux de rétroaction homéostatiques déclenchant les neurones hypothalamiques pour qu'ils s'engagent dans un ensemble complexe de réponses neurophysiologiques, régulant ainsi l'équilibre énergétique.

Le noyau arqué (ARC) de l'hypothalamus est situé au bas du 3^{ème} ventricule, à proximité immédiate de l'éminence médiane, une structure de la barrière hémato-encéphalique qui permet un échange sélectif entre les molécules véhiculées par le sang et le liquide céphalo-rachidien. En raison de leur situation anatomique privilégiée, les neurones de l'ARC ont un meilleur accès aux hormones et nutriments circulants que toute autre région du cerveau. Ainsi, l'ARC est le premier relais hypothalamique qui détecte et intègre dynamiquement les signaux périphériques de faim et de satiété.

Parmi les neurones de l'ARC bien caractérisés, deux populations neuronales interdépendantes et fonctionnellement antagonistes surveillent en permanence les signaux reflétant la disponibilité de l'énergie et régulent de manière coordonnée l'appétit et l'alimentation homéostatique :

- Les neurones orexigènes NPY/AgRP - exprimant le neuropeptide Y (NPY), le peptide lié à l'agouti (AgRP) et l'acide gamma-aminobutyrique (GABA) - stimulent la prise alimentaire.

- Les neurones anorexigènes POMC/CART - exprimant la pro-opiomélanocortine (POMC) et le transcrit lié à la cocaïne et à l'amphétamine (CART) - inhibent la prise alimentaire.

Bien que fonctionnellement opposés, les neurones NPY/AgRP et POMC/CART partagent des connexions efférentes similaires. Ces neurones de premier ordre se projettent vers un large éventail de neurones postsynaptiques exprimant le récepteur de la mélanocortine 4 (MC4R) afin d'assurer une transmission synaptique correcte des signaux liés à l'énergie.

Bien que l'étude des comportements alimentaires soit souvent limitée à la mesure de la consommation alimentaire, l'ingestion de nutriments ne représente que le point final d'une longue séquence de comportements liés à l'alimentation qui comprend une phase appétitive (besoin de nourriture et recherche de nourriture) menant à la découverte de la nourriture (indices sensoriels, phase d'anticipation) et enfin, la phase de consommation et post-ingestive (ingestion de nutriments). Les neurones AgRP sont dynamiquement régulés au cours de chacune de ces phases d'alimentation par des signaux interoceptifs (nutriments ingérés, hormones, ...) ou extéroceptifs (signaux sensoriels liés à la nourriture) qui impliquent différents effets régulateurs sur l'activité des neurones AgRP. En plus de promouvoir l'appétit et de motiver l'animal à rechercher de la nourriture, les neurones AgRP agissent comme des "gardiens" de la survie en protégeant l'animal de la famine par le déclenchement d'un large spectre de comportements et de réponses physiologiques liés à la faim. Par exemple, l'activation des neurones AgRP permet à l'animal de surmonter le stress environnemental et de donner la priorité aux comportements de recherche de nourriture.

En intégrant les signaux alimentaires internes (hormones) et externes (signaux sensoriels), les neurones hypothalamiques AgRP régulent la prise alimentaire et contrôlent les comportements alimentaires. Immédiatement avant l'ingestion de nourriture, l'activité des neurones AgRP peut être modifiée par l'odeur de la nourriture. Après l'ingestion, l'activité des neurones AgRP est modulée par les hormones de la faim et de la satiété telles que la ghréline et la leptine.

Outre l'impact des signaux interoceptifs, des études récentes ont montré l'impact des signaux extéroceptifs sur l'activité des neurones AgRP. Jusqu'à récemment, la notion bien établie de la façon dont les neurones AgRP contrôlent l'alimentation était que, en cas de jeûne, les hormones de la faim activent les neurones AgRP pour initier l'alimentation, puis que l'ingestion de nourriture entraîne la libération de plusieurs hormones liées à la satiété qui inhibent les neurones AgRP pour terminer le repas. Cette notion a été remise en question par l'utilisation de nouvelles avancées techniques permettant l'enregistrement in vivo en temps réel de l'activité des neurones AgRP.

L'évaluation de la dynamique de l'activité des neurones AgRP a réfuté notre hypothèse sur la manière dont ces neurones sont régulés, en révélant que les neurones AgRP sont inhibés en quelques secondes par la simple vue et l'odeur de la nourriture, ou par des indices conditionnés qui prédisent la disponibilité de la nourriture. Ces réponses étaient trop rapides pour être médiées par un signal hormonal, ce qui suggère qu'elles proviennent de changements dans les circuits neuronaux afférents. De manière intrigante, cette inhibition sensorielle rapide mais transitoire des neurones AgRP se produit avant qu'une seule bouchée de nourriture ne soit consommée et persiste ensuite pendant toute la durée du repas qui suit.

Parmi toutes les modalités sensorielles, l'olfaction ou le sens de l'odorat représente l'un des sens les plus anciens en termes d'évolution et joue un rôle majeur dans la survie. En tant que capteur chimique, le système olfactif détecte, code et discrimine des milliers de petites substances chimiques en suspension dans l'air (odorants) qui sont des composés organiques hydrophobes volatils et légers. Cependant, l'odorat n'est pas seulement vital pour identifier et discriminer les substances odorantes environnantes, il déclenche une myriade de comportements, notamment l'alimentation, la recherche de partenaires et la fuite des prédateurs.

L'odorat fonctionne indépendamment et en conjonction avec d'autres sens. Lors de la respiration, les substances odorantes volatiles sont inhalées. Au fond de la cavité nasale, l'épithélium olfactif représente le principal site de détection des substances chimiques en suspension dans l'air. Lorsque nous mâchons et avalons des aliments, les composés organiques atteignent l'épithélium olfactif par les narines (voie orthonasale) et la bouche (voie rétronasale). Manger est en effet l'une des expériences multisensorielles les plus agréables de la vie quotidienne, où la saveur des aliments est une combinaison d'odeur, de goût et de toucher (chimio-détection, système trigéminal).

L'olfaction agit comme un indicateur de la valeur qualitative des aliments, induisant des préférences liées au plaisir (valeur hédonique - palatabilité). Par ailleurs, outre sa fonction première dans le traitement des odeurs, des études récentes suggèrent que le bulbe olfactif (BO) est impliqué dans le maintien de l'équilibre énergétique. En effet, le BO exprime de nombreux récepteurs hormonaux associés à l'appétit et au métabolisme, ce qui suggère un rôle neuroendocrinien en dehors de l'hypothalamus. En outre, des études expérimentales ont établi un lien entre l'altération de l'olfaction et le dysfonctionnement métabolique. Il est donc essentiel d'étudier la nature de cette relation réciproque entre l'olfaction et le métabolisme pour comprendre comment surviennent les troubles olfactifs et métaboliques.

Alors que ces hormones métaboliques clés régulent la prise alimentaire, le poids corporel et le traitement métabolique périphérique par le biais de circuits hypothalamiques, l'accès privilégié du BO à ces hormones suggère que l'état physiologique et nutritionnel du corps module la sensibilité du système olfactif. En effet, des études montrent que la faim augmente la capacité à détecter les odeurs, alors que la satiété réduit cette capacité.

Dans ce contexte, outre l'impact indéniable des signaux périphériques sur l'olfaction, et puisque les neurones AgRP favorisent non seulement la consommation alimentaire mais aussi les comportements qui conduisent à la découverte de nourriture, nous avons proposé comme hypothèse générale de ce travail que l'activité des neurones AgRP hypothalamiques participe à la régulation du traitement des odeurs au sein du BO modulant les capacités olfactives chez la souris. Nous nous sommes particulièrement intéressés à l'activité des cellules granulaires (CGs) du BO car ces cellules sont un point d'entrée massif pour les projections centrifuges qui modulent l'activité du BO.

L'objectif de cette thèse est d'évaluer si les neurones exprimant l'AgRP sont impliqués dans le traitement olfactif et la performance olfactive chez la souris. Pour ce faire, nous avons utilisé deux modèles de souris génétiquement modifiées dans lesquels nous pouvons manipuler spécifiquement les neurones exprimant l'AgRP. Dans notre premier modèle, nous avons éliminé les neurones AgRP par une injection de toxine diphtérique quelques jours après la naissance chez les souris AgRP^{DTR}. Les performances olfactives des souris dépourvues de neurones AgRP et des souris témoins ont été évaluées et comparées dans de multiples tests comportementaux olfactifs et dans différents états métaboliques. La dynamique de l'activité des CGs a été évaluée dans les deux groupes en réponse à des odorants et dans différents états métaboliques. Grâce à ce modèle, nous avons également pu comparer les comportements olfactifs des mâles et des femelles.

Pour garantir la manipulation exclusive des neurones NPY/AgRP du noyau arqué, puis éviter l'ablation des cellules périphériques exprimant l'AgRP et prévenir le mécanisme compensatoire susceptible de se produire, le second modèle a consisté à activer artificiellement, transitoirement et exclusivement les neurones AgRP du noyau arqué à l'aide de la chimiogénétique chez des souris adultes AgRP-cre. L'activité des CGs in vivo et les capacités olfactives ont été évaluées en réponse à différents stimuli olfactifs sous activation des neurones AgRP.

Enfin, l'obésité est associée à une altération de la physiologie hypothalamique et s'accompagne souvent d'une altération des capacités olfactives. Le troisième objectif était donc d'évaluer les capacités olfactives dans un modèle murin d'obésité induite par l'alimentation (DIO). En outre, en appliquant des approches chimiogénétiques, nous avons cherché à déterminer si la stimulation artificielle des neurones AgRP permettrait de rétablir le traitement olfactif et les performances olfactives chez les souris DIO.

Nos résultats combinant les tests comportementaux et l'imagerie calcique *in vivo* démontrent que les neurones AgRP influencent la perception olfactive et peuvent régler l'activité des CGs dans l'BO. Dans l'ensemble, l'originalité de ce travail est qu'il montre qu'au-delà et indépendamment de l'influence déjà bien caractérisée des hormones sur le BO, les neurones AgRP jouent un rôle dans la perception et le traitement olfactifs.

Notre premier objectif était d'évaluer et de comparer les capacités olfactives des souris mâles et femelles AgRP-contrôle et AgRP-ablation dans différents états nutritionnels en réponse à des odeurs liées à la nourriture. Nous avons utilisé différents tests olfactifs pour évaluer différents aspects des capacités olfactives et nous avons trouvé des différences entre les deux groupes. Nous avons trouvé que pour les odeurs inconnues, la détection et la discrimination olfactives étaient différentes selon le statut métabolique (à jeun ou nourri), mais cette spécificité était abolie chez les souris dépourvues de neurones AgRP. Cependant, lorsque des odorants liés à la nourriture sont utilisés dans des contextes appétitifs ou aversifs, seule la discrimination est significativement affectée. Cependant, tous ces tests montrent que l'ablation des neurones AgRP tend à améliorer les capacités olfactives.

L'activité des CGs a été enregistrée dans le BO de souris mâles AgRP-contrôle et AgRP-ablated, nourries ou à jeun, pendant la présentation d'odeur de nourriture familière et de Carvone+ (nouvelle odeur). Comme prévu, l'activité CG a augmenté en réponse à chaque odeur présentée, ce qui indique que la détection de signaux sensoriels olfactifs induit une augmentation de l'activité GABAergique dans le BO.

Nos données suggèrent de plus que des réseaux distincts sont impliqués dans le traitement des odeurs liées à la nourriture. Les résultats concernant le traitement d'odorants inconnus conduisent à l'hypothèse que la faim ou un état de déficit énergétique réduit l'activité du CGs, ce qui entraîne une augmentation de l'activité des cellules mitrales (M/TCS) provoquée par les odeurs, et finalement une meilleure performance olfactive chez les souris mâles témoins

à jeun, comme dans le test comportemental olfactif décrit précédemment. Les neurones AgRP semblent effectivement être impliqués dans la transition des capacités olfactives entre état nourris et à jeun.

Afin d'activer transitoirement et exclusivement les neurones AgRP du noyau arqué tout en évitant la modulation des niveaux hormonaux circulants de faim et de satiété, nous avons utilisé des approches chimiogénétiques (DREADDs) chez des souris mâles AgRP-hM3Dq nourries et nous les avons comparées à leurs cohortes contrôle, les souris AgRP-mcherry nourries. Nous avons confirmé des résultats antérieurs montrant que la stimulation des neurones AgRP augmente la prise alimentaire chez les souris AgRP-hM3Dq nourries.

Les résultats correspondent à ce que nous avons observé précédemment chez les souris mâles contrôle entre nourries et à jeun en termes d'activité des CGs. Nous émettons l'hypothèse que l'activation des neurones AgRP induite par la faim améliore la sensibilité olfactive tout en réduisant l'activation des CG provoquée par les odeurs dans le BO.

Enfin nous avons examiné les réponses olfactives chez des animaux nourris avec un régime obésogène. Nos résultats montrent qu'après 8 semaines d'obésité induite par le high-fat diet (HFD), les souris obèses C57/b16 étaient encore capables de détecter et de discriminer les odeurs de manière significative, qu'elles soient nourries ou à jeun. Cependant, le test de recherche de nourriture enterrée a montré que le seuil de détection olfactive des souris HFD rassasiées était significativement plus élevé (sensibilité olfactive plus faible) par rapport aux souris minces, ce qui indique qu'après 8 semaines de HFD, les souris obèses étaient légèrement hyposmiques par rapport aux souris témoins.

Il semble que 8 semaines de régime obésogène n'aient pas été suffisantes pour induire une altération majeure des performances olfactives des souris. Cependant, les enregistrements par photométrie à fibres optiques ont montré qu'en réponse à la présentation d'une nouvelle odeur, la modulation de l'activité des CGs dans le BO des souris HFD entre les conditions de rassasiement et de jeûne est émoussée. Ce résultat semble être similaire à ce que nous avons trouvé précédemment chez les souris AgRP-ablated, suggérant une altération du circuit alimentaire hypothalamique.

En conclusion, cette étude apporte des éclairages nouveaux sur les interactions complexes entre régulation énergétique, perception sensorielle et comportements alimentaires, ouvrant des perspectives pour la compréhension et le traitement des troubles métaboliques et olfactifs.

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ABBREVIATIONS

ACh	Acetylcholine
abGCs	adult-born Granule Cells
AA	amino-acid
AgRP	Agouti-Related Peptide
aBNST	anterior bed nucleus of the stria terminalis
AON	Anterior Olfactory Nucleus
ARC	Arcuate Nucleus
AP	Area Postrema
ANS	Autonomic Nervous System
A^y	agouti yellow
BF	Basal Forebrain
BBB	Blood-Brain Barrier
BMI	Body Mass Index
BAT	Brown Adipose Tissue
BW	Body Weight
CB1R	cannabinoid receptor 1
CeA	Central nuclei of the Amygdala
MeA	Medial nuclei of the Amygdala
CB1	Cannabinoid 1
CART	Cocaine-Amphetamine Related Transcript
CCK	Cholecystokinin
CNS	Central Nervous System
CNO	Clozapine N-Oxide
CSF	Cerebrospinal Fluid
CoA	Cortical Amygdala
CRH	Corticotrophin Releasing Hormone
cAMP	cyclic Adenosine Monophosphate
dSACs	deep Short-Axon Cells
DT	Diphtheria Toxin
DTR	Diphtheria Toxin Receptor
DMH	Dorsomedial Hypothalamic nucleus
DR	dorsal raphe
DREADD	Designer Receptor Exclusively Activated by Designer drug
EE	Energy expenditure
EECs	Enteroendocrine Cells
EC	Entorhinal Cortex
EPL	External Plexiform Layer
ETCs	External Tufted Cells
FA	Fatty Acids
GABA	Gamma-Aminobutyric Acid
GI	Gastrointestinal

GL	Glomerular Layer
GLP-1	glucagon-like peptide-1
GPCR	G-protein coupled receptors
GCL	Granule Cell Layer
GHS-R	Growth Hormone Secretagogue Receptor
HFHS	High-Fat High-Sugar
HFD	high-fat-diet
HDB	Horizontal limb of the Diagonal band of Broca
IPL	Internal Plexiform Layer
i.c.v.	intracerebroventricular
IGF1	Insulin-like Growth Factor 1
i.p.	intraperitoneal
IR	Insulin Receptor
JGCs	Juxtaglomerular Cells
LH	Lateral Hypothalamus
LOT	Lateral Olfactory Tract
LS	Lateral Septum
LC	Locus Coeruleus
MO	Mineral Oil
MOB	Main Olfactory Bulb
MOE	Main Olfactory Epithelium
ME	Median Eminence
MC1R	Melanocortin 1 Receptor
MC4R	Melanocortin 4 Receptor
M/TCs	Mitral and Tufted Cells
MCs	Mitral Cells
MCL	Mitral Cell Layer
NPY	Neuropeptide Y
NE	Norepinephrine
NTS	Nucleus Tractus Solitarius
NAc	Nucleus Accumbens
Ob	obese gene
OB	Olfactory Bulb
OC	Olfactory Cortex
OE	Olfactory Epithelium
OMP	Olfactory Marker Protein
ONL	Olfactory Nerve Layer
OR	Olfactory Receptor
OSNs	Olfactory Sensory Neurons
OS	Olfactory System
OT	Olfactory Tubercle
OFC	Orbitofrontal Cortex
PBN	Parabrachial Nucleus
PVN	Paraventricular Nucleus
PVT	Paraventricular Thalamic Nucleus
PYY	Peptide YY
PAG	Periaqueductal Gray

PGCs	Periglomerular Cells
PC	Piriform Cortex
PACAP	Pituitary Adenylate Cyclase-Activating Polypeptide
POA	Preoptic Area
POMC	pro-opiomelanocortin
RN	Raphe Nuclei
RER	Respiratory Exchange Ratio
RMS	Rostral Migratory Stream
SubP	Subpostrema area
SVZ	Subventricular Zone
sSACs	superficial Short-Axon Cells
SCN	Suprachiasmatic Area
3rd V	Third Ventricle
TRH	Thyrotropin-Releasing Hormone
TAG	Triacylglycerol
TCs	Tufted Cells
T2D	Type 2 diabetes
VMH	Ventromedial Hypothalamus
Vgat	Vesicular GABA Transporter
VTA	Ventral Tegmental Area
WAT	White Adipose Tissue
α-MSH	α -melanocyte-stimulating hormone

SECTION - I
INTRODUCTION

CHAPTER I

Hypothalamic AgRP neurons: regulators of feeding, metabolism, and behavior

Energy homeostasis results from the dynamic and exquisite balance between energy intake and energy expenditure. The long-term stability of body weight and body fat mass reflects this state of equilibrium, suggesting that, a complex physiological control system is involved in the homeostatic regulation of energy balance over time. The disruption of this regulation gives rise to life-threatening pathophysiological conditions that include anorexia nervosa at one extreme and metabolic syndrome at the other.

1. Central control of food intake

A case report in 1901 was perhaps the first to distinguish the central nervous system (CNS) in the regulation of energy homeostasis: Dr. Albert Fröhlich, a neurologist in Vienna at that time, reported the case of one of his pediatric patients who developed obesity as a consequence of a tumor in the diencephalon (Bruch 1993; Zöllner 1908). Decades later, experimental investigations, using electrical stimulation and ablative lesioning in the diencephalic area, allowed the identification of functionally distinct nuclei of the hypothalamus, involved in food intake regulation (B. K. Anand and Brobeck 1951; Hetherington and Ranson 1940): the ventromedial hypothalamus (VMH) was considered as a “satiety center” since its ablative lesion in rodents resulted in hyperphagia and obesity, whereas electric stimulation of VMH led to starvation and eventually death. On the other hand, the lateral hypothalamus (LH) emerged as a “feeding center” since lesions of the LH led to anorexia, while its stimulation caused voracious eating and obesity (Bal K. Anand and Brobeck 1951). These observations rapidly recognized the role of the hypothalamus as a key regulator of food intake.

Since then, as our knowledge of the neuronal networks and their ramifications has evolved, the notion of hunger and satiety centers has been replaced by a more complex hypothalamic organization: distinct neuronal populations, expressing specific neurotransmitters, were identified in the hypothalamus and were clustered according to their differential actions on food intake and/or energy expenditure (Michael W. Schwartz et al. 2000; Williams et al. 2001).

However, before diving into this chapter, it should be noted that although the hypothalamus is considered as the main structure involved in the maintenance of energy homeostasis, it is definitely not the only brain area running this crucial regulation (Hans-Rudolf Berthoud 2006). Advanced studies showed the existence of a broad and complex neural network connecting multiple brain regions to the homeostatic and/or non-homeostatic feeding process: the parabrachial nucleus (Hajnal, Norgren, and Kovacs 2009), the nucleus accumbens and the amygdala (Will, Franzblau, and Kelley 2003), the area postrema (Truong, Magrum, and Gietzen 2002; Beverly, Gietzen, and Rogers 1990), the cerebral cortex and hippocampus (Davidson et al. 2009) as well as sensory structures such as the visual and the olfactory system (Faour et al. 2022; Riera et al. 2017; Y. Chen et al. 2015).

1.1. The hypothalamus: anatomy and functions

In vertebrates, the hypothalamus is a diencephalic structure located at the base of the brain, below the thalamus, and just above the pituitary gland. It is composed of several nuclei symmetrically duplicated on both sides of the third ventricle (3rd V) (Figure I.1.) (Baroncini et al. 2012).

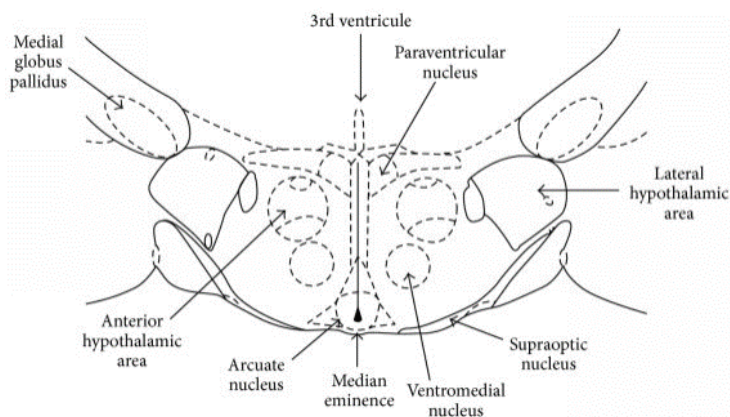


Figure I.1. Bilateral overview of the major hypothalamic nuclei in rats. The arcuate nucleus (1st order nucleus), the paraventricular nucleus, the lateral hypothalamic area, the anterior hypothalamic area, the ventromedial, and the supraoptic nuclei. *Image source: (Bakos et al. 2016).*

The hypothalamus barely constitutes 4 grams out of the 1400 grams of the adult human brain (Saper and Lowell 2014). Yet, this tiny area is characterized by highly conserved neural circuitries involved in the regulation of numerous vital functions including sexual differentiation, reproduction, thermoregulation, circadian rhythm, blood circulation, water and salt intake, and energy balance (from food intake to energy expenditure), the latter being the main focus of this thesis.

To maintain energy homeostasis, the hypothalamus constantly receives neural and hormonal inputs relaying information about the nutritional state of the body. Circulating nutrients, metabolites, and hormones, released by peripheral organs such as the adipose tissue, liver, pancreas, and the gastrointestinal (GI) tract, act as homeostatic feedback signals triggering hypothalamic neurons to engage in a complex set of neurophysiological responses, thereby regulating energy balance (Morton, Meek, and Schwartz 2014).

1.2. The arcuate nucleus of the hypothalamus

The arcuate nucleus (ARC) of the hypothalamus is located at the bottom of the 3rd V in close vicinity to the median eminence (ME), a structure of the blood–brain barrier (BBB) described as a fenestrated and densely irrigated endothelium that has evolved to allow selective exchange between blood-borne molecules and the cerebrospinal fluid (CSF) (Langlet et al. 2013; Norsted, Gömüç, and Meister 2008) (Figure I.2.). Due to their privileged anatomical location, ARC neurons have greater access to circulating hormones and nutrients than any other region of the brain protected by a more fully developed BBB. Thus, the ARC is the first hypothalamic relay dynamically sensing and integrating peripheral hunger and satiety signals. As a result, neurons that reside in the ARC are referred to as “first-order neurons”.

Among the well-characterized ARC neurons, two interrelated and functionally antagonistic neuronal populations continuously monitor signals reflecting energy availability, and coordinately regulate appetite and homeostatic feeding:

- The orexigenic NPY/AgRP neurons - expressing neuropeptide Y (NPY), agouti-related peptide (AgRP), and gamma-aminobutyric acid (GABA) - stimulate food intake.
- The anorexigenic POMC/CART neurons - expressing pro-opiomelanocortin (POMC) and cocaine-amphetamine related transcript (CART) - inhibit food intake.

Despite being functionally opposite, NPY/AgRP and POMC/CART neurons share similar efferent connections (D. Wang et al. 2015). These 1st order neurons project to a wide range of postsynaptic melanocortin 4 receptor (MC4R)-expressing neurons to ensure proper synaptic transmission of energy-related signals (Williams et al. 2001). MC4R is a G-protein coupled, seven-transmembrane receptor that is highly expressed in 2nd order nuclei of the hypothalamus (Mountjoy and Wong 1997): the hypothalamic paraventricular nucleus (PVN), the VMH, and the LH.

The important role of MC4R in regulating food intake is highlighted by the fact that MC4R mutations are the most common cause of monogenic forms of obesity and are associated with early-onset severe obesity in humans (Chami et al. 2020; Vaisse et al. 1998; Huszar et al. 1997). A special focus will be given to MC4R-expressing neurons of the PVN for their crucial involvement in regulating energy balance (Balthasar et al. 2005; M. S. Kim et al. 2000): chemogenetic activation of PVN^{MC4R} neurons reduces food intake after fasting, suggesting that the activation of these neurons is sufficient to promote satiety (Garfield et al. 2015).

In the next section, I will present the canonical melanocortin signaling pathway which integrates hunger and satiety signals to regulate homeostatic food intake.

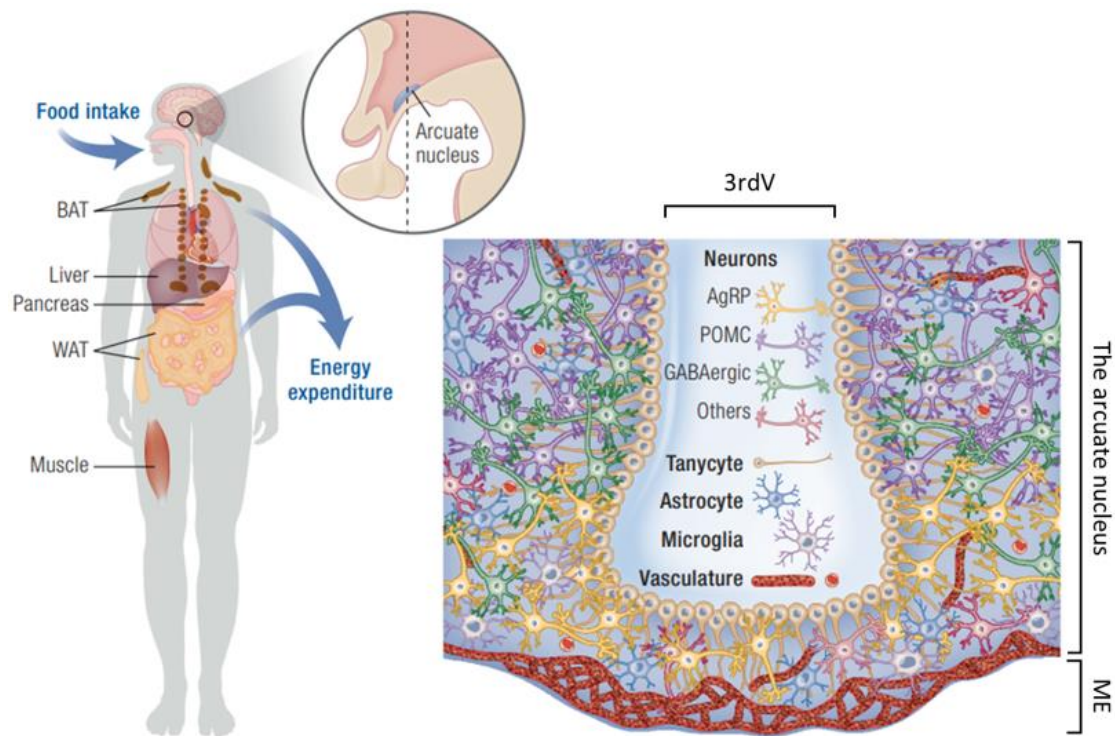


Figure I.2. The arcuate nucleus of the hypothalamus: exquisite location for exquisite regulation. The ARC of the hypothalamus is perfectly positioned, close to a BBB entry point, the median eminence (ME). The ME allows the selective transportation of peripheral signals into the nutrient-sensing hypothalamic nuclei. Moreover, tanyocytes form a barrier between the ARC neurons and the cerebrospinal fluid of the 3rd V. Tanyocytes actively transport circulating molecules from the ME and the 3rd V to the ARC neurons. Among ARC neurons, agouti-related protein (AgRP) and pro-opiomelanocortin (POMC) neurons play a major role in sensing and integrating peripheral signals of hunger and satiety. *Image adapted from (Jais and Brüning 2022) © 2021 Endocrine Society.*

1.3. The melanocortin signaling pathway

1.3.a. POMC neurons

Hypothalamic POMC neurons are mainly stimulated by leptin, an adipocyte-derived hormone signaling high energy availability. Active POMC neurons release multiple cleavage products of POMC, including α -melanocyte-stimulating hormone (α -MSH). α -MSH effectively binds to and activates downstream melanocortin receptors PVN^{MC4R} to suppress feeding (anorexigenic) and enhance energy expenditure (Figure I.3.) (Cone 2005; Fan et al. 1997). In response, PVN neurons release several anorectic neuropeptides such as thyrotropin-releasing hormone (TRH), Corticotrophin Releasing Hormone (CRH), and oxytocin to reduce food intake (Michael W. Schwartz et al. 2000).

Recent studies revealed more complexities in the classical view of the two antagonistic functions of AgRP / POMC neurons. Intriguingly, chemogenetic activation of POMC neurons was found to promote cannabinoid receptor 1 (CB1R)-induced feeding in sated mice (Koch et al. 2015). The precise function of POMC neurons has not been fully elucidated due to their heterogeneous nature (Quarta et al. 2021). POMC neurons are segregated into distinct subpopulations expressing different receptors: leptin receptor, insulin receptor, as well as serotonin receptor (Htr2c) (Lam et al. 2017; J. N. Campbell et al. 2017). Moreover, different POMC neuron subsets express either the inhibitory neurotransmitter GABA, the excitatory neurotransmitter glutamate, or both, depending on their spatial distribution within the ARC (Hentges et al. 2009; Dicken, Tooker, and Hentges 2012; Wittmann, Hrabovszky, and Lechan 2013; Lam et al. 2017). Furthermore, 27% of POMC neurons express high levels of AgRP and NPY mRNA, elucidating functional similarities with appetite-promoting NPY/AgRP neurons (Lam et al. 2017). Apart from food intake/metabolism, heterogeneous POMC neurons are involved in cardiovascular responses, pain, fear, anxiety, and locomotion.

1.3.b. AgRP neurons

On the other hand, hypothalamic AgRP neurons are activated by hormonal and neural signals of low energy availability, such as ghrelin, a gastrointestinal hormone that increases before a meal, and are inhibited by signals of high energy availability, such as leptin and insulin (Cowley et al. 2003). Through the synaptic release of AgRP, a high-affinity antagonist of α -MSH, active AgRP neurons antagonize POMC neuron activity at the level of postsynaptic PVN^{MC4R} neurons to induce appetite (orexigenic) and drive feeding (Figure I.3.) (Büch et al. 2009; Ollmann et al. 1997). AgRP neurons also release the orexigenic neurotransmitter, NPY,

which acts on its receptor Y1R particularly expressed in the PVN (Beck 2006; Donald J. Marsh et al. 1998; Pedrazzini et al. 1998). In addition, AgRP neurons directly inhibit POMC neurons through the release of the inhibitory neurotransmitter, GABA (Horvath et al. 1997; Cowley et al. 2001).

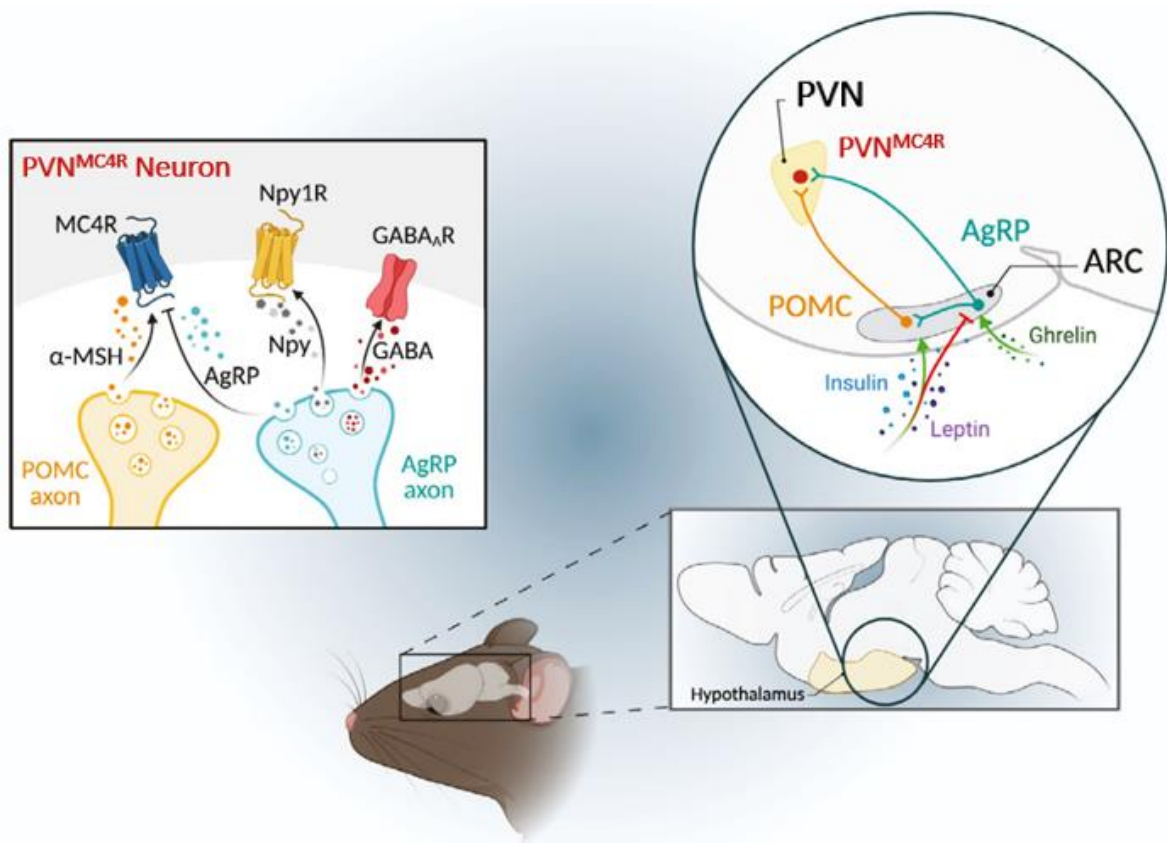


Figure I.3. AgRP and POMC neurons: two intermingled neuronal populations defining the central melanocortin system. The central melanocortin system involves two distinct neuronal populations located in the ARC, expressing either AgRP or POMC. Once activated by leptin and insulin, POMC neurons release α -MSH, an agonist that binds to downstream PVN^{MC4R} to inhibit food intake and induce weight loss. Conversely, AgRP neurons are inhibited by insulin and leptin while being activated by the gastrointestinal hormone, ghrelin. Once activated, AgRP neurons stimulate food intake and reduce energy expenditure. These neurons also express NPY, and the inhibitory neurotransmitter, GABA. AgRP neurons mediate their effects through the release of 1) AgRP, which antagonizes the binding of α -MSH to PVN^{MC4R} neurons, 2) NPY, acting on downstream Y1 and Y5 receptors, and 3) GABA, inhibiting POMC neurons. *Image adapted from (Deem, Faber, and Morton 2022).*

For years, hypothalamic AgRP neurons have been thought to stimulate appetite and promote food intake by inhibiting their neighboring POMC neurons and opposing their actions through AgRP release in post-synaptic targets (PVN^{MC4R}) of the melanocortin signaling pathway (Palmiter 2012; G. J. Morton and Schwartz 2001; Jacobowitz and O'Donohue 1978). Therefore, excessive activation of PVN^{MC4R} represented an attractive explanation for the starvation phenotype observed in adult mice lacking AgRP neurons. Intriguingly, starvation could not be rescued by chronic antagonism of PVN^{MC4R} (Aponte, Atasoy, and Sternson 2011; Wu et al. 2008), demonstrating that AgRP neurons drive feeding independently of the melanocortin signaling pathway (Wu et al. 2008; Aponte, Atasoy, and Sternson 2011). Distinct AgRP subpopulations target different brain regions, several of which are sufficient to independently drive feeding and trigger feeding behaviors (Betley et al. 2013a).

The urge to understand the fundamental mechanisms involved in the homeostatic regulation of food intake has therefore generated a frantic race in the scientific community to unravel the contribution of AgRP neuron activity to the feeding process. My work is particularly focused on the effect of AgRP neuron modulation. Therefore, the rest of this chapter will be dedicated to highlighting recent advances that extend our understanding of arcuate AgRP neurons, including the neural circuits they engage in to regulate food intake, energy expenditure, and behaviors.

2. Peripheral metabolic signals involved in food intake regulation

Homeostatic regulation of food intake relies peripherally on the release of hormones, peptides, and visceral information from the gastrointestinal tract (GI) and the adipose tissue. GI hormones such as GLP-1, CCK, and PYY are secreted in response to food-derived macronutrients (proteins, glucose, lipids) arriving in the gut lumen (Camilleri 2015; Saper, Chou, and Elmquist 2002). The gut/brain axis is indeed a key modulator of feeding behavior. The vagus nerve carries visceral information from the gut to the brainstem, where it is processed and projected to the hypothalamus (and other brain areas) (Hans-Rudolf Berthoud 2008).

In this subchapter, I present distinct peripheral metabolic signals (hormones and nutrients) involved in food intake regulation, while particularly focusing on each's role in modulating hypothalamic AgRP neuron activity (Figure I.4.).

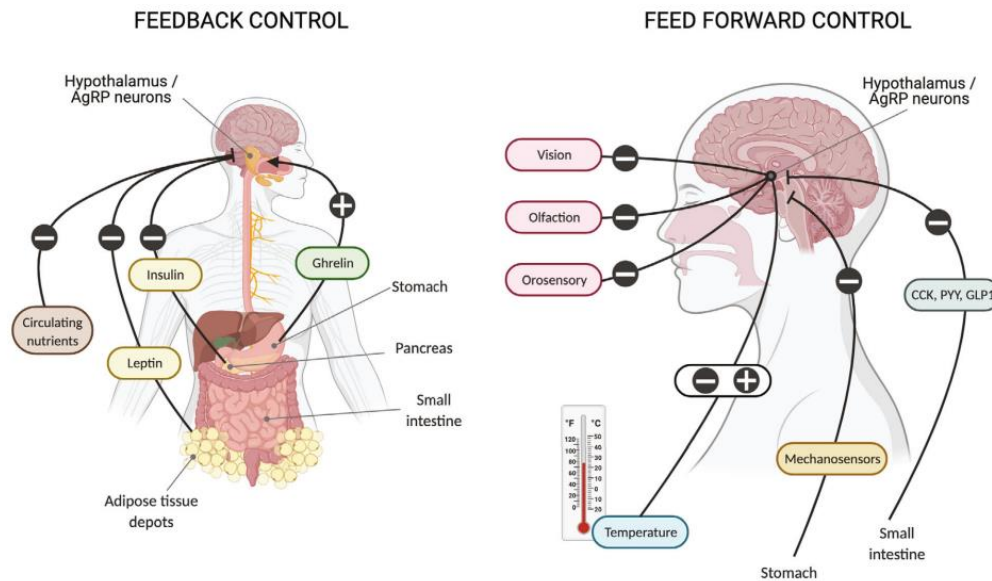


Figure I.4. Feedback and feedforward regulation of AgRP neuron activity. Energy homeostasis is kept through a complex interplay of nutritional, neuronal, and hormonal inputs. Feedback control of AgRP neuron activity is primarily regulated by hormonal (ghrelin, leptin, and insulin) and nutrient-related inputs (glucose, free fatty acids, ...). On the other hand, visual and olfactory sensory cues related to food discovery, as well as orosensory signals related to taste, inhibit AgRP neuron activity early in a meal, while mechanosensation and gastrointestinal-derived hormones (CCK, PYY, GLP1) inhibit AgRP neuron activity as nutrients enter the gut. In addition, ambient temperature modulates AgRP neuron activity in either direction: cold/low temperatures increase AgRP neuron activity whereas warmer/high temperatures reduce their activity. *Image source: (Deem, Faber, and Morton 2022).*

2.1. Hormones:

2.1.a. Ghrelin

The gastrointestinal peptide hormone ghrelin is the only peripheral orexigenic signal known so far (Date et al. 2000). It's famously known as the “hunger hormone” for its role in inducing appetite, enhancing feeding behaviors, and increasing food intake. Circulating ghrelin levels rise significantly in the preprandial phase and fall back to trough levels after eating (Cummings et al. 2001), suggesting that ghrelin is a key regulator of energy homeostasis (Horvath et al. 2001).

Ghrelin is a ligand predominantly produced in the stomach (Ariyasu et al. 2001; Kojima et al. 1999) but exerts its orexigenic effect in the CNS (Cowley et al. 2003) via its functionally relevant receptor, the growth hormone secretagogue receptor (GHS-R). GHS-R is particularly

expressed in the ARC of the hypothalamus: ghrelin injections were found to increase c-fos expression in more than 90% of hypothalamic NPY/AgRP neurons (L. Wang, Saint-Pierre, and Taché 2002). Conversely, knockout of both NPY and AgRP completely abolishes the orexigenic action of ghrelin, indicating that NPY/AgRP signaling pathway is critically essential in mediating the orexigenic effect of ghrelin (H. Y. Chen et al. 2004).

When administered peripherally or centrally to rodents, ghrelin activates hypothalamic NPY/AgRP neurons and increases, in a dose-dependent manner, food intake and body weight (Nakazato et al. 2001; Wren et al. 2000; Tschöp, Smiley, and Heiman 2000).

2.1.b. Leptin

In 1994, the discovery of leptin represented a key milestone in the study of obesity and the understanding of the central control networks involved in maintaining energy balance and body weight (Y. Zhang et al. 1994). Leptin is a 16 kDa satiety hormone encoded by the obese gene (*ob*) and is mainly produced by adipocytes of the white adipose tissue (Martínez-Sánchez 2020; Ahima and Osei 2004).

Leptin-deficient (*ob/ob*) mice were described as being morbidly obese and diabetic with a reduced metabolic rate. However, when administered peripherally, leptin normalized the metabolic status of the *ob/ob* phenotype by effectively lowering food intake and reducing body fat mass (Farooqi et al. 1999; Pelleymounter et al. 1995; Campfield et al. 1995).

Normally, in the bloodstream, leptin levels are directly correlated with body fat mass (Considine et al. 1996). Low circulating leptin levels are perceived as an energy deficit state triggering an increase in appetite and a decrease in energy expenditure (Flier and Maratos-Flier 2017; Weigle et al. 1997). Conversely, high leptin levels signal to the brain an excess of energy leading to a decreased appetite and an increased energy expenditure.

The adipocyte-derived hormone, leptin, is indeed able to cross the blood-brain barrier to exert its anorexigenic effect by targeting, its primary site of action in the CNS, the hypothalamus (Hussain and Khan 2017). Within the arcuate nucleus of the hypothalamus, leptin was found to differentially regulate the activity of NPY/AgRP and POMC/CART neurons primarily through its hypothalamic receptor *ob-Rb*: an acute administration of leptin increases the activity of the anorexigenic POMC/CART neurons and decreases the activity of the orexigenic NPY/AgRP neurons, leading to a reduced appetite in mice (Cowley et al. 2001; Elias

et al. 1999). In addition, leptin decreases the GABAergic inhibition exerted by NPY/AgRP neurons on POMC/CART neurons (Baver et al. 2014).

Finally, common obesity is associated with elevated accumulation of adipose tissue fat stores which results in constantly high levels of circulating leptin. But, isn't that contradictory? Isn't leptin supposed to reduce food intake and body fat mass as in the previously described ob/ob phenotype? The failure of these significantly high endogenous leptin levels (as well as therapeutically administered exogenous leptin) in the obese state to reduce feeding and normalize adipose fat mass has elicited the fact that obesity alters hypothalamic physiology which potentially impairs leptin action and leads to "leptin resistance" (Pan and Myers 2018).

2.1.c. Insulin

Insulin is a 6 kDa polypeptide hormone, produced and secreted by β -cells of pancreatic Langerhans islets. Insulin is best known for its role in reducing circulating levels of glucose 1) by facilitating its absorption mainly in skeletal muscles and adipose tissues and 2) by reducing its production by the liver (Abdul-Ghani and DeFronzo 2010).

To achieve those actions, insulin must first bind to its membrane receptor, IR (insulin receptor), in order to induce a cascade of well-characterized intracellular events (Laplante and Sabatini 2012). In the late 1970s, multiple studies suggested that the CNS constitutes an important target for insulin action (Havrankova et al. 1978): insulin and IRs were found to be highly expressed in distinct brain structures such as the olfactory bulb (OB) (J. M. Hill et al. 1986), the hypothalamus, the hippocampus (Stockhorst et al. 2004) as well as the cerebral cortex (Ghasemi et al. 2013). Insulin affects feeding behavior and body energy stores (Dimitriadis et al. 2011), as well as various aspects of memory (Sędzikowska and Szablewski 2021) and cognition (B. Kim and Feldman 2015).

Of particular note, insulin acts as a catabolic hormone inducing satiety, and increasing energy expenditure (Vogt and Brüning 2013; Varela and Horvath 2012; Gerozissis 2004; Air et al. 2002): pharmacological studies showed that intracerebroventricular (icv) or intranasal administration of exogenous insulin results in reduced food intake and body weight (Hallschmid et al. 2004; Woods et al. 1979; McGowan et al. 1993).

The importance of insulin in energy balance regulation has been highlighted in a mouse model of neuron-specific disruption of IR. The disruption of the central action of insulin leads

to diet-sensitive obesity in female mice, with increased body fat and pronounced insulin resistance (Brüning et al. 2000). However, selective inactivation of IR in either AgRP or POMC neurons does not alter energy balance, suggesting that the anorexigenic effect of insulin is independent of these neurons (Konner et al. 2007; J. W. Hill et al. 2010).

Furthermore, multiple studies elicited that insulin's anorexigenic action is mediated through the mesocorticolimbic dopaminergic pathway (Figlewicz et al. 2003). Under normal physiological conditions, insulin lowers dopamine release in the ventral tegmental area (VTA), reducing palatable feeding behavior (Mebel et al. 2012). Moreover, pathological hyperinsulinemia results in impaired insulin action in the VTA, which contributes to hyperphagia and obesity (S. Liu et al. 2013).

2.2. Gastrointestinal peptides

2.2.a. GLP-1

The satiety signal, glucagon-like peptide-1 (GLP-1), is a cleavage product of proglucagon expressed in the gut, pancreas, and brain (Drucker 2006). Other proglucagon-derived satiation signals include glucagon, GLP-2 (an intestinal growth factor), glicentin (a gastric acid inhibitor), and oxyntomodulin.

GLP-1 is primarily secreted by L-enteroendocrine cells (EECs) in the distal small intestine and colon, in response to ingested nutrients, especially fats and carbohydrates (Brubaker and Anini 2003). It is also secreted in the CNS, mainly by a small subset of neurons located in the nucleus tractus solitarius (NTS) (Holt et al. 2019). Notably, GLP-1 reduces short-term food intake in several species (Donahey et al. 1998; Turton et al. 1996), including humans (Gutzwiller et al. 1999; Näslund et al. 1999), induces glucose-dependent insulin release, inhibits glucagon secretion, enhances pancreatic β -cell growth and delays gastric emptying (J. E. Campbell and Drucker 2013).

The anorexigenic effects of GLP-1 are mediated specifically via its receptor GLP-1R expressed in the gut, pancreas, brainstem, hypothalamus (ARC, PVN), nucleus tractus solitarius (NTS), area postrema (AP), and vagal nerve afferents (Parkinson et al. 2009; Baggio et al. 2004). Activation of hypothalamic GLP-1R reduces feeding without causing illness, whereas GLP-1R activation in the amygdala causes nausea leading to a reduced food intake (Kinzig, D'Alessio, and Seeley 2002).

The mechanisms underlying GLP1-induced satiety are not fully unraveled. However, recent studies using ex-vivo patch-clamp electrophysiology found that, within the ARC, GLP-1R agonists (liraglutide and semaglutide) directly activate POMC neurons and indirectly inhibit NPY/AgRP neurons (Dong et al. 2021; Gabery et al. 2020; Brubaker and Anini 2003; Secher et al. 2014). Meanwhile, in-vivo fiber photometry experiments showed that intraperitoneal (i.p.) injection of GLP-1 and GLP-1R agonists failed to modulate arcuate neuron activity, suggesting that peripheral GLP-1 is rapidly degraded preventing it from reaching the ARC.

2.2.b. CCK

The peptide hormone cholecystokinin (CCK) is secreted by I-EECs located primarily in the upper small intestine. CCK plays a crucial role in the control of digestion and serves as a short-term satiety signal contributing to the termination of a meal in vertebrates (Konturek et al. 2003): when administered to rodents – centrally or peripherally - right before a meal, CCK decreases in a dose-dependent manner meal size and duration (Edwards, Ladenheim, and Ritter 1986; Gibbs, Young, and Smith 1973).

The major nutrients that trigger CCK release are fats and ingested proteins (Y. Wang et al. 2011). CCK-induced satiation is primarily mediated by its receptor CCK-R1 (CCK-A) expressed on vagal afferent fibers (Timothy H Moran 2004): selective vagotomy abolishes or reduces the anorexigenic effect of CCK (G. P. Smith et al. 1981; T. H. Moran et al. 1997). In addition, increased meal size and body weight were observed in rats lacking CCK-R1 (T. H. Moran et al. 1998). Upon ingestion, CCK regulates gastrointestinal motility by delaying gastric emptying, enhancing pancreatic enzyme secretion, and accelerating intestinal transport (Olsson et al. 1999; Andrews, Young, and Bone 1997; Guilloteau et al. 2006).

In the CNS, the gut-brain hormone CCK acts preferentially via its receptor CCK-R2 (CCK-B), particularly targeting the NTS, the LH, and the dorsomedial nucleus of the hypothalamus (Blevins, Stanley, and Reidelberger 2000; Peikin 1989). Within the ARC, in vivo calcium imaging experiments showed that AgRP neuron activity is rapidly suppressed (maximal suppression reached in less than 2 min) in response to CCK i.p. injection (Su, Alhadeff, and Betley 2017a).

2.2.c. PYY₃₋₃₆

The peptide YY (PYY) is a 36 amino-acids (AA) satiety signal secreted by L-ECCs in the distal part of the intestine (ileum and colon), especially in response to short-chain fatty acids (Larraufie et al. 2018). Circulating PYY is a combination of its secreted form PYY₁₋₃₆ and its active form PYY₃₋₃₆ (Batterham et al. 2002). Circulating PYY₃₋₃₆ levels rapidly increase in the postprandial phase, in proportion to the calorie content of a meal, resulting in restrained feeding behavior (Grandt et al. 1994; Adrian et al. 1985). Peripheral PYY₃₋₃₆ administration inhibits food intake and reduces body weight in humans (Batterham et al. 2007). The gut-derived satiety signal PYY₃₋₃₆ controls gastric motility, as well as water and electrolyte uptake in the intestine.

In the CNS, the anorexigenic effects of PYY are mediated primarily via vagus nerve afferents and NPY Y2 receptors (Y2R) expressed in the ARC of the hypothalamus (Baraboi et al. 2010). PYY is indeed able to cross the BBB, directly inhibiting NPY/AgRP neurons, thus activating adjacent POMC neurons (Batterham et al. 2002; Wynne, Stanley, and Bloom 2004). Furthermore, *in vivo* calcium imaging experiments showed that peripheral administration of PYY slowly suppresses AgRP neuron activity - maximal suppression reached almost half an hour post-PYY injection - suggesting that PYY has a slow latency but longer-lasting anorexigenic effect on AgRP neurons when compared to CCK (Su, Alhadeff, and Betley 2017a).

2.3. Circulating nutrients:

2.3.a. Amino acids and proteins

The involvement of dietary proteins and circulating AA in the regulation of appetite and food intake was first elicited in the early 1950s (Mellinkoff et al. 1956). Dietary proteins are described as short-term satietogenic signals, suppressing appetite and inducing weight loss (Weigle et al. 2005; Leidy et al. 2015; Porrini et al. 1997). The work of Mithieux et al. (2005) demonstrated that protein-enriched diets reduce food intake by 1) increasing portal vein glucose levels (mainly from intestinal gluconeogenesis) and 2) triggering the release of anorexigenic gut peptides (CCK, GLP-1, and PYY).

In addition, it has now been shown that out of the 20 essential AA, leucine is a key regulator of hypothalamic nutrient-sensing pathways (Potier, Darcel, and Tomé 2009). Leucine exerts its anorexigenic effect by stimulating POMC neurons in the ARC and other neurons in the NTS of the brainstem, resulting in reduced meal size and number (Blouet et al. 2009).

Conversely, injection of L-threonine into the prepiriform cortex of rats fed a threonine-free diet, increases the firing rate of LH neurons, thereby resulting in increased food intake (Monda et al. 1997).

For decades, protein was regarded as the most satiating macronutrient. However, multiple follow-up studies showed that proteins, fats, and carbohydrates have similar satiating effects on appetite (de Graaf et al. 1992; Potier et al. 2010; Vozzo et al. 2003). Interestingly, obesity lowers the appetite-suppressing effect of fats, most probably as a result of high-fat overconsumption, whereas responses to proteins remain relatively intact (Golay and Bobbioni 1997; Leidy et al. 2015). Hence, high-protein diets are nowadays considered a successful strategy to prevent and treat overweight and obesity.

2.3.b. Glucose

Besides its role as a primary fuel for brain function, glucose acts as a major metabolic signal regulating food intake (Mayer 1996). Blood glucose levels fluctuate during the day depending on meal intake and/or physical activity. In the preprandial phase, hypoglycemia was found to increase appetite and food intake in both humans and animals. Conversely, high blood glucose levels induce satiety after a meal (Thompson and Campbell 1977; G. Smith and Epstein 1969).

In the early 1960s, it was shown that the brain dynamically senses and responds to variations in circulating glucose levels (Oomura et al. 1964). Two populations of glucosensing neurons have been classified as glucose-responsive and glucose-sensitive neurons, based on their response to rising glucose levels by increasing or decreasing their firing rates respectively. These neurons are mainly located in the hypothalamus (ARC, VMH, and LH), but also in the brainstem, the olfactory bulb, the hippocampus, the thalamus, and the amygdala (Routh 2002; Pénicaud et al. 2006).

Multiple studies demonstrated that within the ARC, the majority of glucose-sensitive (or glucose-inhibited) neurons express NPY (Murphy et al. 2009; Fioramonti et al. 2007; Muroya et al. 1999). On the other hand, no study has shown that glucose directly modulates POMC neuron activity. Nevertheless, Parton et al. (2007) found that α -MSH release is increased in response to increased glucose levels, suggesting that POMC neuron activity is modulated by presynaptic inputs rather than a direct glucose detection by POMC neurons themselves (Hu et al. 2014). Finally, glucose sensing by POMC neurons is impaired in obesity (Parton et al. 2007).

2.3.c. Free fatty acids

For decades, the possibility that circulating fatty acids (FA) might impact the brain's activity was neglected because they were thought to not cross the BBB. However, a growing amount of data proved that cerebral lipids originate from local synthesis but also from plasma-derived FA (Rapoport, Chang, and Spector 2001).

Furthermore, it has now been demonstrated using brain uptake index methods that FA uptake in the hypothalamus is up to 10 to 15% compared to less than 2% in other brain areas (Rapoport 2001) and that is due to its privileged location next to the ME of the BBB, making it easily reachable. Interestingly, high FA uptake was also observed in the olfactory bulb (Ueno et al. 1991).

It became increasingly evident that hypothalamic "FA-sensing" is involved in the control of feeding behavior. For instance, oleic acid icv infusion decreases hypothalamic NPY-expression leading to decreased food intake (Obici et al. 2002). Moreover, triglycerides icv infusion alters both insulin secretion and hepatic glucose production in rats (Cruciani-Guglielmacci et al. 2005; Clément et al. 2002).

Deregulation of FA-sensing contributes to the development of metabolic diseases in predisposed subjects when exposed to chronic lipid overload. Hence, such impairment of the central action of FA may be involved in the etiology of obesity and type 2 diabetes (Das 2002, 2; Elmquist and Marcus 2003).

3. AgRP neurons govern a critical aspect of survival: the drive to eat

Although the study of feeding behaviors is often restricted to measuring food consumption, nutrient ingestion only represents the endpoint of a long sequence of feeding-related behaviors which entails an appetitive phase (food craving and foraging) leading to food discovery (sensory cues, anticipatory phase) and finally, the consummatory and post-ingestive phase (nutrient ingestion). AgRP neurons are dynamically regulated across each of these feeding phases by interoceptive (ingested nutrients, hormones, ...) or exteroceptive (encountered food-related sensory cues) signals that imply different regulatory effects on AgRP neuronal activity (Figure I.5.). Besides promoting appetite and motivating the animal to seek food, AgRP neurons act as "gate-keepers" of survival by protecting the animal from starvation through the initiation of a large spectrum of hunger-related behaviors and physiological responses (Gouveia, de Oliveira Beleza, and Steculorum 2021; Deem, Faber, and Morton

2022). For instance, the activation of AgRP neurons allows the animal to overcome environmental stress and prioritize food-seeking behaviors.

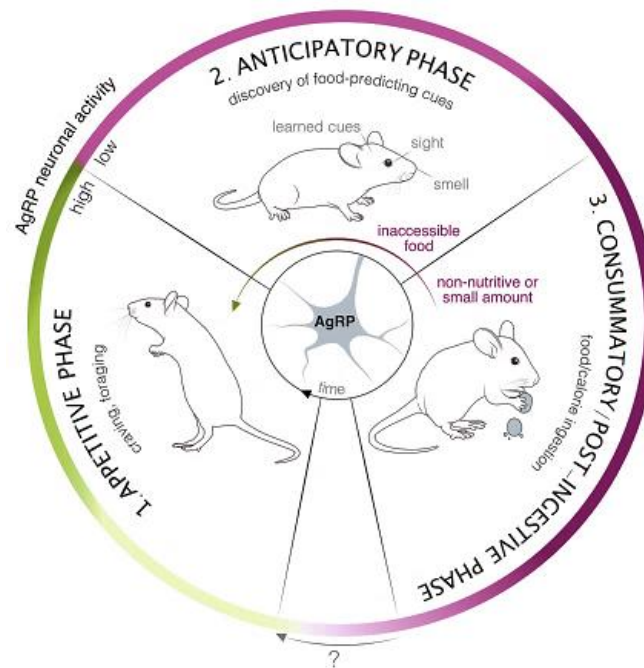


Figure I.5. Dynamics of AgRP neuron activity across feeding behaviors. Feeding behavior is a sequence of 3 main phases: the appetitive (food craving and foraging), anticipatory (food-predicting cues), and consummatory/post-ingestive (food ingestion) phase. During the appetitive phase, the activity of AgRP neurons gradually increases to reach its highest levels to promote craving and subsequent drive to find food. Upon finding food-predicting cues (anticipatory phase), AgRP neurons are instantly inhibited and this inhibition is sustained during the consummatory/post-ingestive phase, where the animal ingests calories. In case of the discovery of inaccessible food or ingestion of an insufficient amount of calories, AgRP neuron activity only transiently decreases. In the transition from the consummatory to the appetitive phase, the dynamics of AgRP neuron activity remain unclear. *Image source: (Gouveia, de Oliveira Beleza, and Steculorum 2021).*

4. Neurotransmitters released by AgRP neurons

Arcuate NPY/AgRP neurons can drive feeding through the synaptic release of 3 distinct neurotransmitters: AgRP, NPY, and GABA (Krashes et al. 2013a; Tong et al. 2008; Hahn et al. 1998). NPY/AgRP neurons are commonly referred to as “AgRP neurons” because, unlike NPY and GABA, which are widely expressed in the CNS, AgRP is a signature molecule exclusively synthesized and released by specific neuronal populations (Broberger et al. 1998).

4.1. AgRP

Here's a brief history of how agouti-related peptide (AgRP) was discovered.

Agouti is a 131 amino-acid (AA) protein normally expressed in the skin where it regulates skin pigmentation: it acts as an antagonist of the melanocortin 1 receptor (MC1R) (Lu et al. 1994), blocking the production of dark black melanin and producing lighter yellow-orange pigmentation in mice (Bultman, Michaud, and Woychik 1992).

However, in 1992, researchers found that a mutation in the agouti gene (A^y : agouti yellow) leads to an excessive and ectopic expression of the agouti protein (Miller et al. 1993; Mountjoy et al. 1992), and results in mice with characteristic yellow skin color and a pronounced metabolic phenotype including severe obesity and diabetes (Yen et al. 1994) (Figure I.6.).

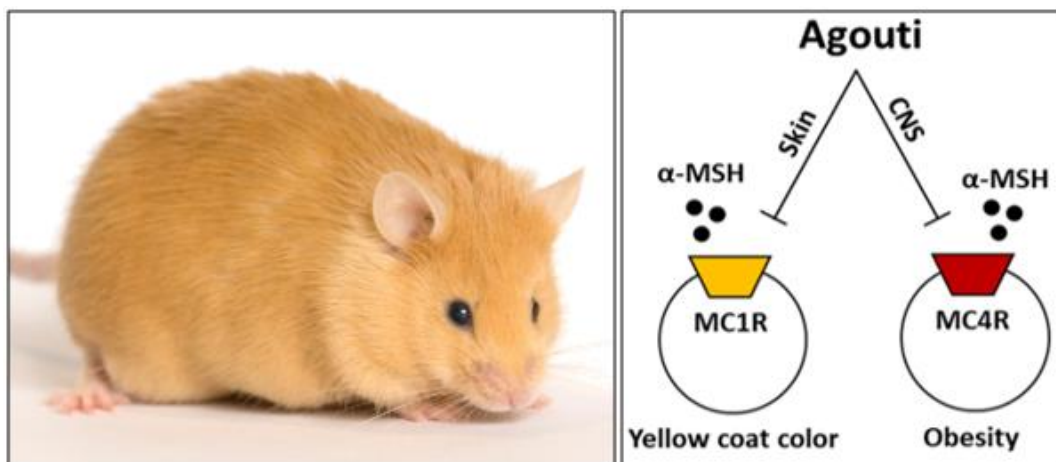


Figure I.6. First genetic model of obesity: the yellow obese mouse. A mutation at the agouti gene locus results in an excessive expression of agouti and a characteristic yellow obese phenotype in mice. Agouti antagonizes MC1R in the skin and MC4R in the CNS leading to yellow pigmentation and pronounced obesity. *Image on the left: Mouse Genome Informatics (MGI). Image on the right is adapted from (Fisher, Yagaloff, and Burn 1999).*

Later on, in 1997, the Agouti-related peptide (AgRP) gene was cloned and identified based on its homology to agouti. AgRP, 132 AA peptide, was found to be expressed in the hypothalamus as a natural antagonist of MC3R/MC4R (Shutter et al. 1997; Ollmann et al. 1997). Hence, the “agouti yellow obese syndrome” was attributed to 1) the excessive antagonism of MC1R in the skin and 2) the antagonism of MC4R in the CNS by agouti (Mountjoy 1994; Mountjoy et al. 1992) (Figure I.6.).

Arcuate AgRP expression levels increase dramatically in response to fasting or food deprivation. AgRP signaling via MC4R drives a sustained increase in food intake (Y. Chen et al. 2019). An icv injection of exogenous AgRP was shown to acutely stimulate feeding (D. J. Marsh et al. 1999). Finally, transgenic mice overexpressing AgRP develop hyperphagia and obesity, similar to the obesity observed in “agouti yellow obese syndrome” (A^y/a mice) (Graham et al. 1997; Ollmann et al. 1997).

In a very recent study published in 2024, extra-hypothalamic AgRP-expressing cells were identified in the brainstem, and more specifically in the hindbrain, at the level of the area postrema (AP), the adjacent subpostrema area (SubP), and the commissural nucleus of the solitary tract (cNTS) - collectively referred to as AgRP^{Hind} neurons. Food deprivation stimulates hindbrain AgRP expression. Moreover, chemogenetic activation of AgRP^{Hind} neurons results in hyperphagia and weight gain in adult mice with or without hypothalamic AgRP neurons, suggesting that AgRP^{Hind} neurons drive feeding independently of hypothalamic AgRP neurons (Bachor et al. 2024).

Other recent studies demonstrated the existence of peripheral AgRP-expressing cells, in the pituitary (S.-M. Liu et al. 2023) and adrenal glands (Gupta et al. 2017), that are also involved in food intake regulation. Chemogenetic inhibition of AgRP-expressing cells of the pituitary gland causes weight loss (S.-M. Liu et al. 2023). Meanwhile, short-term fasting leads to an increase in adrenal AgRP expression (Gupta et al. 2017). Regardless, we still ignore the mechanisms by which these cells may or may not impact energy homeostasis.

In humans and rats, plasma AgRP levels were found to be lower in the fed state when compared to the fasted state. Two hours after breakfast, AgRP levels drop by 39%. Conversely, two hours of fasting are sufficient to increase AgRP concentrations by up to 73%. Taken together, these pieces of evidence support the notion that plasma AgRP may serve as a biomarker for the transition from a fasted to a satiated state (Shen et al. 2002).

4.2. NPY

Neuropeptide Y (NPY) is a 36 AA neurotransmitter (Tatemoto 1982) belonging to the pancreatic polypeptide (PP) family and shares a 70% homology with the gastrointestinal (GI) peptide YY (PYY, described later) (Clark et al. 1984). NPY is the most prevalent and widely distributed neuropeptide in the brain. NPY-positive cells can be found in ARC, PVN, supraoptic nucleus, dorsal medial hypothalamic nucleus (DMH), paraventricular thalamic nucleus, - extra

hypothalamic: hippocampus, amygdala, NTS, nucleus accumbens and cerebral cortex (Morris 1989; Chronwall et al. 1985; Y. S. Allen et al. 1983). In the ARC, 90% of NPY-expressing neurons also express AgRP (Broberger et al. 1998). Peripherally, NPY is expressed in the liver, heart, kidney, spleen, urogenital tract, and vascular endothelial cells (Silva et al. 2005; Cavadas et al. 2001).

Correspondingly, NPY receptors are also widely spread. To date, five NPY G protein-coupled receptors have been cloned and identified in mammals: Y1, Y2, Y4, Y5, and Y6 (Pedragosa-Badia, Stichel, and Beck-Sickinger 2013; Cabrele and Beck-Sickinger 2000; Blomqvist and Herzog 1997). In the human central and peripheral nervous system, NPY receptors are encoded by distinct genes and have different tissue distribution, thereby indicating that NPY binds to its receptors to activate distinct signaling pathways involved in different physiological processes, including food intake regulation, hypothalamic-pituitary signaling, and cardiovascular physiology.

Hypothalamic NPY expression is best known for its orexigenic effects primarily mediated through binding to Y1 and Y5 receptors in PVN oxytocin neurons (Atasoy et al. 2012; Bo et al. 2016), a key target of NPY/AgRP neuron innervation (Stanley and Leibowitz 1985). Food restriction or deprivation correlates with increased NPY concentrations mainly in the ARC and the PVN (Sahu, Kalra, and Kalra 1988; Brady et al. 1990), resulting in enhanced feeding behaviors. Moreover, acute intracerebral administration of NPY induces appetite and promotes food intake (Stanley and Leibowitz 1985). When administered chronically, intra-PVN NPY induces hyperphagia and obesity (Frankish et al. 1995; Sainsbury et al. 1996).

4.3. GABA

The inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), is widely distributed in the CNS. It reduces neuronal excitability by inhibiting signal transmission. GABA, released into post-synaptic nerve terminals, binds to its receptors, subdivided into GABA_A and GABA_B receptors (Sigel and Steinmann 2012). GABA_A is the major inhibitory neurotransmitter receptor in the mammalian brain. It's classified as an ionotropic receptor (ligand-gated ion channel) and is considered in fast synaptic inhibition. On the other hand, GABA_B is a metabotropic receptor (G-coupled protein receptor) and is regarded as a slow synaptic inhibitor (Horvath et al. 1997; M. J. Allen, Sabir, and Sharma 2024).

Intrahypothalamic (PVN and VMH) injection of GABA agonist, muscimol, results in a dose-dependent increase in food intake (Kelly, Rothstein, and Grossman 1979). The importance of GABA in food intake regulation was also highlighted in transgenic mice with an AgRP neuron-specific deletion of vesicular GABA transporter (Vgat). The lack of GABAergic AgRP neurotransmission produced a lean phenotype with an attenuated diet-induced obesity in mice (Tong et al. 2008). Hence, GABA signaling in the PVN contributes at least in part to feeding evoked by chemogenetic stimulation of AgRP neurons.

4.4. Differential impact of AgRP neurotransmitters

A single i.c.v. injection of AgRP increases food intake for up to a week (Hagan et al. 2001; Semjonous et al. 2009), whereas injection of NPY enhances appetite for about an hour, illustrating the complementary but temporally distinct effects of AgRP and NPY on food intake (Stanley, Chin, and Leibowitz 1985). This redundancy may help explain the normal ongoing feeding in mice lacking either AgRP or NPY (Qian et al. 2002). These results are confirmed by a study using DREADD-induced activation of AgRP neurons in KO mice for one of the 3 neurotransmitters: the disruption of one neurotransmitter does not affect food intake, showing the redundancy in their effects. A combination of genetic manipulation and pharmacology showed that GABA or NPY are required for short-term initiation of food intake (<2h) whereas AgRP is involved in chronic food intake (Krashes et al, 2013).

Furthermore, during chemogenetic activation of AgRP neurons, targeted antagonism of either NPY-Y1 or GABA_A signaling at the level of PVN is not sufficient to block acute (~1hr) feeding responses in mice (Atasoy et al. 2012). However, using channelrhodopsin-2, the authors showed that targeted antagonism of either NPY-Y1 or GABA_A signaling during photostimulation of AgRP axons in the PVN inhibited food intake. This result shows that AgRP signaling in the PVN involves both NPY and GABA_A receptors. This difference shows that even though ARC to PVN projections are critical, other AgRP-targeted areas have an impact on food intake.

5. Postsynaptic targets of AgRP neurons : AgRP circuits in feeding

To orchestrate food-seeking and food-intake behaviors, AgRP neurons project to multiple downstream neuronal populations (Figure I.X.). Intrahypothalamic AgRP neuron projections include the lateral, ventromedial, and dorsal hypothalamic areas (LH, VMH, and DMH, respectively), suprachiasmatic area (SCN), preoptic area (POA), and the PVN. AgRP

projections also extend beyond the hypothalamic borders, innervating the central and medial nuclei of the amygdala (CeA and MeA, respectively), the anterior bed nucleus of the stria terminalis (aBNST), the paraventricular thalamic nucleus (PVT), the parabrachial nucleus (PBN), the lateral septum (LS), and the periaqueductal gray (PAG) (Broberger et al. 1998; D. Wang et al. 2015) (Figure I.7.).

The work of Betley et al. (2013a) shows that AgRP neuronal circuits use a segregated, parallel, and redundant output configuration. Activation of AgRP neuron projections in the PVN, aBNST, LH, PVT, medial preoptic area (mPOA), and medial amygdala (MeA) is sufficient to induce feeding (Padilla et al. 2016; Atasoy et al. 2012; Betley et al. 2013a; Li et al. 2019). This distinguished anatomical segregation of AgRP neuron projections points to a core feeding circuit that functions as an interaction hub for various cortical, visceral, and homeostatic inputs mediating different aspects of hunger (Dietrich et al. 2015; Padilla et al. 2016). Ongoing efforts to identify discrete AgRP neuron subpopulations through neurocircuit mapping and interrogation of functional projection sites are crucial to an advanced understanding of these neurons' broad behavioral and metabolic effects.

5.1. Paraventricular nucleus (PVN)

Among all postsynaptic targets of AgRP neurons, PVN receives the densest AgRP neuron projections ($\approx 30\%$) (Betley et al. 2013b; D. Wang et al. 2015). The PVN is an integrative center that represents the main output pathway from the hypothalamus (Sutton, Jr, and Olson 2016). Through direct connection with parasympathetic brainstem neurons or sympathetic neurons in the spinal cord, the PVN has a central position to control autonomic outflow to the liver, white and brown adipose tissue (Kalsbeek and Buijs 2021). It is notably involved in increasing fatty acid oxidation and lipolysis and participates in controlling energy balance and thermogenesis. As described in the previous section, the robust feeding response evoked by stimulating AgRP→PVN terminals underscores the important role of the melanocortin signaling pathway in the control of food intake (Atasoy et al. 2012; Betley et al. 2013b).

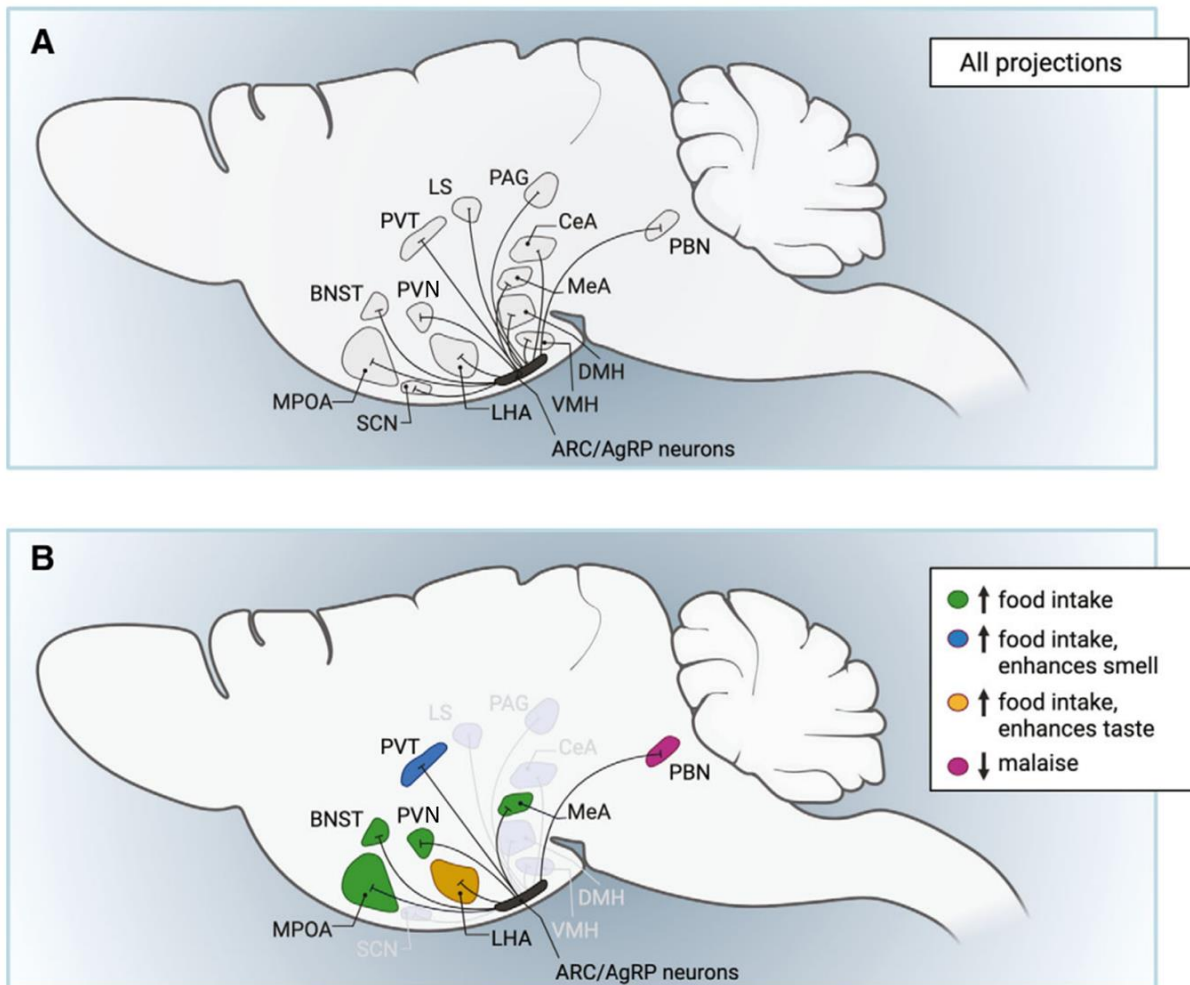


Figure I.7. Mapped illustration of AgRP neuron projections. (A) AgRP neurons project to a broad array of brain areas involved in various behaviors. (B) AgRP neurons projections to the LHA, PVN, BNST, PVT, MeA, and MPOA are involved in the regulation of food intake. Meanwhile, AgRP neurons projections to the PBN suppress discomfort and malaise. **Abbreviations:** AgRP, agouti-related peptide; ARC, arcuate nucleus; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; DMH, dorsomedial hypothalamus; LHA, lateral hypothalamic area; LS, lateral septum; MeA, medial amygdala; MPOA, medial preoptic area; PAG, periaqueductal gray; PVN, paraventricular hypothalamus; PBN, parabrachial nucleus; PVT, paraventricular thalamic nucleus; VMH, ventromedial hypothalamus; SCN, suprachiasmatic nucleus. *Image adapted from (Deem, Faber, and Morton 2022).*

5.2. Lateral hypothalamus (LH)

The LH is a richly heterogeneous structure that contains several genetically distinct cell populations, including GABAergic (LH^{GABA}) and glutamatergic (LH^{Glut}) subpopulations, involved in feeding, motivation, and reward (Stuber and Wise 2016). Activation of LH^{GABA} neurons tends to increase food intake and reward (Jennings et al. 2015), whereas activation of LH^{Glut} neurons suppresses feeding in food-deprived mice and produces aversion (Jennings et al. 2013). Conversely, selective inhibition of either LH^{GABA} or LH^{Glut} neurons elicits opposite responses, implying a key role for both subsets of neurons in food intake regulation (Stuber and Wise 2016).

The LH receives dense AgRP neuron projections. However, we still ignore how AgRP neurons control distinct LH neuronal subsets. Recent work suggests that hunger-induced activation of AgRP→LH^{Glut} neurons enhances sweet taste responsiveness and reduces aversive taste sensitivity (Fu et al. 2019). LH has long been known to regulate feeding behavior and taste processing by interacting with multiple cortical and subcortical gustatory regions (Hans-Rudi Berthoud and Münzberg 2011).

5.3. Paraventricular thalamus (PVT)

The PVT is a structure involved in arousal, but it is also suggested that PVT contains representations of valence (Penzo and Gao 2021). Apart from taste, hunger also enhances the attraction to food odor cues over other olfactory signals (Rolls 2015). Optogenetic stimulation of AgRP→PVT projections selectively promotes attraction to food odors, whereas inhibition of these neurons reduces food odor attraction in food-deprived mice. This effect is mediated by AgRP-neuron-derived NPY and its receptor, within the PVT, the NPY-Y5 receptor (Horio and Liberles 2021). Taken together, these findings suggest that hunger-induced activation of AgRP neurons enhances olfactory and orosensory perceptions, in part, by projecting to the PVT and the LH, respectively.

5.4. Parabrachial nucleus (PBN)

The PBN is a sensory hub for pain and aversion, linking interoceptive (visceral) and exteroceptive (gustatory) signals. It consists of a dozen small subnuclei that integrate signals of malaise, unpleasantness, internal discomfort, and sickness (Kent et al. 1996; Gauriau and Bernard 2002). These signals are provided by excitatory inputs from the NTS and the spinal cord (Wu, Clark, and Palmiter 2012). In contrast, hunger modulates inhibitory inputs arising from hypothalamic AgRP neurons to the PBN (Campos et al. 2017).

Multiple studies highlighted the importance of AgRP→PBN projections in food intake regulation. Firstly, AgRP neuron ablation in adult mice (inducing a loss of AgRP inhibitory GABAergic inputs onto postsynaptic targets) induces pronounced neuronal hyperactivity in the PBN, associated with severe anorexia and life-threatening weight loss (Luquet et al. 2005; Padilla et al. 2016). Conversely, injection of bretazenil (GABA agonist) in the PBN, mimicking AgRP→PBN inhibitory projections, rescued the anorectic phenotype induced by AgRP neuron ablation (Wu, Boyle, and Palmiter 2009), suggesting that the activation of PBN neurons plays a physiological role to suppress feeding when the inhibitory tone from AgRP neurons is reduced (Carter et al. 2013) (Figure I.8.).

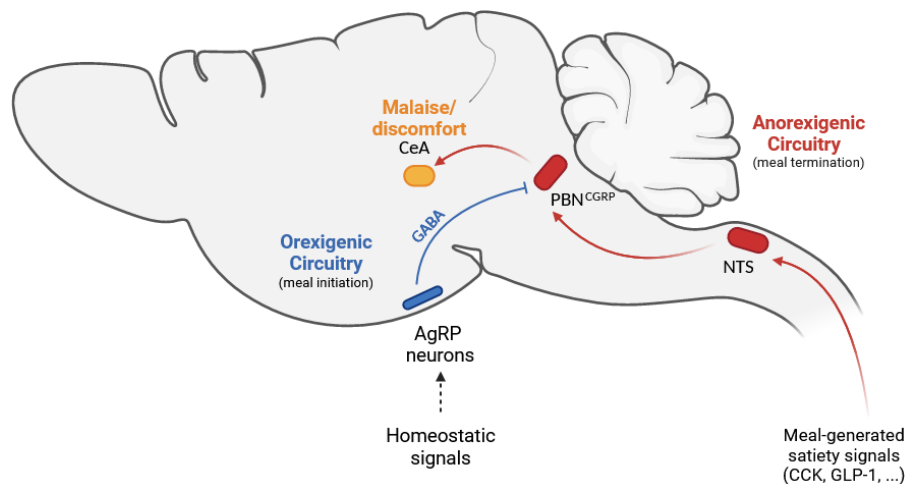


Figure I.8. Hunger-activated AgRP neurons inhibit PBN^{CGRP} neurons to induce appetite. Active AgRP neurons release GABA onto multiple postsynaptic targets including PBN^{CGRP} neurons, leading to enhanced appetite and meal initiation. Conversely, satiety peptides such as CCK trigger the NTS to send excitatory inputs into the PBN. Activation of PBN^{CGRP} projections to the CeA induces malaise/discomfort involved in suppressing food intake. *Image adapted from (Campos et al. 2016).*

Within the external lateral subnucleus of the PBN, AgRP neurons directly synapse with a population of neurons that express calcitonin gene-related peptides (PBN^{CGRP} neurons). The activation of these neurons potently suppresses food intake. When discomfort outweighs appetite, NTS→PBN^{CGRP} projections suppress appetite by stimulating the central nucleus of the amygdala (CeA), a brain region associated with fear, anxiety, and related responses (Carter et al. 2013). Visceral malaise induced by lithium chloride (LiCl), systemic inflammation, or even cancer activates the PBN^{CGRP}→CeA circuit inducing hypophagia (Carter et al. 2013; Campos et al. 2017) (Figure I.8.).

Furthermore, the AgRP→PBN^{CGRP} circuit also regulates meal size. Meal-related signals, such as the gastro-intestinal satiety peptide cholecystokinin (CCK), activate PBN^{CGRP} neurons, leading to a reduced appetite. Stimulation of AgRP→PBN^{CGRP} inhibitory projections is sufficient to downregulate CGRP neuron activity, inducing appetite and increasing food intake following the administration of CCK (Essner et al. 2017). Moreover, functional inactivation of CGRP neurons, mimicking AgRP→PBN^{CGRP} inhibitory projections, potently increases meal size - without affecting total food intake, due to a compensatory decrease in meal frequency (Campos et al. 2016).

6. Arcuate AgRP neurons are sufficient to initiate feeding

Soon after the discovery of AgRP, it was clear that while its overexpression resulted in hyperphagia and obesity (Graham et al. 1997; Mountjoy 1994; Miller et al. 1993), embryonic targeted gene deletion of NPY (J. C. Erickson, Clegg, and Palmiter 1996; Jay C. Erickson, Hollopeter, and Palmiter 1996), AgRP, or both did not alter food intake or reduce body weight (Qian et al. 2002). These contradictory findings implanted a strong confusion in the field!

A decade later, advanced studies reported the selective ablation (or elimination) of AgRP neurons in AgRP^{DTR} mice, a transgenic mouse model in which AgRP neurons are genetically engineered to express the diphtheria toxin receptor (DTR), which allows AgRP neuron ablation by the administration of diphtheria toxin (DT). In agreement with previous results, neonatal ablation of AgRP neurons produced minimal effect on food intake and body weight (Luquet et al. 2005). In stark contrast, acute depletion of AgRP neurons in adult mice inhibited food intake, leading to starvation, life-threatening anorexia, and even death (Luquet et al. 2005; Gropp et al. 2005). Additional models of either progressive postnatal cell death (A. W. Xu et al. 2005) or incomplete ablation (less than 50%) of AgRP neurons (Bewick et al.

2005) consolidate previous findings, showing that post-embryonic ablation of AgRP neurons leads to a lean hypophagic phenotype with reduced adiposity.

These results suggest that network-based compensatory mechanisms can develop after the ablation of AgRP neurons in neonates: the plasticity of the brain allows it to wire and rewire itself in order to adapt to the lack of AgRP neurons, resulting in normal ongoing feeding (“Making the Most of Transgenic Mice” 1998). In fact, AgRP neurocircuitries are not fully formed in mice before day 21 after birth (Nilsson et al. 2005; Bouret, Draper, and Simerly 2004). Thus, neonatal ablation performed prior to this time point is unlikely to inhibit feeding, since projections arising from AgRP neurons have not yet reached upper feeding structures. Taken together, these findings suggest that NPY/AgRP neurons are necessary for feeding in adult mice but their ablation is tolerated in neonates.

However, this well-established notion was recently challenged by a study conducted by Cai et al. (2023). It was reported that complete ablation of arcuate AgRP neurons caused no apparent alterations in daily ad libitum feeding, but impaired refeeding after 24 hours fast, suggesting that AgRP neurons are not indispensable for feeding in adult mice (Cai et al. 2023). It seems that controversies between Luquet et al. (2005) and Cai et al. (2023), concerning whether or not arcuate AgRP neurons are required for feeding and body weight maintenance in adult mice, may be due to methodological differences: both studies employed the DT-induced AgRP neuron ablation in adult AgRP^{DTR} mice. However, in Cai et al. (2023) work, DT was not delivered peripherally (i.p.) but rather centrally (i.c.v. injection) to avoid ablating peripheral AgRP-expressing cells. These observations were made under standard diet, however, when providing high palatability high-fat high-sugar (HFHS) diet, normal feeding response is restored after a fast in AgRP-ablated mice. This result suggests that AgRP neurons are indispensable when food is highly palatable (Denis et al. 2015).

Our understanding of the hypothalamic AgRP neuron role in feeding accelerated with the application of both optogenetic (Mattis et al. 2011) and chemogenetic approaches (Roth 2016). Selective activation of AgRP neurons in sated (ad libitum) mice, enhances food-seeking behavior, induces voracious feeding, and reduces energy expenditure (Aponte, Atasoy, and Sternson 2011; Atasoy et al. 2012; Krashes et al. 2011b). Moreover, chronic activation of AgRP neurons resulted in marked hyperphagia and weight gain (Zhu et al. 2020), whereas acute inhibition of AgRP neurons reduces food intake (Krashes et al. 2011b). Together, these findings demonstrate that AgRP neuronal activity is sufficient to initiate the full feeding sequence.

7. AgRP neurons in energy homeostasis

The appropriate utilization, storage, and conversion of nutrients in peripheral tissues, referred to as nutrient partitioning, is a fundamental process to adapt the body to nutritional and metabolic challenges. Hence, it's critical for maintaining energy homeostasis.

7.1. Energy expenditure and substrate utilization

A common frustration for individuals attempting to lose weight through dieting is the significant compensatory decrease in energy expenditure (EE) observed during fasting or caloric restriction (Weyer et al. 2001). Based on these observations, recent studies investigated the potential involvement of AgRP neurons in regulating peripheral substrate utilization and lipogenesis.

Early studies showed that an i.c.v. administration of amino-terminal (N-terminal) fragments of AgRP (AgRP₂₅₋₅₁ and AgRP₅₄₋₈₂) induces a potent and long-lasting decrease in EE and an increase in adiposity and body weight in rats, despite the absence of hyperphagia and cross-reactivities with MC4R (Goto et al. 2003). This suggests that the N-terminal region of AgRP plays a regulatory role in energy metabolism.

Likewise, an i.c.v. or intra-PVN administration of NPY results in a reduced EE, an effect partially due to reduced sympathetic outflow to thermogenic tissues such as the brown adipose tissue (BAT) (Egawa, Yoshimatsu, and Bray 1990). BAT-mediated adaptive thermogenesis dissipates energy (heat) and protects against obesity in both rodents and humans (Machado et al. 2022; Kajimura and Saito 2014).

Advanced chemogenetic investigations corroborated these early pharmacological studies, and emphasized the role of AgRP neurons in the suppression of EE: AgRP stimulation not only rapidly increases the respiratory exchange ratio (RER), indicating enhanced carbohydrate utilization and reduced lipolysis (Krashes et al. 2011a; Joly-Amado et al. 2012; Cavalcanti-de-Albuquerque et al. 2019; Krashes et al. 2013b; J. Zhang et al. 2023), but also suppresses the browning of the white adipose tissue (WAT) (Ruan et al. 2014). WAT primarily stores energy as triacylglycerol (TAG), commonly referred to as fat or triglycerides (Figure I.9.). its excess lies at the core of obesity and associated metabolic disorders (Straznicky, Nestel, and Esler 2009).

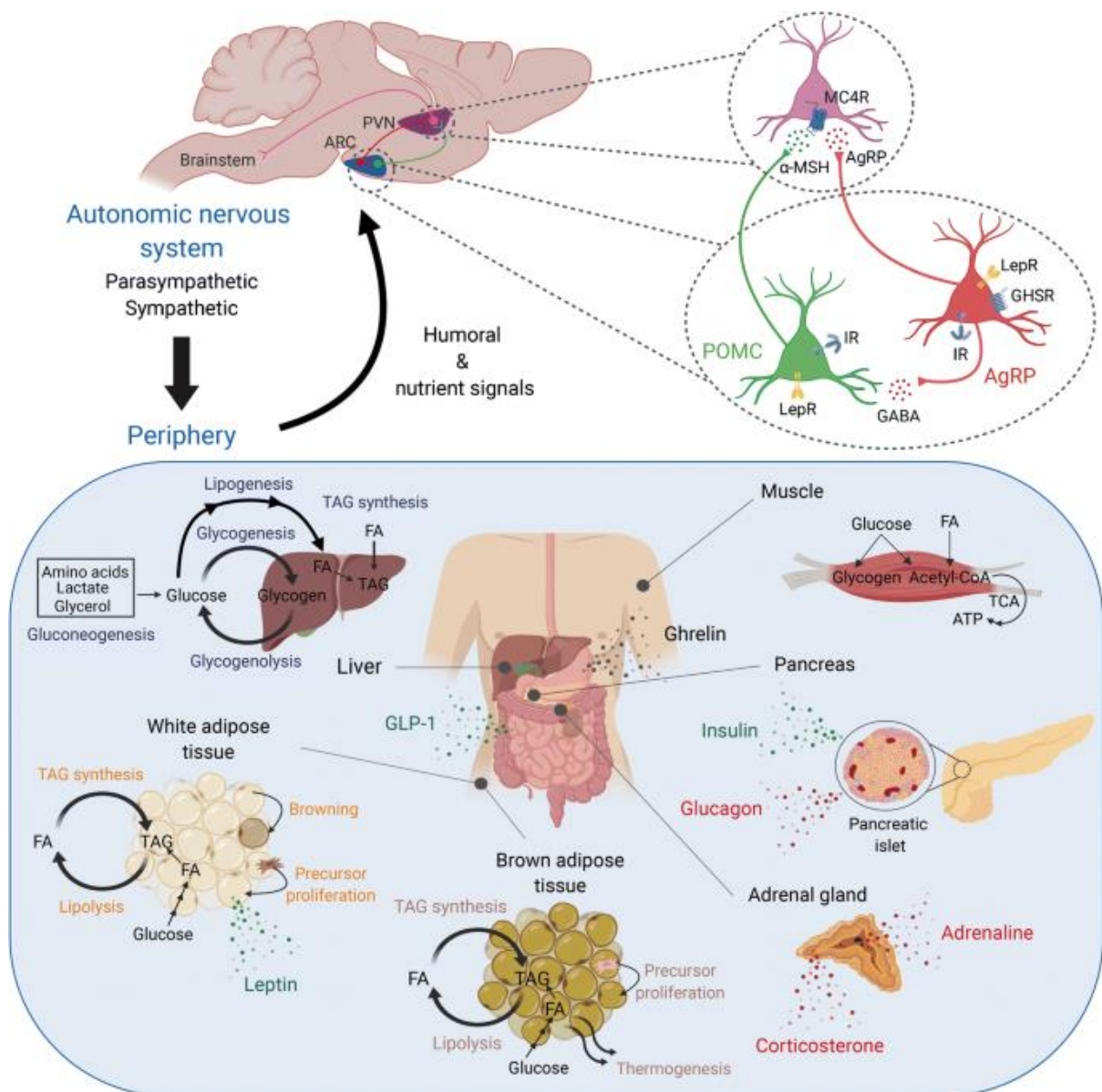


Figure I.9. Central control of peripheral nutrient partitioning. In the ARC, the activity of AgRP and POMC neurons is regulated by circulating metabolic signals. In turn, the release of α -MSH and AgRP modulates the activity of post-synaptic PVN^{MC4R} neurons projecting to the brainstem and regulating the activity of the autonomic nervous system. The autonomic nervous system (ANS) innervates all metabolic tissues and endocrine organs via the parasympathetic and sympathetic systems. These ANS orchestrate the utilization (transport and oxidation), storage (glycogen and TAG), and conversion (gluconeogenesis and lipogenesis) of glucose and fatty acids (FA), and secretion of pancreatic hormones which, in turn, contribute to nutrient partitioning. Moreover, the hypothalamic–pituitary–adrenal axis regulates corticosterone and adrenaline secretion. GLP-1, glucagon-like peptide 1; GHSR, growth hormone secretagogue receptor (ghrelin receptor); IR, insulin receptor, LepR, Leptin receptor; TCA, tricarboxylic acid cycle. *Image source: (Manceau, Majeur, and Alquier 2020).*

Furthermore, evidence of a role for AgRP neurons in nutrient partitioning emerged from studies conducted on adult mice after neonatal ablation of AgRP neurons. Mice lacking AgRP neurons develop late-onset obesity and hyperinsulinemia when fed a regular chow diet (Joly-Amado et al. 2012). Here, obesity was not the result of an increased food intake, but rather, increased hepatic triglycerides synthesis from carbohydrates and a shift in nutrient oxidation that favored lipid (TAG, long-term energy storage) instead of carbohydrate (glycogen, short-term energy storage) utilization. The enhanced lipid utilization in muscles and adipose tissues of AgRP-ablated mice was a metabolic advantage during high-fat feeding, reducing glucose intolerance, fat mass accumulation, and diet-induced obesity (Joly-Amado et al. 2012). In line with these observations, ablation of AgRP neurons in adult mice reduces fat mass accumulation in response to liquid diet infusion (to avoid starvation) when compared to control mice receiving the same amount of infused calories.

Together, these findings demonstrate that fasting-evoked AgRP neuron activation shifts metabolism toward energy conservation and lipid storage (fat accumulation and lipogenesis), mediating adiposity and weight gain (Cavalcanti-de-Albuquerque et al. 2019; Joly-Amado et al. 2012), partly, by reducing EE in peripheral tissues (WAT and BAT). Weight loss strategies aim at preventing this shift to reduced metabolic rate despite restricted caloric intake.

7.2. Glucose homeostasis

In addition to controlling feeding behavior and EE, AgRP neurons were found to regulate glucose homeostasis. Early pharmacological studies show that an i.c.v. administration of NPY results in a reduced peripheral insulin sensitivity that is independent of food intake and body weight. This effect was partly attributed to reduced insulin-induced suppression of hepatic glucose production (Marks and Waite 1997; van den Hoek et al. 2004), a key determinant of fasting blood glucose levels. Moreover, selective inactivation of insulin receptors in AgRP neurons also impairs hepatic glucose production and insulin sensitivity (Könner et al. 2007).

In line with these findings, chemogenetic and optogenetic activation of AgRP neurons induce insulin resistance by impairing insulin-stimulated glucose uptake into BAT (Steculorum et al. 2016). Interestingly, insulin resistance, induced by artificially activating AgRP neurons, does not occur in NPY-deficient mice. However, selective NPY re-expression in AgRP neurons is sufficient to rescue insulin sensitivity. These observations reveal a pivotal role of NPY-signaling in mediating glucose homeostasis induced by the activation of AgRP neurons

(Engström Ruud et al. 2020). Consistent with this interpretation, activation of POMC neurons does not alter insulin sensitivity (Steculorum et al. 2016), suggesting that these metabolic actions are driven by NPY/AgRP neurons independently of the melanocortin signaling pathway.

8. Modulators of AgRP neuron activity

8.1. Afferent input to AgRP neurons

In contrast to our rapidly growing understanding of AgRP neuron projections or efferents and their contributions to food intake, food-seeking behaviors, and metabolism, information regarding afferent input to these neurons remains poorly investigated. Retrograde tracing studies (D. Wang et al. 2015; Krashes et al. 2014) and ex vivo electrophysiological approaches (Krashes et al. 2014; Garfield et al. 2016) show numerous inputs to AgRP neurons, with the largest innervations arising from the PVN and the DMH (Figure I.10.).

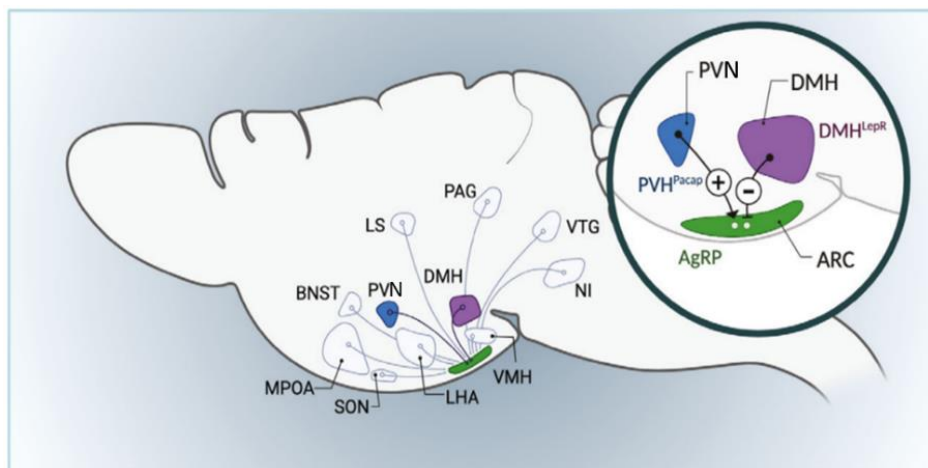


Figure I.10. Functionally mapped AgRP neuron afferents. AgRP neurons receive afferent input from several distinct brain areas, including the PVN and the DMH. PVN sends strong, excitatory, glutamatergic input to arcuate AgRP neurons that stimulate feeding. On the other hand, AgRP neurons receive inhibitory GABAergic input from leptin-receptor-expressing neurons in the DMH that, when activated, inhibit feeding. ARC, arcuate nucleus; AgRP, agouti-related peptide; PVN, paraventricular hypothalamus; PACAP, pituitary adenylate cyclase-activating peptide; DMH, dorsomedial hypothalamus; LepR, leptin receptor; BNST, bed nucleus of the stria terminalis; LS, lateral septum; LHA, lateral hypothalamic area; MPOA, medial preoptic area; NI, nucleus incertus; PAG, periaqueductal gray; PBN, parabrachial nucleus; SON, supraoptic nucleus; VMH, ventromedial hypothalamus; VTG, ventral tegmental area. *Image source adapted from (Deem, Faber, and Morton 2022).*

In the PVN, a small subset of neurons expressing the pituitary adenylate cyclase-activating polypeptide (PACAP) sends strong excitatory glutamatergic afferents to arcuate AgRP neurons. Chemogenetic activation of PVN^{Pacap} activates AgRP neurons and is sufficient to drive food intake in sated mice (Krashes et al. 2014).

In contrast, GABAergic neurons in the DMH that express the leptin receptor (DMH^{GABA/LepR}) send inhibitory input to AgRP neurons (Garfield et al. 2016). Inhibition of AgRP neuron activity upon food presentation in fasted mice is provided, at least in part, by activation of DMH^{GABA/LepR} neurons (Krashes et al. 2014; Garfield et al. 2016). While optogenetic activation of DMH^{GABA/LepR}→ARC afferents rapidly reduces food intake in fasted mice, chemogenetic silencing of these neurons does not increase food intake, suggesting that these neurons are not critically required for maintaining satiety (Garfield et al. 2016). Indeed, besides the direct action of leptin on AgRP neurons themselves, leptin inhibition of AgRP neurons is mediated at least in part by activating upstream leptin-responsive DMH neurons (DMH^{GABA/LepR}).

8.2. Sensory input

Besides the impact of interoceptive signals (cf part Peripheral metabolic signals involved in food intake regulation) recent studies have shown the impact of exteroceptive signals on AgRP neurons activity. Since their discovery, arcuate AgRP neurons have been recognized as pivotal hunger neurons. Until recently, the well-established notion of how AgRP neurons control feeding was that, upon fasting, hunger hormones activate AgRP neurons to initiate feeding, and then, food ingestion leads to the release of multiple satiety-related hormones that inhibit AgRP neurons to terminate the meal (Varela and Horvath 2012; Timper and Brüning 2017).

This notion was challenged by the use of new technical advances allowing real-time in vivo recording of AgRP neuronal activity. Assessing the dynamics of AgRP neuronal activity refuted our long-lasting assumption of how these neurons are regulated, by uncovering that, AgRP neurons are inhibited within seconds by the mere sight and smell of food, or by conditioned cues that predict food availability (Y. Chen et al. 2015; Betley et al. 2015; Mandelblat-Cerf et al. 2015). These responses were too fast to be mediated by a hormonal signal, suggesting that they arise from changes in afferent neuronal input. Intriguingly, this rapid, but transient sensory-driven inhibition of AgRP neurons occurs before a single bite of

food is consumed and then persists for the duration of the ensuing meal (Y. Chen and Knight 2016).

The pre-consummatory inhibition of AgRP neurons reveals that these neurons are not solely inhibited after nutrient ingestion, but they're already inhibited upon food detection (Figure I.11.). This anticipatory regulation of AgRP neuron activity elicits the rapid inhibition of foraging and other appetitive behaviors once food is discovered.

Furthermore, Su, Alhadeff, and Betley (2017b) showed that a single exposure to nutritive food trains the sensory system to drive rapid anticipatory AgRP neuron inhibition in response to future presentations of the same food, demonstrating that the learned associations between sensory cues (e.g., taste, smell) and nutritive value (e.g., calories, post-ingestive metabolic signaling) of foods strongly influences eating behaviors by enhancing the reward value of the nutrient-paired flavor. AgRP neuron activity indeed assimilates sensory and nutritive signals –in a sex-dependent manner- to guide flavor preference, as stated in (Nyema et al. 2023). Moreover, the palatability of food correlates with a greater inhibition of AgRP neuron activity: sensory information with less or no calorie content (such as chow diet or an object, respectively) induces a smaller effect on AgRP neuron activity.

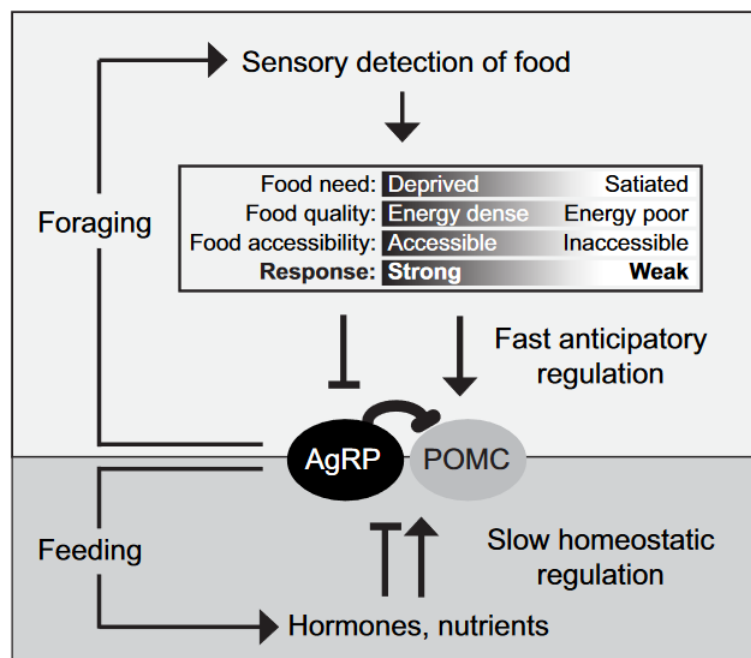


Figure I.11. Fast and slow regulation of arcuate AgRP and POMC neurons. Under energy deficit conditions, active AgRP neurons drive feeding behaviors (foraging and feeding). First, in the appetitive phase, sensory detection of food leads to a fast - but transient (30-minute) -

anticipatory inhibition of AgRP neurons. Then, following the consummatory phase, circulating hormones and nutrients ensure a slow, long-lasting, homeostatic inhibition of AgRP neurons. *Image source:* (Y. Chen et al. 2015).

Besides the sight and smell of food, recent advances suggest that AgRP neurons respond to other sensory inputs, unrelated to the status of body nutritional stores, including environmental temperature (Deem et al. 2020) and circadian rhythms (Mandelblat-Cerf et al. 2015).

8.3. Thermal sensory input

In response to cold exposure, energy expenditure rises in crescendo to maintain core temperature and avoid hypothermia (Rintamäki 2007; Tansey and Johnson 2015). Food intake increases correspondingly to preserve energy homeostasis and body fat mass (Jordan 1995; Cannon and Nedergaard 2004). While the adaptive feeding response to cold exposure has been attributed to signals of negative energy balance, this assumption seems unlikely. Indeed, plasma leptin levels decrease only in response to prolonged cold exposure (Bing et al. 1998; Puerta et al. 2002; Tang, Cui, and Wang 2009) whereas AgRP activation, required for cold-induced food intake, occurs so rapidly as to be incompatible with a compensatory response to a negative energy balance: Deem et al. (2020) reported that selective silencing of AgRP neurons blocks the effect of cold exposure to induce feeding but has no effect on energy expenditure. Instead, cold-induced AgRP neuron activation appears to be part of a predictive feedforward strategy that recognizes future needs for increased calorie intake to meet changing environmental circumstances.

On the other hand, when core body temperature increases (during exercise, exposure to warm environmental temperatures, or even during fever), food intake declines (Brobeck 1985). This heat-induced appetite suppression was attributed to the activation of POMC neurons that express a temperature-sensitive ion channel, the transient receptor potential vanilloid 1 (TRPV1). A slight increase in body temperature leads to a TRPV1-dependent activation of POMC neurons, which suppresses appetite in mice (Vicent, Mook, and Carter 2018).

8.4. Other sources of modulation of AgRP neurons

8.4.a. Circadian input

Circulating levels of nutritionally relevant hormones that regulate AgRP neuron activity (such as leptin, ghrelin, and insulin) are not static. Instead, they follow cyclical patterns associated either with light-dark cycles or with meal time (Oster et al. 2006). Therefore, AgRP neuron activity follows a rhythm that progressively increases across the light cycle inducing food consumption in awake mice (Mandelblat-Cerf et al. 2015). AgRP neurons integrate time-of-day information of past feeding experiences with current metabolic needs to predict circadian feeding time (Sayar-Atasoy et al. 2024). Recent work suggests that autonomous circadian regulation of AgRP neurons is required for transcriptional rhythmic responses involved in feeding (Cedernaes et al. 2019).

8.4.b. Reproductive hormones

Reproductive hormones play a role in regulating appetite, feeding behaviors, and energy metabolism (Figure I.12.). Impaired levels of reproductive hormones are implicated in the development of eating disorders and obesity. For instance, estrogen suppresses food consumption, while progesterone and testosterone promote appetite (Hirschberg 2012).

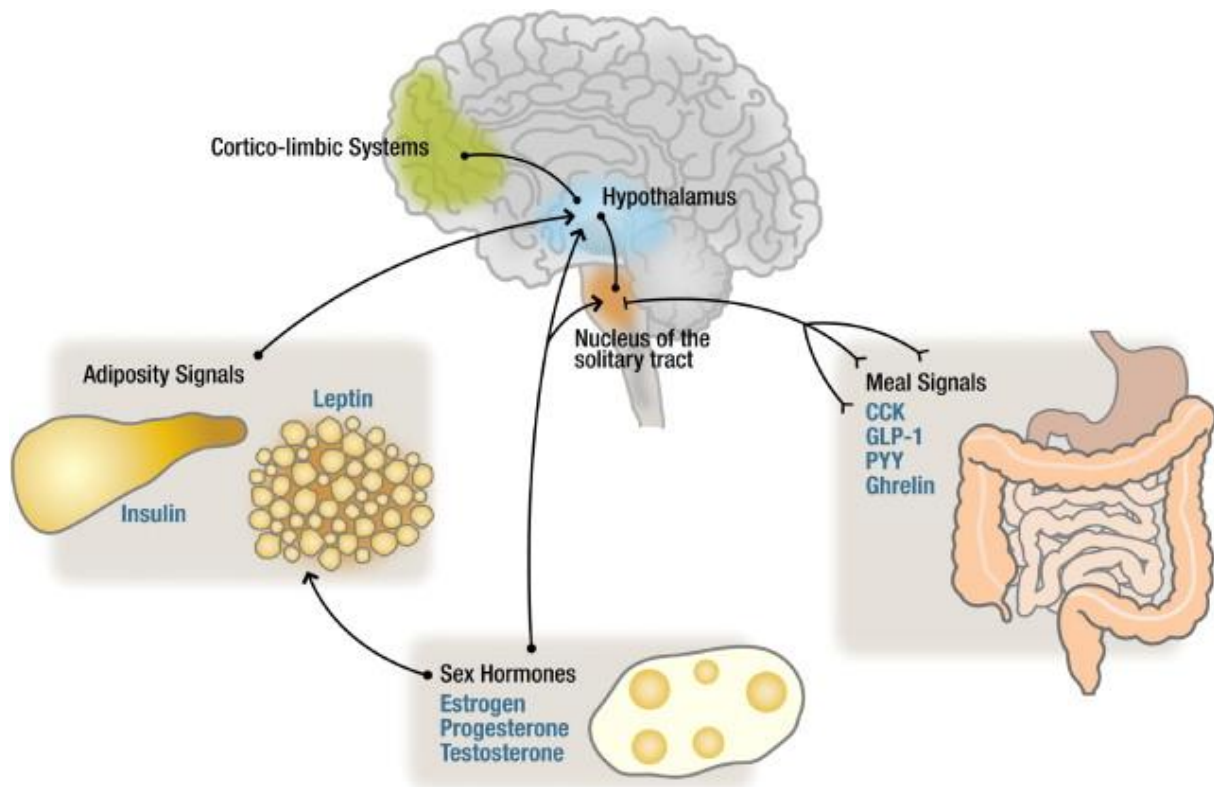


Figure I.12. Afferent mechanisms involved in regulating appetite and energy balance. The peripheral feeding system generates gastrointestinal peptides that decrease short-term food intake. These signals travel via vagal afferent fibers to the nucleus of the solitary tract in the brainstem. Subsequently, this information is relayed to the hypothalamus, activating anorexigenic systems. Long-term regulation of food intake involves circulating adiposity signals, providing negative feedback to the brain, and reducing food intake. Additionally, sex hormones influence appetite and energy metabolism coordination with reproductive states through both central mechanisms and peripheral effects on adipose tissue. The cortico-limbic system is involved in the hedonic response to food. *Image source: (Hirschberg 2012).*

Here, I describe recent findings regarding interactions between estrogen levels and AgRP neuron activity. Other hormones influence food intake but there's no evidence in the literature linking their actions to a direct modulation of AgRP neuron activity.

Estrogen, a crucial hormone essential for sexual reproduction, is involved in feeding and energy balance regulation. In female mammals, feeding decreases during the peri-ovulatory period of the ovarian cycle, which coincides with a significant increase in circulating estrogen levels (Asarian and Geary 2006) (Figure I.13.).

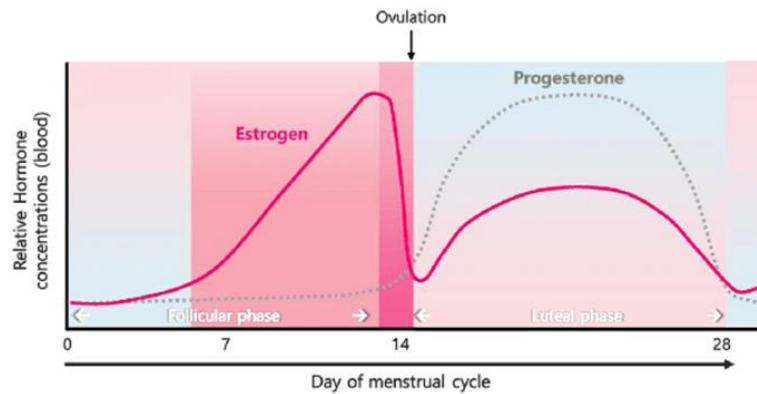


Figure I.13. Estrogen level during the menstrual cycle. The menstrual cycle is divided into two phases in humans: the follicular phase and luteal phase. *Image adapted from (Hong and Choi 2018).*

Estrogen deficiency results in increased feeding and adiposity in both rodents and humans (Heine et al. 2000; Wade and Gray 1979). In addition, ovariectomy increases food intake and body weight, which can be normalized by an adequate estrogen treatment (Asarian and Geary 2002; Gao et al. 2007). Centrally, selective silencing of the estrogen receptor alpha ($ER\alpha$) in the VMH results in severe obesity and metabolic syndrome (Musatov et al. 2007).

Interestingly, a study conducted by Olofsson, Pierce, and Xu (2009) showed that cyclic changes in feeding coincide with changes in hypothalamic expression of AgRP and NPY. These cyclic changes in feeding are abolished in mice with degenerated AgRP neurons – but menstrual cycles remain intact. Furthermore, central administration of 17β -estradiol suppresses fasting-induced cfos activation of neurons expressing AgRP and NPY, leading to a decreased food intake in controls but not in mice lacking AgRP neurons. However, NPY/AgRP neurons do not express the key mediator of estrogen’s anorexigenic effects, $ER\alpha$, suggesting that estrogen may regulate these neurons indirectly via presynaptic neurons that do express $ER\alpha$. Hence, NPY/AgRP neurons are essential mediators of estrogen's anorexigenic function.

Nevertheless, Sotonyi et al. (2010) reported an increased POMC neurons excitatory activity in response to estradiol, resulting in a suppressed appetite. Changes in AgRP neuron activity during other physiological conditions such as pregnancy, lactation (Makarova, Kochubei, and Bazhan 2010), or growth, require further investigation.

9. AgRP neurons in metabolic diseases

The worldwide prevalence of obesity has been increasing at an alarming rate in the past 50 years, reaching pandemic levels (Blüher 2019). Obesity is associated with several chronic pathophysiological diseases, such as type 2 diabetes, hypertension, cardiovascular diseases, certain types of cancers, and neurodegenerative disorders (Global BMI Mortality Collaboration et al. 2016), collectively referred to as metabolic syndrome.

9.1. Obesity

Having more than doubled since 1980, obesity is inflicting a significant public health burden, that according to the World Health Organization (WHO) (<http://www.who.int/mediacentre/factsheets/fs311/en/>) originates mostly from an increased consumption of energy-dense food and a sedentary lifestyle. The primary measurement method used to assess overweight and obesity is the body mass index (BMI), obtained by dividing an individual's weight in kilograms by the square of their height in meters (kg/m^2). An individual is categorized as overweight with a BMI exceeding 25, and as obese with a BMI surpassing 30.

Given that both diet and obesity cause dysfunction in CNS mechanisms responsible for regulating energy balance (Michael W. Schwartz et al. 2017), impairments in the intrinsic sensitivity of NPY/AgRP neurons to hormonal or nutrient signals, or disruptions in the connectivity between the internal energy state and AgRP neurons may explain this dysfunction.

High fat-induced obesity leads to significantly increased mRNA levels of AgRP (Harrold, Williams, and Widdowson 1999; Lin, Storlien, and Huang 2000). Correspondingly, electrophysiological investigations revealed that AgRP neurons are hyperactive in long-term high-fat-diet (HFD)-fed mice and that this effect is dependent on the diet composition but not the calorie content (Wei et al. 2015). The same authors showed that AgRP to PVN projections are significantly decreased after 3 weeks of a high-fat diet.

A recent study conducted by Beutler et al. (2020) shows that high fat-induced obesity in mice changes the dynamics of AgRP neuron activity in response to surrounding food cues. Interestingly, subsequent weight loss restores the sensitivity of AgRP neurons to external sensory cues. HFD also attenuates AgRP neuron responsiveness to circulating hormones including ghrelin (Briggs et al. 2010), leptin (Wei et al. 2015), and cholecystokinin (Beutler et al. 2020). Remarkably, diet-induced obesity abolished the inhibition of AgRP neurons in

response to intragastric infusion, whereas intragastric infusion of sugar or protein does not affect this modulation (Beutler et al. 2020). Weight loss fails to rescue AgRP responsiveness to intragastric infusion of lipids, ghrelin, or CCK. Nonetheless, other studies showed that leptin sensitivity could be restored if high-fat mice were switched back to a standard chow diet (Wei et al. 2015) or by simply reducing the fat content in their diets (Enriori et al. 2007). These findings reveal that obesity triggers a broad dysregulation of hypothalamic AgRP neuron activity that is only partially reversed by weight loss and may contribute to the hardships of maintaining reduced body weight (Mazzone et al. 2020).

Finally, Ca^{2+} recording using fiber photometry showed that, although AgRP neurons continue to increase their activity in response to a fast, HFD exposure reduces AgRP neuron basal activity (Mazzone et al. 2020).

9.2. Type 2 diabetes

Type 2 diabetes (T2D), also known as "diabetes mellitus", is a chronic metabolic disorder characterized by high blood sugar levels (hyperglycemia), insulin resistance, and relative insulin deficiency. It typically develops in adulthood, although it can occur in children as well. In type 2 diabetes, the body either doesn't produce enough insulin or becomes resistant to the effects of insulin.

AgRP neurons may contribute to diabetic hyperglycemia, as their activity is increased across several rodent models of diabetes, including leptin-deficient ob/ob mice (M W Schwartz et al. 1996), Zucker diabetic fatty rats (Park et al. 2005) and mice with streptozotocin-induced diabetes mellitus (STZ-DM) (Havel et al. 2000). STZ is a chemical agent that is selectively toxic to pancreatic β -cells and causes loss of insulin.

In the STZ-DM model, chemogenetic inhibition of AgRP neurons reduces diabetic hyperphagia and hyperglycemia (J. Xu et al. 2018). Unexpectedly, the amelioration of diabetic hyperglycemia by central leptin administration is associated with the suppression of hypothalamic AgRP mRNA expression (Hwang et al. 2022; Bentsen et al. 2020). Finally, CRISPR-mediated deletion of leptin receptor in AgRP neurons causes severe obesity and diabetes and is required for leptin's antidiabetic effects in STZ-DM (J. Xu et al. 2018), these findings collectively indicate that inappropriate activation of AgRP neurons contributes to diabetic hyperglycemia.

CHAPTER II

The Olfactory System

Among all sensory modalities, olfaction or the sense of smell represents one of the oldest senses in terms of evolution and plays a major role in survival. As a chemical sensor, the olfactory system (OS) detects, encodes, and discriminates among thousands of small airborne chemicals (odorants) which are volatile, light-weighted, organic hydrophobic compounds (Breer 2003). However, the OS is not only vital for identifying and discriminating surrounding odorants, it elicits myriad of behaviors including feeding, finding mates, and escaping predators.

The sense of smell operates independently and in conjunction with other senses. With a sniff, volatile odorants are inhaled into the nasal cavity by the respiratory airflow. In the rooftop of the nasal cavity, the olfactory epithelium (OE) represents the main site for the detection of airborne chemicals (Figure II.1.). When we chew and swallow food, organic compounds reach the OE through both the nostrils (orthonasal pathway) and the mouth (retronasal pathway) (Blankenship et al. 2019). Eating is indeed one of the most pleasant multisensory experiences of everyday life, where the flavor of food is a combination of smell, taste, and touch (chemosensing, trigeminal system). If the sense of smell is impaired, a condition called anosmia, one must rely almost solely on the sense of taste. As a result, food may not be perceived as richly flavored as it should be.

In comparison to other senses, the sense of smell was historically regarded as unimportant and often dismissed as the most “ungrateful” sense, leading to neglect in research pursuits. However, interest in understanding how humans perceive smell has increased after the discovery of the olfactory receptor (OR) superfamily of genes by Buck and Axel (Nobel Prize in Physiology or Medicine 2004) which represented a significant scientific breakthrough.

In this chapter, we discuss multiple findings for a better understanding of how the OS detects and encodes odorants while focusing on the role of the olfactory bulb (OB) in processing the olfactory information.



Figure II.1. The mammalian olfactory system. Comparative representation of rodent and human olfactory systems. Volatile molecules are detected by the olfactory epithelium, located at the rooftop of the nasal cavity. The olfactory message is then transmitted to the olfactory bulb to be processed. Finally, the olfactory bulb transmits the encoded olfactory message to the olfactory cortex.

1. The olfactory epithelium

1.1. Detection of volatile molecules in the main olfactory epithelium

Prior to reaching higher ascending olfactory pathways, smell starts when airborne odorants enter the nose. At the back of the nasal cavity of most mammals resides the main olfactory sensory organ, the OE, which is critically essential to the detection of odorant molecules. The OE comprises primarily olfactory sensory neurons (OSNs) and support cells, including sustentacular cells, basal and horizontal globose cells, and olfactory ensheathing cells (Figure II.2.). While support cells mainly secrete mucus and sustain the integrity of the epithelium and mucosa, OSNs are bipolar neurons responsible for the detection of volatile chemicals. Their apical dendrites extend, through the supporting cell layer, to reach the surface of the main olfactory epithelium (MOE), where the olfactory cilia are immersed in mucus, exposed to inhaled molecules. These molecules are solubilized in the mucus of the OE, where they can bind to the surface of OSNs via G-protein coupled receptors (GPCR), referred to as olfactory receptors (ORs) (Buck and Axel 1991).

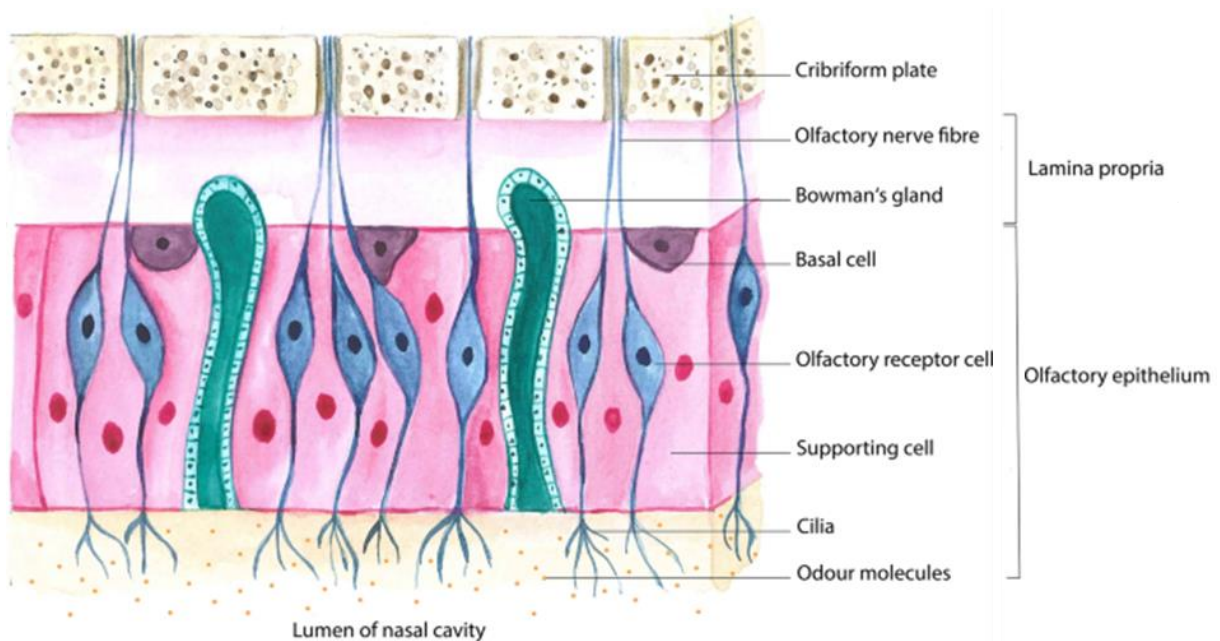


Figure II.2. Anatomy of the olfactory mucosa. The olfactory mucosa (OM) consists of the olfactory epithelium (OE) and the lamina propria. The OE is in direct contact with the external environment. Olfactory receptor cells, also known as olfactory sensory neurons, detect inhaled odor molecules and send olfactory input to the OB via distinct olfactory nerve fibers. *Image adapted from: Olfactory Epithelium Anatomy.*

Unlike other CNS neurons, OSNs are directly exposed to the external environment. While this direct contact allows OSNs to detect odors, it also exposes them to toxins, bacteria, and viruses leading to cell death. Therefore, the capacity for neurogenesis and the replacement of OSNs is critical for maintaining a functional olfactory sensory system throughout the adult lifespan (Browne, Crespo, and Grubb 2022). In mice, it takes almost 12 weeks to completely regenerate all OSNs (Cheung et al. 2014).

1.2. Olfactory receptors

The understanding of the functional organization of the olfactory system was made possible by the work of Linda Buck and Richard Axel (Buck and Axel 1991). The OR family is the largest mammalian gene family known so far. An estimated 1500 olfactory-receptor-encoding genes exist in the mouse genome (X. Zhang and Firestein 2002; Young et al. 2002), and over 900 distinct genes in humans (Glusman et al. 2001). The African elephant has the largest family of OR genes known to date, with nearly 1950 genes (Niimura, Matsui, and Touhara 2014).

Each OSN expresses only one specific OR gene: once a functional OR gene is expressed, the OR itself exerts a negative feedback control blocking the expression of other OR genes within the same OSN (S et al. 2003; Nishizumi and Sakano 2015). However, despite expressing only one type of OR, a single OSN can recognize several odorant molecules by detecting a particular feature of the molecule (e.g. chemical groups, carbon chain length, spatial configuration, aromatic cycles, ...). If the molecule has several characteristic features, it simultaneously activates distinct OR subtypes (Mombaerts 2004; Chess et al. 1994). Nevertheless, the odorant-receptor interaction varies in affinity. Some receptors exhibit a high affinity for an odorant while others show a low affinity to this same odorant, but in the end, all of them contribute to the coding process (Patel and Hallem 2022). Hence, one OSN recognizes multiple odorants and one odorant can be recognized by multiple OSNs.

Each odorant indeed activates a unique set of OSNs, suggesting that every odorant has a unique “code”. Thus, different odorants are recognized by different combinations of ORs - different codes - that allow thousands of volatile chemicals to be perceived as having distinct odors (Malnic et al. 1999). Odorant coding at the level of the olfactory receptors is combinatorial (Figure II.3.)

Odorant receptors	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Description
A rancid, sour, goat-like					●										
B sweet, herbal, woody		●				●									
C rancid, sour, sweaty	●			●	●		●			●	●				
D violet, sweet, woody		●			●	●									
E rancid, sour, repulsive	●			●	●		●	●		●	●	●			
F sweet, orange, rose				●	●		●			●					
G waxy, cheese, nut-like	●			●	●		●	●		●		●		●	
H fresh, rose, oily floral				●	●		●			●		●			

Figure II.3. Combinatorial receptor codes. The odorant receptor family operates in a combinatorial manner to detect odorants and encode their unique identities. Distinct odorants are detected by different combinations of ORs, resulting in unique receptor codes for each odorant. These codes are interpreted by the brain, giving rise to a wide range of odor perceptions. The vast array of potential receptor combinations forms the foundation of our capacity to distinguish and remember over 10,000 different odorants. *Image source: The Nobel Prize in Physiology or Medicine 2004. NobelPrize.org.*

1.3. Odorant signal transduction: from chemistry to electricity

Once an odorant molecules diffuse in the mucus of the MOE, each molecule binds to several ORs - located on the cilia of distinct OSNs – based on its chemical structure, conformation, and concentration (Malnic et al. 1999). This binding triggers the chemical transduction, initiating the transmission of an olfactory signal.

The signal transduction process is a typical GPCR-mediated response (Figure II.4.). When an odorant binds to its corresponding receptor, an olfactory neuron-specific G-protein, called “Golf”, is activated (Jones and Reed 1989), subsequently activating adenylyl cyclase III, which produces cyclic adenosine monophosphate (cAMP). The second messenger cAMP opens cyclic-nucleotide-dependent ion channels, leading to the influx of Ca^{2+} and Na^{+} ions into the cell. The signal transduction ultimately results in membrane depolarization, with the entry of Ca^{2+} (third messenger) triggering the opening of chloride channels (Menini 1999), which further amplifies this mechanism (Takeuchi and Kurahashi 2005; Reisert et al. 2003). Recent

work have shown that olfactory marker protein (OMP), a cytoplasmic protein expressed in OSNs also participates in the early stage of odorant transduction by modulating the kinetic of cAMP (Dibattista et al. 2021).

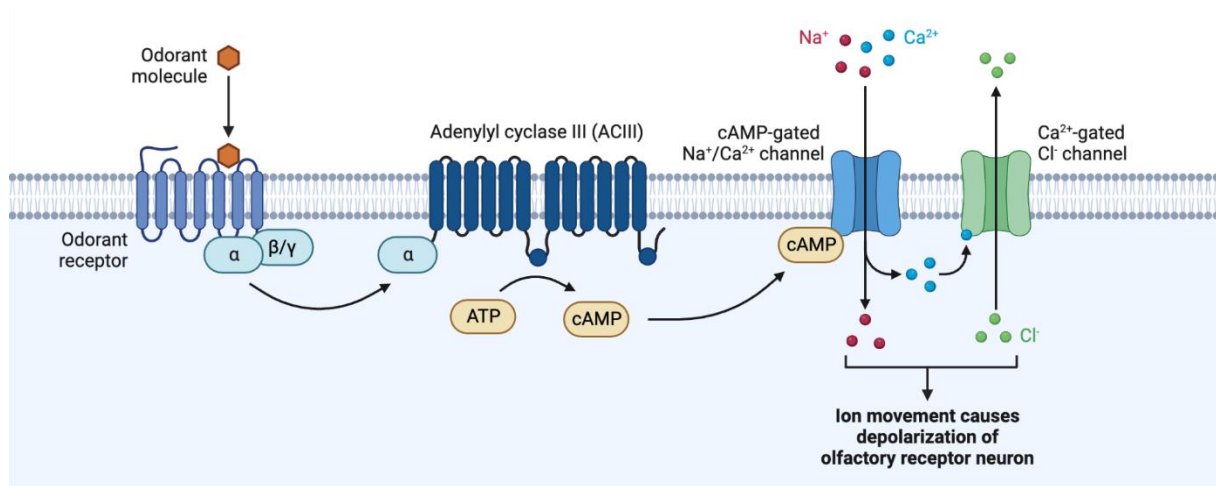


Figure II.4. Mechanism of odorant signal transduction. An odorant molecule binds to its receptor, which stimulates the adenylyl cyclase III, leading to an increase in cAMP expression. In its turn, cAMP induces the opening of cAMP-gated Na⁺/Ca²⁺ channels, resulting in a cation influx and a Cl⁻ efflux. Together, these ion exchanges depolarize the membrane of the OSN. *Image source: Biorender Science Templates.*

Despite these robust responses to a single odorant molecule, it appears that the binding of a single molecule to a receptor is insufficient to activate the Golf protein, which alone is also unlikely to initiate an effective response. Unlike other sensory pathways, such as vision, where a single photon is sufficient to trigger a response, proper olfactory detection requires a high number of odorants binding to Golf protein because the odorant dwell time is very short. Therefore, signal amplification in olfactory transduction seems indispensable (Bhandawat, Reisert, and Yau 2005). Several factors influence the ability to elicit a response, including the sniffing frequency (Connelly et al. 2015), binding of odorants to odorant-binding proteins abundantly expressed in the nose (Mastrogiacomo et al. 2014), the density of expression of a given receptor or ion channel (Reisert et al. 2003), ...

Finally, ion movement, in response to odorant-receptor binding, contributes to OSN depolarization. The depolarization spreads passively to the soma of the OSN, generating an action potential that will transmit the encoded olfactory message along the OSN axons to reach the first cortical relay of the olfactory system: the main Olfactory Bulb (OB) (Firestein 2001).

2. The olfactory bulb: first central relay of the olfactory system

In rodents, the main OB is the most anterior neural structure of the brain. It represents the first central structure for odor information processing: following the activation of ORs and their corresponding OSNs by an odorant molecule, the olfactory signal is sent to the OB where it can be encoded by generating a specific spatiotemporal representation of the perceived odor: neurons in the OB indeed establish spatial odor maps (chemotopic organization of olfactory glomeruli) and temporal changes occurs in their oscillatory patterns arising from the interplay between mitral and granule cells (for more details regarding OB oscillations and olfactory memory, please refer to the review of (Martin and Ravel 2014)). Interestingly, OSNs synapse directly into the olfactory bulb without the thalamic relay contrarily to all other sensory systems.

2.1. Concentric organization of the MOB

Despite size differences across species, OBs share a common layered concentric organization (Nagayama, Homma, and Imamura 2014). The OB consists of 2 symmetrical hemi-bulbs with 6 distinct concentric layers of which only 4 comprise cell bodies (Figure II.5.). From here on, I specifically describe the structural organization of the rodent OB, from the most superficial to the deepest layer (Tavakoli et al. 2018).

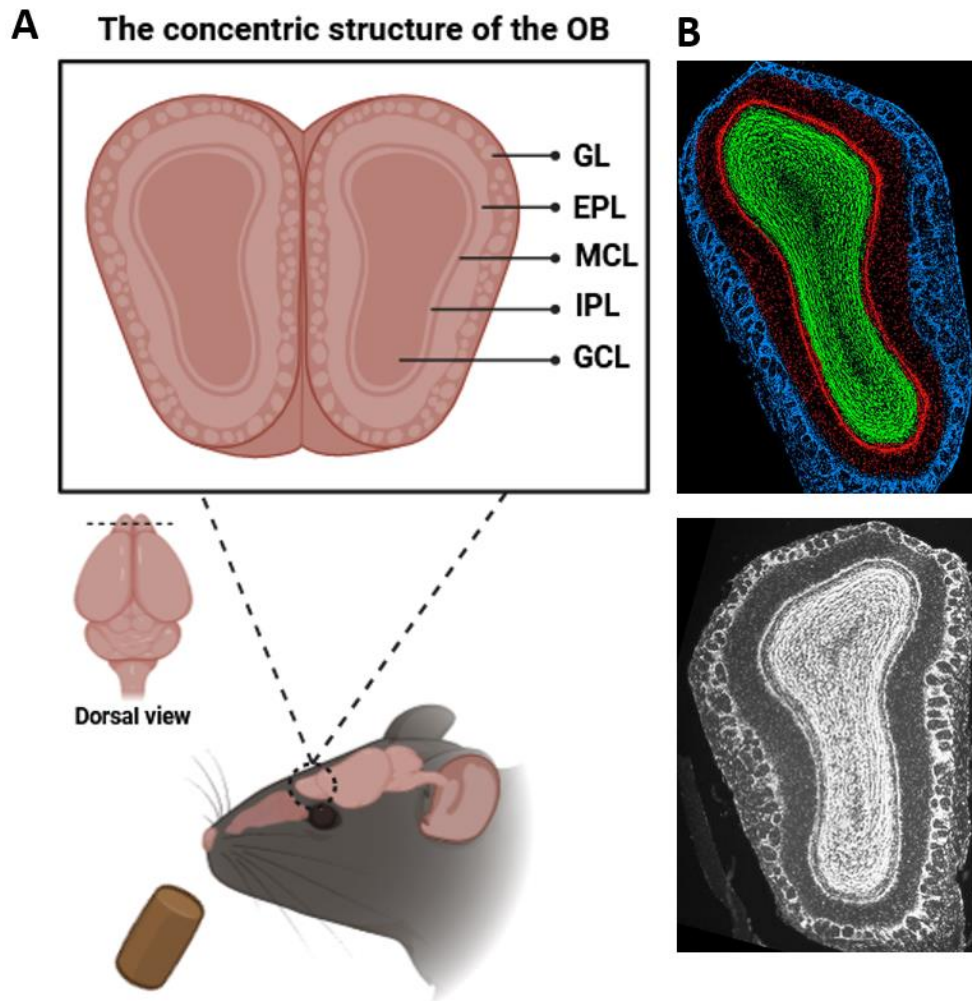


Figure II.5. Anatomy of the mouse olfactory bulb. (A) Coronal view of the layered organization of the olfactory bulb (OB), from the most superficial to the deepest layer : the glomerular layer (GL), the external plexiform layer (EPL), the mitral cell layer (MCL), the internal plexiform layer (IPL) and the granule cell layer (GCL). *Image created with biorender.* (B) Coronal section of the olfactory bulb of an adult male mouse. Image taken with confocal microscopy. False colors indicate the 3 main anatomical layers: Blue - GL, Red – EPL and MCL, Green – IPL and GCL. *Image source : Matt Valley and (Hasegawa-Ishii et al. 2020).*

2.1.a. The olfactory nerve layer

The Olfactory Nerve Layer (ONL) represents the most external layer. It comprises the distal endings of OSN axons. These axons extend along the lamina propria, pass through the cribriform plate of the ethmoid bone, and terminate in the glomeruli of the OB where they establish synapses with different cell types (Q. Liu and Zhang 2019).

2.1.b. The glomerular layer

The Glomerular Layer (GL), the first site of olfactory information processing, contains the functional modules of the OB, the olfactory glomeruli. Glomeruli are small spherical structures where the main feedforward excitatory synapses are established between OSN axons and dendrites of mitral and tufted cells (Pinching and Powell 1971). These glutamatergic synapses are regulated by surrounding astrocytes, juxtglomerular cells (JGCs), and centrifugal afferents (Daumas-Meyer et al. 2018). Three types of JGCs were identified in the GL based on their morphological and functional variability: the periglomerular cells (PGCs), the external tufted cells (ETCs), and the superficial short-axon cells (sSACs) (Pinching and Powell 1971; Tavakoli et al. 2018).

2.1.c. The external plexiform layer

In the External Plexiform Layer (EPL) reside the cell bodies of Tufted Cells (TCs). These cells project their large apical dendrite to a single glomerulus where they form synapses with OSN terminals (K. Mori, Kishi, and Ojima 1983). TC axons then carry the output signal to the olfactory cortex.

2.1.d. The mitral cell layer

The Mitral Cell Layer (MCL) is a thin single-cell layer composed of densely packed somas of mitral cells (MCs). Mitral (MCs) and tufted cells (TCs) (referred to as M/TCs) represent the main output neurons of the OB. They both share similar morphology (pyramidal shape) with a greatly developed single apical dendrite extending through the superficial layers to reach a single glomerulus. Nevertheless, several MCs (20 to 25) can converge into one single glomerulus. In the EPL, secondary dendrites of MCs interact dendrodendritically with GCs (G. Liu et al. 2019). Moreover, MC axons establish several divergent synaptic connections with distinct neurons of the olfactory cortex (Sosulski et al. 2011).

2.1.e. The internal plexiform layer

The Internal Plexiform Layer (IPL) is an extremely thin layer mainly composed of the axons of M/TCs, which converge together to form the Lateral Olfactory Tract (LOT). This layer also contains a small population of deep Short-Axon Cells (dSACs) (Burton et al. 2017), as well as GC dendrites and afferent axons from top-down cortical projections.

2.1.f. The granule cell layer

Finally, the Granule Cell Layer (GCL) contains the somas of GABAergic GCs. In the OB, GCs are the most abundant interneurons and are continuously regenerated via adult neurogenesis (Lois and Alvarez-Buylla 1994). GCs don't have axons, but instead develop a highly dense dendritic arborization that enables them to establish key synaptic connections with MCs secondary dendrites within the EPL. The GCL also contains somas of dSACs which project their axons to reach the MCL and the EPL (J. L. Price and Powell 1970).

2.2. Topographic organization of the MOB

The layered organization of the OB underlies another spatial organization. In the OS, the segregation of the olfactory information begins with the convergence of OSN axonal subpopulations onto specific glomeruli (Figure II.6.). Early labeling experiments revealed that glomeruli do not receive random inputs from various OSNs, instead, all OSNs expressing the same OR converge onto two distinct glomeruli (one medial and one lateral) in each bulb, four in total, thereby creating an odor map (Miyamichi et al. 2011; Vassar et al. 1994). These findings suggest that each odorant is associated with a specific topographic pattern of activity within the OB. Moreover, each glomerulus enables the synaptic connection of nearly 25,000 OSN terminals to roughly one hundred M/TC dendrites, allowing additional massive olfactory signal convergence in the OB (Najac et al. 2011).

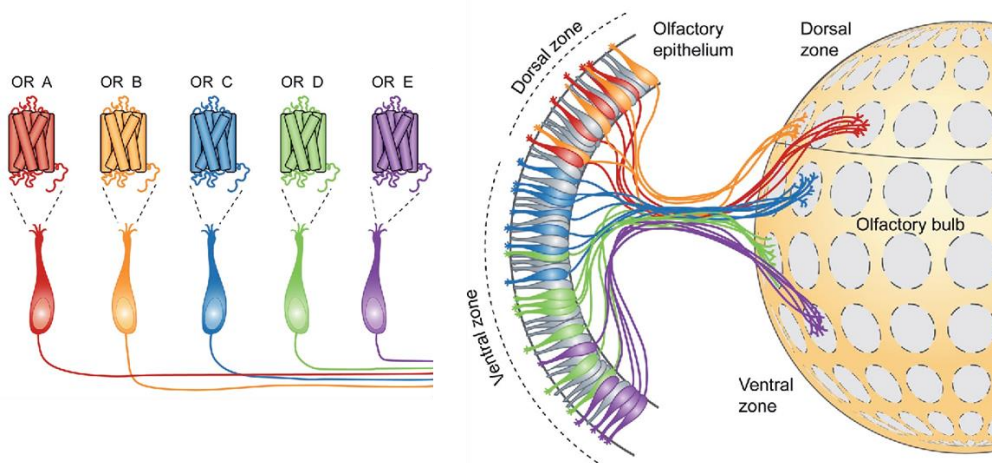


Figure II.6. Direct input from the olfactory epithelium to the olfactory bulb. Each OSN of the olfactory epithelium expresses one specific odorant receptor (OR A, B, C,...). Neurons expressing a specific odorant receptor (OR) are arranged along the dorsal-ventral axis of the olfactory epithelium and converge onto common glomeruli at corresponding dorsal-ventral zones in the olfactory bulb. Consequently, each glomerulus receives input from sensory neurons

expressing a single OR, establishing the anatomical foundation of the olfactory spatial sensory map. *Image source: (DeMaria and Ngai 2010).*

Another level of organization is evident with a columnar map between the MOE and the MOB. In the MOE, OR expression is segregated into four distinct zones, labeled from I to IV dorsoventrally (Willhite et al. 2006; Sullivan, Ressler, and Buck 1995). Within each zone, only a subset of ORs is expressed by their corresponding OSNs. In parallel, this map is also roughly conserved in the GL of the MOB. Interestingly, this level of organization might contribute to the coding of innate and learned odorant hedonics, hence it may have a behavioral relevance. The rodent ventral OB appears to have a greater response to pleasant odorants, whereas the dorsal part seems to have an aversive odorant-related activity (Miyamichi et al. 2011; Kermen, Mandairon, and Chalençon 2021). For instance, ablation of dorsal glomeruli results in impaired avoidance behavior to innate aversive cues (Kobayakawa et al. 2007).

Together, these studies reveal that the olfactory sensory message is first broadly segregated and organized in the nose and is subsequently transformed in the OB into a stereotyped and highly organized spatial map, critical for odor information processing.

2.3. Intrinsic modulation of OB function: Olfactory processing

Now that we've established a clear understanding of the anatomical and topographic organization of the OB, it's essential to examine the journey of the electrical olfactory message, tracing it from its arrival at OSN terminals to the axonal output of OB projection cells.

MCs have a high spontaneous firing activity generally modulated by the respiratory rhythm which can serve as a temporal frame for odorant coding (Fukunaga et al. 2012). Odorant-evoked response was characterized as a change in distinct temporal spike patterns carrying odorant information which would be decoded by downstream neurons of the piriform cortex (Uchida, Poo, and Haddad 2014).

The complex temporal pattern of M/T cells are modulated at two stages of OB processing, at the glomerular layer and granule cells layer (Figure II.7.).

2.3.a. Olfactory processing in the glomerular layer

The activation of a single OR type induces the activation of corresponding OSNs. In each glomerulus, OSN terminals establish direct excitatory (glutamatergic) synapses with M/TCs and ETCs (Jan et al. 2009) (Figure II.7.). Recording experiments demonstrated that active ETCs provide monosynaptic excitatory inputs to MCs and other JGCs (PGCs and sSACs) (Hayar et al. 2004). Hence, MCs receive direct and indirect polysynaptic excitatory inputs from OSNs (Najac et al. 2011).

Nevertheless, after receiving excitatory inputs from ETCs, PGCs and sSACs establish inhibitory dendrodendritic synapses with M/TCs, resulting in reduced M/TCs activity. PGCs are indeed inhibitory interneurons with no axon. By releasing GABA, PGCs provide feedforward inhibition to M/TCs, but also feedback inhibition to ETCs and lateral inhibition of neighboring PGCs (Murphy, Darcy, and Isaacson 2005). Moreover, PGCs also provide presynaptic inhibition of OSNs by acting on GABA_B receptors of OSN terminals and preventing them from releasing glutamate (Aroniadou-Anderjaska et al. 2000). On the other hand, despite their name, superficial short-axon cells, have extra-long axons that can activate inhibitory PGCs up to 20-30 glomeruli away from their original location, which provides additional lateral inhibition (Aungst et al. 2003). It is suggested that this two-stage interglomerular inhibitory network enhances spatiotemporal responses to odors associated with olfactory coding.

Finally, the work of J. S. Isaacson (1999) demonstrated that MCs are capable of self-excitation. In the OB, MC dendrites release glutamate only at synapses formed with dendrites of inhibitory GCs. Dendritic glutamate release from individual MCs causes self-activation via activation of local receptors (NMDA receptor; AMPA receptor). This simultaneous activation of neighboring MCs by a diffuse action of glutamate underlies the synchronized lateral activation of principal olfactory projection cells. Once the signal exits the glomerulus, M/TC axons project to the piriform cortex and higher-order cortical structures conveying the olfactory information to the CNS (Nagayama, Homma, and Imamura 2014).

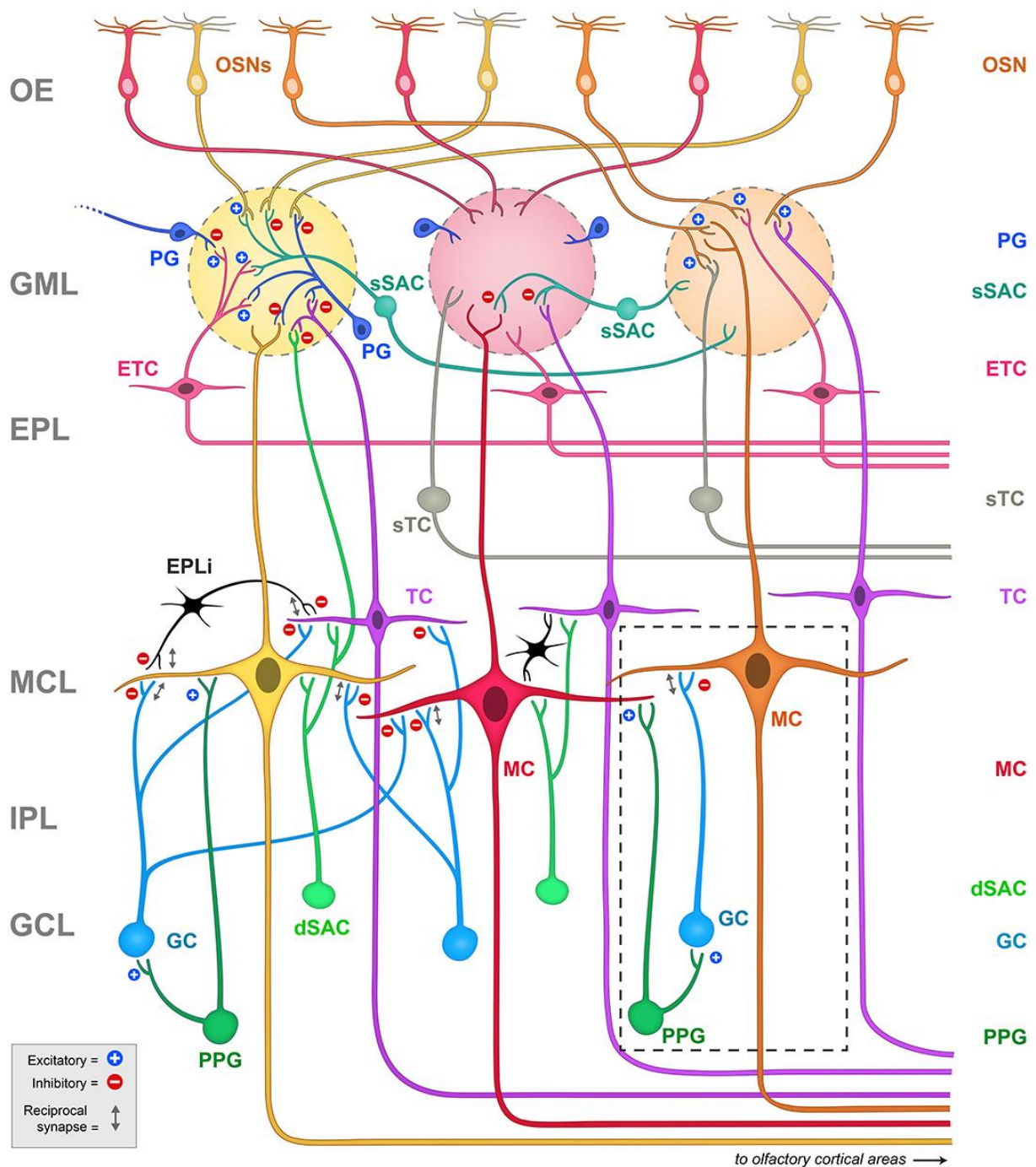


Figure II.7. Schematic representation of synapses established between the principal projection neurons and interneurons of the OB. The olfactory signal is transferred from OSNs to mitral (MC) and tufted (TC) cells. Axons of both M/TCs project to the olfactory cortex conveying the information to other brain areas. Both M/TCs send an apical dendrite into a single defined glomerulus, where they establish reciprocal synapses with OSN terminals and with several juxtglomerular (JG) cells that include periglomerular (PG) neurons, external tufted cells (ETCs) and short axon cells (SACs). ETCs do not project out of the OB (Zhou and Belluscio 2008). MCs dendrites form synapses with granule cell (GC) dendrites whose somas

are located in the granule cell layer (GCL). The GCL also contains deep short axon cells (dSACs). Preproglucagon neuron (PPG) is a subset of dSACs located within the GCL. It has excitatory synaptic connections with both GCs and MCs. GCs establish a dendro-dendritic reciprocal synapse onto MCs leading to MCs inhibition. Abbreviations: OE, olfactory epithelium; GML, glomerular layer; EPL, external plexiform layer; MCL, mitral cell layer; IPL, internal plexiform layer; GCL, granule cell layer; OSNs, olfactory sensory neurons; PG, periglomerular cell; sSAC, superficial short-axon cell; ETC, external tufted cell; sTC, superficial tufted cell; TC, tufted cell; MC, mitral cell; dSAC, deep short axon cell; GC, granule cell; EPLi, interneuron of the external plexiform layer. *Image source: (Huang et al. 2021).*

2.3.b. Granule cells, central role in olfactory processing

GCs were first described by Golgi in 1875 as an enigmatic cell with no axon. GCs constitute the most abundant inhibitory cell type in the OB (Parrish-Aungst et al. 2007). Their long apical dendrites expand through the IPL, MCL, and EPL to connect with other bulbar cell types. In 1983, the work of Orona, Scott, and Rainer (1983) showed that GCs can be divided into different subpopulations with different dendritic patterns depending on their location in the GCL: superficial GCs were found to predominantly innervate the superficial EPL while deep GCs exclusively act on the bulbar output neurons, the M/TCs, through reciprocal dendrodendritic synapses (Shepherd 1963) (Figure II.8.). Given that TC secondary dendrites extend into the superficial EPL while dendrites of most MCs ramify in the deep EPL, it was stated that superficial and deep GCs may preferentially modulate the responses of TCs and MCs respectively.

Reciprocal dendrodendritic synaptic connections between glutamatergic M/TCs and GABAergic GCs allow inhibitory feedback input on M/TCs secondary dendrites: the glomeruli-generated action potential propagates from the somas of M/TCs to their dendrites located in the EPL. Once the depolarization gets to these dendrites, glutamate is released into the synaptic cleft on GC dendritic spines, subsequently triggering the depolarization of GC dendrites. In return GCs release GABA, resulting in the inhibition of M/TCs (via GABA_A receptors) in a quick feedback loop (Wellis and Kauer 1993; Wilfrid Rall et al. 1966; Jahr and Nicoll 1982) (Figure II.8.). GC dendritic depolarization also results in the inhibition of other M/TCs that are closely in contact with the same GC, hence ensuring additional lateral inhibition (W. Rall and Shepherd 1968).

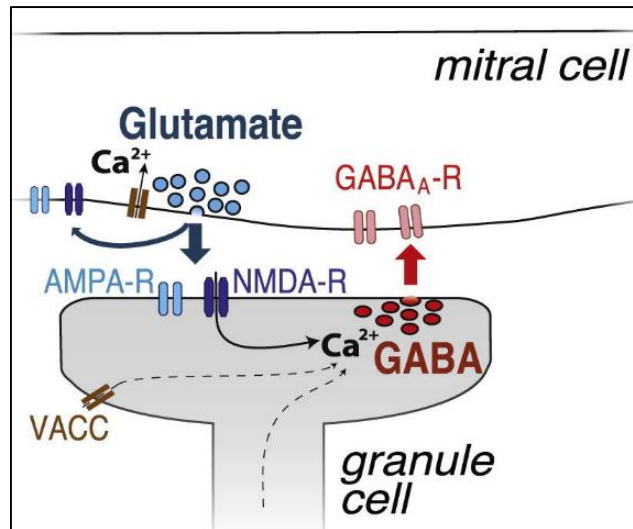


Figure II.8. Schematic representation of the dendrodendritic synapse established between MC and GC. In the dendrite of MC, the action potential triggers Ca^{2+} entry and glutamate release in the synaptic cleft. Glutamate activates postsynaptic AMPA-R and NMDA-R in the GC dendrite. In turn, Ca^{2+} entry from NMDA-R and voltage-gated Ca^{2+} channels (VACC) triggers GABA release and postsynaptic activation of $\text{GABA}_A\text{-R}$. Moreover, glutamate release also activates local glutamate receptors expressed on MC dendrites. Image source: (Lepousez and Lledo 2013).

Moreover, besides dendrodendritic synapses, M/TCs and GCs interact via axodendritic synapses in the IPL and GCL: MC axon collaterals are indeed able to activate GCs, triggering the excitation of these latter and resulting in an action potential originating from GCs somas (Pressler and Strowbridge 2019; Schoppa 2006). Again, this results in the inhibition of M/TCs that are directly connected to the activated GC (Pressler and Strowbridge 2020).

GC-mediated inhibition appears to play a crucial role in shaping the contrast between two odor-evoked responses, as demonstrated by the work of (Yokoi, Mori, and Nakanishi 1995). This inhibition synchronizes M/TCs activity, thus enhancing spatiotemporal fine-tuning of odor-evoked responses (Urban 2002) and contributing to accurate stimulus-dependent odor discrimination (Nunes and Kuner 2018; Egger and Kuner 2021).

If recurrent activation of GCs occurs in response to redundant odorant stimulation, GCs induce self-inhibition by acting on the slow synaptic inhibiting receptor, GABA_B receptor, expressed on the dendritic membrane of M/TCs (Jeffrey S. Isaacson and Vitten 2003). GABA_B receptors modulate the dendrodendritic inhibition of M/TCs primarily by reducing GABA release in the synaptic cleft connecting GC and M/TC dendrites (Egger and Kuner 2021) to normalize GC firing rates (Burton and Urban 2015).

Finally, in addition to early olfactory processing in the GL, granular processing in the GCL plays a key role in the refinement of the olfactory message by controlling M/TCs output to other cortical structures. It is important to note that GC activity is not solely modulated by intra-bulbar inputs, but it also receives centrifugal inputs arising from other brain regions to the OB (described later in the “centrifugal projections to the OB” paragraph).

2.3.c. Differentiation and integration of adult-born GCs in the OB

The OB is one of the few regions of the mammalian brain where neurogenesis has been observed in adulthood. Most of the studies comes from rodent models. OB interneurons (PGCs and GCs, mainly) are produced during embryogenesis and continuously regenerated during adulthood through adult neurogenesis. These interneurons mostly originate from the ventral telencephalon, particularly from the subventricular zone (SVZ) (Batista-Brito et al. 2008) that borders the lateral ventricles.

In the SVZ, neural stem cells are referred to as B cells (Codega et al. 2014). Once activated, B cells proliferate by symmetric cell division: 20-30% of cell divisions result in B cells self-renewal, while 70-80% undergo additional divisions resulting in C-type cells, which are fast-dividing neural progenitors that help amplify the progenitor pool (Obernier et al. 2018; Ponti et al. 2013). After a few additional divisions, C-type cells differ into A-type cells (Doetsch, García-Verdugo, and Alvarez-Buylla 1997). A-type cells (neuroblasts) are migrating neuronal progenitors that continuously travel from the SVZ toward the OB (Thomas, Gates, and Steindler 1996) (Figure II.9.).

Neuroblasts first migrate via the rostral migratory stream (RMS, tangential migration) to reach the MOB (estimated time of migration = 7 days) (Davenne et al. 2005). Then, after arriving at the center of the bulb, they migrate radially to differentiate into interneurons and integrate the olfactory network. Most neuroblasts (95%) migrate radially to reach the GCL, while some (5%) continue migrating to reach the GL and differentiate into PGCs (Luskin 1993). From here on, we mainly focus on the development of adult-born GCs (abGCs).

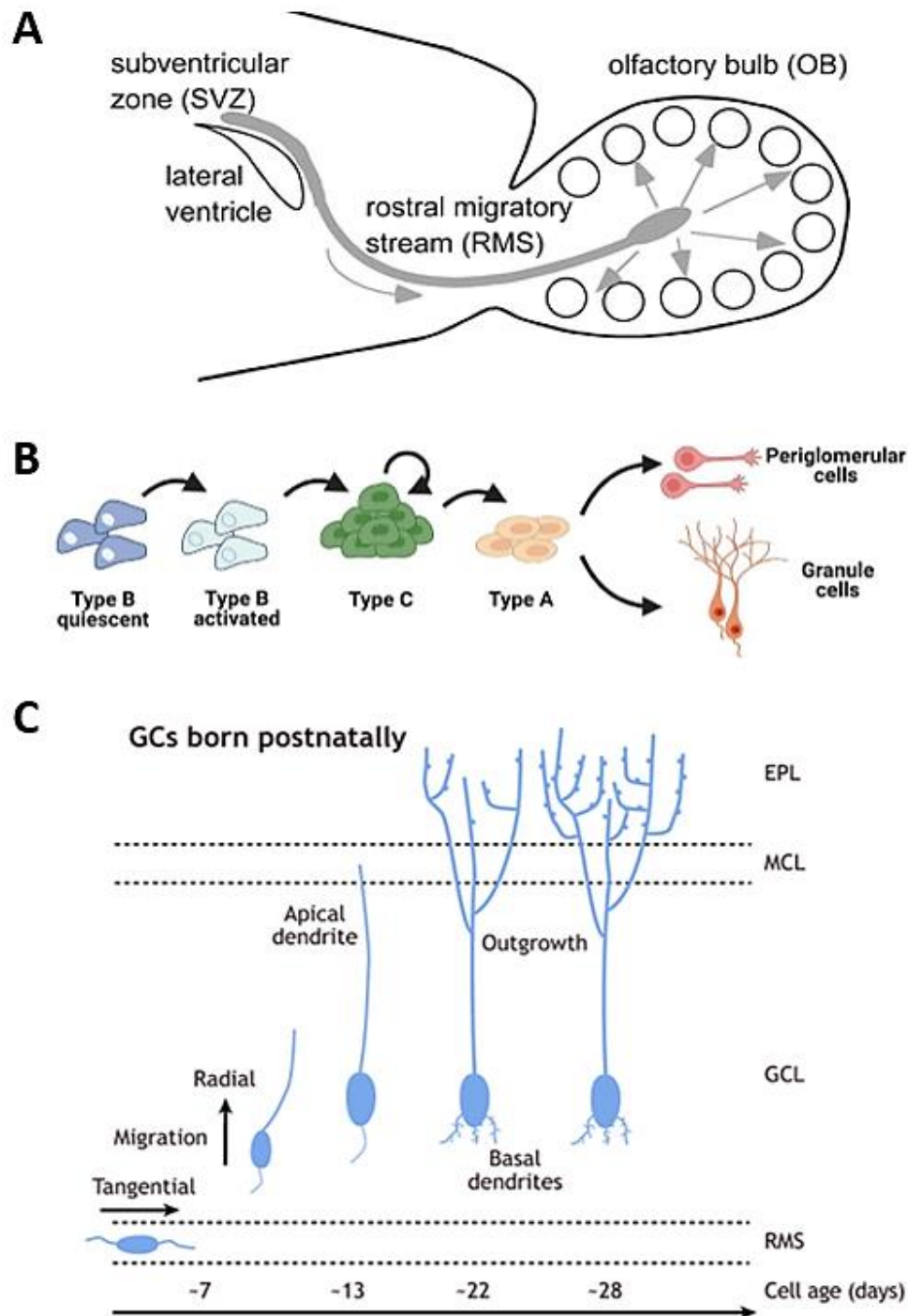


Figure II.9. Adult neurogenesis in the olfactory bulb. (A) neural stem cells migrate from the subventricular zone (SVZ) to the olfactory bulb (OB) via the rostral migratory stream (RMS). Stem cells then migrate radially within the OB. (B) Stem cells differentiate progressively into periglomerular cells and granule cells. (C) Granule cells (GC) born postnatally develop apical reaching the superficial layers (MCL and EPL), and basal dendrites mainly located in the GCL. Morphological maturation of adult born GCs takes around 28 days. *Image source:* (A) (Yamaguchi 2014); (B) (Ceanga et al. 2021); (C) (Tufo et al. 2022).

The location of mature abGCs is regulated by the activity of glutamatergic projection neurons in the OB through the activity-dependent release of glutamate (Breton-Provencher et al. 2016). These findings suggest that adult-born GCs undergo different experience-dependent synaptic modifications compared with the pre-existing mature GCs (Saghatelian et al. 2005). Thus, experience shapes the morphological maturation of abGCs.

Besides the morphological maturation, abGCs also undergo functional and electrophysiological changes crucial for their integration into the olfactory network. During the tangential migration, GCs first express GABA_A receptors, which are essential for guiding and controlling their migration. GCs then express AMPA receptors, with the expression of NMDA receptors occurring only during the radial migration (Carleton et al. 2003).

Once their dendritic arborization is fully developed, newborn GCs exhibit voltage-dependent Na⁺ currents capable of triggering action potentials in response to olfactory nerve stimulation (Belluzzi et al. 2003). At this stage, newborn GCs can be effectively activated by odor presentation (Carlén et al. 2002). Furthermore, abGCs can develop long-term glutamatergic potentiation shortly after their arrival at the OB, but with maturation, this property fades away (Nissant et al. 2009). Hence, adult neurogenesis of GCs promotes synaptic plasticity in the OB.

It is important to note that, even before the apical dendritic arborization becomes fully functional, these newly generated GCs already receive both GABAergic (from the basal forebrain, BF) and glutamatergic synaptic inputs from OB intrinsic neurons and centrifugal afferents (Panzanelli et al. 2009). These inputs play a crucial role in the survival and maintenance of abGCs in the OB (Hanson, Swanson, and Arenkiel 2020). This sheds light on the significant role of cortico-bulbar inputs in shaping OB neurogenesis.

As the dendrites mature, abGCs develop both input and output synapses with their corresponding targets: excitatory postsynaptic loci first emerge on the apical dendrite in abGCs, later extending to both the basal dendrite and the distal part of the apical dendrite. Reciprocal synapses start to develop on the distal segment of the apical dendrite after reaching the EPL (Kelsch, Lin, and Lois 2008). These synapses appear to reach their full functional capacity for inhibiting MCs 5 to 7 weeks after GCs generation (Bardy et al. 2010). On the other hand, newly generated GCs already receive centrifugal inputs from the anterior olfactory nucleus (AON) and the piriform cortex (PC) by the second week (Deshpande et al. 2013).

The survival of abGCs is significantly reduced by sensory deprivation, indicating that odor-evoked activity contributes to the maintenance of at least a portion of abGCs (Yamaguchi and Mori 2005; Saghatelian et al. 2005; Sawada et al. 2011). In contrast, enriched odor stimulation appears to promote the generation of adult-born interneurons, and to improve odor memory (Rocheffort et al. 2002; Bonzano et al. 2014). Together, these findings point to the strong link between olfactory activity and OB neurogenesis, from stem cell proliferation to the survival of newly integrated cells.

2.3.d. Conveying the olfactory signal to the olfactory cortex

The olfactory signal is conveyed from the OB to the olfactory cortex (OC) by axons of M/TCs via the LOT (Kensaku Mori and Sakano 2011). Early tracing studies showed that the OB directly projects to the nucleus of the lateral olfactory tract (nLOT), the anterior olfactory nucleus (AON), the olfactory tubercle (OT), the piriform cortex (PC), the cortical amygdala (CoA), the horizontal limb of the diagonal band of Broca (HDB), the dorsal raphe (DR), and the entorhinal cortex (EC) (Joseph L. Price 1985; De Carlos, López-Mascaraque, and Valverde 1989; Sosulski et al. 2011) (Figure II.10.).

The piriform cortex, a key structure for olfactory memory, is the region that receives the most afferents from the MOB. The PC can be divided into two subparts structurally and functionally different (J. L. Price and Powell 1970; Sosulski et al. 2011): the anterior piriform cortex (aPC) and the posterior piriform cortex (pPC). These regions receive differential and overlapping parallel inputs from OB output cells, the M/TCs (Nagayama et al. 2010; Igarashi et al. 2012), and encode distinct aspects of odor information (Choi et al. 2011). The aPC contains significantly more feedback-projecting neurons compared to the pPC (Padmanabhan et al. 2016). The PCx is a plastic and integrative structure. A study by Richard Axel's group in 2011 showed that the repeated association between optogenetic activation of an arbitrary set of just 500 pyramidal neurons and a reward or punishment is sufficient to form a memory that can then be evaluated by behaviour (Choi et al. 2011).

Interestingly, TCs route odor information with a shorter latency to the most anterior cortical structures, particularly to the AON and the anterior PC, while MCs response to odor information is slower but conveyed by collateral projections to all OC regions cited above (Igarashi et al. 2012). Then, the olfactory signal is transmitted to higher brain structures. The

posterior PC projects to the insula, the thalamus, the hippocampus, the frontal cortex, the orbitofrontal cortex (OFC), and the hypothalamus (J. L. Price, Slotnick, and Reviel 1991).

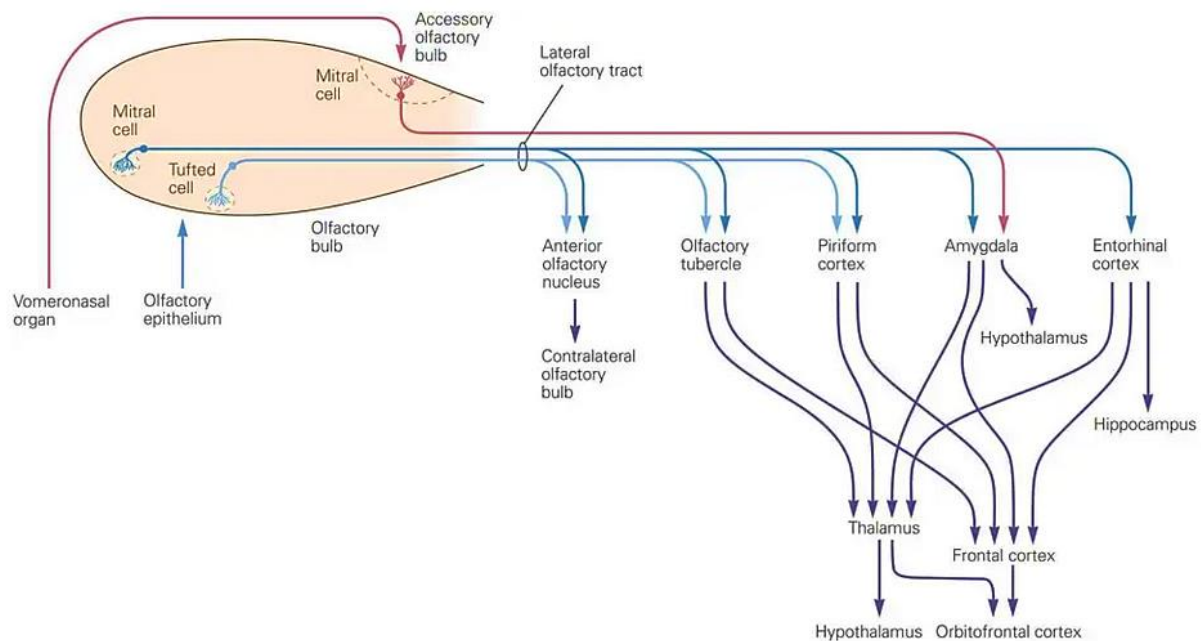


Figure II.10. Olfactory sensory pathways. The bulbar output neurons, mitral and tufted cells, project via the lateral olfactory tract to several structures of the primary olfactory cortex: anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala, and entorhinal cortex. The primary olfactory cortex connects to other brain regions including the thalamus, the hypothalamus (more precisely, the lateral hypothalamic area), and the hippocampus. *Image source:* neuroscience & graphic design.

Multisensory integration occurs across multiple brain regions including the PC, the insula, the amygdala, and the OFC. By integrating olfactory, gustatory, and trigeminal information, the insula plays a central role in flavor perception during food consumption (Rolls 2016). On the other hand, the EC receives olfactory input from the AON and projects it into the hippocampus which is involved in spatial navigation guided by olfactory cues (Canto, Wouterlood, and Witter 2008). Moreover, the OT projects to the nucleus accumbens (NAc) implicated in hedonic and motivated feeding (Murata 2020). Consequently, the OB serves as a direct and potent source of sensory input to the limbic system, which controls emotions and memory. The "Madeleine de Proust" phenomenon, characterized by sudden vivid recollection of specific events triggered by food-related odors and tastes, is attributed to a direct amygdala stimulation (Bray 2013). Odors serve as powerful cues that evoke episodic memories and strongly activate the limbic system, as discussed in (Saive et al. 2014).

Except for the OT, bidirectional modulation occurs between the OB and the higher processing areas of the OC (Zandt et al. 2019). These structures send direct centrifugal projections to the OB, via the LOT, mainly targeting the inhibitory interneurons of the GL and the GCL (Matsutani and Yamamoto 2008; Markopoulos et al. 2012; Padmanabhan et al. 2019). These feedback loops play a crucial role in optimizing olfactory processing and memory. In this following subpart, I describe the feedback projections arising from the OC to the OB, while primarily focusing on centrifugal projections to the GCL.

3. Extrinsic modulation of OB function: Centrifugal projections

Sensory inputs are sufficient to trigger bottom-up feedforward neuronal activity in the cortical structures. However, sensory processing is not a strict one-way feedforward process. Several studies have shown that M/TC activity is determined not only by the sensory stimulus but also by the environment or the animal's internal state (Rinberg, Koulakov, and Gelperin 2006; Kay and Laurent 1999). Multiple cortical structures project back to the first site of olfactory processing, the OB, in a top-down centrifugal manner. Centrifugal inputs from downstream areas carry information about the individual internal state as well as expected outcome from memories of previous experiences. In this subpart, we focus on the cortical projections innervating the MOB, allowing the subsequent integration and processing of the sensory information before its transmission to cortical regions. While significant progress has been made to characterize these cortical top-down projections, their functional role in olfactory processing remains largely unsolved.

Centrifugal fibers can be divided into two main categories. First, neuromodulatory regions of the brain project axons to the OB, which expresses numerous neuromodulator receptors, allowing a drastic change in the intrinsic properties of OB neurons in specific contexts. On the other hand, most of the cortical targets of the MOB send feedback projections to this structure (Figure II.11.).

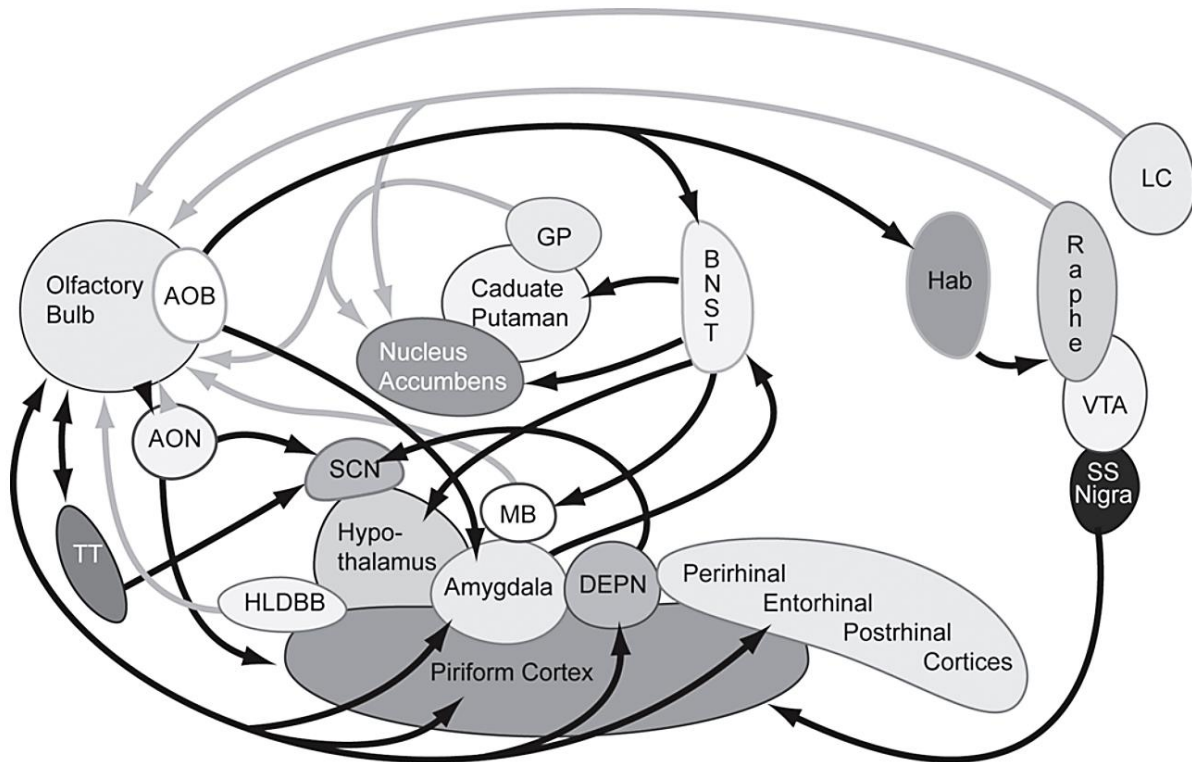


Figure II.11. Olfactory bulb (centrifugal and efferent) projections. Black arrows indicate efferent projections and double-headed black arrows indicate two-way communication. Grey arrows indicate centrifugal input to the olfactory bulb. AOB = accessory olfactory bulb, AON = anterior olfactory nucleus, BNST = bed nucleus of the stria terminalis, DEPN = dorsal endopiriform nucleus, GP = globus pallidus, Hab = habenula, HLDBB = horizontal limb of the diagonal band of Broca, LC = locus coeruleus, MB = mammillary bodies, Raphe = dorsal and medial raphe nuclei, SS nigra = substantia nigra, SCN = suprachiasmatic nucleus, TT = taenia tecta, VTA = ventral tegmental area. *Image source: (Palouzier-Paulignan et al. 2012).*

3.1. Neuromodulatory centrifugal projections

The OB serves as a target for various neuromodulatory brain regions, including the Locus Coeruleus (LC), the Raphe Nuclei (RN) – known as the dorsal and median raphe – and the HBD located in the Basal Forebrain (BF). These 3 regions project to numerous brain areas and release distinct neuromodulators that modulate bulbar activity in a state-dependent manner: norepinephrine (NE), serotonin (5-HT) and acetylcholine (ACh) (Matsutani and Yamamoto 2008).

The LC sends rich projections mainly to the GCL and the IPL of the OB (Shipley, Halloran, and de la Torre 1985). These projections release NE known for its involvement in

attention, arousal, and various physiological and cognitive processes (Schwarz and Luo 2015). Studies on the modulation of OB cell activity by NE were initially inconsistent. NE in the OB can lead to either excitatory or inhibitory effects. NE exerts complex effects within the BO: in addition to its excitatory role on M/TC (Ciombor, Ennis, and Shipley 1999), it can modulate the GABAergic inhibitory drive of GC on M/TC in a concentration-dependent manner (Nai et al. 2009). The GL also receives inputs from the LC and recent studies suggest that NE release in the OB also contributes to the suppression of odor-evoked responses at the synapse between the olfactory receptors and M/TCs (Eckmeier and Shea 2014). Consistent with the effects on the OB network, data from behavior experiments showed that NE in the OB would improve signal-to-noise ratio leading to enhanced odor detection and better olfactory memory performances (Linster et al. 2020; Doucette, Milder, and Restrepo 2007). Optogenetic manipulation of NE fibers, which resulted in a transient decrease in NE release in the OB impaired both the stability and specificity of odor learning memory (Linster et al. 2020).

Serotonin (5-HT) is best known for its involvement in various neuropsychological processes (Berger, Gray, and Roth 2009). It mainly originates from the RN and diffuses throughout the entire brain. In the OB, serotonergic afferents modulate olfactory processing by acting primarily on the level of the GL (McLean and Shipley 1987). In 2009, the work of Petzold, Hagiwara, and Murthy (2009) demonstrated that serotonin impairs odor-evoked activation of glomeruli by increasing the inhibitory input of PGCs into OSN terminals. In contrast, selective optogenetic activation of RN axons in the OB led to an increased activation of M/TCs through dual release of serotonin and glutamate (Kapoor et al. 2016; Brill et al. 2016). In conclusion, serotonin differentially modulates olfactory inputs by acting on distinct targets in the OB.

ACh terminals, mainly originating from the HBD and the magnocellular preoptic area (MCPO) of the BF (Ichikawa and Hirata 1986; Rothermel et al. 2014), preferentially target the JGCs of the GL, as well as MCs (K. A. Carson 1984; Le Jeune and Jourdan 1993), resulting in their excitation (Castillo et al. 1999). ACh robustly enhances M/TCs spontaneous odorant responses (Rothermel et al. 2014).

Finally, a particular feature of the MOB is that it receives little extrinsic afference from the dopaminergic systems. Only a recent axonal tracing study showed the presence of projections from neurons of the Substantia Nigra to the EPL and GCL of the MOB (Höglinger et al. 2015). The MOB is one of the few brain regions that contains dopaminergic neurons

outside of the midbrain. Indeed the SAC from the GL co-release GABA and dopamine, with modulatory effects on the first stage of odor processing (S. Liu et al. 2013) In contrast, the PC and especially the OT, which is part of the ventral striatum, are largely modulated by dopaminergic projections from the substantia nigra and the striatum (Xiong and Wesson 2016; W. Zhang et al. 2019).

3.2. Centrifugal projections from other brain regions

Centrifugal projections in the olfactory system play a crucial role in both olfactory processing and behavior. Multiple tracing studies helped identifying the principle centrifugal projections to the MOB: AON, PC, BF, nLOT, CoA, LC, EC, RN, and Hippocampus (Padmanabhan et al. 2019; Wang et al. 2023; Schneider et al. 2020). Cortico-bulbar projections, mainly glutamatergic, are strongly involved in plasticity and would be strengthened during odor learning, subsequently shaping odor representation within the MOB (Otazu et al. 2015; Boyd et al. 2015; Chae et al. 2022). Consistent with the distinct projections of MC and TC described above, data suggest that feedback from the AON preferentially modulates TC, whereas the PC specifically shapes MC responses. Thus, two distinct olfactory processing loops (TC/AON and MC/PC) would operate distinctly and sub serve different functions (Trejo et al. 2023; Chae et al. 2022). In addition, the OB receives massive cholinergic and GABAergic long-range projections from the BF, which have target-specific actions on various OB neuron subtypes and modulate olfactory sensitivity, discrimination and memory. Then, we particularly focus on the role of AON, PC and BF projections to the OB.

3.2.a. Anterior olfactory nucleus

The majority of cortico-bulbar projections originates from the AON (Keith A. Carson 1984). The AON is involved in numerous functions, including odor percept formation (Haberly 2001), olfactory memory representation (Aqrabawi and Kim 2020), social interaction (Wacker et al. 2011), food intake regulation (Soria-Gómez et al. 2014), and integration of bulbar activity, within (ipsilateral) and between the two OBs (contralateral) (Reyher, Schwerdtfeger, and Baumgarten 1988; Grobman et al. 2018) have shown that feedback from AON preferentially control the gain of TC.

. Glutamatergic AON-derived axons project to multiple layers of the OB, including the GCL, the EPL, and the MCL (Padmanabhan et al. 2016). Moreover, AON projections are bilateral, meaning that they project to both the ipsilateral and contralateral OB via the anterior

commissure (Wen et al. 2019; Illig and Eudy 2009). The AON can be divided into two major subparts: pars principalis (AONpP) and pars externa (AONpE) (Brunjes, Illig, and Meyer 2005).

Few studies investigated the influence of centrifugal AONpP projections on OB activity. The work of Rothermel and Wachowiak (2014) found that AONpP provides sensory-evoked feedback to the OB. Moreover, activation of glutamatergic AONpP-derived fibers activates GCs, resulting in a strong inhibition of spontaneous as well as odor-evoked activity of M/TCs, suggesting a gating function (Medinaceli Quintela et al. 2020; Markopoulos et al. 2012). On the other hand, AONpE neurons receive inputs from ipsilateral and contralateral OB (Kikuta et al. 2010). However, AONpE projections appear to be exclusively contralateral (Yan et al. 2008; Schoenfeld and Macrides 1984): their projections to the OB link mirror-symmetric M/TCs from each bulb to each other, possibly contributing to the unity of odor perception (Grobman et al. 2018).

Furthermore, a study conducted by Mazo et al. (2022) recently discovered an additional inhibitory feedback circuit to the OB, originating from a subpopulation of GABAergic neurons in the posterior AON. These inhibitory projections target almost all cell types of the OB except GL interneurons (PGCs and sSACs). Disruption of the GABAergic inputs impairs discrimination of similar odor mixtures.

3.2.b. Piriform cortex

Top-down projections from the PC to the OB mainly target GCs and dSACs in the GCL, and to a lesser extent, the GL, targeting PGCs and sSACs (Otazu et al. 2015; Boyd et al. 2015). Glutamatergic projections from the PC to the OB primarily activate GCs (J. L. Price and Powell 1970; Davis and Macrides 1981; Davis et al. 1978; Boyd et al. 2012), which subsequently inhibit M/TCs (Balu, Pressler, and Strowbridge 2007; Strowbridge 2009). PC feedback would be critical for decoding odor identity. Functional investigations into PC-derived fiber activity suggest a potential role for PC fibers in decorrelating MC activity and sensory gating (Boyd et al. 2012). In addition, in the context of odor learning, imaging of calcium signals from feedback projections from PC in the MOB revealed rapid changes according to reward contingency of the odorant (Trejo et al. 2023).

3.2.c. Basal Forebrain

The BF is composed of multiple subcortical nuclei, including the medial septum, vertical and horizontal limbs of the diagonal band (HDB), the MCPO, and the substantia innominata. BF neuromodulatory systems enhance sensory processing, amplify the signal-to-noise ratio of relevant responses, and play a crucial role in attentional modulation of sensory processing and cognitive coordination (Picciotto, Higley, and Mineur 2012; Goard and Dan 2009; Sarter et al. 2005).

Most of these functions were attributed to cholinergic signaling (described previously). However, most BF nuclei also comprise GABAergic as well as glutamatergic projecting neurons (Gritti et al. 2006; Henny and Jones 2008; Agostinelli, Geerling, and Scammell 2019). Centrifugal inputs from the BF to the OB mainly originate from the HDB (Gielow and Zaborszky 2017; X. Li et al. 2018; Gracia-Llanes et al. 2010; Záborszky et al. 1986). Cholinergic and GABAergic HDB-derived projections display an overlapping but largely segregated pattern in the BF and innervate the OB layers differently (Zaborszky et al., 1986; Bohm et al. 2020). GABAergic axon terminals strongly innervate the GL and GCL with weaker innervation of the EPL and MCL (Böhm, Brunert, and Rothermel 2020; Nunez-Parra et al. 2013).

Regarding GABAergic fibers, little is known about their role in the OB. However, recent studies showed that optogenetic activation of GABAergic fibers in the OB inhibits spontaneous and weak sensory activity while increasing odor-evoked responses, indicating a role in signal-to-noise ratio modulation or high-pass filtering of weak sensory inputs (Böhm, Brunert, and Rothermel 2020). GABAergic HDB-derived fibers to the OB selectively innervate interneurons, mainly GCs, sSACs and PGCs (Nunez-Parra et al. 2013; Sanz Diez, Najac, and De Saint Jan 2019), and is likely to modulate the OB output via disinhibition of principal cells. Interestingly, disrupting GCs inhibition impairs olfactory sensitivity and affects odor discrimination, indicating the important role of the inhibitory GABAergic afferents in olfactory processing (Nunez-Parra et al. 2013).

Conversely, cholinergic BF-derived fibers have been demonstrated to enhance odor discrimination ability (Chaudhury, Escanilla, and Linster 2009; Mandairon et al. 2006; G. Li and Cleland 2013) and facilitate olfactory learning and memory (Ross, Bendahmane, and Fletcher 2019; Linster and Devore 2012). Further research is needed to elucidate the specific

functions and interactions of the cholinergic and GABAergic systems originating from the BF, their impact on OB odor processing, and their influence on olfactory-guided behaviors.

Moreover, activating BF projections to the AON results in increased AON inputs to the OB, independently of odorant stimulation (Rothermel and Wachowiak 2014).

CHAPTER III

Crosstalk between Metabolism, Food Intake, and Olfaction

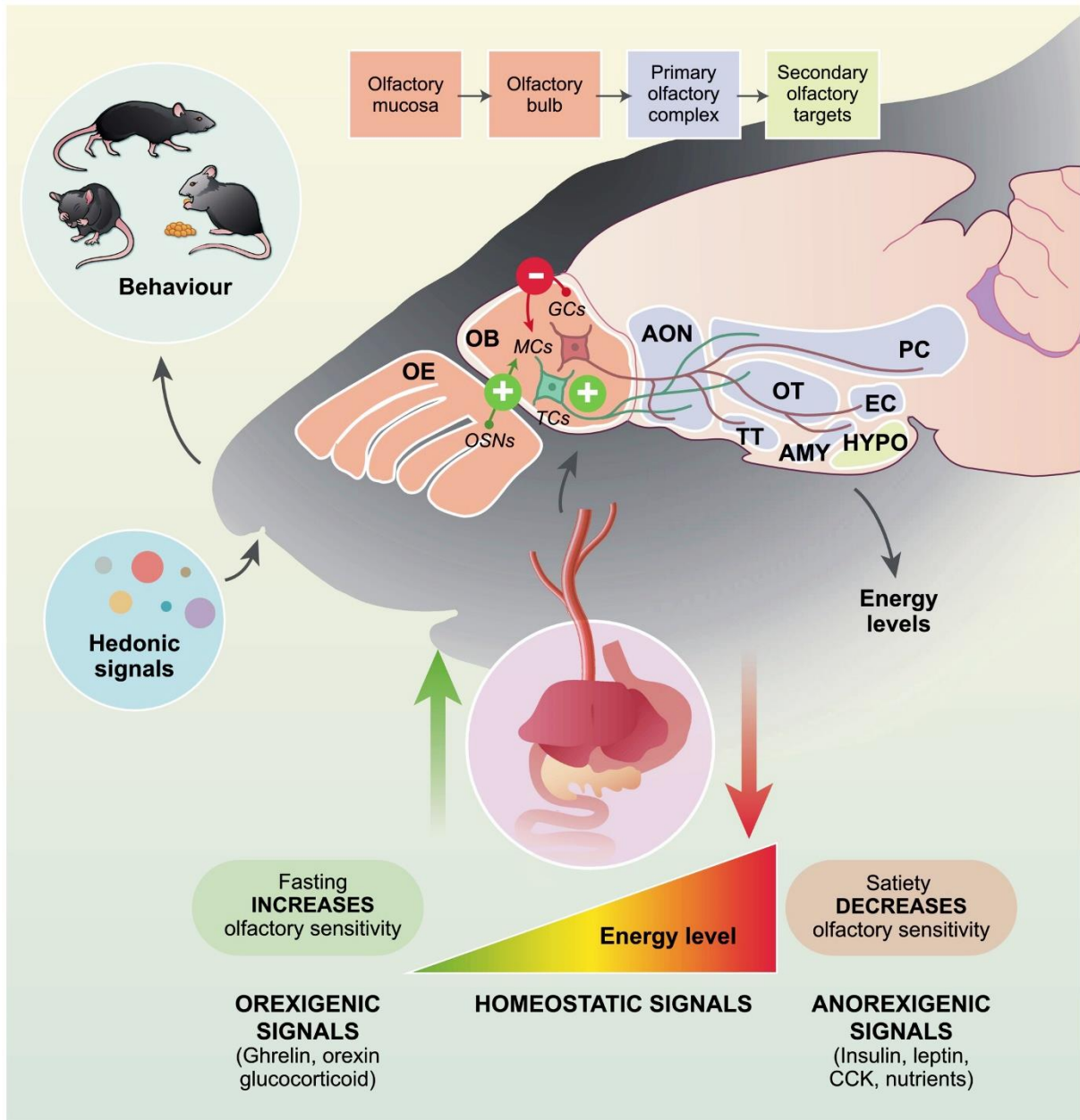


Figure III.1. The OB is at the crossroads between olfactory processing and metabolic sensing. Food-related hedonic olfactory signals are detected in the OE by OSNs. OSN terminals project to the OB and activate (mitral cells) MCs and (tufted cells) TCs. Glutamatergic M/TCs are the main projecting neurons of the OB, conveying the olfactory signal to various regions of the olfactory cortex involved in odor recognition and processing, and modulating the activity

of secondary olfactory structures, such as the hypothalamus (HYPO). MCs axons project to all olfactory cortex structures, including the anterior olfactory nucleus (AON), piriform cortex (PC), olfactory tubercle (OT), tenia tecta (TT), amygdala (AMY), and entorhinal cortex (EC). On the other hand, TCs only innervate anterior parts of the AON, OT, and PC. Within the OB, M/TCs interact with inhibitory GABAergic granule cells (GCs). In addition to its role in olfactory sensing, the OB is a metabolic sensor, sensing peripheral homeostatic signals (hormones and nutrients). Hence, the OB integrates internal and external feeding signals and drives physiological and behavioral responses (cognition, digestion, and metabolism) by modulating the olfactory perception and acting on higher cortical brain regions. *Image source: (Stark, n.d.).*

Olfaction acts as an indicator of the qualitative value of food, inducing preferences linked to pleasure (hedonic value - palatability). Furthermore, in addition to its primary function in odor processing, recent studies suggest that the OB is involved in maintaining energy balance. Indeed, the OB expresses numerous hormone receptors associated with appetite and metabolism, suggesting a neuroendocrine role outside of the hypothalamus. Furthermore, experimental investigations linked impaired olfaction and metabolic dysfunction. Therefore, studying the nature of this reciprocal relationship between olfaction and metabolism is essential to understanding how olfactory and metabolic disorders arise.

1. How does olfaction influence food intake and metabolism?

Odorants are a powerful cue to the hedonic value of food. Studying the influence of odors on food palatability, Le Magnen introduced the concept of "specific sensory satiation" in 1949 (Le Magnen 2001), after noticing that rats consumed 2 to 3 times more food when presented with successive portions of the same food, each with a different odor. Although satiated for a food with a given odor, rats will ingest the same food with a different odor (Le Magnen 2001). The concept of specific sensory satiation was then studied in humans and characterized as the differential reduction in the pleasantness of a consumed food relative to other foods (Yeomans 2006; Rolls and Rolls 1997). Taken together, these studies have demonstrated that olfactory cues play an important role in satiety (end of the meal), and may as well be critical for initial appetite stimulation.

This idea has been reinforced by examination of behavior in the context of bulbectomy (removal of the OB) which is associated with changes in behavior and eating habits (Kelly,

Wrynn, and Leonard 1997). Rats that have had their OB removed show altered food intake, with a total food intake unchanged, but smaller meals eaten more frequently (Primeaux, Barnes, and Bray 2007).

As described in Chapter I (Part 8.2. Sensory input), recordings from AgRP and POMC neurons have shown that these neurons are immediately affected by olfactory signals and that the magnitude of the change in activity is dependent on the hedonic value of the odorant. This fast regulation of hypothalamic neurons has similarities with a mechanism that has long been known: the cephalic phase response. The best-known example is the dog that starts salivating at the sound of a bell, usually associated with the arrival of food (Pavlov (1927) 2010). This "conditioned reflex" shows that, following a learning mechanism, sensory cues are capable of controlling the autonomic nervous system. It has been reported in humans that the secretion of saliva or gastric acid can be triggered by the sight, the smell of food, or simply a discussion about food (Pangborn, Witherly, and Jones 1979; Fine and Riera 2019).

How the olfactory signal is able to exert control over the peripheral tissues is still under investigation. A recent study in our laboratory by Montaner et al. (2023) provided further insight into the GLP-1/GLP-1R system in the MOB. The study found that this system is required for the olfactory-evoked cephalic phase release of insulin by the pancreas. The effect was inhibited by injection of exendin-9, a GLP-1R antagonist. Furthermore, a study aimed at characterizing the link between olfactory system activity and energy homeostasis by either disrupting or enhancing the activity of the OE in response to odorants. Metabolic parameters were assessed in mice whose olfactory perception was impaired following ablation of olfactory sensory neurons (OSNs) located in the olfactory mucosa and expressing olfactory receptors (Riera et al. 2017). The data show that these mice were more resistant to diet-induced obesity and had increased energy expenditure (lipolysis and thermogenesis). Then, the authors used engineered mice with improved olfaction to test the opposite effect. IGF1 is a factor involved in neuronal regeneration, its ablation is responsible for enhanced neurogenesis and improved olfaction. Contrary to the previous results, loss of IGF1R in the OE increases adiposity and insulin resistance. In contrast, Kolling et al. (2022) used CRISPR gene editing to increase the excitability of OB M/TCs and showed that this correlated with resistance to DIO, whereas reducing M/TC excitability using chemogenetics (DREADDs) led to an obesogenic metabolic profile. Thus, olfactory activity has an impact on whole-body metabolism, but further studies are needed to understand its specific effects.

2. How do food intake and metabolism influence olfaction?

In turn, the processing of olfactory information is affected by the body's physiological and nutritional state (Jovanovic and Riera 2022). Early studies have shown that the olfactory system is more reactive to odors (P. Aimé et al. 2007; Prud'homme et al. 2009; Pager et al. 1972), and that the hedonic value of a food odor is greater (Albrecht et al. 2009), under conditions of hunger and its activity is reduced after satiety. Indeed, mitral cells in a starving rat respond better to odors with food value (palatability) than to neutral odors, whereas in a satiated rat, this difference in reactivity disappears (Pager et al. 1972). This palatability can be induced by habituating the animal to the odorized food. The author demonstrates that an odor associated with a food can acquire a 'food value' as well as the capacity to activate more strongly the main neurons of the OB, the mitral cells (Pager 1974). Then internal state would modulate the reward value of an odorant, with the consequence of moving the body's physiological state toward homeostasis. The term "allesthesia" has been proposed to describe the fact that the same stimulus can be perceived as pleasant or unpleasant depending on the internal state (Duclaux, Feisthauer, and Cabanac 1973).

1.1. Feeding-related centrifugal projections to the OB

The representation of odor in the OB is state and context dependent and is strongly modulated by cortical feedback, as described in Chapter II. In the context of feeding and energy homeostasis, the OS receives inputs from numerous brain regions involved in both homeostatic regulation and motivation/reward processing (Palouzier-Paulignan et al. 2012; Debra Ann Fadool and Kolling 2020). One such brain region is the hypothalamus which sends out far-reaching projections to multiple brain areas (as described in Chapter I), including projections to the OB (Zandt et al. 2019; Schneider et al. 2020; Broadwell and Jacobowitz 1976). Retrograde trans-synaptic tracing studies showed that the OB receives both direct and indirect projections from the LH, with some direct projections originating from distinct orexin neurons (Qi, Fadool, and Storace 2023; Peyron et al. 1998; Nambu et al. 1999; Gascuel et al. 2012; Schneider et al. 2020), which receive direct projections from AgRP and POMC neurons. Indirect projections (involving two to three synapses) from multiple hypothalamic nuclei, including the ARC, VMH, PVN, and DMH extending toward the OB have also been observed (Schneider et al. 2020).

Similarly, the NTS that is implicated in energy homeostasis also connects via indirect projections with the OB (Schneider et al. 2020).

In addition to homeostatic signals, eating is strongly influenced by the rewarding effect of food. Among the reward pathways, the ventral tegmental area (VTA) and ventral pallidum (VP) send direct projections, whereas indirect projections originate from the nucleus accumbens (NAc) and the lateral habenular nucleus (LHb). Other studies also reported projections from the substantia nigra to the OB (Höglinger et al. 2015). Direct projections originating from homeostatic and hedonic brain regions, such as the LH (Bernardis and Bellinger 1996) and the VTA respectively, suggest that these projections may act together to modify the odor value depending on the energy state of the body (fasted/satiated) (Schneider et al. 2020). Other food-related indirect projections were observed from the laterodorsal tegmental nucleus (LDT, involved in reward processing) as well as the basolateral amygdaloidal nucleus (BLA, implicated in positive and negative odor memory formation) (Schneider et al. 2020). All these brain areas (NAc, LHb, LDT, and BLA) connect with the VTA and thus contribute to the brain reward network. Further centrifugal direct projections arise from primary olfactory regions (PC, nucleus of the LOT, anterior cortical amygdaloidal area, dorsolateral EC), as described in Chapter II. A direct projection to the OB was also identified from the ventral hippocampus and might be potentially involved in the processing of fear- and aversive-related odors (Schneider et al. 2020).

1.2. Adapting olfactory perception to metabolic needs

The OB is a key site for the access of interoceptive metabolic signals due to the highly permeable local BBB (Chaigneau et al. 2003; Ueno et al. 1991). Hormones and nutrients act on the OS by adjusting olfactory physiology and structure, olfactory function, olfactory capacities and ultimately feeding behaviors (Figure III.2.). Therefore, it is not surprising that the OB has high expression of numerous metabolic hormone receptors, such as ghrelin, leptin, insulin, and GLP-1 (Julliard et al. 2017; Palouzier-Paulignan et al. 2012; Debra Ann Fadool and Kolling 2020; Montaner et al. 2023). Orexigenic hormones, such as ghrelin, tend to increase olfactory sensitivity - by lowering the olfactory detection threshold -, whereas anorexigenic hormones, such as leptin, most likely decrease olfactory sensitivity. Indeed, hunger and energy deprivation increase olfactory discrimination and sensitivity (P. Aimé et al. 2007; Cameron, Goldfield, and Doucet 2012; Hanci and Altun 2016; K. R. Tucker et al. 2012), whereas satiety reduces the pleasantness of food-related odors (Rolls and Rolls 1997).

While these key metabolic hormones regulate food intake, body weight, and peripheral metabolic processing through hypothalamic circuits, the privileged access of the OB to these

hormones suggests an important and unexplored influence of olfactory information to regulate feeding behaviors and feeding-related neural circuits. Indeed, hormonal uptake into the OB is faster than anywhere else in the brain making it an ideal neuroendocrine regulator of appetite and metabolism.

Furthermore, manipulating feeding-related receptors in the OB modulates olfactory activity, indicating the impact that metabolic hormones have on olfactory performance (Kuczewski et al. 2014; Savigner et al. 2009; D. A. Fadool et al. 2000; Debra Ann Fadool, Tucker, and Pedarzani 2011; Apelbaum, Perrut, and Chaput 2005; Hardy et al. 2005). For instance, the work of Hardy et al. (2005); Apelbaum, Perrut, and Chaput (2005) showed that orexin-induced hyperpolarization of MCs – significant decrease in MCs spontaneous firing activity and changes in odor responsiveness - was partly mediated through GABA_A receptors in the OB. And, since GCs and PGCs express orexin receptors and are GABAergic cells, they could be both involved in this hyperpolarization.

Moreover, the OB is a metabolic sensor for nutrients, such as amino acids, glucose, and fats, which may fine-tune olfactory neuronal activity to metabolic requirements (Julliard et al. 2017). For instance, OB neurons are involved in glucose sensing (K. Tucker et al. 2010; 2013; Pascaline Aimé et al. 2014; Al Koborssy et al. 2014; Kovach et al. 2016) and several glucose transporters are expressed in the OS (GLUT1,3,4; SGLT1,4; Kv1.3; mTORC1) (Julliard et al. 2017). However, the presence of metabolic processing in the OB is not limited to glucose processing and includes amino acid transporters, receptors, and intracellular molecules, as well as various fatty acid solute carrier transporters (Julliard et al. 2017). Hence, glucose-, protein- and lipid-sensing in the OB are all likely to play a pivotal role in regulating metabolic-related olfactory function.

Finally, the endocannabinoid system is involved in the regulation of food intake and is closely linked to reward mechanisms. The CB1 receptor is widely expressed in both glutamatergic and GABAergic cortical neurons (Marsicano and Lutz 1999; Marsicano and Kuner 2008). Activation of the CB1 receptor is strongly associated with the intake of palatable and fatty foods (DiPatrizio and Simansky 2008). At the level of the MOB, CB1 receptor activity directly modulates the action of centrifugal glutamatergic projections from the AON and PC that project onto GCs. By activating CB1 receptors on these fibers, endocannabinoids reduce glutamatergic transmission to GCs, indirectly leading to disinhibition of M/TCs.

Pharmacological activation of CB1 receptors, specifically at the level of the MOB, is sufficient to increase food intake and improve olfactory performance (Soria-Gómez et al. 2014).

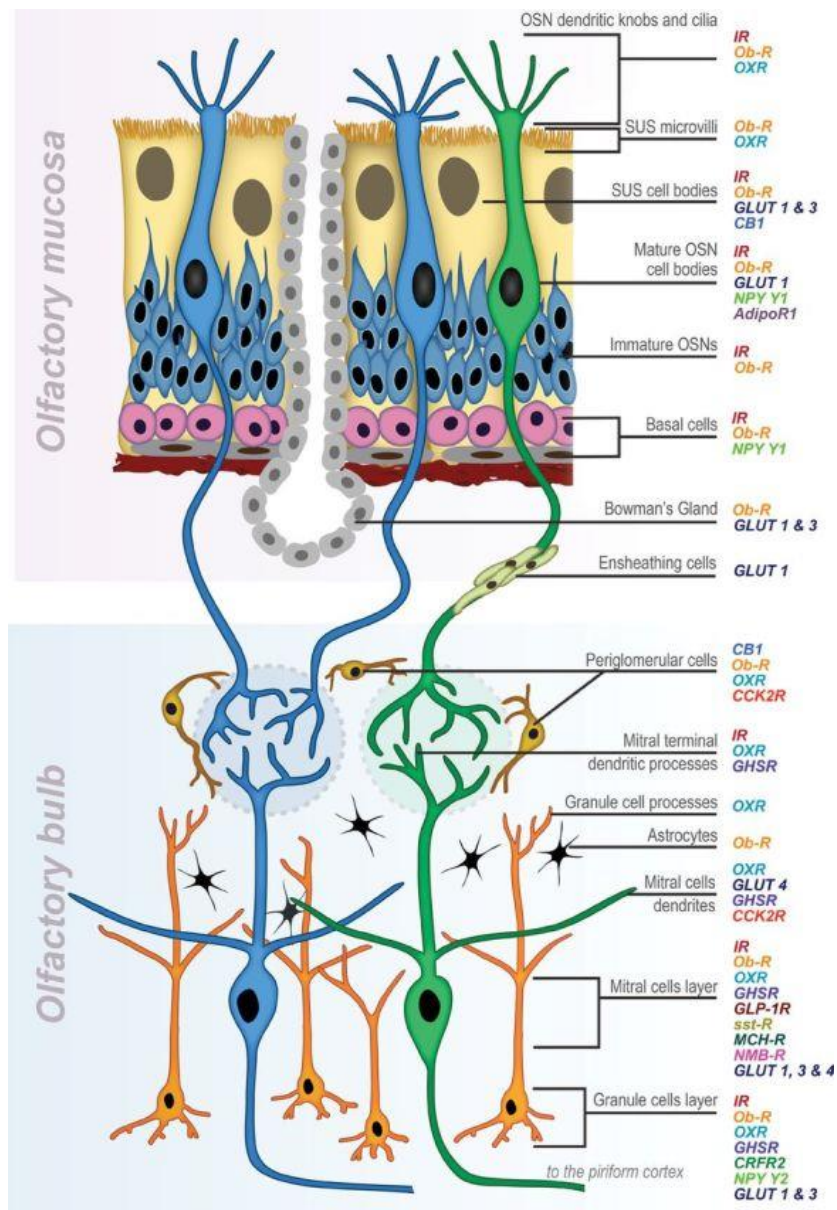


Figure III.2. Schematic representation of the broad expression of energy-related receptors for the major metabolic factors in the OS. Cellular and subcellular distribution of metabolic factors in the olfactory mucosa and the olfactory bulb. Abbreviations: SUS = sustentacular cells, NPY Y1 = neuropeptide Y receptor Y1, OXR = orexin receptor, GLUT = glucose transporter, CB1 = cannabinoid receptor 1, IR = insulin receptor kinase, Ob-R = leptin receptor, AdipoR = adiponectin receptor, CCK2R = cholecystokinin receptor 2, GHSR = ghrelin receptor, GLP-1R = glucagon-like protein receptor-1, sst-R = somatostatin receptor, MCH-R = melanin-concentrating hormone receptor, CRFR2 = corticotropin-releasing factor receptor-2, NMB-R = neuromedin B receptor. *Image source: (Palouzier-Paulignan et al. 2012).*

		Major source	Receptor	Expression peptide/receptor	Olfactory bulb	Olfactory effects
Orexigenic	Neuropeptide Y (NPY)	Brain (hypothalamus, amygdala, hippocampus, cerebral cortex), and periphery (adrenal medulla)	NPY Y2R	+/+	GL, GCL, EPL	Neuroproliferation, ↓ pre-synaptic excitatory transmission between OB neurons
	Orexin A and B (hypocretin-1/2)	Hypothalamus	OX1, OX2	+/+	GL, MCL, GCL, periglomerular cells, MC/TC and GC dendrites and processes	Modulates MC activity ICV orexin: ↓ MCs firing and ↑ olfactory sensitivity
	Melanin-concentrating hormone (MCH)	Hypothalamus	MCH-R	+/+	GL, MCL, GCL, cilia of interneurons in GL and GCL	Maternal behaviour, food-seeking
	Galanin	Brain (dorsal raphe nucleus, locus coeruleus, hypothalamus, amygdala, paraventricular nucleus, and supraoptic nucleus) and periphery (gastrointestinal tract)	GALR1, GALR2	+/+	GL, GCL, external plexiform layer, tufted cells, short-axon neurons	Modulates cholinergic transmission, promotes neuronal differentiation
	Endocannabinoids	Brain (hippocampus, cortex, cerebellum) and periphery	CB1R	?/+	GL, GCL, periglomerular cells	↑ odour sensitivity and processing, ↑ feeding behaviour, modulates synaptic plasticity, influences excitation state of M/TC
	Endogenous opioids (e.g., enkephalins, endorphins, endomorphins, dynorphins, and nociception/orphanin)	Brain (hypothalamus, pituitary gland, brainstem) and periphery	Mu, delta, kappa opioid receptors	+/+		Olfactory learning, neurogenesis
	Dopamine	Brain (substantia nigra, VTA, hypothalamus)	DR1, DR2	+/+	GL, MCL, GCL, EPL	Cue-reward associations, motivation, modulation of olfactory information transmission, processing, and odour intensity perception D1R and D2R ↑ cytoplasmic Ca ²⁺ in astrocytes DR1: ↓ odour detection/discrimination, ↓ granule cells GABA/Cl ⁻ currents and indirectly inhibits M/TCs DR2: ↓ odour detection/discrimination, ↓ odour information transduction, ↓ presynaptic glutamate release, ↓ postsynaptic M/TCs excitability, ↑ GABA/Cl ⁻ currents in M/TCs
Anorexigenic	Corticotropin-releasing hormone (CRH)	Hypothalamus	CRHR1, CRHR2	+/+	GCL	Mice lacking CRHR1 show olfactory bulb circuit dysfunction and impaired olfactory behaviours
	Cocaine- and amphetamine-related transcript (CART)	Brain (hypothalamus, amygdala, nucleus accumbens) and periphery	Undisclosed	+/?	?	Regulator of body weight, reward and food intake
	Oxytocin	Hypothalamus	OXTR	-/+	GL, MCL, GCL, periglomerular cells	↓ MC excitability, ↓ GC activity, ↑ odour responses and discrimination of MC/TCs, modulates neural networks during sensory processing, ↑ signal-to-noise ratio, synaptic maturation and circuit formation, ↑ social behaviour, ↑ social learning
	Brain-derived neurotrophic factor (BDNF)	Brain (OB, cortex, hippocampus, hypothalamus, brainstem)	Tropomyosin receptor kinases (TrkB)	+/+	GL, MCL, GCL, periglomerular cells	BDNF ↓ Kv1.3 current, TrkB ↑ Kv1.3 channel expression, modulates appetite regulators (e.g., Insulin, leptin), olfactory learning and memory, neurogenesis
	Vasopressin	Hypothalamus	V1aR, V1bR, OXTR	+/+	GL, MCL, GCL, EPL	↓ MC firing, olfactory and behavioural modulation, social odour processing
	Neuropeptide FF	Brain (spinal cord, brainstem)	NPFFR1, NPFFR2	-/+		Pain modulation and opiate tolerance
	Melatonin	Pineal gland in the brain	MT1, MT2	+/+	GL, MCL, GCL	Circadian rhythm, odour discrimination, modulation of olfactory function and depressive behaviour
	Gonadotropin-releasing hormone (GnRH)	Hypothalamus, OB Hypothalamus and periphery	GnRHR1, GnRHR3	+/+ +/+	MCL, GCL GL, MCL, GCL	Reproduction, modification of olfactory information, olfactory sensitivity to pheromones

Table 1. Appetite and olfactory performance regulating neuropeptides/hormones and their receptors expressed in the OB. Abbreviations: -, absent; ?, unknown; +, present. *Image source: (Stark, n.d.).*

		Major source	Receptor	Expression peptide/receptor	Olfactory bulb	Olfactory effects
Orexigenic	Ghrelin	Stomach	Growth hormone secretagogue receptor (GHSR)	+/+	GL, MCL, GCL, MC dendrites, mitral terminal dendritic processes	↑ olfactory sniffing behaviours, exploration and food searching
	Glucocorticoid	Adrenal gland	GR	?/+	GL, MCL, GCL, EPL	Acute stress is associated with improved olfactory performance Chronic intranasal delivery: neuronal degeneration, olfactory impairment
	Insulin	Pancreas	IR	+/+	GL, MCL, GCL, EPL, mitral terminal dendritic processes	Neurogenesis, modulation of potassium channel Kv1.3 and MC excitability, ↓ olfactory performance depending on odour quality ICV insulin: ↓ odour detection, ↓ sniff frequency in response to food odour. Intranasal insulin: ↑ odour discrimination, ↓ odour sensitivity, ↓ decision making time, improved memory Chronic intranasal insulin: ↓ body weight
Anorexigenic	Leptin	Adipose	LepR	+/+	GL, MCL, GCL, astrocytes, periglomerular cells	Inhibits GC and M/TCS, ↓ calcium response, ↓ odour discrimination, slows reaction time, ↓ olfactory sensitivity, ↓ food-seeking, ↓ OB coding and activation of higher centres
	Cholecystokinin (CCK)	Intestine, brain, OB	CCK-1R, CCK-2R	+/+	CCK-1R: internal granular layer CCK-2R: GL, MCL, EPL, M/TCS, MC dendrites, periglomerular cells	Excitation and suppression of MC firing, whereas CCK-1R ↑ and CCK-2R ↓ olfactory performance CCK-1R regulates neurogenesis, female sexual behaviour CCK-2R activation ↓ M/TCS inhibition, modulates olfactory recognition and memory
			NPY Y2R	?/+	GCL	
	Peptide tyrosin-tyrosin (PYY)	Gut L-endocrine cell				No effect on olfactory responsiveness
	Bombesin-homologs: Neuromedin B	Gastrointestinal tract	NMB/Bombesin receptor 1	+/+		Gastrointestinal hormone release and gastrointestinal motility, involved in stress, fear and social behaviour
	Gastrin-releasing peptide (GRP)		GRPR/Bombesin receptor 2	-/+		
	Glucose-dependent insulinotropic polypeptide (GIP)	Intestine	GIPR	?/+		Controls insulin secretion and glucose tolerance, is involved in explorative behaviour
	Amylin	Pancreas, brain	AMY1, AMY2, AMY3	+/?		Overexpression of human amylin affects olfactory processing in <i>Caenorhabditis elegans</i>
	Glucagon-like peptide (GLP-1)	Intestine, OB	GLP-1R	+/+	MCL, GCL, Cajal cells	Modulates microcircuits and fine-tunes olfactory information transmission, ↓ Kv1.3 activity, ↑ MCs firing frequency, ↑ olfactory sensitivity, food-seeking behaviour, ↑ odour-evoked cephalic phase insulin release
	Vasoactive intestinal peptide (VIP)	Gastrointestinal tract, pancreas, brain	VPAC1, VPAC2 (also binds PAC1)	+/+	VPAC1: EPL VPAC2: GCL	Circadian rhythms in olfactory performance, modulates olfactory output, ↓ M/TCS activity, odour processing and olfactory behaviours
Orexigenic/ anorexigenic	Adiponectin	Adipose	adipoR1, adipoR2	?/+	GL, MCL, GCL, periglomerular cells, neurons, astrocytes, blood vessels	Modulates OB insulin signalling

Table 2. Appetite and olfactory performance regulating hormones and their receptors expressed in the OB. Abbreviations: -, absent; ?, unknown; +, present. *Image source: (Stark, n.d.).*

3. Olfaction in the context of obesity and diabetes

Metabolic disorders such as obesity are associated with impaired olfactory performance, giving further evidence that olfaction is modulated by metabolic factors (Palouzier-Paulignan et al. 2012), including adiposity, blood glucose and endocrine feedback (Jovanovic and Riera 2022; Zigman, Bouret, and Andrews 2016). This subpart is discussed in detail in the following review (Faour et al. 2022).

Olfaction in the context of Obesity and Diabetes: insights from animal models to humans

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Olfaction in the context of Obesity and Diabetes: insights from animal models to humans

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Highlights

- Olfaction and feeding behaviors are tightly connected.
- Olfactory functions are impaired in obesity and type 2 diabetes both in humans and animal models.
- Decreased olfactory sensitivity could be due to hormonal and nutrients variations, inflammation, and reward system changes.
- Bariatric surgery rapidly restores olfactory sensitivity in anosmic morbid obese patients.

Abstract

The olfactory system is at the crossroad between sensory processing and metabolic sensing. In addition to being the center of detection and identification of food odors, it is a sensor for most of the hormones and nutrients responsible for feeding behavior regulation. The consequences of modifications in body homeostasis, nutrient overload and alteration of this brain network in the pathological condition of food-induced obesity and type 2 diabetes are still not elucidated. The aim of this review was first to use both humans and animal studies to report on the current knowledge of the consequences of obesity and type 2 diabetes on odorant threshold and olfactory perception including identification discrimination and memory. We then discuss how olfactory processing can be modified by an alteration of the metabolic homeostasis of the organism and available elements on pharmacological treatments that regulate olfaction. We focus on data within the olfactory system but also on the interactions between the olfactory system and other brain networks impacted by metabolic diseases.

Keywords: anosmia, diet-induced obesity, hyposmia, metabolic regulation, olfactory bulb, olfactory mucosa, type 2 diabetes

Abbreviations

AgRP neurons = Agouti-Related Peptide-expressing neurons

ARC = arcuate nucleus

BMI = Body mass index

BS = bariatric surgery

DIO = diet-induced obesity

GLP-1 = Glucagon-like peptide-1

HFD = high fat diet

HFHSD = high fat and high sucrose diet

HOMA-IR = Homeostatic model assessment of insulin resistance

LOT = lateral olfactory tract

MOB = main olfactory bulb

MMSE = Mini-Mental State Examination

M/T = mitral and tufted

NPY = Neuropeptide Y

OM = olfactory mucosa

ORN = olfactory receptor neurons

OT = olfactory tubercle

PCx = piriform cortex

POMC = Pro-opiomelanocortin

RYGB = Roux en-Y gastric bypass

SG = sleeve gastrectomy

SST = Sniffin' Sticks Test

T2D = type 2 diabetes Mellitus

TRPV = transient receptor potential vanilloid ion channels

TDI = olfactory threshold (T) odor discrimination (D) and identification (I)

UPSIT = The University of Pennsylvania Smell Identification Test

1. Introduction

1.1. Metabolic disease and food intake

Metabolic diseases are driven by a combination of genetic predisposition and environmental factors. They eventually result in a dysregulation of energy balance driven by a reduced energy expenditure failing to compensate for enriched calorie intake. Obesity is a chronic relapsing disease characterized by a dysfunction of the biological system that regulates energy balance (Box 1). The prevalence of overweight and obesity is constantly increasing in Western countries (Czernichow et al., 2021) and it is suggested that by 2030 nearly 1 in 2 adults in the United States will be obese (Ward et al., 2019). This disease has deleterious consequences on many physiological factors and is associated with cardiovascular diseases, musculoskeletal disorders and cancers (O'Neill and O'Driscoll, 2015). Obesity dramatically increases the risk of type 2 diabetes (T2D) since one major consequence of an ectopic accumulation of triglycerides is insulin resistance and glucose intolerance that can lead to chronic hyperglycemia when pancreatic β -cell dysfunction occurs (Chatterjee et al., 2017).

Box 1

Obesity and Type 2 Diabetes Mellitus (T2D)

Obesity, a chronic and relapsing disease, is a medical condition described as excess body weight due to adipose tissue expansion following triglyceride storage. In addition, ectopic accumulation of triglycerides outside adipose tissue and low-grade inflammation can lead to severe health problems by the functional impairment of major organs such as the pancreas, the liver, the heart and the brain. T2D, hypertension, dyslipidemia, cardiovascular disease, stroke, sleep apnea, gallbladder disease, gout, osteoarthritis as well as certain cancers are among the comorbidities associated with obesity. Two billion adults overweight, of those 650 million are considered to be affected by obesity (Body Mass Index ≥ 30 kg/m²). 13% of the world's adult population (11% of men and 15% of women) were obese in 2016. The prevalence of obesity across the world nearly tripled since the 1970's and continues to rise (source: <https://www.worldobesity.org/>; <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>).

Type 2 Diabetes Mellitus (T2D) (90% of all diabetes cases) is also known as insulin-resistant diabetes, non-insulin dependent diabetes, and adult-onset diabetes. It is characterized by a cascade of events, usually starting by the appearance of insulin resistance (due to ectopic storage of triglycerides and low-grade inflammation in insulin-sensitive tissues, such as liver and skeletal muscles) which leads to impaired glycemia regulation, to exhausted pancreatic beta cell function and insulin secretion and finally to chronic hyperglycaemia. T2D is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. T2D is most commonly diagnosed in older adults, but is increasingly seen in children, adolescents and younger adults due to rising levels of obesity (80% of individuals with T2D are obese), physical inactivity and poor diet. The number of people with diabetes rose from 108 million in 1980 to 422 million in 2014 with a prevalence rate of approximately 4.4% (source: <https://www.diabetesatlas.org/>; <https://www.who.int/news-room/fact-sheets/detail/diabetes>).

Metabolic causes of obesity include lipotoxicity of fatty acids *per se* chronically elevated in the blood, ectopic accumulation of triglycerides in the liver and skeletal muscles and also proinflammatory cytokines release from adipose tissue altogether leading to insulin resistance. However, obesity is also characterized by a bidirectional cross-talk between the brain and the periphery. Energy homeostasis regulation is supported by a large network of brain areas, including the brainstem, hypothalamus, and mesocorticolimbic structures, constantly monitoring the internal milieu through nutrient and hormone sensing. These parameters are integrated to generate adaptive food intake and autonomic/endocrine responses determining energy balance (Berthoud, 2004). However, these brain regions are highly susceptible to changes in peripheral signals in the context of obesity. As a result, many clinical studies showed an association between obesity and T2D and neurological impairments including structural effects with atrophy of brain regions, vascular and metabolism hypoactivity. Obesity impairs cognition and increases the prevalence of some neurodegenerative disorders such as Alzheimer's disease (O'Brien et al., 2017; Mattson, 2019). As described by (Herbert and Pollatos, 2014), obese individuals have a reduced interoceptive sensitivity to internal hunger and satiation signals. Hence, their food consumption and food preferences are mainly driven by external food related cues. Indeed, the metabolic control of energy balance is not restricted to the management of internal milieu but is rather embedded in a much larger set of functions from sensations to hedonic (Saper et al., 2002; Berthoud and Morrison, 2008). Food has potent reinforcing and motivational properties and feeding is associated with sensations and is influenced by the context. The evaluation of pleasure provided by food and the metabolic consequences of their ingestion requires learning ~~in order~~ to associate sensory features with nutritional properties. Therefore, sensory inputs are decisive cues in assessing whether a food is palatable, i.e. provides a pleasant sensation when consumed; or if a food could be harmful or poisonous. In this context, smell is one of the most relevant sensory cues predicting food characteristics and is likely to play a key role in food choice and consumption (Boesveldt and de Graaf, 2017). The relation between olfaction and food intake has been established early on (Le Magnen, 2001; Yeomans, 2006) but did not receive much attention in the context of metabolic diseases until recently.

Several diseases as well as normal aging can impair the sense of smell either partially (hyposmia) or totally (anosmia) (Boesveldt et al., 2017). Hyposmic or anosmic patients report a severe alteration of the quality of life. It is estimated that 5% of the population has altered olfaction. This number increases with age as more than 50% of the population aged between 65 and 80 years old show signs of olfactory impairment, increasing to up to 75% above 80 years old (Landis et al., 2004; Doty and Kamath, 2014). We have relatively poor knowledge of the consequences of obesity and T2D on olfactory brain regions and olfactory processing. Even though this problem has been underestimated it is important to understand what are the factors associated with metabolic syndrome that could be responsible for olfactory dysfunctions. The objective of this review is twofold: 1/ to increase awareness on olfactory impairments related to metabolic disease to improve medical care; 2/ to propose that probing olfactory function could be used as an early biomarker in clinics predicting the fate of energy metabolism disorders and cognitive impairments.

1.2. Flavor, smell and food intake

The flavor of food is determined by the cerebral integration of taste, chemesthesis and smell, 3 senses closely linked by the location of their receptors on the face, and their convergent pathways and integration in the brain. Taste (sweet, salty, bitter, sour and umami) is detected by buds located on the tongue. Chemesthesis, is the activation of the somatosensory system by chemical stimuli. It relies on the activation of the trigeminal nerve which innervates the tongue, the olfactory epithelium, and also the main olfactory bulb (MOB) via a family of transient receptor potential vanilloid (TRPV) ion channels (Brand, 2006). For instance, the activation of the trigeminal nerve is responsible for the freshness of mint or the heat of spices. Finally, the sense of smell is predominant in the evaluation of flavor and determines all its richness and finesse: many people experiencing anosmia report a loss of “taste” even though the gustatory system remains fully functional.

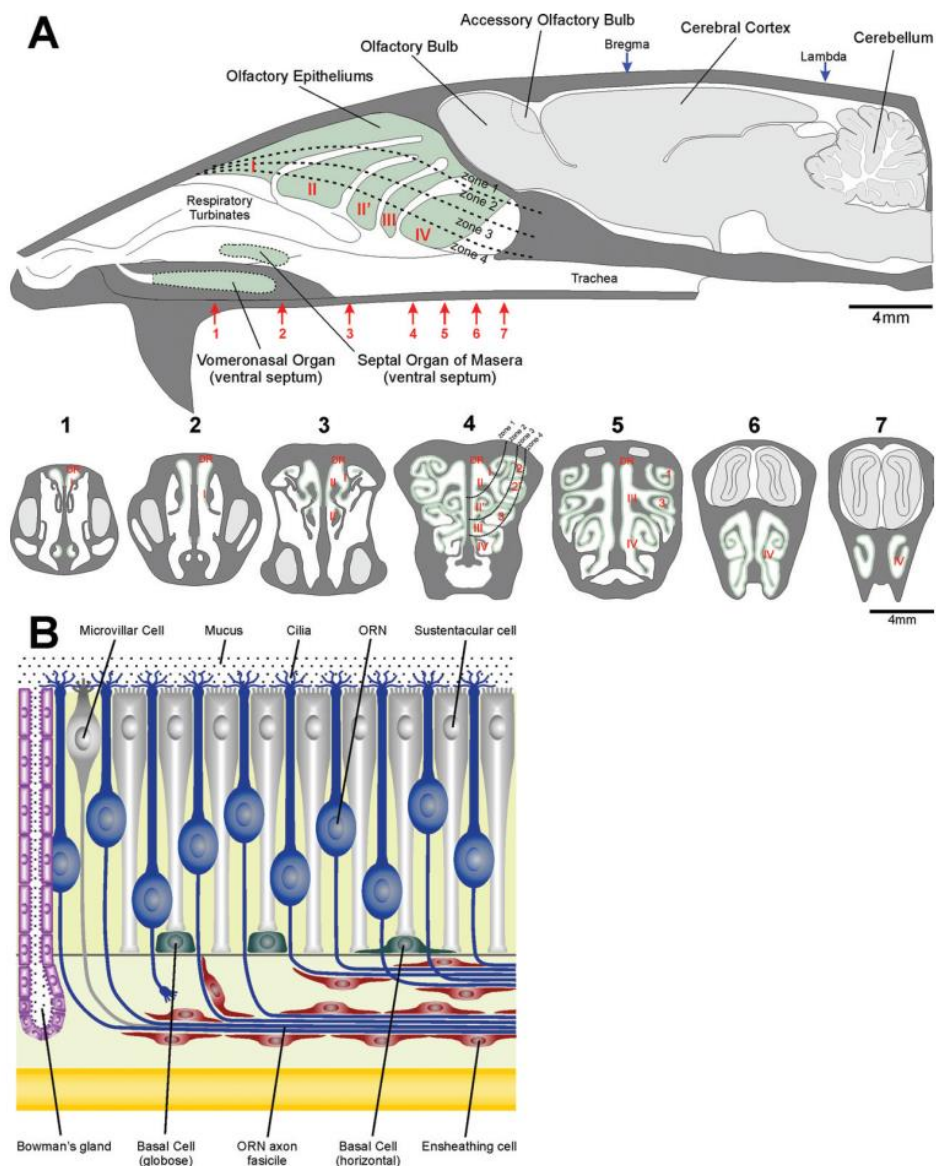


Figure 1. Diagram of the rat nasal cavity and brain shown in a lateral view and cross section diagrams through the nasal cavity at the location of the arrows. The main sensory epithelium is divided into four zones on the basis of the expression of different odorant receptor genes.

Dashed lines show these approximate positions of these zones in the adult. Two other smaller regions of sensory neuroepithelium are present within the nasal cavities: the septal organ of Masera and the vomeronasal organ. (B) Schematic illustration of the olfactory epithelium showing the major cell types present in the rat epithelium. Reproduced from (Shipley et al., 2004) with permission from Pr Adam C. Puche.

Olfaction is a chemical sense, which means that odorants are only perceived if there is physical contact between the olfactory receptor neurons (ORN) of the olfactory mucosa (OM) -located in the superior part of the nasal cavity- and the odor molecules carried by breathing (Firestein, 2001). The inner recesses of the human nasal cavity, defined laterally by three pairs of turbinates (or conchae) and medially by the nasal septum that bisects it, is lined by a mucous membrane called the respiratory mucosa (Moran et al., 1982) (figure 1). Human respiratory and olfactory mucosa present distinct anatomo-functional features, especially because the OM is the only (very small) region containing ORN (figure 2B). Inspiration captures molecules in the ambient air and conveys them to the OM through the orthonasal pathway, expiration allows odorants present in the mouth and released by chewing, to reach ORN via the retronasal route. There are about 400 different types of receptors on the surface of ORN in humans. Each receptor can recognize several molecules, and each odorant can activate several receptors. Because of its organization the olfactory receptor system has an extensive ability to decode odorant traits (Mori and Sakano, 2011; Mainland et al., 2014; Kurian et al., 2021). Projections from the ORN converge and form the olfactory nerve (cranial nerve I) that crosses the cranial bone at the level of the ethmoid bone. The olfactory information is then processed by the projection cells of the MOB (mitral and tufted (M/T) cells; figure 2AC) at the level of the olfactory glomerulus, a functional module responsible for modulating the signal from the receptors (figure 2C). M/T cells directly project through the lateral olfactory tract (LOT) to brain regions identified as the olfactory cortex, including the piriform cortex (PCx) the anterior olfactory nucleus and the olfactory tubercle (OT), but also the entorhinal cortex and the anterior and posterolateral cortical amygdala (Price, 1985)(figure 3). The absence of thalamic relay and the anatomical proximity with the limbic system confer a unicity in the organization of the olfactory circuits explaining why the sense of smell is imprinted by emotions, has a unique evocative power, and is characterized by robust and lasting memory capacity (Uchida et al., 2014). Except for the OT, structures of the primary olfactory cortex project back to the MOB by sending centrifugal fibers through the LOT to the granular and the glomerular layers (Matsutani and Yamamoto, 2008; Padmanabhan et al., 2019; Zandt et al., 2019): these forward-feedback loops conveyed by the LOT are essential for olfactory processing and memory.

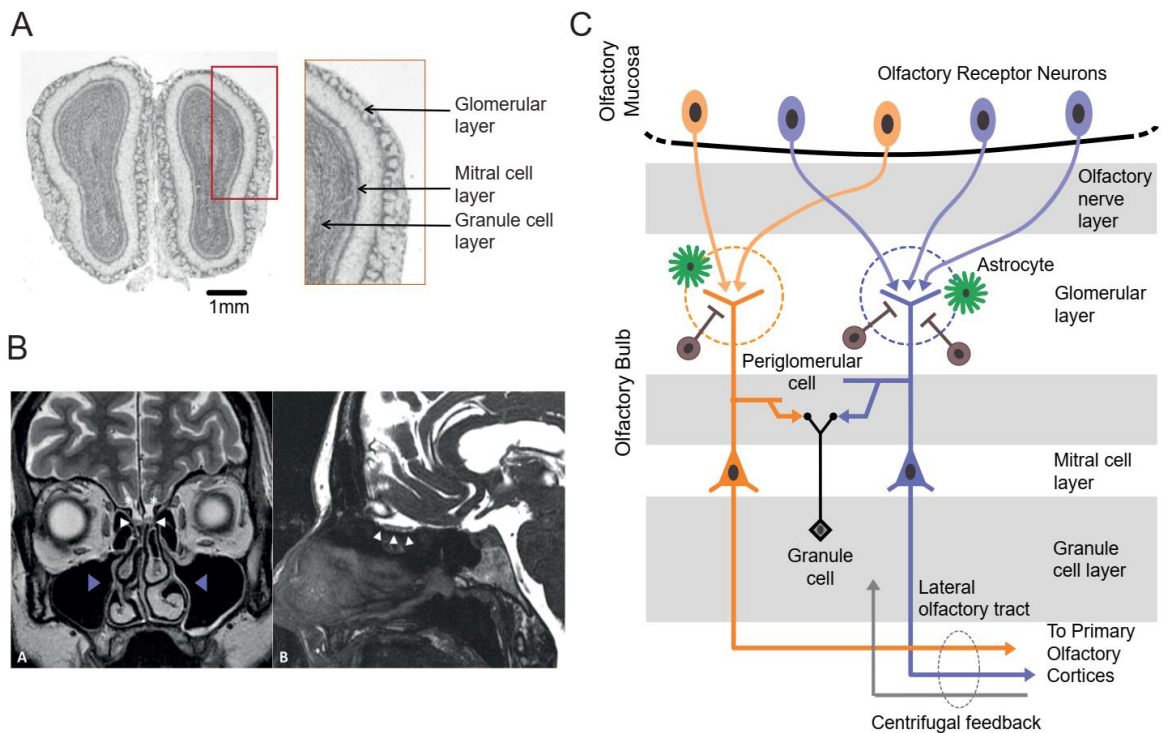


Figure 2

A: Coronal section of the adult main olfactory bulb in the rat. B: MRI of normal-size olfactory bulbs (white arrowheads) on coronal T2-weighted imaging (left) and sagittal Fast Imaging Employing Steady-state Acquisition, FIESTA (right). Turbinates of the nasal cavity are also observed (blue arrowheads). With permission from (Braun et al., 2019). C: Organization of the main olfactory bulb in rodents. In the olfactory mucosa, olfactory receptor neurons which express the same odorant receptor converge onto the same glomeruli in the MOB. Olfactory glomeruli located at the surface of the MOB enclose the synapses between the olfactory sensory neurons and the M/T cells, principal output cells (glutamatergic) of the structure. Granule and periglomerular cells (GABAergic interneurons) and glial cells (astrocytes) modulate olfactory information processing. Centrifugal fibers are constituted of inputs from the primary olfactory cortex and the neuromodulatory nuclei (noradrenaline, acetylcholine, serotonin). They contact granule and periglomerular interneurons.

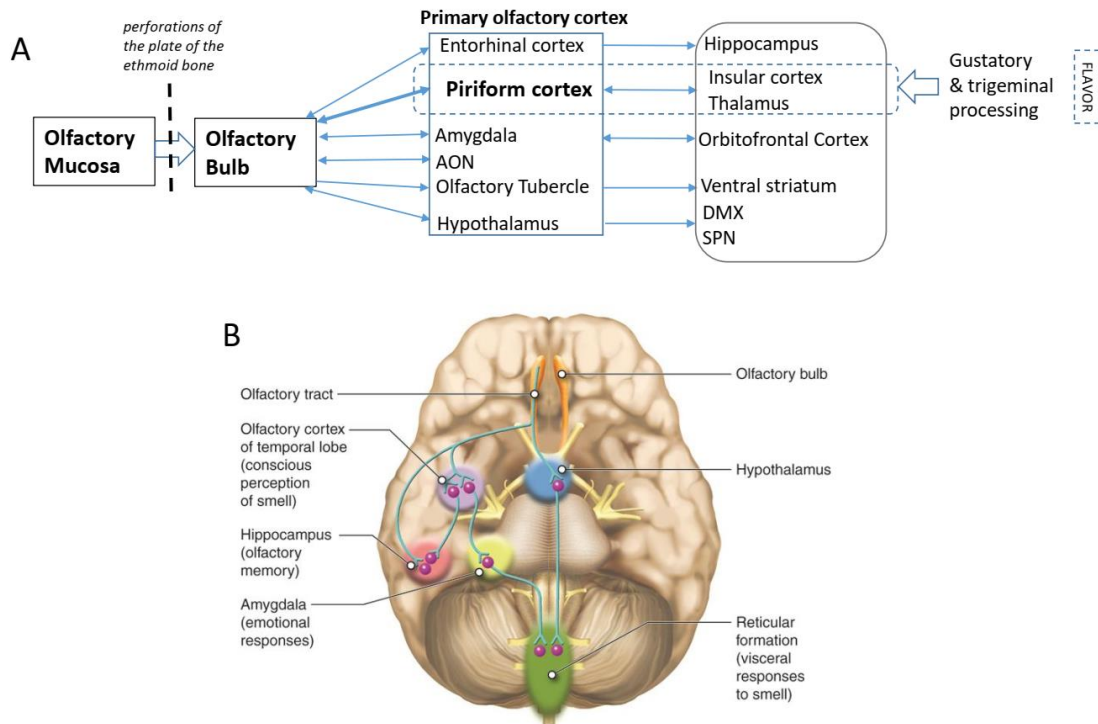


Figure 3

A: Diagram of the neuroanatomical connections within the olfactory pathways. AON: Anterior Olfactory Nucleus. DMX: dorsal motor nucleus of the vagus nerve. SPN: sympathetic preganglionic neurons. Dorsal B: Ventral aspect of human brain, in colors are brain regions that receive information from the main olfactory bulb. This work by Cenveo is licensed under a Creative Commons Attribution 3.0 United States (<http://creativecommons.org/licenses/by/3.0/us/>).

1.3. Bidirectional modulation between olfaction and food intake regulation

Even though it is recognized in humans that smell strongly influences appetite and satiety (Yeomans, 2006), the crosstalk between olfaction, food intake and energy metabolism in physiological conditions is still largely unknown.

First, seemingly to the hypothalamus, the MOB is highly sensitive to modulation by hormonal signals related to metabolism and food intake. In this structure, the blood-brain barrier is more permeable than in other brain regions, and the density of blood capillaries in the superficial layers of the MOB, at the level of the glomeruli is particularly high (Ueno et al., 1991; Lecoq et al., 2009). In addition, the olfactory system (OM, MOB and PCx) express high levels of receptors for the main peptidergic and hormonal factors that accompany energy homeostasis, whether they are orexigenic (promote food intake) or anorexigenic (inhibit food intake). Many arguments suggest that the MOB in addition to processing odors can be considered as a metabolic sensor (see for a review Palouzier-Paulignan et al., 2012).

Second, recent studies gathered evidence that odorants influence brain areas regulating food intake and energy expenditure and particularly the hypothalamus. This structure, primary integrator of circulating signal of hunger and satiety, contains autonomic neurons that regulate

sympathetic and parasympathetic outflow (Luquet et al., 2010; Sladek et al., 2015). Chen et al. showed that in fasted mice, the mere presentation of food (and not its ingestion) has the same effect on the activity of the neurons of the arcuate nucleus (ARC) than the presence in the circulation of nutrients or hormones related to food intake. Food odorants inhibit Agouti-Related Peptide-expressing (AgRP) neurons and activate pro-opiomelanocortin (POMC) neurons within seconds. Contrary to predictions, this result shows that sensory cues previously associated with food by animals (particularly its smell and sight) are processed by neurons of the ARC as a real-time information on the availability of food in the environment (Betley et al., 2013; Chen et al., 2015). Direct projections from the MOB to the ARC (Russo et al., 2018) could be involved in this connection albeit pathways from the MOB to the hypothalamus are still not clarified (Price et al., 1991; Garrison and Knight, 2017). There is no evidence for a direct projection from the ARC to the olfactory system but both POMC and AgRP neurons project onto orexin neurons located in the lateral hypothalamus, and interestingly these neurons have a direct projection to the MOB (Peyron et al., 1998; Shibata et al., 2008; Schneider et al., 2020). Moreover, cholinergic neurons of the basal forebrain have recently been found to have a powerful role in modulating feeding behavior (Herman et al., 2016), and could also be involved in the variation of olfactory perception. In mice, optogenetic stimulation of the basal forebrain glutamatergic projections to the lateral hypothalamus elicited both hypophagia and a potent food-odor avoidance behavior (Patel et al., 2019).

Third, odorants are the most powerful distal cues to evaluate food palatability and hedonic content. Early work by Jacques Le Magnen in the 1960's examined the influence of odors on food palatability and introduced the notion sensory specific satiety. When he fed rats with odorized food for 2 hours every day, he noticed that rats consumed 2 to 3 times more food when differently odorized food were presented in succession compared to the same amount of food associated with a single odorant (Le Magnen, 1956, 2001). Indeed, the same odorant can be very appetizing on an empty stomach, acquire lower pleasantness after consumption and even become completely repulsive at satiety. But this affects specifically the consumed odorant (Cabanac and Duclaux, 1973; Duclaux et al., 1973; Rolls et al., 1981; Le Magnen, 2001). Then, the olfactory system came into focus as an important contributor to unintentional weight gain (Morquecho-Campos et al., 2020). Given that olfactory signals are perceived before food consumption (orthonasal route), food odor exposure has been shown to enhance sensory-specific appetite (McCrickerd and Forde, 2016; Boesveldt and de Graaf, 2017), initiate cephalic phase responses (Ferriday and Brunstrom, 2011) and even increase meal sizes (Ferriday and Brunstrom, 2008) and calorie intake.

To summarize, the olfactory system, the hypothalamus and mesocorticolimbic circuits form an integrative network for the regulation of energy metabolism and food intake. This network is deeply impacted by metabolic syndrome and specifically obesity and fat and carbohydrate enriched food consumption. However, the outcomes of metabolic syndrome on olfaction are still unclear. Until recently, olfactory testing lacked the proper standardization necessary to have an overview of olfactory characteristics linked with metabolic diseases. In the past few years, an increasing amount of data have been gathered on this topic. In parallel, studies of the link between olfaction and food intake in animal models have also expanded, arguing that the olfactory system is at the crossroad between sensory processing and metabolic sensing (Palouzier-Paulignan et al., 2012). Here we review evidence in humans and in rodents of the

cost of energy rich food intake leading to metabolic diseases and its associated deleterious impact on olfactory processing.

2. Characteristic of olfactory modifications in the context of metabolic diseases

2.1. Obesity and olfaction in humans

Surprisingly enough, it is difficult to find a clear answer about the impact of obesity on olfactory ability and performances. Two systematic reviews are specifically focused on psychophysics evaluation of olfaction in obesity (Islam et al., 2015; Peng et al., 2019). As pointed by (Islam et al., 2015), a lot of studies have technical limitations regarding the lack of sample power, the gender balance, the age of the patients, accurate control groups and the procedures used to scale smell. With improvement of procedures used to test olfaction, and the increasing number of studies taking smell into account, more data are available on changes in olfactory processing in obese individuals.

2.1.1. Clinical Olfactory Tests

Different tests have been used in clinical studies, we will focus our review on the 3 main and standardized tests. In the last decade, the “Sniffin’ Sticks” test (SST) has become the most widely used tool for assessment of orthonasal olfactory performance, based on pen-like odor dispensing devices (Hummel et al., 1997). It assesses three subtests of olfactory function: olfactory threshold (T), odor discrimination (D) and odor identification (I), the sum of the results for the three subset being the “TDI” score. Odorant detection measure the lowest concentration of odorant detected by the subjects; the discrimination assesses if the subject can distinguish between different odorant compounds. Alteration on both parameters reflect an alteration of olfactory function, but discrimination can be impacted with an intact detection threshold. The identification is the ability to name an odorant from a list of 4 alternatives for each odor. Based on obtained TDI score, functional anosmia is defined as a TDI score ≤ 16.5 , normosmia as a TDI score > 30.5 and hyposmia as a score between these two values. The University of Pennsylvania Smell Identification Test (UPSIT) is another commonly-used test. Participants are required to identify the smell of 40 “scratch-and-sniff” microencapsulated odorant strips from a forced choice of 4 possible answers. The total number of smells correctly identified is normalized by age and sex (Doty et al., 1984; Joseph et al., 2019).

As described previously, odors are perceived through two classic pathways: via the nostrils, during sniffing, referred to as orthonasal olfaction, and via the mouth, during eating and drinking, referred to as retronasal olfaction. The perceived smell of food is based on the interaction between these two pathways (Bojanowski and Hummel, 2012). Contrarily to the tests described above the Candy Smell Test can test retronasal olfactory performances. It consists in placing a small candy with one target food-related aroma on the tongue. Subjects are then asked to suck or chew the candy and name the target aroma out of a list of four to five possible answers in a forced choice manner (Renner et al., 2009; Besser et al., 2020). After each candy, subjects must rinse their mouth with water. The maximal attainable test score is 21 for

the applied 21-item version of the Candy Smell Test also known as 'Flavor Test' (Nettore et al., 2020). A total of 21 aromatics (including one blank, water) are administered. The flavor score (FS) is calculated as the sum of correctly identified aromatics and ranged from 0 to 21.

2.1.2. Relation between orthonasal and retronasal olfaction and the BMI

According to the World Health Organization (<https://www.who.int/health-topics/obesity>; 2021), overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Body Mass Index (BMI) is a simple numeric measuring tool representing the weight in kilograms divided by the square of height in meters. An individual is considered overweighted when its BMI is comprised between 25 and 30 kg/m², a BMI above 30 falls in the obesity range and a BMI of 40 or higher is categorized as severe or morbid obesity.

Overall, pathologically high or low body weight or BMI is associated with reduced olfactory capacities. Aschenbrenner et al found that patients with eating disorders, such as anorexia nervosa or low weight bulimia nervosa (BMI<18), have lowered olfactory sensitivity that improved with increasing BMI (Aschenbrenner et al., 2008). Subjects suffering from severe obesity (BMI >45 kg/m²) are more likely to have olfactory dysfunction than moderately obese individuals (BMI>30 kg/m²), and elevated BMI is associated with a significantly increased olfactory thresholds (Obrebowski et al., 2000; Richardson et al., 2004; Simchen et al., 2006; Skrandies and Zscheschang, 2015; Fernández-Aranda et al., 2016; Fernandez-Garcia et al., 2017; Peng et al., 2019; Poessel et al., 2020b). Consistent results were reported in 2015 by (Patel et al., 2015) who found that 80% of individuals (19-85 years) with impaired olfactory performances were overweighted or obese. In middle-aged stage I-II non-diabetic obese patients (31 women, 29 men, 50-70 years; 30>BMI>40 kg/m², with metabolic characteristics in the physiological range including normoglycemia after fasting), olfactory deficit is significantly prevalent (Campolo et al., 2021). If the BMI is a simple measure of obesity, it is also correlated with hormonal changes which could be the cause of olfactory impairment, but very few studies have controlled these parameters. The relation between hormonal levels and olfaction will be discussed in the section 3.1.

Flavor perception mostly relies on retronasal rather than orthonasal olfaction (Shepherd, 2006), but most of the studies investigating the effects of olfactory dysfunction on body weight essentially focused on orthonasal abilities, while only a few of them examined the retronasal odor perception. In a recent study conducted by (Besser et al., 2020), retronasal olfactory abilities were assessed in a group of 74 subjects (47 females, 27 males; 20–63 years) using the SST while retronasal olfactory functions were tested in a group of 66 subjects (42 females, 24 males; 20–59 years) using the Candy Smell Test. The author found that both orthonasal and retronasal olfactory abilities declined with higher BMI and suggested the need of further studies using established retronasal tests. A similar study by (Nettore et al., 2020), investigated retronasal olfaction using the Flavor test, a series of 20 aromatic extracts, in 420 subjects belonging to different BMI classes (276 females and 144 males, >18 years old; BMI 25.9 to 43.7). In those subjects, higher BMI inversely correlated with Flavor Score (FS): individuals with obesity had lower FS when compared to normal weight or overweight subjects. This effect

was significant between 18 and 47 years old); whereas it was absent in older individuals (>47 years old).

2.1.3. Homeostatic and Hedonic regulation of olfaction in normal weighted vs obese individuals: discrepancy between food vs non-food odors

It was hypothesized that the olfactory detection threshold was lower when hungry compared to being satiated, which would support the notion that a decrease in sensitivity is part of the food intake control mechanism. Older studies have shown that odor acuity was higher with higher degrees of hunger and that a meal was preceded by a period of decreased olfactory thresholds whereas a period of increased olfactory thresholds was observed after food intake (decreased sensitivity) (Goetzl et al., 1950; Hammer, 1951). However, available data are not consistent and it is still unclear whether the satiety or the circadian clock are responsible for olfactory acuity change over daytime (Koelega, 1994). These discrepancies are also confounding for studies in obese individuals. When using neutral odors, Stafford and Welbeck observed that olfactory sensitivity was indeed higher in the non-satiated versus satiated state, and that this difference was increased in high BMI group compared to the low BMI group (19-49 years) (Stafford and Welbeck, 2011). The authors did not show a difference in olfactory acuity in response to food odors in the satiated versus non-satiated state in a low BMI group. Nevertheless, the high BMI group had greater acuity for food odor in the satiated versus non-satiated state (Stafford and Welbeck, 2011). These data point to opposite effects in the relation between internal state and acuity for food and non-food odorants, and this effect is enhanced in obese individuals. Moreover, obese subjects (BMI>30; 18-24 years) were found to have higher sensitivity to odors with high hedonic value (pleasantness ratings) like chocolate odor (Stafford and Whittle, 2015). However, (Poessel et al., 2020b) used 3 different odors (n-butanol, chocolate and grass) in 75 young healthy participants (26 normal weight, 25 overweight, and 24 obese; 37 women; 18-35 years). They found no significant interaction between the quality of odor (food vs. non-food), hunger state, and BMI. Thus when focusing on chocolate odor, they found that higher BMI is associated with an increase in odor sensitivity in the sated state, but only on metabolically healthy individuals (optimal Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) ≤ 1). Although data are not sufficient to conclude, there may be a dysregulation of the modulation of central odor sensitivity with nutritional state in obese.

From a different perspective and at the other end of the hedonic spectrum, (Trellakis et al., 2011) found that pleasantness rates for the generally disliked odor of black pepper oil were significantly lower in obese compared with non-obese subjects (20-45 years). This implies that the association of an odor to bitter tasting foods of generally lower calorific value is more negative in obese versus non-obese individuals. Taken together, these findings show that obese individuals show increased sensitivity and preference for an odor associated with palatable energy dense foods.

2.1.4. Effect of age and sex on olfactory performances in obese vs non-obese individuals

Nutrition is an important factor to consider in elderly people. Given that metabolic rate, energy intake, and macronutrient intake decrease with age, food selection can become critically

important in maintaining energy balance (Wakimoto and Block, 2001). Age-related declines in various aspects of olfactory function have been documented and older individuals show higher olfactory detection thresholds: (Cain et al., 1990; Murphy et al., 2002).

Most adults gain weight between the third and the sixth decade of life. After the sixth decade, a moderate weight loss is observed (Addison and Ryan, 2018). In line with these physiological facts, (Simchen et al., 2006) found that in adults (<65 years), an increasing body weight or BMI was associated with a lower odor identification and odor detection capacities. However, surprisingly, among elderly subjects (>65 years) higher relative body weight or BMI was associated with higher odor identification and odor detection abilities.

In addition to BMI and age, the subject's gender has been found to be associated with changes in olfaction. Within a normosmic population (85 males and 76 females; ages 19-95), women had better olfactory capabilities when compared to men of the same age (Ship et al., 1996). The effect of high BMI (obesity) on olfactory performances had been studied in both female and male groups but no study compared the interaction between BMI and gender on olfactory perception. Hence, further studies are needed to answer this question.

2.2. Type 2 diabetes and olfaction

Changes in olfactory sensitivity were reported both in type 1 insulin-dependent Diabetes (mild hyposmia in T1D which is insulin-dependent but obesity-independent, (Yilmaz et al., 2019)) and T2D (Weinstock et al., 1993). Since most T2D patients are obese (~80%) and the pandemic of obesity explains the incidence and prevalence of T2D, we decided here to focus on smell function in T2D patients (Eckel et al., 2011). Indeed, a progressive rise in insulin resistance and a consequent progressive defect in insulin secretion makes the metabolic state of obese patients worsens slowly towards T2D (Box1).

Smell dysfunction in diabetes was first reported 60 years ago (Jørgensen and Buch, 1961): even tested roughly and only with coffee, 35 over 69 patients were found hyposmic and 7 anosmic. No gustatory problems were detected confirming the lack of dysgeusia previously reported in T2D subjects (Bonatti, 1950). Finally, no correlation between hyposmia and age or severity/duration of diabetes was observed but it was not indicated whether patients were T1D or T2D. Two excellent reviews thoroughly revisited studies dealing with the fate of olfaction in T2D patients from the 1960's (13 articles reviewed in (Zaghloul et al., 2018)) and with a further systemic analysis (11 articles reviewed in (Kim et al., 2019)). Although most of the studies have checked for the absence of any Ear, Nose and Throat (ENT) problems, results are controversial: some studies clearly found olfactory deficits while others describe only mild impairment of olfactory function or intact smell. Methodological issues on the poor follow-up of olfactory function (Jørgensen and Buch, 1961; Hawkins and Pearlson, 2011; Seraj et al., 2015) on one side and the imprecise description of the metabolic state of patients (for example T1D not distinguished from T2D in (Jørgensen and Buch, 1961; Patterson et al., 1966; Brämerson et al., 2004; Gascón et al., 2013) on the other seem to be the two major source of discrepancies. In addition, the correlation between BMI and smell defects in T2D observed by Naka et al. (2010) was not confirmed by Gouveri et al. (2014). However, if a classification of smell deficit is made according to the severity of T2D, results are more homogeneous: in fact, patients suffering from uncomplicated T2D had the least olfactory impairment (Naka et al., 2010) while accumulation of complications (retinopathy, neuropathy, hypertension...) was

accompanied by profound hyposmia (Floch et al., 1993; Brady et al., 2013; Gouveri et al., 2014; Yazla et al., 2018; Catamo et al., 2021). In that sense, very recently, (Catamo et al., 2021) showed that 22% of 94 T2D patients had identification impairment in salt taste and 55% odor recognition (SST) compared to control subjects. In the same study, 65% of hypertensive T2D subjects presented smell identification impairment compared to 18% of non-hypertensive patients underlying again the fact that T2D with complication strongly impairs olfaction. Micro- (retinopathy, Gouveri et al., 2014) and macro- (coronary artery disease and peripheral vascular disease with a likely ischaemic damage to the olfactory system, (Weinstock et al., 1993)) vascular damages were shown to be associated with poor olfactory scores and could contribute to the overall olfactory impairment described so far. Self-reported profound hyposmia was also observed for T2D patients on aggressive treatments including oral and insulin treatment, i.e. with higher severity, compared to those who reported no use of drug treatment (Chan et al., 2018). Like earlier, these authors did not find an association between diabetes duration and prevalence of olfactory dysfunction. However, noteworthy, in elderly populations (age 68-77), altered olfaction in T2D patients was reported to be associated with cognitive decline (Sanke et al., 2014).

2.3. Bariatric surgery and olfaction

Laparoscopic bariatric surgery (BS) procedures are used to induce weight loss in patients with extreme, morbid obesity (BMI > 40). These techniques mainly consist in Roux en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) (Angrisani et al., 2015). RYGB involves cutting the stomach in two to create a pouch out of the smaller proximal portion of the stomach, attaching it to the jejunum, thus bypassing a large part of the stomach and all the duodenum. In SG, the stomach is reduced to about 1/3 of its original size, by surgical removal following the major curve, leaving the stomach with a banana shape also called a “sleeve-like” shape. SG permanently reduces the size of the stomach. Both RYGB and SG induce rerouting of nutrients to the small intestine, directly and faster respectively. They allow significant improvement in weight loss outcomes and obesity-related comorbidities such as diabetes (Koliaki et al., 2017). They were shown to trigger profound changes in peripheral secretions, including the levels of key hormones involved in energy metabolism such as gut hormones ghrelin and GLP-1, leading to a lower weight set point (Moffett et al., 2021). Since it was observed that food preferences of obese patients after BS is significantly changed (Coluzzi et al., 2016), suggesting a modification of sensory perception, the influence of BS on chemical senses was explored recently. Since taste is not the focus of the present review, we advise to refer to the comprehensive reviews of (Nance et al., 2020) and (Ahmed et al., 2018) for the follow-up of gustatory performances after BS.

Using a semi-quantitative taste and smell questionnaire, (Graham et al., 2014) reported that in 104 patients (24–63 years) with RYGB, sensory changes in appetite, taste and smell were noted in 97, 73 and 42 % of patients, respectively. With SST and focusing on morbidly obese patients, about 22.7 % of the 44 patients were found hyposmic by Holinski and colleagues (Holinski et al., 2015), showing significantly lower TDI scores than healthy controls. The same percentage of this cohort (15 RYGB and 15 SG patients) presented dysgeusia probed with the taste strip test. Six months after BS, half of the hyposmic patients presented better olfactory function.

Hancı et al., (2016) performed SST in 54 morbidly obese patients (19–57 years) before SG then patient's weight losses and TDI were estimated at first, third and sixth month post SG. Median TDI scores of the patients were significantly increased from 25 preoperatively to 32 in the first month, 37 in the third month, and 41 in the sixth month showing improvement in olfactory sensitivity in SG patients (as a reminder a TDI score >30.5 is the threshold for normosmia). Jurowich et al., (2014) found a positive correlation between body weight loss and TDI scores after SG (15 patients) but not in 15 RYGB patients. In fact, some controversies exist on the higher impact of SG over RYGB on smell. First, SG patients showed no improvement in their olfactory function tested by SST (39 patients tested 24 hours post SG in (Pisarska-Adamczyk et al., 2021); 8 patients in (Enck et al., 2014)). In opposition, (Makaronidis et al., 2016) analyzed subjective appetite, taste, smell and food preferences before and after BS and reported olfactory improvement in 41% of the 98 RYGB patients compared to 28% of the 155 SG patients. In addition, (Melis et al., 2021) recently reported that both SG (n=21) and RYGB (n=26) had positive effects on taste and smell (tested by SST recovery) after surgery. These discrepancies may be due to multiple methodological reasons ranging from the number of patients in the cohort (from a dozen to more than 100) to the success rate of BS. In fact, successful surgery in the long term is a crucial factor in sensory recovery and food preference changes (Guyot et al., 2021).

Surprisingly, changes in sensory sensitivity post-surgery could reach hyper-reactions leading to food aversions in patients who experienced more post BS weight loss and reduction in BMI (Makaronidis et al., 2016). Graham et al., (2014) reported that 73% patients had aversion to specific foods after surgery, with meat products the most commonly cited. Tichansky et al., (2006) also observed that many patients developed aversions to sweet foods and meats and that food preferences changed right after surgery (in 68% of 82 RYGB patients). Sensory aversion is a complex emotional reaction implicating at least the amygdala and the insular cortex (Miranda, 2012; Münzberg et al., 2015), two brain areas that receive massive olfactory information (Mainland et al., 2014). Extended effects of BS on the overall sensory-limbic networks should be studied by brain imaging techniques to give access to the brain activity underlying changes in olfactory aversion after surgery.

2.4. Preclinical model of diet-induced obesity and olfaction

Although most of the knowledge regarding impairment of olfaction in metabolic syndromes has been collected in humans, animal models are required to decipher the origin and mechanisms and to understand what is the biological substrate of olfactory defects. Diet-induced obesity (DIO) models are considered the most accurate in reproducing the multidimensional aspect of human obesity combining genetic and environmental factors. They are based on the fact that in rodents –as in human- the consumption of food enriched in fat (using diet having a different percentage of fat and sucrose content) is positively correlated with body weight gain and elevation of plasma glucose, cholesterol, triglyceride, free fatty acid, and leptin levels (Ghibaoudi et al., 2002; Hariri and Thibault, 2010).

Numerous alterations in olfactory performances have been observed in diet-induced obesity (DIO) models in rodents. Behavioral testing shows that mice under high fat diet (HFD) have poorer olfactory performances. Lietzau et al have followed the evolution of olfactory performances during the development of obesity by comparing 3 groups of mice fed either with standard diet, HFD (54% energy from fat, 29% from carbohydrate) or western diet (42% energy

from fat, 43% from carbohydrate). After 1 month of HFD or western diet, no difference in the olfactory performances was observed compared to a regular diet fed group. After 3 months of diet, the HFD group started to spend more time to find a buried food (a piece of a protein bar), and the difference was significant for the 2 groups (HFD and western diet) after 8 months of diet (Lietzau et al., 2020). Another study in mice revealed different outcomes according to the sensory properties of the food used in the test (Tucker et al., 2012b). Using a peanut butter cracker, whose odorant properties were close to the fat food used for the diet they obtain an increase of the time necessary to uncover the food. However, this impairment was abolished when a chocolate treat was buried. The authors suggest that the fat diet impaired the detection of fat related odorants. Moreover, the same test performed on a genetically obese strain MC4R-null mice fed on regular diet did not show olfactory impairment (Tucker et al., 2012b). However, they did not find impairment in the capacity of obese mice to discriminate between two fatty acids (oleic and linoleic acid) that represent a large proportion of the used diet when tested in the habituation dishabituation test. This is in agreement with the study by Lietzau, in which HFD mice were impaired in novel odor recognition paradigm but not in discrimination capacity in habituation dishabituation test. Interestingly, mice fed with western diet, enriched in carbohydrate and rather associated with T2D, were impaired earlier than mice fed on HFD, suggesting a higher impact on cognitive processes. On the opposite, using HFD (Takase et al., 2016) found that performances in a habituation dishabituation task were impaired for DIO mice. Moreover, a similar deficiency was found in mice fed with the same fat diet but whose quantity eaten was adjusted based on their body weight each day so that their body weights were kept similar to those of the chow diet group, i.e. in non-obese non-diabetic mice consuming low quantities of HF (Takase et al., 2016). Finally, when challenged in an olfactory go-no go operant conditioning paradigm, HFD (60% fat) or mild FD (32% fat) fed mice failed to show good discriminatory performances compared to animals fed on chow diet (13% fat). In addition, obese mice were not able to learn the reversal of the task rules (Thiebaud et al., 2014). However, the limitation of these tests is that the olfactory component is only partial, they rather involve cognitive functions such as flexibility and memory that are known to be impacted in obesity (Yang et al., 2018).

Findings on olfactory performances are similar in rat models. Lacroix et al 2015 compared the behavior of obesity-prone Sprague-Dawley rats fed with a high-fat/high-sucrose diet (HFHSD) with those of obese-resistant ones fed with normal chow. Obese rats also have poorer performances in finding a hidden almond cookie (Lacroix et al., 2015). After several consecutive days of testing, rats became more efficient to find the buried food, however this learning was affected in obese animals that did not improve their performances over time. On the other hand, when challenged in a conditioned odor aversion test, in which the animals have to choose between a bottle containing plain water or a bottle containing water odorized with the conditioning odor, obese rats drank less odorized water even for low odor concentration suggesting that they have an increased odor sensitivity. Authors also report modification in exploratory behavior. In lean rodents, the duration of exploration of a food odor is increased in fasted compared to fed animals (Prud'homme et al., 2009). However the difference according to the metabolic status is largely diminished in obese animals who spent less time sampling a food odor when fasted compared to lean controls (Badonnel et al., 2014; Lacroix et al., 2015). Interestingly, in the conditioned odor aversion test, the obese rats spent more time sniffing the bottles, odorized or not (Lacroix et al., 2015).

The consequences of T2D on olfaction have been assessed using the same tests (buried food and habituation-dishabituation) in the model of Goto-Kakizaki rats. The results recapitulated the clinical observations in humans with T2D showing an alteration of both olfactory detection and memory (Lietzau et al., 2018).

Taken together these data are in agreement with results obtained in humans that metabolic alterations negatively impact olfactory performances. Moreover, the choice of the odorant and the type of odorant (food or neutral odor) could affect the outcome of the tests, in agreement with the results presented above in humans (cf 2.3.1). In addition, the use of precisely controlled food in rodents brings evidence suggesting that the energy content of food independently of the body mass or fat accumulation in the animals is a major contributor of olfactory defects, however, more studies are needed to support this hypothesis.

3. What are the causes of olfactory defects related to metabolic diseases?

Many hypotheses can be raised to explain how metabolic status and/or circulating nutrients concentration affect olfaction. Until recently, the human MOB was not accessible to functional imaging. Since it is now technically possible to use non-invasive technologies to examine this structure in patients, the field will expand (Fournel et al., 2020; Iravani et al., 2020). For now, most of the information we have on modification of the MOB functions by obesity or energy enriched food is provided by animal models. Even if studies on the consequences of obesity on the structures of the olfactory pathway are scarce, we can gain insight from the existing information on the link between the metabolic status and olfactory functions in physiological condition. As mentioned above, the olfactory system is strongly influenced by hormones and nutrients which regulation is profoundly altered in the context of obesity. For a complete review of the link between endocrine mechanisms and olfaction, the reader is referred to (Palouzier-Paulignan et al., 2012). We will mention here more recent results and hormonal changes related to obesity.

3.1.Changes in hormone and nutrients levels can account for olfactory impairment

3.1.1. Insulin and glucose

Obesity is characterized by the elevation of blood glucose and insulin resistance. Insulin, insulin receptors and the insulin dependent glucose transporter GLUT4 are found in olfactory regions, from the mucosa (Lacroix et al., 2008) to the PCx (Zhou et al., 2017; Al Koborssy et al., 2019), the anterior olfactory nucleus, and the OT (Hill et al., 1986). In the MOB, GLUT4 co-localized with ~~IR~~ insulin receptors in M/T cells and glomeruli (Julliard et al., 2017). Moreover, the MOB is the area of the central nervous system containing the highest concentration of insulin (Baskin et al., 1994), the highest concentration of insulin receptors (Hill et al., 1986) and the highest rate of degradation (Banks et al., 1999). Insulin transport through the blood brain barrier at the level of the MOB is 2 to 8 times faster than for other regions of the brain (Banks et al., 1999). Interestingly, using extracellular fluid glucose concentrations measured in the MOB of Zucker leptin-receptor-deficient rat (fa/fa), Aimé et al, 2014 reported that the MOB contained twice the amount of glucose found in the cortex, and in both regions, the values are 2-fold higher in obese fa/fa compared to lean rats. Finally, M/T cells in the MOB

are responsive to glucose concentration (Tucker et al., 2010; Aimé et al., 2014; Al Koborssy et al., 2014) and express molecular hallmarks of glucose sensing cells (Julliard et al., 2017). Taken together, these elements add to the hypothesis of the MOB as a metabolic sensor.

In obese Sprague-Dawley rats fed with HFHSD, the number of insulin receptors in the MOB is decreased (Lacroix et al., 2015). However, the lack of sensitivity to insulin in DIO mice could also be explained by the physiological properties of the M/T cells of the MOB. In lean mice, insulin application enhances M/T cell excitability by increasing neuronal firing activity of M/T cells and decreasing interspike interval (Savigner et al., 2009; Fadool et al., 2011; Kuczewski et al., 2014). In DIO animals, the basal firing pattern of M/T cells is altered, formed by shorter action potential clusters separated with long pauses. In this condition, acute insulin stimulation dampened M/T cell modulation observed in lean animals (Fadool et al., 2011).

In obese and lean young women (18-35 years), (Poessel et al., 2020b) measured insulin reactivity in response to food intake and found that insulin resistance was negatively correlated with food-related odorant (chocolate) independently of BMI. In elderly human population (>50 years), it was shown that insulin resistance (but not fasting blood glucose, glycohemoglobin or serum insulin levels) was linked to an increased risk of olfactory defect (Min and Min, 2018). Chronic administration of insulin in rodents was used as a way to recapitulate the insulin elevation in obese individuals. However, in mice models, long-term administration of insulin delivered intranasally did not affect olfactory behaviors, energy expenditure, or object memory recognition (Bell and Fadool, 2017). Hence, a possible mechanism of how high-fat diet perturbs insulin modulation of M/T cells has been extensively studied by the laboratory of D. Fadool. They investigated the role of Kv1.3, a voltage-dependent, insulin-regulated potassium channel predominantly expressed in M/T cells and a main contributor to the total potassium current in these neurons (Fadool et al., 2000). As expected, the deletion of Kv1.3 has metabolic impacts. Mice double mutant for Kv1.3 show improved insulin sensitivity and an elevated energy expenditure. When challenged with a moderately high-fat diet of 32% fat for 26 weeks, Kv1.3^{-/-} mice fail to gain weight compared to their wild-type counterparts. Interestingly, removal of the MOB reverses their DIO resistance (Tucker et al., 2012b). These metabolic effects are accompanied by changes in the olfactory capacities as Kv1.3^{-/-} mice are “Super-smeller”, they have improved olfactory performances (Fadool et al., 2004). Interestingly, a polymorphism in the human Kv1.3 gene that functionally elicits a gain in function has been associated with impaired glucose tolerance, lower insulin sensitivity, and impaired olfactory ability in male homozygous carriers (Tschrutter et al., 2006).

3.1.2. Ghrelin

GHRSR-1a type of ghrelin receptor is expressed in the different layers of the MOB and in the PCx. In lean rats, intracerebro-ventricular injection of ghrelin increases odor detection as tested in a conditioned odor aversion test (Tong et al., 2011). Ghrelin has also been related to sniffing as intra cerebroventricular injections of the compound enhanced exploratory sniffing in both rodents and humans (Tong et al., 2011). Then ghrelin could act to increase olfactory sensibility and performance before a meal. Ghrelin level is decreased in obese individuals (Tschöp et al., 2001) and HFD in mice has also been shown to trigger central ghrelin resistance by reducing both receptors' expression in the hypothalamus and AgRP neuronal responsiveness to ghrelin (Briggs et al., 2010; Naznin et al., 2015). Since ghrelin level is negatively correlated to BMI it is also negatively correlated to smell capability (Fernández-Aranda et al., 2016). Even

though the independence of these two factors cannot be established, we can hypothesize that ghrelin resistance in HFD mice would reduce the impact of the metabolic status (fasted/fed) on olfactory perception and decrease its sensitivity.

3.1.3. Leptin

Leptin-dependent obesity has only been observed in rare cases of obesity in humans (O'Rahilly, 1998). Nevertheless, obesity has typically been associated with leptin resistance, i.e. the inability of exogenous or endogenous circulating leptin to initiate metabolic outcomes, such as anorexigenic effects in obese individuals. Yet, the role of leptin in obesity especially in humans is still unclear (Myers et al., 2010).

Early studies have used animal models of leptin signaling dysfunctions to test odor perception. The ob/ob mice strain results from a spontaneous autosomal recessive mutation of the ob gene that produces leptin (Zhang et al., 1994). Very quickly during their growth these mice become overweight and develop metabolic syndromes characteristic of obesity: hyperphagia, transient hyperglycaemia and elevated plasma insulin concentration associated with an increase in the number and size of islet beta cells (Coleman, 1982). Obesity in ob / ob mice and the associated phenotypical alterations can be restored with exogenous administration of leptin. An analog model in rats is the Zucker strain, a genetic model of obesity driven by a mutation of the leptin receptor. The mutation causes an increase of food intake, a decrease of energy expenditure, hyperglycemia and insulin resistance (Yamashita et al., 1997). Contrarily ob/ob mice, the production of leptin is maintained but its affinity for the receptor is decreased.

Obesity caused by leptin signaling deficiency is associated with an increased olfactory detection threshold as seen for a buried food test in ob/ob mice (Getchell et al., 2006) as well as in Ob Zucker leptin-receptor-deficient rat (fa/fa) in a conditioned odor aversion protocol (Aimé et al., 2014). The Zucker fa/fa rats also exhibited greater food-seeking behavior when exposed to both novel and familiar olfactory cues (Thanos et al., 2008). In DIO rats, no difference in leptin receptors either in the mucosa or in the MOB was found (Lacroix et al., 2015). In addition, ob/ob mice performed better than wild-type (WT) mice in the early stage of an odor discrimination task, and brain rhythms associated with odorant processing, which mark the functional dialogue between the MOB and the PCx, were largely modified (Chelminski et al., 2017). This suggests a broader role for leptin that would modulate neuronal activity, functional connectivity and memory between brain structures within the olfactory system.

3.2. Alteration of the olfactory structures: from the olfactory mucosa to associative brain regions

In obesity, a chronic caloric excess will lead to non-subcutaneous ectopic fat mass accumulation. An excess of adipose tissue favors inflammatory cascades associated with the secretion of pro-inflammatory adipokines. This condition is associated with impaired insulin signaling triglyceride storage and basal lipolysis and an increase in the circulating fatty acids. When present in excess in the circulation, fatty acids become lipotoxic and promote dyslipidaemia, while fat deposition in the muscle promotes insulin resistance and fat deposition in the pancreas promotes β -cell dysfunction and insulin resistance (O'Brien et al., 2017). This type of inflammation can have dramatic consequences in the brain. Since we showed in this review that olfactory function is blunted in obese patients (with or without T2D, (Campolo et

al., 2021)), in non-obese diabetic patients (Zhang et al., 2019), and even, in animal models, in non-obese non-diabetic mice fed with low quantities of HF (Takase et al., 2016), we postulate that inflammatory mechanisms in the brain are the main and early pathological events that could impair the olfactory system in all these different metabolic contexts. Noteworthy, fast HFD-induced inflammation is sufficient to decrease insulin sensitivity in non-obese non-diabetic rats (Cruciani-Guglielmacci et al., 2005).

3.2.1. The olfactory mucosa

Enriched diet has highly deleterious consequences on brain structures and functions. This is well documented in the hypothalamus where (De Souza et al., 2005) noted abnormal concentration circulating inflammatory markers including proinflammatory cytokines, IL-1, TNF α and IL-6 after 16 weeks of high fat diet. Hypercaloric diet intake related inflammation triggers changes in astrocytes, microglia and tanycyte physiology as well as vascular hyperpermeability (García-Cáceres et al., 2019). Moreover, inflammation processes and astrogliosis appear to start after only a week of hypercaloric diet availability, well before fat accumulation and systemic inflammation (Thaler et al., 2012; Clyburn and Browning, 2019).

In the olfactory system, highly deleterious inflammation has been documented as early as in the OM in mice fed with enriched food upon weaning and for 24 weeks. Similar effects on the mucosa are obtained using a high-fat diet (HF; 60%) or moderately high-fat diet (MHF;32%). Both conditions show an increase of microglial marker Iba-1 and a decrease in the number of ORNs in the OM due to apoptosis (Thiebaud et al., 2014; Fardone et al., 2019). The consequence of the reduced number of ORN is a decreased number of axonal projections to the glomerulus. Accordingly, apoptosis level is also increased in the OM of obesity-prone Sprague-Dawley rats fed with a HFHSD compared to obese-resistant rats fed with normal chow (Lacroix et al., 2015). This effect is accompanied by a decreased expression of insulin receptors, glucocorticoid receptors GLUT3 and GLUT4 and MCT1 type of monocarboxylate receptor with no difference in leptin receptors (Lacroix et al., 2015).

The extracellular electrical activity of olfactory receptors in the mucosa can be measured by electro-olfactogram recordings. HFD exposure causes a decrease in the amplitude of the electro-olfactogram in response to odorants (Thiebaud et al., 2014), showing an alteration of signal transduction and suggesting poorer signal transmission to the MOB. In addition, four weeks of enriched fructose diet triggered diabetes in mice which exhibited a decline in olfactory behavior correlated to decreased electrophysiological responses of olfactory neurons recorded as a population and individually (Rivière et al., 2016).

3.2.2. The main olfactory bulb

At the level of the MOB, DIO has a clear structural impact. In transgenic mice carrying a GFP tag on the M72 odorant receptor, (Fardone et al., 2019) have described that in the medial but not the lateral glomerulus targeted by the M72 receptors, the number of juxta-glomerular cells is decreased in mild or HFD compared to control mice. The excitability of those cells is also impaired. Under olfactory activation, juxta-glomerular cells surrounding the lateral glomerulus exhibited a loss of activity that was stronger for HFD than for mild FD, whereas juxta-glomerular cells surrounding the medial glomerulus were equally impaired for both diets (Fardone et al., 2019). No effect in MOB neurogenesis was reported after 8 months of HFD

(Lietzau et al., 2020), on the other hand, a reduction of neurogenesis was reported in the T2D model of Goto-Kakizaki rats (Lietzau et al., 2018). Furthermore, (Soleimanzad et al., 2019) examined resting state and odor-evoked hemodynamic profiles in the MOB of 3 months and 8 months HFD-fed mice by laser speckle contrast imaging. They observed that the relative changes in cerebral blood flow during olfactory stimulation were impaired in HFD compared to lean mice. This impairment was independent of blood vessel density a parameter not altered in HFD mice, but could rather be due to the impact of insulin resistance on neurovascular coupling.

In humans, a reduction in the MOB volume has previously been observed in pathologies presenting olfactory dysfunction, such as Parkinson's disease, Alzheimer's disease, schizophrenia, and depression (Turetsky et al., 2000; Thomann et al., 2009; Negoias et al., 2010). As observed in animals, an alteration of peripheral olfactory function can cause a reduction of the MOB volume associated with an alteration of olfactory performances (Gudziol et al., 2009). Thus, using MRI volumetric measurements in humans, studies have found a correlation between MOB volume and olfactory functions including odor threshold, odor discrimination, and odor identification (Rombaux et al., 2006; Buschhüter et al., 2008; Mazal et al., 2016). A recent study conducted by Poessel et al., (2020a) showed that obesity and increasing BMI were associated with reduced MOB volume. Accordingly, the size of the MOB was negatively correlated with several metabolic indexes, i.e. HOMA-IR, levels of plasma leptin and body fat percentage.

3.2.3. The piriform cortex

Very few studies have examined neuroplasticity associated with metabolic diseases in the PCx. No effect on the volume of the PCx nor on the neuronal activation of piriform neurons assessed using c-fos were reported after 8 months of HFD (Lietzau et al., 2020). However, in T2D Goto-Kakizaki rats, (Lietzau et al., 2018) observed a decrease in parvalbumin expression and impairment of neuroblast differentiation in the PCx. In addition, the number of GABAergic CB+ interneurons in the PCx dramatically decreased (~ 50%) in middle-aged T2D rats versus age-matched Wistar rats as a consequence of Calbindin-D28k down-regulation (Lietzau et al., 2016). Cellular stress characterized by increased JNK phosphorylation was also enhanced in T2D rats. Interestingly, both effects are counteracted by chronic treatment with the GLP-1 receptor agonist exendin-4 (Lietzau et al., 2016).

Besides, the expression of monocarboxylate transporters (MCTs) MCT1 and MCT2 was increased in the neurons of PCx of HFD-fed mice (Pierre et al., 2007). In HFD mice cortical neurons presented a high expression of both MCT1 and 2 whereas in normal conditions, neurons express mainly MCT2 and only low levels of MCT1. This increase was also observed in ob/ob mice fed with chow diet, ruling out a direct effect of nutrients. MCTs are membrane transporters that carry lactate, pyruvate and ketone bodies allowing the import and use of alternative energy substrates in conditions of metabolic challenges. The authors suggest that overexpression of MCTs in neurons could be a response obesity-related brain insulin resistance and its impact on the utilization of energy substrates by brain cells. This is consistent with the action of insulin and glucose on neuronal activity in the PCx (Al Koborssy et al., 2019).

3.3. Beyond the olfactory system: involvement of broader networks encompassing food intake regulation and reward

3.3.1. Alteration of the mesocorticolimbic system could impact food odor perception and attraction

Numerous evidence point to a clear link between pathways governing hedonic control of food intake and overconsumption of palatable food in the context of metabolic syndrome and obesity. Maladaptive modifications within the mesocorticolimbic system could lead to olfactory impairment and alter the hedonic value of food odor cues.

In both hungry and satiated lean individuals, food odors presentation leads to higher activation of the anterior cingulate cortex, the insula and the putamen (ventral striatum) than the presentation of non-food odors. In addition, in hungry individuals both food and non-food odor stimulation activates the ventral tegmental area (Sorokowska et al., 2017). The frontostriatal loop, the connections between the striatum and the prefrontal cortex is dependent on dopamine release. This network subserves adaptive behavior hence alteration in the mesocorticolimbic system in the context of obesity is likely to change the perception of odorants. This pathway is known to be differentially activated in response to food-cue between obese and normal weighted individuals (Rothmund et al., 2007; Bragulat et al., 2010). More recently, a study by (Han et al., 2021) showed that despite their lower olfactory performances, obese individuals had enhanced activation of reward regions toward an odor of high energy content food (chocolate) compared to the odor of a low calories food (cucumber). More broadly, changes in general attentional processes in obesity could change the orientation of individuals toward food odor. Indeed, overweight and obese individuals present an enhanced reactivity to food cues (Hendrikse et al., 2015). In the context of an olfactory priming paradigm, evoked potentials measured when subjects perceived visual food cues primed by food odors were different between groups with different BMIs (Zsoldos et al., 2021).

In rodents, studies have shown that the OT could play a determining role in tagging the hedonic value of an odor (Millman and Murthy, 2020). This structure receives direct projections from the MOB and also from the ventral striatum, thus it belongs both to the olfactory cortex and the reward circuits. It receives projections from dopaminergic neurons in the ventral tegmental area which would be involved in the formation of olfactory preferences (Zhang et al., 2017). Likewise, increased activity of projections from the MOB towards the OT (Midroit et al., 2021) or from the Ventral Tegmental Area to the OT during odor exposure increases the attractiveness of odors (Zhang et al., 2017). Whereas it was not clearly established early on, the MOB receives direct projections from the reward system. Even though the tubercle does not project back to the MOB, there is some evidence for direct projections from the substantia nigra (Höglinger et al., 2015) and from Tyrosine Hydroxylase positive neurons of the Ventral Tegmental Area and the ventral pallidum (Schneider et al., 2020) to the MOB.

To summarize, cerebral patterns elicited by food cues, and neural processing of hedonic olfactory information may be different in obese subjects. This difference could lead to a different level of awakening, including attention and motivation, provoked by food odor in obese individuals.

3.3.2. A potential retrograde impact of the hypothalamus on odor processing

It was shown that M/T cells project to ARC (Russo et al., 2018) and that Para Ventricular Nucleus cells in the hypothalamus respond to electrical stimulation of MOB (Guevara-Aguilar et al., 1988). Even though there is no evidence for a direct projection back from the arcuate nucleus of the hypothalamus to the olfactory system, we know that both POMC and AgRP neurons project onto orexin neurons located in the lateral hypothalamus, and interestingly there are direct projections of these orexin neurons to the MOB (Peyron et al., 1998; Gascuel et al., 2012). Besides, a recent work revealed the key role of the AgRP to paraventricular hypothalamus projections for hunger dependent food attraction by comparison with pheromone attraction (Horio and Liberles, 2021). The authors pointed the role of one of the principal neurotransmitters released by AgRP neurons, Neuropeptide Y (NPY) and its receptor NPY5R. Indeed, fasted NPY-KO mice failed to be more attracted by food odor than by pheromone contrarily to control mice, an effect reversed by injections of NPY5R agonists in the paraventricular hypothalamus. On the contrary, injection of NPY5R antagonists into the paraventricular thalamus blocked food-odor attraction in fasted mice. Then, the hypothalamo-olfactory network would control alliesthesia for food cues, i.e. the modulation in the reward value of a stimulus according to the internal state and the potential to move the body's physiological state towards homeostasis. An alteration of this mechanism in obesity could be responsible for the modification of olfactory processing of food vs. non-food odors in obesity.

3.3.3. Decrease in olfactory acuity by functional changes in the trigeminal activation

Obesity also results in functional changes to the trigeminal system. In obese HFD mice, photophobic behavior is induced by a lower dose of capsaicin intradermally injected in the face and functional TRPV1 expression is altered in trigeminal ganglion neurons (Rossi et al., 2016). Data are lacking regarding the activity of neurons connecting the trigeminal ganglion and the olfactory system in obese mice. However, in a study conducted in teenagers (10-16 years old) the impact of obesity on olfaction was shown to be stronger for odorants that stimulate both olfactory and trigeminal nerves than for odorants stimulating only the olfactory nerve (Obrebowski et al., 2000).

As mentioned previously, the trigeminal nerve activation participates to olfactory processing since the majority of odorants also activate the TRPV family of cation channels expressed at the endings of trigeminal fibers. Thus, alteration of the trigeminal system could cause olfactory changes observed in obese patients and contribute to a perceptual and cognitive difference in obesity as compared to non-obese individuals.

4. Pharmacological treatments

Type 1 endocannabinoid receptor (CB1R) is expressed in the CNS and is notably implicated in the regulation of food intake. It was targeted by rimonabant, a CB1R inverse agonist investigated as a treatment for obesity and the metabolic syndrome, which triggered deleterious side effects on mood in some obese patients and was withdrawn in 2008, two years after its approval. The role of CB1R in food intake inhibition *via* the MOB has been studied: CB1R are implicated in the regulation of glutamatergic feedback projections to the MOB, more specifically those originating from the anterior olfactory cortex (Soria-Gómez et al., 2014b). These projections play a determining role in the activity of granule cells, the main GABAergic interneurons of the MOB. Hunger induces the activation of the CB1R in the olfactory circuits,

and pharmacological activation of the CB1R, specifically at the level of the MOB, is sufficient to increase food intake, with an improvement in olfactory performance (Soria-Gómez et al., 2014a). CB1R are also highly expressed in the PCx and are required for the expression of appetitive olfactory memory (Terral et al., 2019). Moreover, the endocannabinoid system is involved in the regulation of food intake in connection with the mechanisms of reward (Lau et al., 2017). The deletion of CB1 in dorsal telencephalic excitatory neurons regulating hedonic feeding behavior protects against pathological effects of DIO (Ruiz de Azua et al., 2021). However, the rescue of CB1 expression in glutamatergic neurons locally in the olfactory cortex partly restored DIO. The tone of the endocannabinoid system is enhanced in obesity and CB1 inhibitors are a strategy for obesity management (Behl et al., 2021). Consistently, elevated fasting plasma concentrations of the circulating endocannabinoid 2-arachidonoylglycerol (2-AG) in obesity are linked to a lower TDI score (Pastor et al., 2016). The modified activity of the endocannabinoid system and its connection with the mechanisms of sensory perception and reward could explain why obese individuals have different sensibility toward food and non-food odorants, and between neutral and appealing odorant compared to lean counterparts. A promising pharmacological approach would be to use CB1R as a target for both olfactory (Bhatia-Dey and Heinbockel, 2020) and metabolic (Hirsch and Tam, 2019) problems but this strategy will need further clinical research in olfactory perception under cannabinoid therapeutics. Interestingly, a dual approach, combining a peripheral CB1R antagonist with a GLP-1R agonist was shown to potentiate the anti-obesity and anti-diabetic effects of GLP-1R agonist alone in DIO mice (Zizzari et al., 2021).

Recent research studying the fate of smell in T2D has shown that Glucagon Like Peptide-1 (GLP-1) was an interesting hormonal target linking olfaction and T2D: (Zhang et al., 2019) studied 35 obese and 35 non-obese people with T2D compared to 35 control subjects matched for age (35-70 years), sex, and education. Among the 35 obese subjects, 20 obese individuals with diabetes with inadequate glycemic control and metformin monotherapy received GLP-1 receptor agonist treatment (liraglutide and exenatide) for 3 months. Obese T2D subjects demonstrated lower score in the cognitive test (Mini-Mental State Examination, MMSE) and in the olfactory test (increase in olfactory threshold revealed by UPSIT) compared to non-obese participants with diabetes (these diabetic patients also present an impairment compared to control subjects). Interestingly, with 3-month GLP-1 receptor agonist treatment, obese subjects with diabetes exhibited improved cognitive and olfactory scores. This olfactory rescue by GLP-1 analogs can be explained by the expression of GLP-1R by M/T cells that are strongly excited by GLP-1 in *in vitro* mouse MOB slices (Thiebaud et al., 2016, 2019). In addition, chronic exendin-4 treatment reduced neuronal alterations observed in the PCx in the genetic model of T2D rats (Lietzau et al., 2016). In the same model, chronic treatment with the Dipeptidyl peptidase-4 inhibitor (DPP-4i) linagliptin did not improve odorant detection, nevertheless, it normalized T2D-induced effects on neurogenesis in the MOB (Lietzau et al., 2018). Overall, GLP-1R expressed in the olfactory system is a good candidate for pharmacological treatment to rescue olfaction in the context of obesity and T2D.

Table 1. Mechanisms linking impaired smell to obesity and type 2 diabetes

	Olfactory mucosa	Main Olfactory Bulb	Olfactory Cortex
Neurodegeneration or alteration of neuronal response from inflammatory processes	Fardone et al., 2019; Lacroix et al., 2015; Rivière et al., 2016; Thiebaud et al., 2014	Lietzau et al., 2018, 2020; Fardone et al., 2019; Poessel et al., 2020a	Lietzau et al., 2018
Insulin resistance		Fadool et al., 2004; Aimé et al., 2012; Tucker et al., 2012a; Lacroix et al., 2015; Min and Min, 2018; Poessel et al., 2020b	Al Koborssy et al., 2019
Leptin resistance		Getchell et al., 2006; Chelminski et al., 2017	
Vascular dysfunction		Soleimanzad et al., 2019	
Glucotoxicity		Zaghloul et al., 2018	Al Koborssy et al., 2019

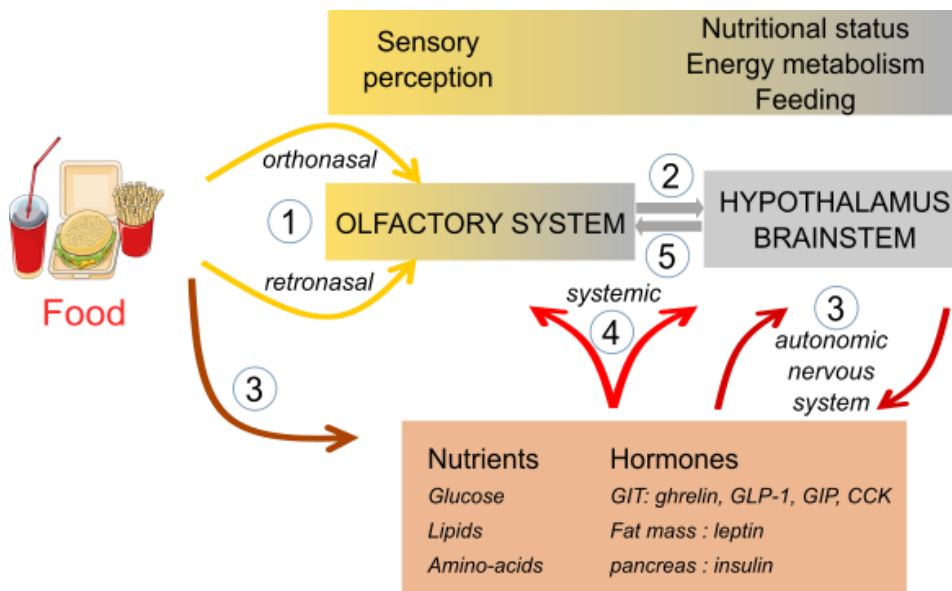


Figure 4

Summary and chronology of the events related to food intake which directly or indirectly impact the olfactory system, and which may be altered in condition of T2D or obesity. GIT = gastro-intestinal tract; GLP-1 = Glucagon-like peptide-1; GIP = Gastric inhibitory polypeptide; CCK = Cholecystokinin.

5. Perspectives

Data reviewed in both humans and animal models converge to show that obesity and T2D are associated with an alteration of olfactory performances. Confounding factors such as age and sex have to be taken into account when considering olfactory testing. It appears that there might also be differences in alteration between the type of odorant and their hedonic content.

Indeed, we noticed that obese and lean individuals have dissociated performances toward neutral versus food related odorants.

Processing odors and particularly food related odor involves a large set of brain structures, hence finding the origin of olfactory defect in obesity and T2D is not trivial. As the metabolic causes and consequences of metabolic disorders, olfactory impairment are likely to be multifactorial (table 1 and figure 4). Since improvement in metabolic health is correlated with better olfactory performances, treatment of the symptoms of obesity are likely to be associated with better olfactory abilities. For instance, physical exercise has been shown to reduce the risk of olfactory impairment in older adults (Schubert et al., 2013). However, we lack controlled studies for the evaluation of such parameters in the context of obesity or T2D probably because they require longitudinal studies on the long term.

It is unlikely that olfactory changes could be the cause of obesity, nevertheless one can wonder to what extent it could accentuate the disease. A difference in olfactory perception between obese and normal-weighted individuals has been suggested to cause differences in food choice and energy consumption (Drewnowski et al., 1995, 1997). We have described how the MOB has many characteristics of a metabolic sensor and olfactory activity would also participate in the modulation of peripheral energy metabolism in mice (Riera et al., 2017). Further probing of the olfactory function in longitudinal studies starting in overweight subjects would be needed to describe the possible participation of smell deficiency in the causal mechanisms leading to dysregulated food intake and obesity. More generally it is acknowledged that olfactory deficits dramatically impair general quality of life, mainly because of the consequences on food intake (enjoyment of food, increase/decrease appetite) (Croy et al., 2014). One treatment option available for post-viral olfactory dysfunction is daily olfactory training, that shows significant olfactory improvement (Denis et al., 2021). We suggest that the same training could be used in obese patients with the aim of improving olfactory and feeding dysregulations.

In the light of recent findings, we propose that probing olfactory perception can be used as a biomarker in clinics to check the fate of energy metabolism (Midorikawa et al., 2021). First, it was shown that among T2D obese patients, those with olfactory problems have also the most severe cognitive dysfunction (Zhang et al., 2018). In that sense, Sanke et al. (2021) recently showed that olfactory impairment is a predictive clinical sign of dementia in elderlies with T2D. Hence, olfactory defects could be used as an early biomarker to detect incoming cognitive problems in the context of metabolic diseases. In addition, a promising avenue is the use of olfactory monitoring among the factors assessing metabolic and weight recovery after bariatric surgery (Guyot et al., 2021).

To conclude, based on information presented in this review, we advocate that both olfactory assessment and reeducation could be more generally used in the context of metabolic diseases and may bring a significant added value to multidisciplinary team-based obesity treatment.

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SECTION - III
THESIS
STATEMENT

THESIS STATEMENT

As described in the first chapter of the introduction, by integrating internal (hormones) as well as external (sensory cues) feeding signals, hypothalamic AgRP neurons regulate food intake and control eating behaviors. Immediately before food ingestion, AgRP neuron activity can be modified by food odor. After ingestion, AgRP neuron activity is modulated by hunger and satiety hormones such as ghrelin and leptin.

While these key metabolic hormones regulate food intake, body weight, and peripheral metabolic processing through hypothalamic circuits, the privileged access of the OB to these hormones suggests that the body's physiological and nutritional state modulates the sensitivity of the olfactory system (Jovanovic and Riera 2022). Evidence shows that hunger increases the ability to detect odors, whereas satiety reduces this ability.

In this context, besides the undeniable impact that peripheral signals have on olfaction, and since AgRP neurons promote not only food consumption but also behaviors that lead to food discovery, as a general hypothesis of this work, we have proposed that hypothalamic AgRP neuron activity participates in the regulation of odor processing within the OB modulating olfactory capacities in mice. We focused particularly on the activity of GCs because, as seen in Chapter II, this layer is a massive entry point for centrifugal inputs that modulate the activity of the OB.

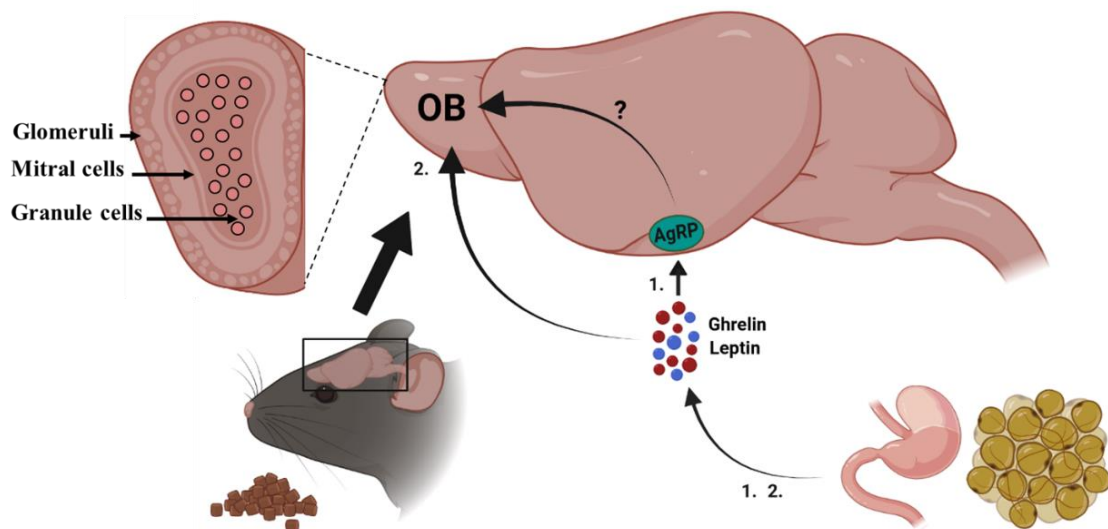


Figure III.1. Schematic representation of the influences on the OB linked to metabolism and food intake

Our objective was to assess whether AgRP-expressing neurons are implicated in olfactory processing and olfactory performance in mice. In order to do this, we have used two genetically engineered mouse models in which we can specifically manipulate AgRP-expressing neurons. In our first model, we ablated AgRP neurons by an injection of diphtheria toxin few days after birth in AgRP^{DTR} mice. The olfactory performance of mice lacking AgRP neurons and control mice were evaluated and compared in multiple olfactory behavioral tests and under different metabolic states. The dynamics of GC activity were assessed in both groups in response to odorants and under different metabolic states. Using this model, we were also able to compare male and female olfactory behaviors.

To guarantee the exclusive manipulation of the arcuate nucleus NPY/AgRP neurons, then avoid ablating peripheral AgRP-expressing cells and to prevent the compensatory mechanism that may occur, the second model was to artificially, transiently, and exclusively activate arcuate AgRP neurons using chemogenetic in adult AgRP-cre mice. In vivo GC activity and olfactory capacities were evaluated in response to different olfactory stimuli under AgRP neuron activation.

Finally, obesity is associated with impaired hypothalamic physiology and is often accompanied by altered olfactory capacities. Hence, the third objective was to assess olfactory capacities in a mouse model of diet-induced obesity (DIO). Furthermore, by applying chemogenetic approaches, we aimed to determine whether artificially stimulating AgRP neurons would rescue olfactory processing and olfactory performance in DIO mice.

SECTION - III

Materials and Methods

MATERIALS AND METHODS

1. Animal Maintenance

All animal care and experimental procedures were performed with the approval of the Animal Experimentation Ethical Committee Buffon (CEEA-40) - Université Paris Cité - following the guidelines of the French Ministry of Research legislation (APAFIS: #15638, #32496). Mice were maintained in a temperature- ($22\pm 1^\circ\text{C}$) and humidity- (50-70%) controlled facility with a 12hrs light/dark cycle (light hours: 7 a.m. till 7 p.m.) and ad libitum access to standard chow diet (Safe diets, A04) or high-fat diet (HFD Kcal 60% fat for 8 weeks, D12492, Research diets) - unless stated otherwise - and water. Mice were group housed (3-5 per cage), in individually ventilated cages, to prevent isolation stress - unless stated otherwise - and they were accustomed to daily manipulation.

2. Mouse models

2.1. AgRP-ires-cre mice

For most experiments, heterozygous AgRP-ires-cre mice ($AgRP^{cre/+}$ - C57Bl/6 genetic background), were bred with C57Bl/6 wild-type $AgRP^{+/+}$ mice to obtain $AgRP^{cre/+}$ heterozygotes. This transgenic mouse model is characterized by the expression of Cre recombinase specifically in hypothalamic AgRP neurons.

2.2. AgRP-DTR mice: DT treatment

For most experiments, homozygous $AgRP^{DTR/DTR}$ (C57Bl/6 genetic background) were bred with C57Bl/6 wild-type $AgRP^{+/+}$ mice, such that all the offspring would be $AgRP^{DTR/+}$ heterozygotes. Heterozygous $AgRP^{DTR/+}$ mice with the Diphtheria Toxin Receptor (DTR) targeted to the AgRP locus have been previously described by (Luquet et al. 2005).

Newly born $AgRP^{DTR/+}$ (male and female) mice, 2-15 days old, received a subcutaneous (interscapular) injection (12.5 $\mu\text{l/g}$ of BW) of either DT (Diphtheria Toxin, Sigma D0564-1mg, 10 $\mu\text{g/Kg}$, diluted 1000x, 12,5ng/ μl) or PBS 1X. Consequently, these mice were divided into 2 groups named “AgRP-ablated” and “AgRP-control” mice respectively. Two months later, both groups of mice received a second injection (i.p. injection, 12.5 $\mu\text{l/g}$) of the same substance (DT/ PBS 1X) previously injected.

3. Viral constructs

All Adeno-associated viruses (AAV) were generated by Addgene vector core (<http://www.addgene.org/>): pAAV-hSyn-DIO-hM3D(Gq)-mCherry was a gift from Bryan Roth (Addgene plasmid # 44361-AAV9 ; <http://n2t.net/addgene:44361> ; RRID:Addgene_44361). pAAV-hSyn-DIO-mCherry was a gift from Bryan Roth (Addgene plasmid # 50459-AAV8 ; <http://n2t.net/addgene:50459> ; RRID:Addgene_50459). pAAV.Syn.GCaMP6f.WPRE.SV40 (AAV9) was a gift from Douglas Kim & GENIE Project (Addgene plasmid # 100837 ; <http://n2t.net/addgene:100837> ; RRID: Addgene viral prep # 100837-AAV9).

4. Surgical and stereotaxic procedures

Prior to all surgical procedures, mice received an intraperitoneal injection (i.p.) of Buprécare® (Buprenorphine 0.3 mg/ml, diluted 1/100 in PBS 1X). Animals were then anesthetized using gaseous isoflurane (Iso-Vet, isoflurane 1000 mg/g) at 2.5% (induction, at a rate of 2 L/min) and maintained at 1.5% (at a rate of 0.8 L/min) throughout the surgery. Lastly, mice received an i.p. injection of Ketofen® (Ketoprofen, 100 mg/ml, diluted 1/100 in PBS 1X) to provide pain relief (analgesia) and reduce inflammation in case of mild to moderate pain.

Mice were placed in the stereotaxic device (David Kopf Instruments, model 940, California, USA) equipped with an electric heating pad (37°C) to maintain their body temperature throughout the surgery. The skull was held on the sides by ear bars and a drop of physiological water (NaCl) was applied to the eyes to prevent corneal damage. Mice were then head shaved and disinfected with VETEDINE® Solution (Vétoquinol).

An incision was made at the center of the scalp (1-2 cm). The skull was then rubbed with 10% hydrogen peroxide (H₂O₂) exposing “bregma”. Bregma is the principal stereotaxic reference point for the identification of all brain regions according to the Mouse Brain Atlas (Paxinos and Franklin 2003). After the skin incision, a hole was drilled in the skull at the corresponding stereotaxic coordinates. A syringe needle (Hamilton, volume 10µl, 33 gauge, model gastight 1701 RN) was then lowered into the hole to reach the target brain area.

After viral injection with a nanoliter infusion pump, the needle was kept at the injection site for at least 1 minute to allow an effective spread of the virus at that exact target region. The needle was then gradually lifted and carefully removed. The skin was sutured with Ethilon®

6.0 non-absorbable surgical monofilament suture (Ethicon, Inc, USA), and, once again, the scalp was disinfected with VETEDINE® Solution (Vétoquinol).

4.1. DREADDs injections in the ARC of *AgRP^{cre/+}* mice

Designer receptors exclusively activated by designer drugs (DREADDs) represent a family of artificially engineered protein receptors that are selectively activated by exogenous ligands. DREADDs are widely used to remotely control cellular signaling, neuronal activity and behavior in different species including mice (Roth 2016).

Bilateral injections of 0.3µl Cre-dependent AAV vectors encoding for stimulatory DREADDs (pAAV-hSyn-DIO-hM3D(Gq)-mCherry, ligand: Clozapine N-oxide (CNO) - titer $\geq 1 \times 10^{13}$ vg/ml, working dilutions 1:5) were performed in the ARC (bregma, L= ± 0.1 mm; AP= -1.4 mm; DV= -5.8 mm) of 2 month-old *AgRP^{cre/+}* mice at a rate of 50 nl/min for 6 minutes. These mice are referred to as “AgRP-hM3Dq” mice.

Control *AgRP^{cre/+}* mice received bilateral injections (0.3µl) of control mcherry virus (pAAV-hSyn-DIO-mCherry, titer $\geq 1 \times 10^{13}$ vg/ml, working dilution 1:5) in the ARC at a rate of 50 nl/min for 6 minutes. These mice are referred to as “AgRP-mcherry” mice.

Following DREADDs injections in the ARC of the hypothalamus, AgRP-hM3Dq and AgRP-mcherry mice received a second stereotaxic injection and a fiber probe implantation into their MOB (as described below). Cre-driven DREADDs expression in AgRP neurons was confirmed via post-mortem immunohistochemistry.

4.2. Optical fiber implantation for in vivo fiber photometry recordings in the OB

Unilateral injection of 1µl pAAV.Syn.GCaMP6f.WPRE.SV40 (AAV9) (titer $\geq 1 \times 10^{13}$ vg/ml, working dilutions 1:5) - encoding GCaMP – was performed in the right OB at the level of GCL (bregma, L= -0.8 mm; AP= 5 mm; DV= -1.55 mm) in all groups of mice (AgRP-ablated, AgRP-control, AgRP-hM3Dq, and AgRP-mcherry mice) at a rate of 0.1 µl /min for 10 minutes. We focused our injections on the GCL previously described as a main target of centrifugal afferents (Matsutani and Yamamoto 2008).

During the same surgery session, and after GCaMP viral injection, mice were implanted with an optical fiber for calcium imaging into the GCL of the right OB. A chronically implantable fiber-optic cannula (Doric Lenses, MFC_400/430-0.48_3mm_MF1.25(g)_FLT,

Québec, Canada) composed of a bare optical fiber (400 μm core, 0.48 N.A.) and a fiber ferrule was implanted 50 μm above the location of the viral injection site (L= - 0.8 mm; AP= 5 mm; DV= -1.5 mm). The fiber was then fixed onto the skull using dental cement (Super-Bond Universal Kit, Sun Medical, and Unifast Trad).

Following the surgery, mice were placed in a cage on a heating pad and were given 30 minutes to recover from anesthesia. Two weeks later, mice underwent multiple metabolic and behavioral tests described later.

5. Feeding experiments

For all feeding assays, mice were transferred into individual cages supplied with water and were allowed to acclimate for 3 days before the test: body weight was measured every morning (around 9 a.m.) and mice were given a saline (0.9% NaCl) injection once/day (at noon) for those 3 days. Their food intake was measured 2hrs and 4hrs post-injection.

5.1. CNO test: AgRP^{cre/+} mice

In AgRP-hM3Dq mice, Clozapine N-Oxide (CNO) injection should stimulate AgRP neurons inducing an increased food intake in fed ad-libitum mice. To check if the stereotaxic injection of DREADD-Gq was a success, a CNO test is performed prior to any olfactory behavioral test. All fed (ad libitum) AgRP-hM3Dq mice were isolated in individual cages and were food deprived for 3hrs/day (from 9 a.m. till 12 p.m.). Mice received a daily (at noon) i.p. injection (0.1ml/10g of BW) of either saline solution, from day 1 to 3, or CNO (0.6 mg/kg of body weight, Tocris Bioscience, #4936, UK) on day 4. Their food intake (chow diet, A04) was measured 2 and 4hrs post-injection and was compared with food intake of their cohort AgRP-mcherry fed mice. Fed AgRP-hM3Dq mice showing no response to CNO injection were excluded.

This test was reproduced in fed AgRP-hM3Dq and AgRP-mcherry mice after 8 weeks of HFD-induced obesity. However, this time, HFD consumption (g) was measured after CNO injection.

5.2. Ghrelin test: *AgRP^{DTR/+}* mice

As previously described, ghrelin (GHL) is a hormone that increases food intake by stimulating AgRP neurons. To assess ghrelin sensitivity, AgRP-ablated and AgRP-control fed (ad libitum) mice received an i.p. injection (0.1ml/10g of BW) of either PBS, for the first 3 days, or acetylated GHL (mouse ghrelin, 0.5 mg/kg), on day 4. Their food intake was measured 2 and 4hrs following the injections. AgRP-ablated fed mice showing an increased food intake in response to GHL injection were excluded.

6. Fiber photometry: in vivo calcium imaging in the GCL of the OB

Fiber photometry is a calcium imaging technique that monitors neuronal activity in the brain of freely moving behaving animals (in vivo). It relies on the expression of genetically encoded calcium indicators (GECIs) which can be targeted to specific cell types (neurons or astrocytes). The most commonly used calcium indicator for fiber photometry is GCaMP6f (Green Fluorescent Protein) (Nakai, Ohkura, and Imoto 2001). In neurons, action potentials induce neurotransmitter release at axon terminals by opening voltage-gated Ca^{2+} channels, allowing for Ca^{2+} influx. Following this influx of ions, GCaMP fluoresces upon Ca^{2+} binding and the change in fluorescence corresponds proportionally to intracellular calcium changes: increased fluorescence emission correlates with increased calcium influx. As a result, GCaMP is used to measure increases in intracellular [Ca^{2+}] in neurons as a marker for neuronal activity in mice.

Fiber photometry collects a cumulative and combined signal from all neurons labeled with GCaMP. Thus, instead of visualizing individual neurons, fiber photometry enables us to visualize the activity of a large neuronal population.

This technique involves the surgical implantation of a fiber-optic cannula – previously described - that delivers excitation light to a group of neurons labeled with GCaMP, and upon binding to Ca^{2+} , GCaMP emits its own light in return, which travels back through the same fiber, allowing the recording of calcium dynamics across time. Thus, GCaMP is directly tied to neuronal response patterns.

6.1. Technical description

Real-time fluorescence emitted from the calcium sensor GCaMP6f expressed by neurons of the GCL was recorded using fiber photometry. Fluorescence was collected using a single optical fiber for both delivery of excitation light streams and collection of emitted fluorescence.

The fiber photometry setup used 2 light emitting LEDs: a 405 nm LED sinusoidally modulated at 330 Hz and a 465 nm LED sinusoidally modulated at 533 Hz (Doric Lenses) merged in an FMC4 MiniCube (Doric Lenses) that combines the 2 wavelengths excitation light streams and separate them from the emission light. The MiniCube was connected to a fiber optic rotary joint (Doric Lenses) connected to the cannula. An RZ5P lock-in digital processor controlled by the Synapse software (Tucker-Davis Technologies, TDT, USA), commanded the voltage signal sent to the emitting LEDs via the LED driver (Doric Lenses). The light power before entering the implanted cannula was measured with a power meter (PM100USB, Thorlabs) before the beginning of each recording session. The light intensity to capture fluorescence emitted by 465 nm excitation was between 25-40 μ W, for the 405 nm excitation this was between 10-20 μ W at the tip of the fiber.

The fluorescence emitted by the GCaMP6f activation in response to light excitation was collected by a femto-watt photo-receiver module (Doric Lenses) through the same fiber patch cord. The signal was then received by the RZ5P processor (TDT). Online real-time demodulation of the fluorescence due to the 405 nm and 465 nm excitations was performed by the Synapse software (TDT). A camera was synchronized with the recording using the Synapse software.

6.2. In vivo recording of GC's activity in the OB of *AgRP^{cre/+}* mice

At least two weeks after viral injections, mice were habituated to the test: *AgRP-hM3Dq* and *AgRP-mcherry* mice received an i.p. injection of saline solution (NaCl 0.9%) and were placed individually in the test cages, with clean litter, for them to explore a minimal-odor environment. The implanted optical fiber was then connected to its cable and mice were allowed to freely move and explore for about 20 min.

All mice were previously habituated to eat, along with their chow diet (A04), a sugary cumin cookie. Thus, the odor of cumin was associated with palatable food. Cumin cookies were prepared as follows: a mixture of 15ml dH₂O, 10g sucrose, 15g wheat flour, and 1g of ground cumin.

On the day of the test, fed *AgRP-hM3Dq* mice received an i.p. injection (0.1 ml/10g of BW) of CNO. Mice were then placed in the minimal-odor cages and multiple odors (chow A04 pellet, cumin cookie, C+, 5 μ l urine from the opposite sex) were presented - one by one - to both groups of mice while recording the calcium dynamics of their GC's activity.

6.3. In vivo recording of GC's activity in the OB of *AgRP^{DTR/+}* mice

AgRP^{DTR/+} mice received a unilaterally viral injection of GCaMP as well as a fiber probe implantation into the GCL of their right OB. At least two weeks later, mice were habituated to the test conditions: *AgRP*-ablated and *AgRP*-control mice were placed individually in the test cages, with clean litter, for them to explore a minimal-odor environment. The implanted optical fiber was then connected to its cable and mice were allowed to freely move and explore for about 20 min.

On the day of the test, fed (ad libitum) vs. overnight-fasted *AgRP*-ablated and *AgRP*-control mice were placed in the test cages and multiple odors (A04 pellet, cumin cookie, C+, 5 μ l urine from the opposite sex) were presented - one by one - to both groups of mice while recording the calcium dynamics of their GC's activity.

6.4. Data analysis

Signals were exported and analyzed offline using pMAT (The Barker Lab pMAT v1.2 (Bruno et al. 2021)). The timing of events (sniffing the odor up close) was extracted from the video. After careful visual examination of all trials to discard artifacts, for each session, signal analysis was performed at two time intervals: one extending from -2 to 0 sec (before the first odor sniff) and the other from 0 to +2 sec (first odor sniff).

For each trial, the signal (465 nm) was normalized to the baseline fluorescence (405 nm) of the trial using the $\Delta F/F$ ratio. $\Delta F/F = (465 \text{ nm signal} - 405 \text{ nm baseline})/405 \text{ nm baseline}$. To compare signal variations between the two conditions (before vs. after the first odor sniff), for each mouse, the time corresponding to the first close-up sniff of the odor was set at zero. $\Delta F/F$ values were then normalized by the mean of $\Delta F/F$ [-2 to -1 sec]. The signals are also converted into a z-score from which the area under the curve (AUC) and 'Peak' or maximum value of individual signals are collected.

7. Odor preparation for in vivo olfactory behavioral experiments

Seven different odorants were used for the in vivo experiments. They were divided into two different categories: Food-odor cues and neutral odorants. The odorant cumin (ground cumin, Ducros), (R)-(+)-Limonene (97%) and (S)-(-)-Limonene (≥ 99.0 %) were adopted as food-odor cues. Odorized cookies were prepared as follows: a mixture of 15ml dH₂O, 10g sucrose, 15g wheat flour, and either 50 μ l (R)-(+)-Limonene or 50 μ l (S)-(-)-Limonene

respectively. On the other hand, all neutral odorants ((+)-carvone, $\geq 98.5\%$; (-)-Carvone, 98%; Hexanal, 98%; Heptaldehyde, 95%) were dissolved to 15%, 15%, 0.5% and 0.5% respectively in mineral oil and were presented, on a cotton swab, in the olfactory behavioral experiments described below. Except for cumin, all odorants were purchased from Sigma-Aldrich.

8. Olfactory Behavioral Tests

Mice were subjected to multiple olfactory behavioral tests to assess their olfactory performance.

8.1. Olfactory Habituation – Dishabituation test

This test is commonly used to assess olfactory detection and discrimination capacities in different species, including mice (Yang and Crawley 2009). It relies on the animal's natural tendency to explore novel odor cues. Therefore, there is no odor training in advance in order to avoid olfactory exploration biased by learning and memory.

Fed (ad libitum) vs. fasted (overnight) AgRP-ablated and AgRP-control mice as well as C57bl6 Chow and HFD (8 weeks) mice were subjected to this test. All mice were individually habituated to the test cages, filled with fresh clean cage bedding, for 2 days (1hr/day) prior to testing. A metallic perforated ball, containing a dry cotton swab, was placed on the cage grid during the pre-test acclimation period and mice were allowed to freely move and explore both the cage and the perforated ball.

On the day of the test, mice were once again habituated to the test conditions for at least 30 min. The olfactory habituation–dishabituation test consists of successive presentations of different odors. Odors, diluted in mineral oil (MO), applied to a cotton swab (20 μ l) and inserted in a perforated ball, are presented in the following order: a sequence of MO, odor A, and odor B (MO – MO – MO – A – A – A – B – B – B). Each odor was presented to the mouse in 3 consecutive trials for a duration of 1 min each and a 3 min inter-trial time interval. The first three presentations of MO – odorless - familiarize the subject with the testing procedure and serve as a negative control. Then, an unknown odor A is presented for 1 minute to the mouse who will explore it. For each presentation, as the mouse becomes familiar with odor A, the sniffing time decreases. The olfactory habituation process is indeed defined as a progressive decrease in olfactory exploration (sniffing) toward a repeated presentation of the same odor stimulus. Then a new odor - odor B - is presented. If it is recognized as new, the mouse will

explore it for a longer time. Olfactory dishabituation is defined as a significant increase in sniffing when a novel odor B is presented for the first time.

Odors used in this test were prepared as follows: Structurally-different odorant pairs - (+)-carvone/ (-)-carvone (2 enantiomers of C₁₀H₁₄O) and hexanal/heptanal (C₆H₁₂O / C₇H₁₄O) - were diluted to 15% and 0.5% respectively in mineral oil. The order of odor presentation was constantly changed: A-(+)-carvone/ B-(-)-carvone or A-(-)-carvone / B-(+)-carvone or A-hexanal/ B-heptanal or A-heptanal/ B-hexanal.

Behaviors were performed in a clean cage, equipped with a video camera, and under a lab hood constantly supplying clean fresh air. The starting point of each odor presentation was when the perforated ball was placed on the grid on top of the cage. Time of active exploration/ sniffing of the odor was recorded over a 1-minute trial period for a total of 9 trials/mouse.

Videos were then carefully analyzed (in milliseconds) using the Kinovea video tool (<https://www.kinovea.org/>). Sniffing was only scored when mice had their head oriented directly toward the perforated ball. All recorded times were normalized to the mean sniffing duration of the 3 MO trials to minimize between-animal variance. Sniffing time (sec) was measured to evaluate the olfactory detection and discrimination capacities in mice. To assess olfactory detection capacities, we compared the sniffing time between the last presentation of MO and the first presentation of odor A. Similarly, we compared the last presentation of odor A with the first presentation of the new odor (B) to assess the olfactory discrimination capacities in mice.

8.2. Buried Food-Seeking Test: Olfactory Detection Capacities

The buried food-seeking test measures how quickly the mouse can detect or find a small piece of familiar palatable food that is hidden underneath a layer of bedding, by relying on olfactory cues for foraging. The main parameter observed in this test is the latency (min) or the time spent by the mouse to uncover the hidden palatable food.

All mice were given cookies (cereals, 0.35g, Nestlé) daily in their home cages, for three days prior to testing to familiarize them with the odor and minimize neophobia. Mice then underwent a pre-test acclimation where they were allowed to explore the testing cages in order to reduce novelty stress.

On the day of the test, mice were placed in the testing cages 20 minutes before hiding the food in the cage bedding. The testing cages were covered with 2-3 cm of fresh cage bedding and were equipped with a video camera. Mice were then transiently transferred into an empty clean cage, the time needed to hide the food cue in the test cage: one piece of cookie was buried 2 cm beneath the surface of the bedding at a random corner of the cage. Mice were then individually retransferred into the test cages and were given a maximum time of 10 min each to search for and retrieve the mini-smarty. A stopwatch was used to monitor the test duration.

The latency (min) to find the cookie was measured by analyzing the video footage of the test, using the Kinovea video tool. Mice with enhanced olfaction can normally find the hidden food within a few minutes or even a few seconds (Low latency). Contrarily, high latency is associated with olfactory impairment. If a mouse failed to find the buried food after 10 minutes have elapsed, the test is put to an end, and “10 min” is recorded as the mouse’s latency score.

The test was performed on all groups of mice. However, fed AgRP-hM3Dq and AgRP-mcherry mice both received an i.p. injection of CNO, at least 20 minutes prior to the test.

8.3. Conditioned Odor Aversion Test: Olfactory Discrimination Capacities

The aversive odor test aims to determine whether *olfactory-based memory* would have a positive impact on olfactory discrimination abilities. The olfactory stimuli used in this test are two enantiomers of the same volatile molecule called Limonene (C₁₀H₁₆): *R*-(+)-limonene and *S*-(-)-limonene are respectively referred to as (Li+) and (Li-). The conditioning procedure consists of two distinct phases: the attraction phase (Li+) and the aversion phase (Li-).

In the attraction phase, mice were offered sweet non-aversive cookies odorized with (Li+) for 2 consecutive days (days 1-2). (Li+)-odorized cookies were pre-weighed and placed in the homepage of single-housed AgRP-ablated and AgRP-control fed mice. Food intake (g) of (Li+)-odorized cookies was measured after a 1-hr period. Mice then received an i.p. injection of saline solution (NaCl 0.9%).

Contrarily, in the aversion phase, mice were offered sweet non-aversive cookies odorized with (Li-) for 2 consecutive days (days 3-4). Food intake of (Li-)-odorized cookies was measured after 1hr. However, this time, mice received an i.p. injection of LiCl (Lithium Chloride, 0.19g/kg, Sigma-Aldrich) solution inducing an aversive gastric reaction.

After conditioning (day 5), the test was conducted on fed mice of both groups in order to assess their finest olfactory discrimination capacities. Fed mice were offered, simultaneously, two non-sweet (sugar-free) cookies each odorized with one enantiomer of limonene (Li+ vs. Li-), and their corresponding odorized-food consumption (g) were measured after 1hr.

The aversion ratio (AR) is defined as their (Li-)-odorized food consumption (g) divided by their total food intake (TFI) after the 1hr test. $AR = (Li-)/TFI$. High AR values are associated with impairment in olfactory discrimination capacities.

9. Histology

9.1. Mice Perfusion

All mice were transcardially perfused with saline solution (PBS1X) followed by 4% paraformaldehyde solution (PFA, fixation solution, pH 7.4) in order to drain the blood and preserve the brain tissue for immunostaining (Wu et al. 2021). The brains were extracted from the skull and post-fixed overnight in 4% PFA. They were then incubated in a cryoprotectant solution (30% sucrose, 0.09% sodium azide in PBS1X) until the tissue sank (24-48 hours). Brains were then cut on a cryostat (Leica, 1800) into 30 μ m-thick coronal sections and stored in a cryoprotectant solution at -20°C. Brain slices were later used for immunohistochemistry (IHC) staining as described below.

9.2. Immunohistochemistry (IHC): AgRP, NPY and c-fos immunostaining

Free-floating sections were washed 3 times for 10 min in PBS1X, incubated in 0.2% Triton X-100 solution for 20 min, rinsed again in PBS (3x10min), and blocked for 1hr in 3% BSA (Bovine Serum Albumin, Sigma-Aldrich, A2153). The slices were then incubated with the primary antibody (anti-AgRP 1/500 or anti-NPY 1/4000 or anti-cfos 1/1000) in a blocking solution of 1% BSA for 72hrs at 4 °C. They were later washed 3 times for 10 min in PBS1X before being incubated with the secondary antibody (diluted 1/500 in PBS1X) for 1hr at room temperature. Finally, the samples were rinsed 3 times for 10 min in PBS1X, stained with DAPI (diluted 1/10000 in PBS1X, Sigma D9542) for 10 to 15 min, washed twice for 10 min with PB1X (0.1M) and mounted onto microscope slides (SuperFrost[®] Plus adhesive microscope slides, 25x75mm) with an antifade mounting medium (Vectashield[®]) before being coverslipped. References for all antibodies used in this study were listed in the key resources table attached below.

9.3. IHC: amplification of mCherry-tagged viruses

In order to visualize the mCherry-tagged viruses, immunohistochemistry was performed on ARC sections to amplify mCherry fluorescence in AgRP-hM3Dq and AgRP-mcherry. Free-floating ARC sections were washed 3 times for 5 min in TBS1X, blocked in “TBS-Plus Goat” containing 3% normal goat serum and 0.3% Triton-x in 1X TBS for 30 min at room temperature, and incubated in mCherry primary antibody, rabbit polyclonal anti-mCherry at a dilution of 1/500 in 0.3% Triton-X in 1X TBS for 24 h at 4 °C. The slices were then washed 3 times for 5 min in TBS1X before being incubated in the secondary antibody Alexafluor 488 Goat anti-Rabbit at a dilution of 1:1000 for 2 h at room temperature. Finally, these slices were rinsed 3 times for 5 min in TBS1X, stained with DAPI (diluted 1/10000 in PBS1X, Sigma D9542) for 10 to 15 min, washed twice for 10 min with TB1X (0.1M) and mounted onto microscope slides (SuperFrost® Plus adhesive microscope slides, 25x75mm) with an antifade mounting medium (Vectashield®) before being coverslipped.

9.4. Confocal microscopy

Confocal images (LSM 700, Zeiss) were obtained with 10X or 40X immersion objective (Zeiss) and were visualized, using the ICY software for bioimage analysis (Image J), in order to evaluate AgRP, NPY and cfos expression in the ARC, PVN and OB of AgRP-ablated vs. AgRP-control mice.

On the other hand, non-stained OB sections, from all mouse models, were inspected to check the GCaMP viral expression and evaluate the correct position of the optical fiber within the OB (GCL). Animals, in which histological examination showed no viral expression or inaccurate recording sites, were excluded from the analysis.

10. Western Blot: AgRP neurons in the ARC of *AgRP^{DTR/DTR}* vs. *AgRP^{+/+}* mice

Mice were euthanized by cervical dislocation and their heads were immediately cut and soaked in liquid nitrogen for 3-4 seconds. The brain was then removed and dissected on an ice-cold surface: the ARC was extracted to assess and compare the expression of AgRP, a 14kda peptide synthesized by AgRP neurons, in the hypothalamus of homozygous *AgRP^{DTR/DTR}* vs. *AgRP^{+/+}* overnight fasted mice.

ARC samples were sonicated and homogenized in 200 μ l lysis buffer (ThermoFisher Scientific, Invitrogen, Tissue Extraction Reagent II – catalog #FNN0081) supplemented with protease inhibitor (ROCHE 05056489001, Inhibiteur de Proteases Complete, EDTA-free, diluted 250X). Brain samples were then centrifuged at 4°C for 20min. Aliquots (2 μ l) of the homogenates were added to 200 μ l of Bradford (Bio-Rad Protein Assay Dye Reagent Concentrate – cat. 5000006 – stored at 4°C) and were used for protein quantification. Equal amounts of proteins (30 μ g/ μ l) for each sample were loaded onto 4-15% polyacrylamide gels (BIO-RAD mini-protean TGX, stain-Free Gels, cat #4568084; 4-15%).

Proteins were separated by SDS-PAGE and transferred to PVDF membranes (Trans-blot Turbo Mini PVDF, #1704156, 0.2 μ m, Bio-Rad). These membranes were blocked in 5% milk for 1hr and were then incubated overnight with the following primary antibodies (diluted in 1% milk) at 4°C: rabbit anti-AgRP (1:1000, Abcam ab254558) and mouse monoclonal anti- β -actin (1:1000, Sigma A5316). Membranes were then washed 3 times for 10 min in PBS-T (Tween 20, Sigma-Aldrich – P9416-100ml) and were incubated in HRP-coupled secondary antibody binding: anti-rabbit IgG HRP-linked antibody (1/5000, Cell Signaling 7074) and anti-mouse IgG HRP-linked antibody (1/5000, Cell Signaling 7076).

Proteins were revealed and detected via enhanced chemiluminescence using an ECL kit (Clarity Western ECL Substrate – BIO-RAD - 1705061). Images were acquired with a GE Amersham Imager 600 (UV 29083463 Luminescent Image Analyzer).

11. Statistical Analysis

Statistical analyses were performed using GraphPad Prism 9.5. Data are represented as mean \pm SEM (Standard Error of the Mean). Two-way ANOVA analysis were applied when more than two groups were compared. A Student's paired or unpaired t-test was used when two groups were compared. Parametric tests are used on data that follow a normal distribution. A p-value lower than 0.05 (*) was considered statistically significant. The number of animals used in each experiment and for each analysis are specified in each figure.

KEY RESOURCES TABLE

Reagent or Resource	Source	Identifier
Mouse Models		
<i>AgRP^{cre/+}</i> mice or <i>AgRP^{tm1(cre)Low1/J}</i> or <i>AgRP-Ires-cre</i> mice from Jackson laboratory		
<i>AgRP^{DTR/+}</i> mice		
Diets		
Chow Diet	Safe diets	A04
High Fat Diet (HFD)	Research diets	D12492
Viral Constructs and Optical Fiber		
pAAV-hSyn-DIO-hM3D(Gq)-mCherry	Gift from Brian Roth	Addgene viral prep #44361-AAV9
pAAV-hSyn-DIO-mCherry	Gift from Bryan Roth	Addgene viral prep #50459-AAV8
pAAV.Syn.GCaMP6f.WPRE.SV40 (AAV9)	Gift from Douglas Kim & GENIE Project	Addgene viral prep #100837-AAV9
Mono Fiber-optic Cannula	Doric, Canada	MFC_400/4300.48_3mm_MF1.25(g)_FLT
Ligands		
Diphtheria Toxin, 10 µg/Kg	Sigma	D0564
Ghrelin (GHL) –acylated	Millipore	494127-M
Clozapine N-Oxide (CNO)	Tocris Bioscience	#4936
Odors		
Mineral Oil	Sigma-Aldrich	M8410
(R)-(+)-Limonene, 97%	Sigma-Aldrich	183164
(S)-(-)-Limonene, ≥ 99.0 %	Sigma-Aldrich	62128
(+)-Carvone, ≥ 98.5 %	Sigma-Aldrich	22070
(-)-Carvone, 98%	Sigma-Aldrich	124931
Hexanal, 98%	Sigma-Aldrich	115606
Heptaldehyde, 95%	Sigma-Aldrich	H2120
Antibodies for IHC and Western Blot		
DAPI (>98%, Mice)	Sigma-Aldrich	D9542
AgRP (Agouti-Related Protein, Rabbit)	Abcam	AB254558-1001
α-NPY (Anti-Neuropeptide Y, Rabbit)	Sigma-Aldrich	N9528
c-fos (Guinea Pig)	Synaptic Systems	226308
CY3 AffiniPure (Donkey Anti-Rabbit)	Jackson Immunoresearch	711-165-152
CY3 AffiniPure (Donkey Anti-Guinea Pig)	Jackson Immunoresearch	706-165-148
Rabbit polyclonal anti-mCherry antibody	Abcam	Ab167453
AlexaFluor 488 Affinipure Goat anti-Rabbit	Jackson Immunoresearch	111-545-144
Anti-AgRP rabbit (0.536mg/ml)	Abcam	ab254558
Mouse monoclonal Anti-B-Actin (Clone AC-15)	Sigma-Aldrich	A5316
Anti-rabbit IgG, HRP-linked Antibody	Cell Signaling Tech	7074S
Anti-mouse IgG, HRP-linked Antibody	Cell Signaling Tech	7076S
Other Chemicals		
Euthasol ® Vet (400 mg/ml)	Dechra	Dechra
PFA (paraformaldehyde 95%)	Sigma-Aldrich	158127
LiCl (Lithium Chloride)	Sigma-Aldrich	L4408
NaCl 0.9%	VERSOL	0459

EDTA-free Protease Inhibitor Cocktail	Roche	05056489001
DPBS 10X (no CaCl ₂ , no MgCl ₂)	ThermoFisher Scientific	14200-067
Tissue Extraction Reagent II (lysis buffer)	ThermoFisher Scientific	FNN0081
Triton X-100	Sigma-Aldrich	X100
Normal Goat Serum	Equitech-Bio	SG30-0500
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A0281
Sucrose (>99.5%)	Sigma-Aldrich	S7903
Sodium azide (>99.5%)	Sigma-Aldrich	S2002
TWEEN® 20	Sigma-Aldrich	P9416
Protein Assay Dye Reagent Concentrate	Bio-Rad	5000006
4–15% TGX Stain-Free™ Protein Gels	Bio-Rad	4568084
Trans-Blot Turbo Mini 0.2 µm PVDF Transfer	Bio-Rad	1704156
4x Laemmli Sample Buffer	Bio-Rad	161-0747
Clarity Western ECL Substrate	Bio-Rad	1705061
Page Ruler Prestained Protein Ladder	Thermoscientific	26620
Other Products and machines		
SuperFrost® Plus adhesive microscope slides	Epredia	J1800AMNZ
Vectashield, antifade mounting medium	Vector laboratories	H-1000
Cryostat	Leica	1800
UV 29083463 Luminescent Image Analyzer	Amersham Imager 600	AI 600
Confocal Zeiss LSM 700	Zeiss	Zeiss

SECTION - IV
RESULTS

RESULTS

1. Validation of AgRP neuron ablation in the ARC of AgRP-ablated mice

AgRPDTR/+ mice express the human diphtheria toxin (DT) receptor under the control of AgRP (Luquet et al. 2005). Newly born AgRPDTR/+ (male and female) mice received a subcutaneous injection of either PBS1X (AgRP-control) or DT (AgRP-ablated) solution. Two months later, both groups received a second injection of the same substance (PBS1X/DT, i.p.) previously injected: if the first injection has failed, adult AgRPDTR/+ mice that still have AgRP neurons will die following the second injection (Luquet et al. 2005). We first measured the body weight (BW) in g of AgRP-control and AgRP-ablated male (Figure IV.1A) and female (Figure IV.1D) mice at 2-3 months old. No significant difference was observed in the BW of AgRP-control vs. AgRP-ablated male mice. On the other hand, the BW of female AgRP-ablated mice was significantly lower than their cohort, female AgRP-control mice. Furthermore, a longitudinal study of body composition showed that even if their BW is similar, AgRP-ablated mice have more fat mass at the expense of lean mice than the controls (Figure IV.1C)

Before starting any behavioral tests on AgRP-ablated mice, we aimed to assess AgRP neuron responsiveness to ghrelin in both groups. Ghrelin is an orexigenic hormone that increases food intake by stimulating AgRP neurons. Fed/satiated (ad libitum) AgRP-control and AgRP-ablated male (Figure IV.1B) and female (Figure IV.1E) mice received an i.p. injection of either saline solution (sal, from day 1-3) or ghrelin (ghr, day 4) and their food intake was measured 4hrs following the injection. Ghrelin injection significantly increased the food intake of AgRP-control mice compared to saline injection, whereas no significant difference was observed in the food intake of AgRP-ablated mice 4hrs following ghrelin injection. In addition, a significant difference was observed in the food intake of male and female AgRP-control vs. AgRP-ablated mice following saline injection (Figure IV.1B,E).

Moreover, we used western blot to assess AgRP expression in overnight fasted AgRP-control vs. homozygous AgRP-ablated mice (Figure IV.1F-G). No AgRP expression was detected in the ARC of AgRP-ablated mice whereas AgRP expression was observed in the ARC of AgRP-control mice.

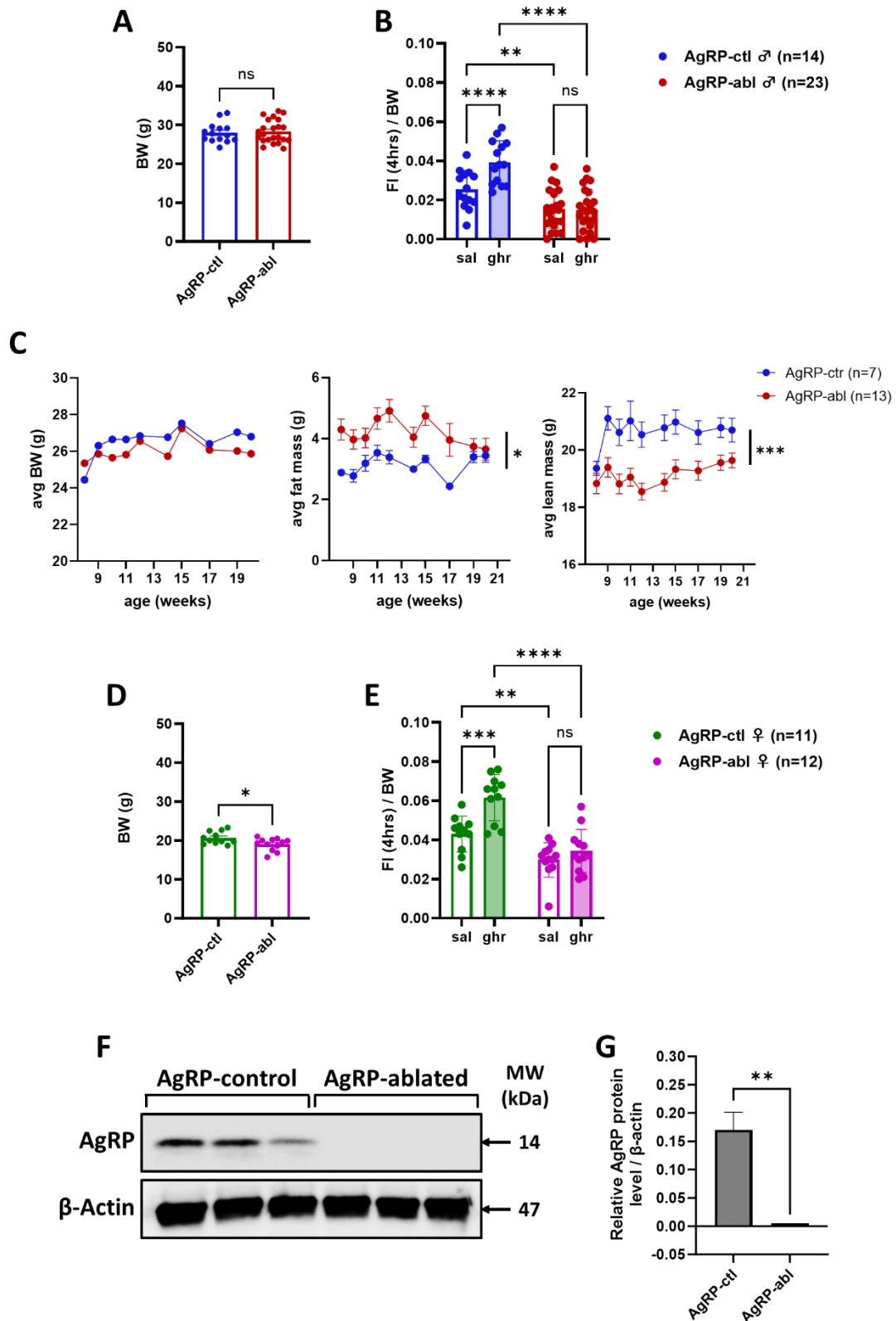


Figure IV.1. Validation of AgRP neuron ablation in the ARC of AgRP-ablated mice.

A- Body weight (g) of 2-3 months old AgRP-control (AgRP-ctl, in blue; n=14) vs. AgRP-ablated (AgRP-abl, in red; n=23) male mice (unpaired parametric t-test).

- B-** Ghrelin test: cumulative food intake (g) normalized on body weight (g) of AgRP-control (n=14) vs. AgRP-ablated (n=23) fed (ad libitum) male mice 4 hrs. post i.p. injection of either saline solution (sal) or ghrelin (ghr) (2way Anova test).
- C-** Averaged body weight and corresponding absolute fat mass and lean body mass measured longitudinally in AgRP-control (n=7) vs. AgRP-ablated (n=13) male mice (2way Anova test)
- D-** Body weight (g) of 2-3 months old AgRP-control (green, n=11) vs. AgRP-ablated (purple, n=12) female mice (unpaired parametric t-test).
- E-** Ghrelin test: cumulative food intake (g) normalized on body weight (g) of AgRP-control (n=11) vs. AgRP-ablated (n=14) fed (ad libitum) female mice 4 hrs. post i.p. injection of either saline solution (sal) or ghrelin (ghr) (2way Anova test).
- E, F-** Representative Western Blot and quantification of AgRP protein levels in the ARC of AgRP-control vs homozygous AgRP-ablated mice after overnight fasting (n=3) (unpaired parametric t-test). β -actin is used as a loading control.

Data are presented as mean \pm SEM. * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexel.

Furthermore, since NPY/AgRP neurons release distinct neurotransmitters such as AgRP and NPY, we applied postmortem AgRP- and NPY-immunostaining at the level of the ARC and the PVN (Figure IV.2A-B) of AgRP-control vs. AgRP-ablated overnight fasted male and female mice. We first observed reduced to no AgRP-expression at the level of both the ARC and the PVN in AgRP-ablated male and female mice. On the other hand, NPY-expression was extremely reduced - but not completely abolished - in AgRP-ablated mice compared to AgRP-control mice. Finally, we assessed the neuronal activation of arcuate neurons by applying cFos-immunostaining at the level of the ARC (Figure IV.2D). A reduced neuronal activation was observed in the ARC of AgRP-ablated mice when compared to the activation level of arcuate neurons in AgRP-control groups.

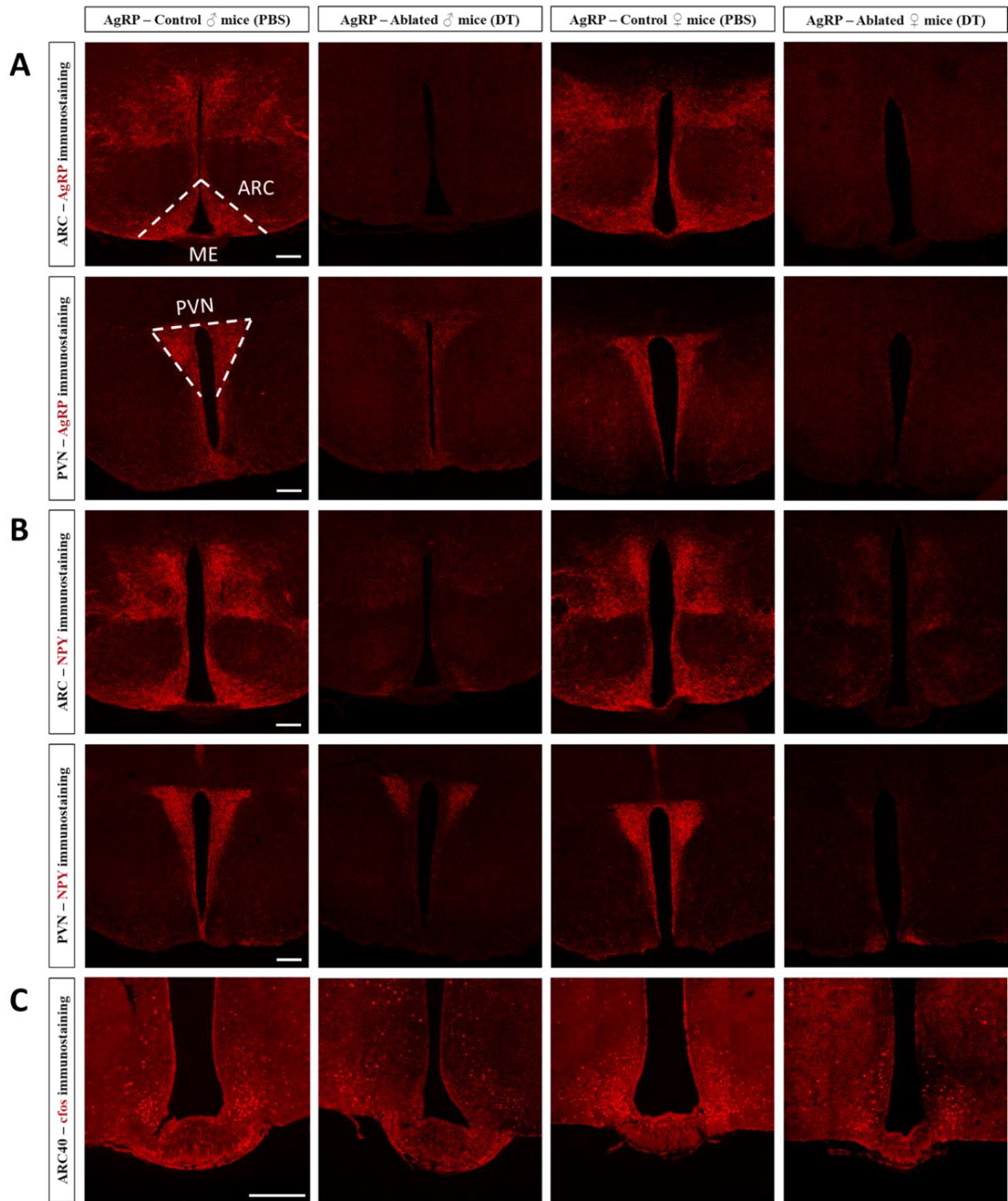


Figure IV.2. Post-mortem validation of AgRP neuron ablation in AgRP-ablated mice. Immunofluorescence staining of AgRP (A), NPY (B), and cFos (C) at the level of the ARC (1st row) and the PVN (2nd row) of the hypothalamus in overnight fasted AgRP control vs. AgRP-ablated male and female mice (scale bar = 200µm). ME: medial eminence.

2. Modulation of the olfactory behavior in AgRP-control vs. AgRP-ablated male and female mice by the metabolic state

AgRP-control and AgRP-ablated male (Figure IV.3.) and female (Figure IV.4.) mice were compared and subjected to multiple behavioral tests. We first started with a habituation dishabituation test (Figure IV.3A). This test consists of the following 3 steps. In a perforated ball, we first present mineral oil (MO), which is odorless and serves as a control solution. Then, an unknown odor A is presented for 1 minute to the mouse who will explore it. For each presentation, as the mouse becomes familiar with odor A, the sniffing time decreases. This process is called olfactory habituation. Then, a new odor - odor B - is presented. If it is recognized as new, the mouse will explore it for a longer time but if it is not discriminated then the mouse will not explore it. Two pairs of odorants were used in this test: (+)-carvone/ (-)-carvone (2 enantiomers of C₁₀H₁₄O) and hexanal/heptanal (C₆H₁₂O / C₇H₁₄O) diluted to 15% and 0.5% respectively in mineral oil.

Sniffing time (sec) was measured to evaluate the olfactory detection and discrimination capacities in mice. To assess olfactory detection capacities, we compared the sniffing time between the last presentation of MO and the first presentation of odor A. Similarly, we compared the last presentation of odor A with the first presentation of the new odor (B) to assess the olfactory discrimination capacities in mice.

We first compared the exploration time of AgRP-control vs. AgRP-ablated fed and fasted male (Figure IV.3B) and female (Figure IV.4A) mice by assessing the average sniffing duration of the 3 MO presentations for each group. No significant difference was observed in the exploration time between these groups.

We realized the habituation dishabituation test on AgRP-control mice to compare their olfactory capacities between fed and fasted conditions (Figure IV.3C). We found that when fasted, control male mice were able to significantly detect (Figure IV.3D) and discriminate (Figure IV.3E) the odorants. However, when fed, their olfactory capacities drastically dropped. By comparing the detection and discrimination spikes between both conditions, we found a very significant difference in olfactory detection (Figure IV.3F) and discrimination (Figure IV.3G) capacities, where fasted mice seem to have significantly greater sniffing (exploration) time in comparison with fed mice.

Next, we realized the same test on mice lacking AgRP neurons (Figure IV.3H). We noticed that AgRP-ablated male mice showed significant olfactory detection (Figure IV.3I) and discrimination (Figure IV.3J) capacities whether fed or fasted, with no significant difference in their detection (Figure IV.3K) or discrimination spikes (Figure IV.3L).

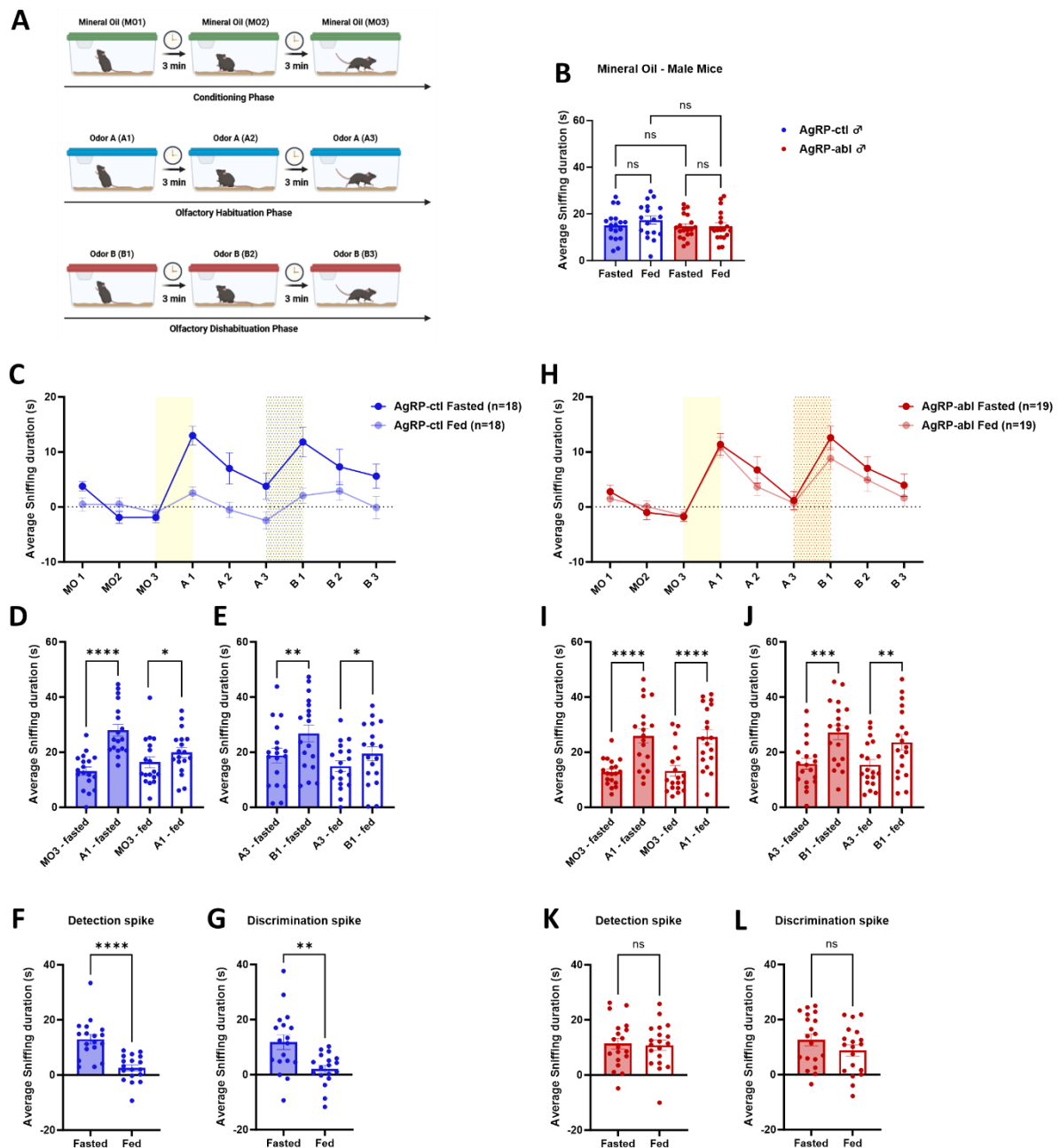


Figure IV.3. Modulation of olfactory behavior in AgRP-control vs. AgRP-ablated male mice by the metabolic state.

A- Schematic illustration of the habituation-dishabituation test (successive presentations of mineral oil, odor A, and odor B). See details in the Methods section.

- B-** Average sniffing duration of the 3 mineral oil (MO) presentations for AgRP-control (blue, n=18) and AgRP-ablated (red, n=19) fed vs. fasted male mice (paired/unpaired parametric t-test).
- C,H-** Average sniffing duration for each odor presentation normalized by the mean sniffing duration of the 3 MO presentations in AgRP-control (C; represented in blue) and AgRP-ablated (H; represented in red) fed vs. fasted male mice (2way Anova).
- D,I-** Detection capacities: comparison of the average sniffing duration between the last presentation of MO (MO3) and the first presentation of odor A (A1) in fed vs fasted AgRP-control (D) and AgRP-ablated (I) male mice (paired parametric t-test).
- E,J-** Discrimination capacities: comparison of the average sniffing duration between the last presentation of odor A (A3) and the first presentation of the new odor or odor B (B1) in fed vs fasted AgRP-control (E) and AgRP-ablated (J) male mice (paired parametric t-test).
- F,K-** Detection spike: comparison between the normalized values of A1 (normalized on the average sniffing duration of the corresponding 3 MO presentations) sniffing duration in fasted vs. fed AgRP-control (F) and AgRP-ablated (K) male mice (paired parametric t-test).
- G,L-** Discrimination spike: comparison between the normalized values of B1 (normalized on the average sniffing duration of the corresponding 3 MO presentations) sniffing duration of fasted vs. fed AgRP-control (G) and AgRP-ablated (L) male mice (paired parametric t-test).

Data are displayed as mean \pm SEM. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexel.

The same test was then realized on AgRP-control (Figure IV.4B) and AgRP-ablated (Figure IV.4G) fed and fasted female mice. We found that fasted control female mice were able to significantly detect odor A (Figure IV.4C) and tended to discriminate between odor A and odor B (Figure IV.4D). However, when fed, AgRP-control female mice had no significant detection (Figure IV.4C) and discrimination (Figure IV.4D) capacities. By comparing the detection and discrimination spikes between both conditions, we found no significant difference in olfactory detection capacities (Figure IV.4E). Meanwhile, fasted AgRP-control female mice showed significantly greater discrimination capacities when compared to the fed control group (Figure IV.4F).

Finally, we realized the same test on female mice lacking AgRP neurons (Figure IV.4G). We noticed that fasted AgRP-ablated female mice show significant olfactory detection capacities (Figure IV.4H) but no significant discrimination capacities (Figure IV.4I). Interestingly, fed AgRP-ablated female mice were able to significantly detect (Figure IV.4F)

and discriminate (Figure IV.4G) the odors. By comparing the detection and discrimination spikes between both conditions, we found a significant difference in olfactory detection capacities (Figure IV.3J) but no significant difference in the discrimination capacities (Figure IV.3K) between fed and fasted AgRP-ablated female mice.

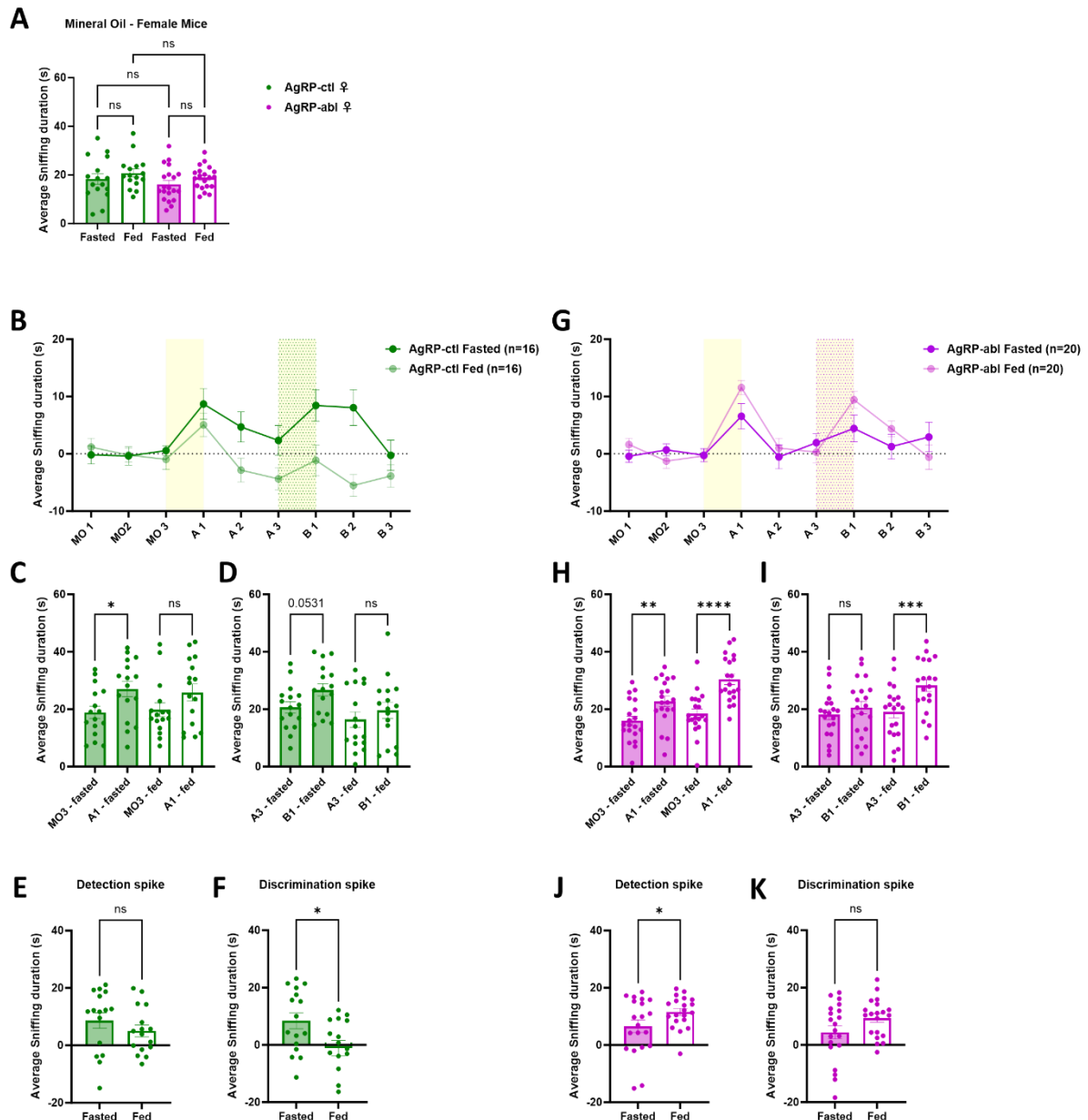


Figure IV.4. Modulation of olfactory behavior in AgRP-control vs. AgRP-ablated female mice by the metabolic state.

A- Average sniffing duration of the 3 mineral oil (MO) presentations for AgRP-control (green, n=18) and AgRP-ablated (purple, n=19) fed vs. fasted female mice (paired/unpaired parametric t-test).

- B,G-** Average sniffing duration for each odor presentation normalized on the mean sniffing duration of the 3 MO presentations in AgRP-control (B; represented in green) and AgRP-ablated (G; represented in purple) fed vs. fasted female mice (2way Anova).
- C,H-** Detection capacities: comparison of the average sniffing duration between the last presentation of MO (MO3) and the first presentation of odor A (A1) in fed vs fasted AgRP-control (C) and AgRP-ablated (H) female mice (paired parametric t-test).
- D,I-** Discrimination capacities: comparison of the average sniffing duration between the last presentation of odor A (A3) and the first presentation of the new odor or odor B (B1) in fed vs fasted AgRP-control (D) and AgRP-ablated (I) female mice (paired parametric t-test).
- E,J-** Detection spike: comparison between the normalized values of A1 (normalized on the average sniffing duration of the corresponding 3 MO presentations) sniffing duration in fasted vs. fed AgRP-control (E) and AgRP-ablated (J) female mice (paired parametric t-test).
- F,K-** Discrimination spike: comparison between the normalized values of B1 (normalized on the average sniffing duration of the corresponding 3 MO presentations) sniffing duration of fasted vs. fed AgRP-control (F) and AgRP-ablated (K) female mice (paired parametric t-test).

Data are displayed as mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexel.

3. Assessment of the olfactory performance in AgRP-control vs. AgRP-ablated male and female mice

AgRP-control and AgRP-ablated male mice were subjected to the buried food-seeking test to assess their capacities to detect food, based on its odor. The latency (min) to find the buried cookie was measured. Both groups, whether fed or fasted, were able to find the buried cookie in less than 5 min with no significant difference in latency between the groups (Figure IV.5A-C).

Fed male and female mice were then subjected to the conditioned odor aversion test to assess their olfactory discrimination capacities when odorants are associated with a positive or negative hedonic value (Figure IV.5D). Two enantiomers of the same molecule, Limonene, were used in this test: (Li+) and (Li-). In the attraction phase, mice were offered sweet cookies odorized with (Li+) for 2 consecutive days (days 1-2, 1hr/day). Mice then received an i.p. injection of saline solution. On the other hand, in the aversion phase, mice were offered sweet cookies odorized with (Li-) for 2 consecutive days (days 3-4, 1hr/day). However, this time, mice received an i.p. injection of LiCl solution inducing an aversive gastric reaction.

Finally, on day 5, fed mice were simultaneously offered two non-sweet (sugar-free) cookies each odorized with one enantiomer of limonene (Li+ vs. Li-), and their corresponding odorized-food consumption (g) were measured for 1hr. The aversion ratio (AR) represents their (Li-)-odorized food consumption (g) divided by their total food intake (TFI) after the 1-hour test.

We measured the total food intake (g) of odorized (Li+/-) cookies for 1hr per day for AgRP-control and AgRP-ablated fed male and female mice (Figure IV.5E-H). The total food intake continuously and significantly increased from day 1 to day 5. We then assessed the total food intake (of 1hr) normalized on the body weight (g) of individual mice on the day of the test or day 5. We found no significant difference between the food intake of both male and female AgRP-control vs. AgRP-ablated fed mice (Figure IV.5I and K, respectively). However, their food choices were different. AgRP-control male mice were not able to discriminate between both odors (Li+/-) (Figure IV.5J). Their aversion ratio is almost equal to 0.5 whereas AgRP-ablated male mice have a significantly lower aversion ratio indicating that they consumed significantly less aversive cookies and that they were able to discriminate the two odorants.

On the other hand, AgRP-control female mice ate significantly less from the aversive Li (-)-odorized cookie compared to the non-aversive Li (+)-odorized cookie but no significant difference was observed in the aversion ratio of AgRP-control vs. AgRP-ablated fed female mice (Figure IV.5L).

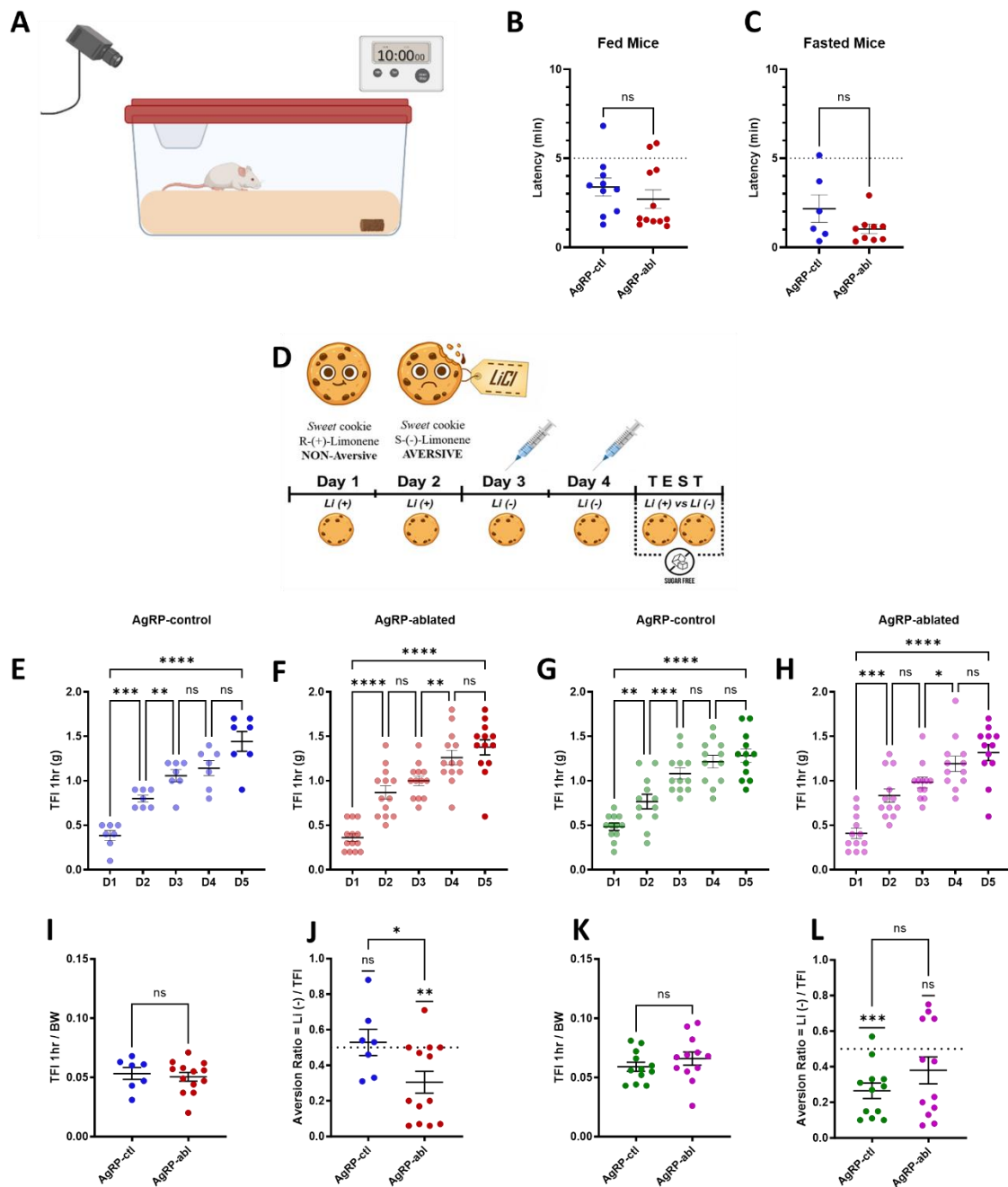


Figure IV.5. Assessment of the olfactory performance in AgRP-control vs. AgRP-ablated male and female mice.

A- Graphical illustration of the buried food-seeking test. See details in the Methods section.

B,C- Latency (in min) to find the buried cookie by AgRP-control vs. AgRP-ablated fed (B) and fasted (C) male mice (unpaired parametric t-test).

D- Schematic illustration of the protocol of the olfactory aversion conditioning aversive odor test.

E,G- Total food intake (g) of 1hr per day from day 1 to day 5 for AgRP-control fed male (E) and female (G) mice (2way Anova test).

F,H- Total food intake (g) of 1hr per day from day 1 to day 5 for AgRP-ablated fed male (F) and female (H) mice (2way Anova test).

I,K- Total food intake (TFI) of 1hr (g) normalized on the body weight (BW, in g) of individual AgRP-control vs. AgRP-ablated fed male (I) and female (K) mice on the day of the test, day 5 (unpaired parametric t-test).

J,L- Aversion ratio of AgRP-control vs. AgRP-ablated fed male (J) and female (L) mice (One-sample t-test, unpaired parametric t-test).

Data are displayed as mean \pm SEM. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexe1.

Summary 1

AgRP-control mice:

- Hunger or an energy deficit state is associated with increased AgRP neuronal activity and enhanced olfactory capacities.
- Satiety or the excess of energy is associated with reduced AgRP neuronal activity and weak olfactory capacities.

AgRP-ablated mice:

- By ablating AgRP neurons, the fed/fasted modulation of olfactory capacities is abolished.
- Ablating AgRP neurons tends to permanently improve olfactory capacities in response to both food and non-food-related odors.

4. Modulation of granule cell activity in the OB of AgRP-control vs. AgRP-ablated male mice under distinct metabolic states

AgRP-control and AgRP-ablated male mice received a unilateral viral injection of pAAV.Syn.GCaMP6f.WPRE.SV40 as well as a fiber probe implantation into the GCL of their right OB (Figure IV.6A).

Recording of in vivo calcium dynamics across time allows real-time assessment of GC neuronal activity in response to the first sniff of Chow (food-related odor) and C+ (novel odor) in AgRP-control and AgRP-ablated fed vs. fasted male mice. We measured the area under the curve (AUC) between $t=0$ sec (odor presentation) and $t=1$ sec (the duration of a sniff). GC activity increased in response to every presented odor, whether chow or C+, in AgRP-control and AgRP-ablated fed and fasted mice (Figure IV.6B-I) indicating an increased activation of GCs in the OB in response to odorant presentation.

A significant difference was observed in the activity of GCs in response to chow odor between the fed and the fasted state in both AgRP-control and AgRP-ablated mice (Figure IV.6B-E). Fasted mice of both groups displayed a significantly higher GC activation when presented with chow odor than fed mice.

Conversely, AgRP-control mice showed higher GC activity in response to C+ odor when fed compared to the fasted state (Figure IV.6F,G). Meanwhile, no significant difference was observed in the GC activity between fed and fasted AgRP-ablated mice in response to C+ odor presentation (Figure IV.6H,I).

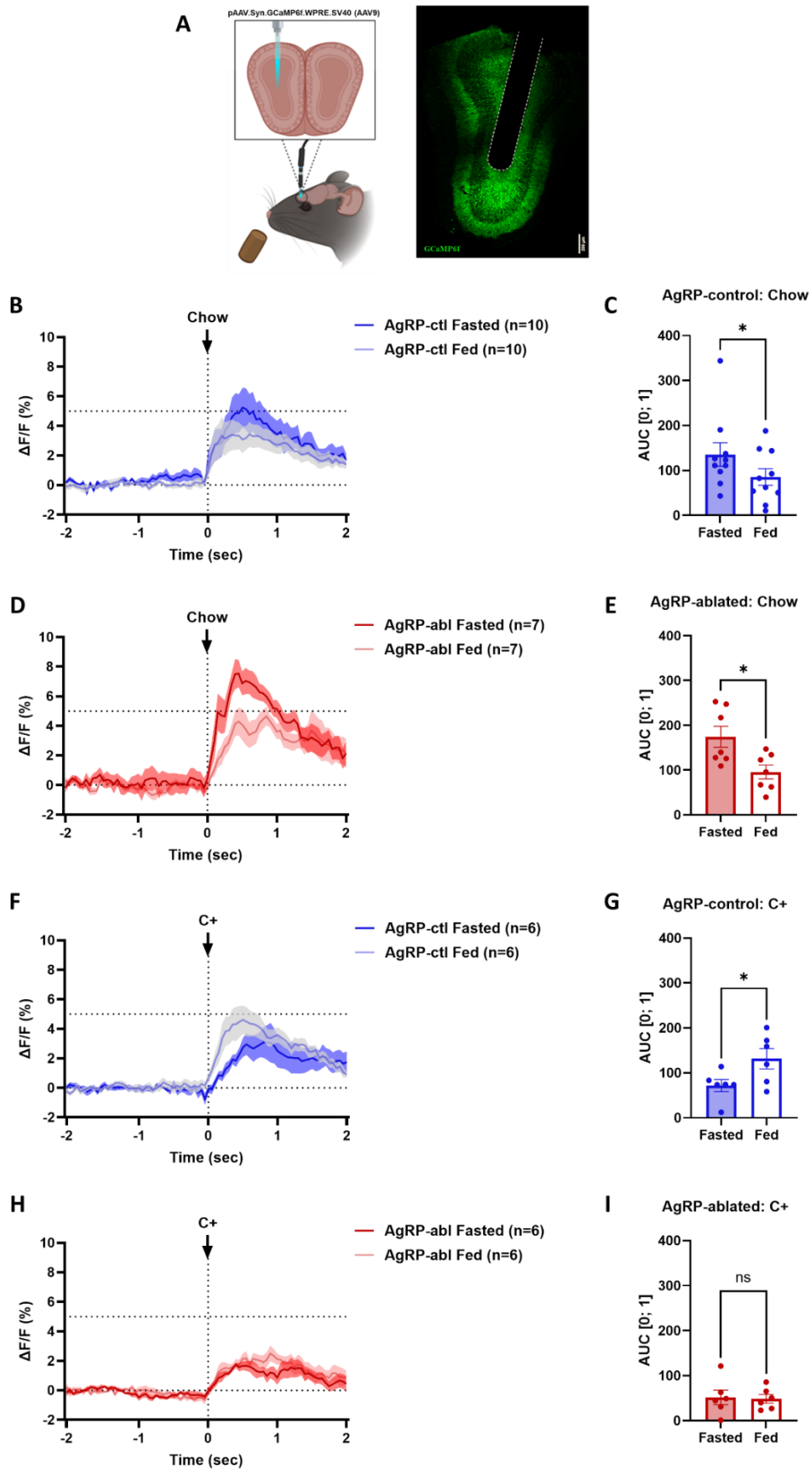


Figure IV.6. Recording of granule cell activity in the OB of AgRP-control vs. AgRP-ablated male mice under distinct metabolic states.

A- Schematic illustration of the viral injection and the optical fiber implantation for in-vivo calcium recording in the OB using fiber photometry. Example of GCaMP6 expression in the granule cell layer of the OB and position of the optic fiber (scale bar 200 μ m).

B,D,F,H- Dynamics of in vivo calcium recording averaged across mice in AgRP-control (B,F) and AgRP-ablated (D,H) fed vs. fasted male mice for a total of 4sec [-2; 2]. Time 0 corresponds to the first sniff of the presented odor: food chow odor (B,D) or neutral C+ odor (F,H). Data are represented as the percent change of fluorescence over the mean fluorescence (% $\Delta F/F$) (paired t-tests).

C,E,G,I- Area under the curve (AUC) for a duration of 1 sec following odor presentation onset [0; 1] in AgRP-control and AgRP-ablated fed vs. fasted male mice (paired parametric t-test).

Data are displayed as mean \pm SEM. * $p < 0.05$; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annex1.

Summary 2

Odor detection always triggers GC activation.

Distinct neuronal networks may be involved in the olfactory processing of food-related vs. non-food related odors.

The results for the novel odor cue lead to the hypothesis that hunger or an energy deficit state reduces GC activity, resulting in an ultimately better olfactory performance, as seen in the olfactory behavioral tests previously described.

On the other hand, ablating AgRP neurons abolishes the fed/fasted modulation of GC activity in response to novel odor cues.

5. Stimulating AgRP neurons increases olfactory food-seeking behaviors and food intake while reducing granule cell activity in the OB

AgRP-ires-cre mice express the Cre recombinase in AgRP neurons. They received a bilateral injection of cre-dependent virus, either control virus “pAAV-hSyn-DIO-mCherry” (AgRP-mcherry) or the DREADD(Gq) virus “pAAV-hSyn-DIO-hM3D(Gq)-mCherry” (AgRP-hM3Dq), in their ARC (Figure IV.7A). Both groups also received a unilateral injection of pAAV.Syn.GCaMP6f.WPRE.SV40 (AAV9) and a probe implantation in the GCL of their right OB (Figure IV.7B) to assess the real-time neuronal calcium dynamics of GC activity in response to stimulating AgRP neurons in AgRP-hM3Dq mice while presenting distinct olfactory stimuli (Chow, cumin, female urine).

To validate the stereotaxic injection of DREADD(Gq), a CNO test is realized. CNO is an exogenous ligand used to remotely and acutely activate DREADD(Gq) receptors leading to the stimulation of AgRP neurons in AgRP-hM3Dq mice. We measured the food intake (g) of AgRP-mcherry and AgRP-hM3Dq mice 4hrs following either SAL or CNO i.p. injection. Food intake (g) was normalized by the body weight (g) (Figure IV.7C). Food intake of AgRP-mcherry fed mice did not increase in response to CNO injection when compared to food intake of the same group injected with saline. Meanwhile, food intake of fed AgRP-hM3Dq mice increased significantly in response to CNO injection indicating that CNO stimulates AgRP neurons in fed AgRP-hM3Dq mice to increase food intake. Mice that did not show CNO-induced food intake were discarded from other tests (data not shown).

Fed AgRP-mcherry and AgRP-hM3Dq mice were then subjected to a buried food-seeking test to assess their olfactory detection capacities. Both groups were fed ad libitum and injected with CNO 20 min before testing. Stimulating AgRP neurons in AgRP-hM3Dq mice led to significantly lower latency to find the buried cookie in comparison with fed AgRP-mcherry mice (Figure IV.7D).

We next assessed the modification of GC activity during the first sniff of chow, cumin (palatable food odor), and urine (social odor) while measuring the AUC [0; 4] for fed AgRP-mcherry and AgRP-hM3Dq mice following a CNO i.p. injection (Figure IV.7E-J). GC activity was significantly reduced in fed AgRP-hM3Dq mice compared to fed AgRP-mcherry mice in response to almost every presented odor.

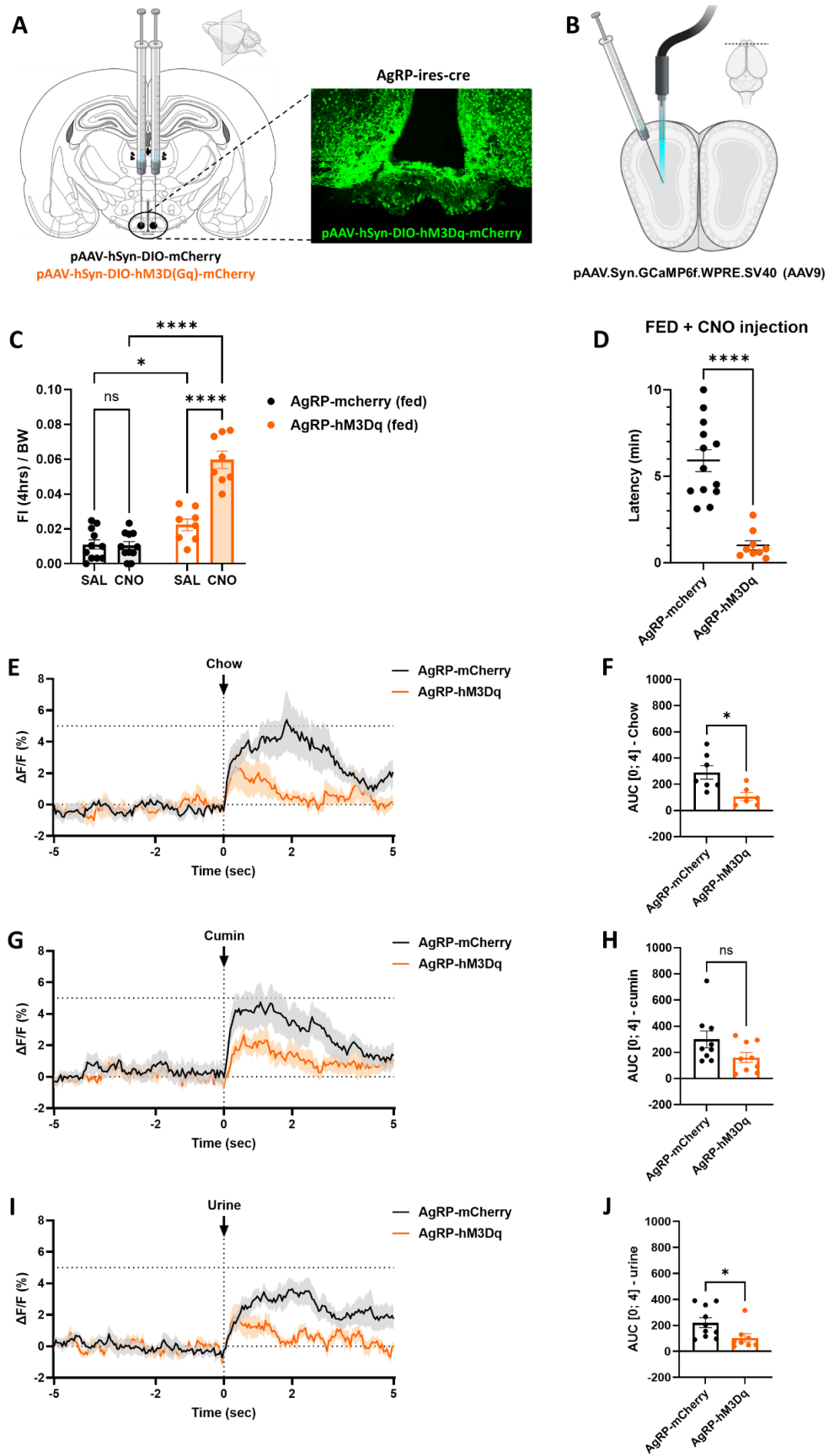


Figure IV.7. Stimulating AgRP neurons increases food-seeking behaviors and food intake while modulating granule cell activity in the OB.

- A-** Graphical illustration of the bilateral viral injection of pAAV-hSyn-DIO-mCherry virus or pAAV-hSyn-DIO-hM3D(Gq)-mCherry virus in the ARC of *AgRP-ires-cre* male mice. Example of the Gq-mCherry expression in the ARC.
- B-** Graphical illustration of the unilateral viral injection of pAAV.Syn.GCaMP6f.WPRE.SV40 in the GCL of the OB in *AgRP-ires-cre* male mice.
- C-** Cumulative food intake (g) normalized over the body weight (g) of fed (ad libitum) AgRP-cherry (black, n=11) and AgRP-hM3Dq (orange, n=8) 4hrs following saline (SAL) or CNO i.p. injection (2way ANOVA test).
- D-** Latency (in min) to find the buried cookie following CNO i.p. injection in AgRP-cherry (n=13) and AgRP-hM3Dq (n=9) fed male mice (unpaired parametric t-test).
- E,G,I-** Dynamics of in vivo calcium recording averaged across mice in AgRP-cherry and AgRP-hM3Dq fed male mice following CNO i.p. injection. Time 0 corresponds to the first sniff of the presented odor: chow (D), cumin (F), and female urine (H). Data are represented as the percent change of fluorescence over the mean fluorescence (% $\Delta F/F$) (paired t-tests).
- F,H,J-** Area under the curve (AUC) for a duration of 4 sec following odor presentation [0; 5] in AgRP-cherry and AgRP-hM3Dq fed male mice (unpaired parametric t-test).

Data are displayed as mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexe1.

Summary 3

These results suggest that the activation of AgRP neurons increases food intake, enhances food-seeking behaviors while simultaneously decreasing GC activity in response to different olfactory cues.

6. Olfactory performance of 8-week chow- vs. HFD-fed mice.

C57/bl6 male mice were divided into two groups and fed either chow or HFD. After 8 weeks of diet, the body weight and the percentage of fat mass in HFD-fed mice were significantly higher than the BW and fat mass of their cohort chow-fed mice (Figure IV.8A,B).

Chow and HFD mice were then subjected to a buried food-seeking test under fasted and fed conditions, and their olfactory detection capacities were assessed and compared (Figure IV.8C). Both groups showed significantly lower latency (in min) to find the buried cookie when fasted. No significant difference was observed in the fasted state between the two groups. However, when fed, chow mice were able to find the hidden cookie significantly faster than the HFD group.

A habituation dishabituation test was then realized to assess the olfactory capacities of both groups under different metabolic states. We first compared the exploration time of chow and HFD, fed and fasted mice (Figure IV.8D) by assessing the average sniffing duration of the 3 mineral oil presentations for each group. No significant difference was observed in the exploration time between these groups.

We realized the habituation dishabituation test on Chow mice to compare their olfactory capacities between fed and fasted conditions. Based on their sniffing duration (sec) or the time they spent exploring the ball, we measured their olfactory capacities (Figure IV.8E). We found that when fasted, control male mice were able to significantly detect (Figure IV.8F) and discriminate (Figure IV.8G) the odors. However, when fed, chow mice showed no significant olfactory detection (Figure IV.8F) and discrimination (Figure IV.8G) capacities. By comparing the detection and discrimination spikes between both conditions, we found that fasted chow mice have a significantly higher detection spike when compared to fed chow mice (Figure IV.8H) but no significant difference was observed in the discrimination spikes between the fed and the fasted states (Figure IV.8I).

Next, we realized the same test on HFD mice (Figure IV.8J). We noticed that HFD mice were able to significantly detect (Figure IV.8K) and discriminate (Figure IV.8L) the odors when fasted. Meanwhile, under fed conditions, the HFD group showed no significant detection capacities (Figure IV.8K) but very significant discrimination capacities (Figure IV.8L). In addition, no significant difference was observed in the detection (Figure IV.8M) and discrimination spikes (Figure IV.8N) of fed vs. fasted HFD group.

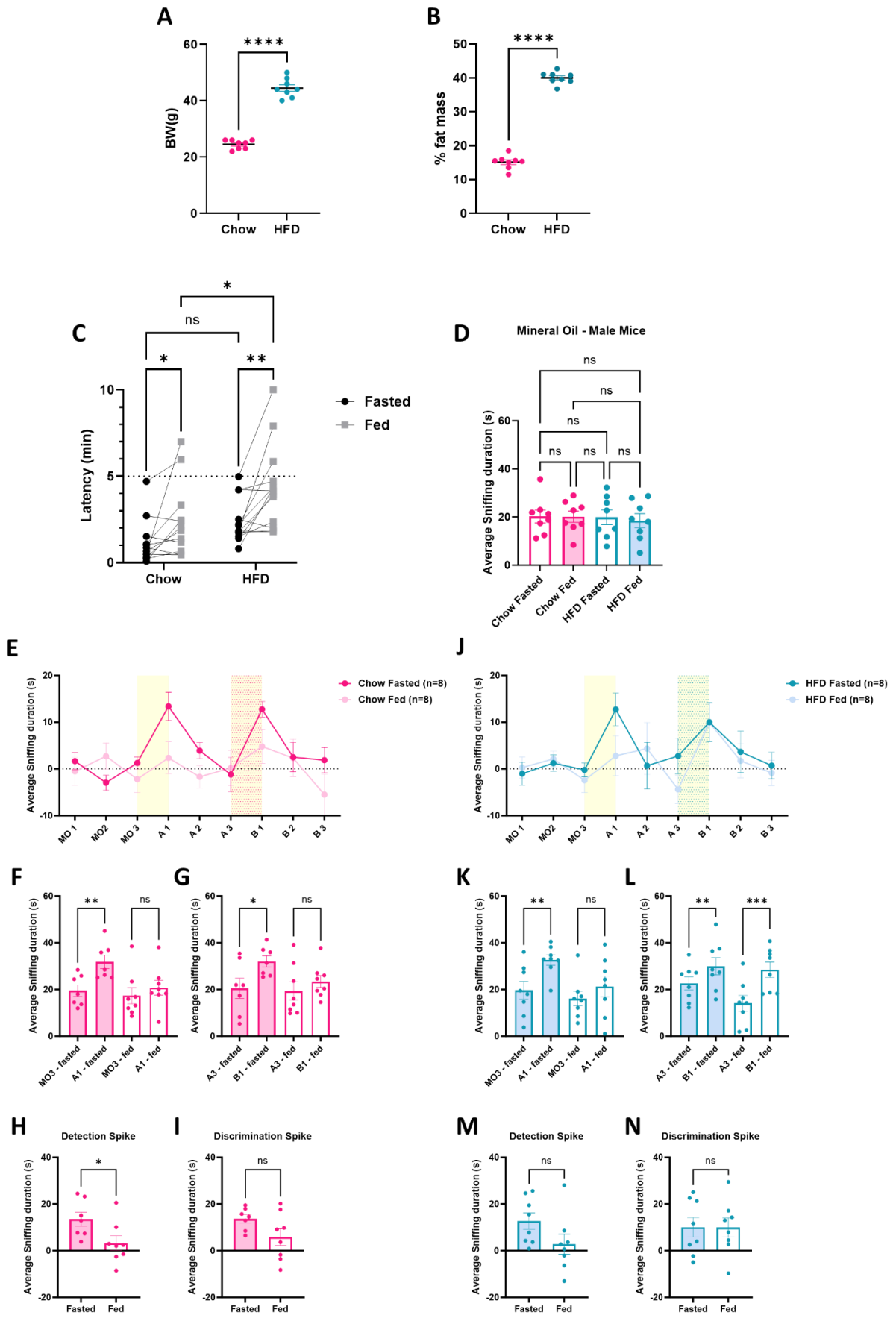


Figure IV.8. Olfactory performance of 8-week chow- vs. HFD-fed mice.

- A-** Body weight (g) of chow- (n=8) vs. 8 weeks HFD-fed (n=8) mice (unpaired t-test).
- B-** Percentage of fat mass in chow- (n=8) vs. 8 weeks HFD-fed (n=8) mice (unpaired t-test).
- C-** Latency (in min) to find the buried cookie by chow- and HFD-fed male mice (2-way ANOVA test).
- D-** Average sniffing duration of the 3 mineral oil (MO) presentations for chow- (n=8) and HFD- (n=8) fed vs. fasted male mice (2way Anova test).
- E,J-** Average sniffing duration for each odor presentation normalized on the mean sniffing duration of the 3 MO presentations in chow (E; represented in pink) and HFD (J; represented in blue) fed vs. fasted male mice (2way Anova test).
- F,K-** Detection capacities: comparison of the average sniffing duration between the last presentation of MO (MO3) and the first presentation of odor A (A1) in fed vs. fasted chow and HFD male mice (paired parametric t-test).
- G,L-** Discrimination capacities: comparison of the average sniffing duration between the last presentation of odor A (A3) and the first presentation of the new odor or odor B (B1) in fed vs. fasted chow and HFD male mice (paired parametric t-test).
- H,M-** Detection spike: comparison between the normalized values of A1 (normalized on the average sniffing duration of the corresponding 3 MO presentations) sniffing duration in fasted vs. fed chow and HFD male mice (paired parametric t-test).
- I,N-** Discrimination spike: comparison between the normalized values of B1 (normalized on the average sniffing duration of the corresponding 3 MO presentations) sniffing duration of fasted vs. fed chow and HFD male mice (paired parametric t-test).

Data are given as mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexe1.

Summary 4

In a recent study, Beutler et al. (2020) demonstrates that exposing mice to 6 weeks of high fat diet is sufficient to attenuate the response of AgRP neurons to nutritionally-relevant stimuli including olfactory food-related cues and hormonal signals, such as the orexigenic hormone ghrelin.

Thus, given that obesity is associated with impaired hypothalamic AgRP neuronal activity, my goal was to assess olfactory capacities as well as olfactory processing in a mouse model of diet-induced obesity.

Although, according to the work of Beutler et al. (2020), 6 weeks of HFD are sufficient to reduce AgRP neuron responsiveness to multiple food-related stimuli, it seems that 8 weeks of HFD exposure were not sufficient to induce significant olfactory impairments in obese C57/bl6 mice.

We next assessed the modification of GC activity in response to C+ odor presentation in fed vs. fasted chow and HFD groups (Figure IV.9.). Fasted chow mice showed significantly lower GC activity in response to C+ odor presentation when compared with fed chow mice. However, no significant difference was observed in GC activity in response to C+ odor presentation in HFD mice whether fed or fasted.

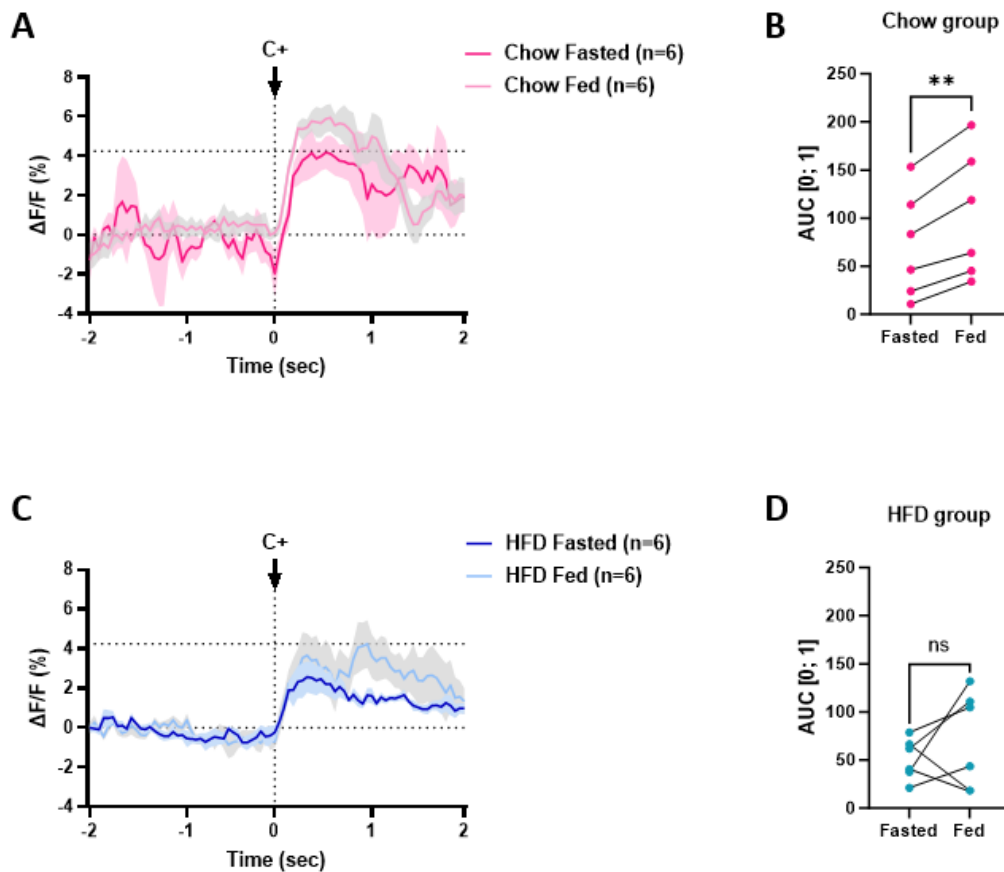


Figure IV.9. Modulation of in vivo granule cell calcium activity in chow- vs. HFD-fed mice under different metabolic states.

A,C- Dynamics of in vivo calcium recording in chow- (A) and HFD- (C) fed vs. fasted male mice. Time 0 corresponds to the first sniff of the presented odor: C+. Data are represented as the percent change of fluorescence over the mean fluorescence (% $\Delta F/F$) (unpaired t-test).

B,D- Area under the curve (AUC) for a duration of 1 sec following odor presentation [0; 1] in chow- and HFD- fed vs. fasted male mice (paired parametric t-test).

Data are given as mean \pm SEM. ** $p < 0.01$; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexel.

7. Stimulating AgRP neurons increases food intake while reducing granule cell activity in the OB of AgRP-hM3Dq obese mice

After 8 weeks of HFD, *AgRP-ires-cre* mice received a bilateral injection of the cre-dependent DREADD(Gq) virus “pAAV-hSyn-DIO-hM3D(Gq)-mCherry” (AgRP-hM3Dq), in their ARC. AgRP-hM3Dq mice also received a unilateral injection of pAAV.Syn.GCaMP6f.WPRE.SV40 and a probe implantation in the GCL of their right OB to assess the real-time neuronal dynamics of GC activity in response to stimulating AgRP neurons in AgRP-hM3Dq while presenting distinct olfactory stimuli (Chow, HFD, C+, and urine).

After 8 weeks of diet-induced obesity (Figure IV.10A), HFD-fed AgRP-hM3Dq mice were subjected to a CNO test. We measured the HFD food intake (g) of AgRP-hM3Dq fed obese mice 4hrs following either SAL or CNO i.p. injection (Figure IV.10B). Food intake of satiated AgRP-hM3Dq mice significantly increased in response to CNO injection when compared to food intake of the same group injected with saline.

Satiated AgRP-hM3Dq obese mice were then subjected to a buried food-seeking test and their olfactory detection capacities were assessed by measuring the latency or the time these mice spent to find the buried cookie following either saline or CNO i.p. injection (Figure IV.10C). CNO injection induced a significant reduction in the latency to find the buried cookie in obese AgRP-hM3Dq fed mice.

We next assessed the modification of GC activity during the first sniff of chow, HFD (palatable food odor), C+ (novel odor), and female urine (social odor) while measuring the AUC [0; 4] for fed AgRP-hM3Dq obese mice following either a saline or a CNO i.p. injection (Figure IV.10D-K). GC activity was significantly reduced in satiated AgRP-hM3Dq obese mice injected with CNO in response to the odor of chow, HFD and C+. However, this modification of GC activity was not observed in response to the presentation of the odor of female urine.

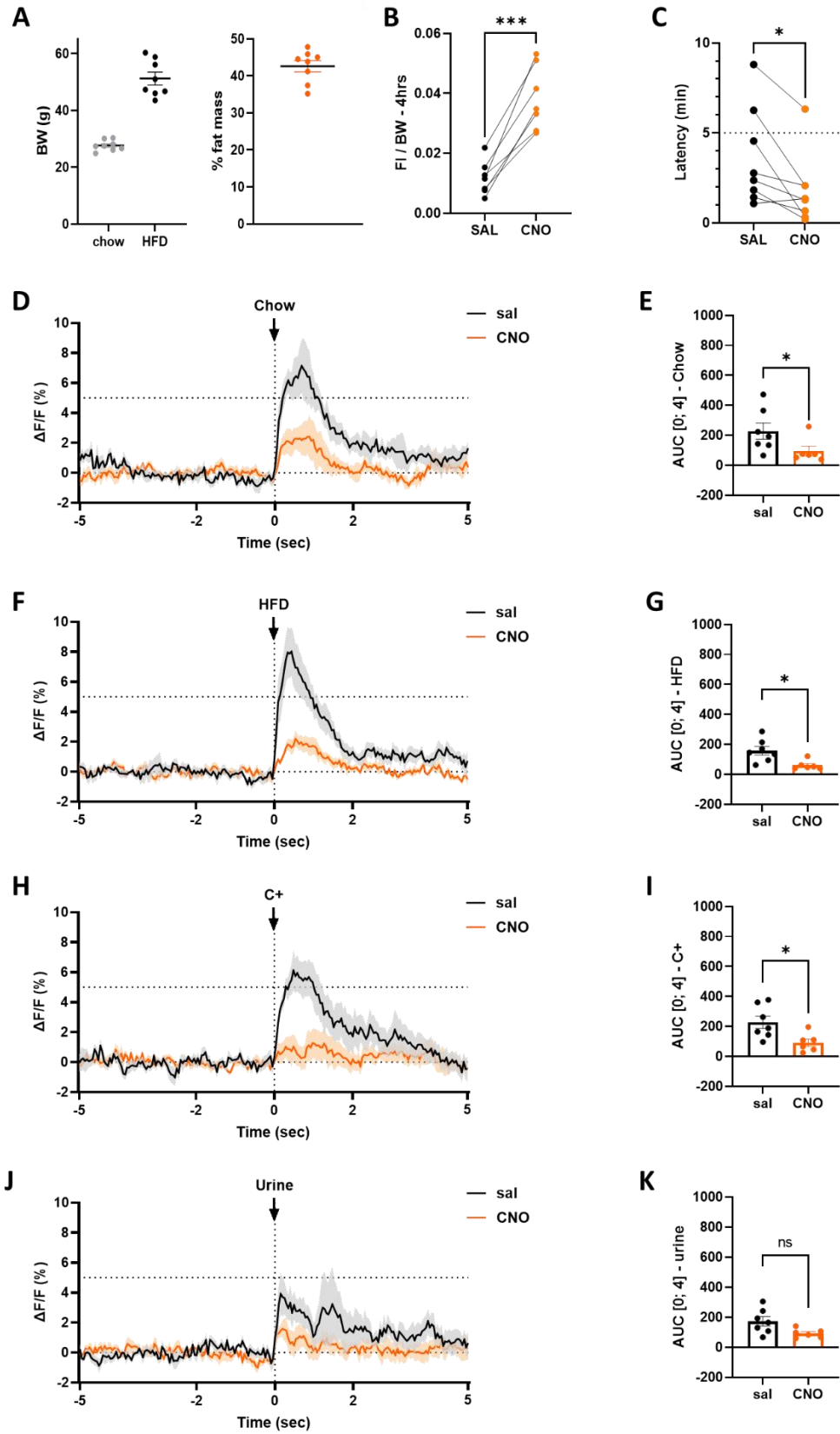


Figure IV.10. Stimulating AgRP neurons increases food intake while modulating granule cell activity in the OB of 8-week HFD-fed mice.

- A-** Body weight (g) difference between lean and obese AgRP-hM3Dq mice after 8-weeks of HFD. % fat mass is displayed only for HFD fed mice (unpaired t-test).
- B-** Food intake (g) normalized over the body weight (g) of HFD-fed (ad libitum) AgRP-hM3Dq (n=7) 4hrs following saline (SAL) or CNO i.p. injection (paired t-test).
- C-** Latency (in min) to find the buried cookie following saline vs. CNO i.p. injection in AgRP-hM3Dq (n=8) HFD-fed male mice (paired parametric t-test).
- D,F,H,J-** Dynamics of in vivo calcium recording in AgRP-hM3Dq (n=7) male mice following an i.p. injection of either saline or CNO. Time 0 corresponds to the first sniff of the presented odor: chow (D), HFD (F), C+ (H), and urine (J). Data are represented as the percent change of fluorescence over the mean fluorescence (% $\Delta F/F$) (paired t-test).
- E,G,I,K-** Area under the curve (AUC) for a duration of 5-sec odor presentation [0; 4] in AgRP-hM3Dq HFD-fed male mice (paired parametric t-test) following an i.p. injection of either saline or CNO (paired t-test).

Data are given as mean \pm SEM. *p < 0.05; **p < 0.01; ***p<0.001; ns, not significant. n represents the number of mice per group. Detailed statistics can be found in Annexe1.

Summary 5

By applying the same chemogenetic approach but this time in AgRP-cre obese mice, I intended to determine whether artificially activating AgRP neurons, (bypassing their low responsiveness to ghrelin), would help rescuing the altered olfaction observed in the context of obesity (described in the literature).

However, no olfactory impairment was observed after 8 weeks of HFD. In alignment with previous results, the activation of AgRP neurons increases food intake, enhances food-seeking behaviors while simultaneously decreasing GC activity in response to different olfactory cues.

SECTION - V
DISCUSSION

DISCUSSION

In this section, I will summarize the main experimental results and then discuss the contribution of our results to the understanding of the involvement of arcuate AgRP neuron activity in the modulation of olfactory processing in the OB, under different nutritional states, in physiological and pathophysiological conditions. Finally, I will discuss the limitations of our study and propose perspectives for future work.

To evaluate the impact of AgRP neurons on olfaction, we performed behavioral tests and physiological recordings in two different genetic mouse models and in different metabolic conditions: *AgRP^{DTR/+}* mice, in which we ablated all AgRP neurons from birth, and AgRP-cre mice, in which we used chemogenetics to transiently activate AgRP neurons in adults. For this last model, we have tested AgRP neuron activation in both lean and diet-induced obese mice. Our results combining behavioral testing as well as *in vivo* calcium imaging demonstrate that AgRP neurons influence olfactory perception, and can tune the activity of GCs in the OB. Overall this work is original in that it shows that, indeed, beyond and independently of the already well-characterized influence of hormones on the OB, AgRP neurons plays a role in olfactory perception and processing.

1. The OB function is modified in the absence of AgRP neurons

Our first objective was to evaluate and compare olfactory capacities in male and female AgRP-control and AgRP-ablated mice under different nutritional states in response to food-related odorants. We used different olfactory tests to assess different aspects of olfactory capacities and found differences between the two groups. In our first mouse model, neonatal injection of DT in *AgRP^{DTR/+}* mice completely abolished AgRP-expression (Figure IV. 1E,F) and reduced NPY-expression by 90% in the ARC of the hypothalamus (Figure IV. 2A,B), given that 90% of NPY neurons in the ARC co-express AgRP and NPY. In agreement with previous studies (Luquet et al. 2005; Gropp et al. 2005; Xu et al. 2005; Bewick et al. 2005), neonatal ablation of AgRP neurons was tolerated and did not lead to starvation. Moreover, AgRP neuron responsiveness to ghrelin was abolished in AgRP-ablated male and female mice (Figure IV.1B,D).

The habituation-dishabituation test is commonly used to assess olfactory detection and discrimination capacities in different species, including mice (Yang and Crawley 2009; Kolling et al. 2022). It relies on the animal's natural tendency to explore novel odor cues. Odors used in this test were structurally similar to assess the finest olfactory capacities in mice.

First, by measuring the average sniffing duration of the 3 mineral oil presentations in fed vs. fasted AgRP-control and AgRP-ablated male and female mice (Figure IV.3B; 4A), we found no difference in their olfactory exploration time.

Fasted AgRP-control male and female mice were able to significantly detect and discriminate odors whereas, when fed, their olfactory capacities drastically dropped (Figure IV.3C; 4B). Our results perfectly matched the literature suggesting that hunger and energy deprivation increase olfactory sensitivity and olfactory discrimination capacities (Aimé et al. 2007; Cameron, Goldfield, and Doucet 2012; Hanci and Altun 2016; Tucker et al. 2012), whereas satiety reduces the olfactory sensitivity by increasing the olfactory detection threshold (Rolls and Rolls 1997). It is not surprising that the olfactory performance is adapted to the nutritional state of the body since the OB highly expresses numerous metabolic hormone receptors, such as ghrelin, leptin, insulin, and GLP-1 (A.-K. Julliard et al. 2017; Palouzier-Paulignan et al. 2012; Debra Ann Fadool and Kolling 2020; Montaner et al. 2023). Hormones and nutrients act on the OB by adjusting olfactory physiology, olfactory capacities, and ultimately olfactory performance (Palouzier-Paulignan et al. 2012).

On the other hand, AgRP-ablated male and female mice showed high olfactory detection and discrimination capacities whether fed or fasted (Figure IV.3H; 4G). Indeed, AgRP neurons act as a major sensor of nutrient availability, and it seems that, in the absence of AgRP neurons, the olfactory system is disconnected from the metabolic needs of the body. To examine if the performance for odor detection is identical when it comes to food-related odorant, mice were next subjected to a buried food-seeking test. Both AgRP-control and AgRP-ablated mice displayed low olfactory detection threshold by finding the buried palatable cookie in a significantly short time (latency) whether fed or fasted, indicating a high olfactory sensitivity to palatable-food-related odors. These two olfactory tests suggest that AgRP-ablated mice have better olfactory detection and discrimination capacities than AgRP-control mice, but this was not observed for odorant associated to appetitive hedonic value.

Then, we assessed if their olfactory discrimination capacities are also impacted in an aversive context. Fed AgRP-control and AgRP-ablated male and female mice were subjected to a conditioned odor aversion test using two enantiomers of the same molecule, Limonene (Li+/Li-). Enantiomers possess the same functional groups and chemical properties but differ only in stereo-configuration. Thus, they would be expected to have similar spatiotemporal representations of their molecular features in the OB, with some pairs spontaneously perceived as different odors (Heth et al. 1992; Leitereg et al. 1971; Friedman and Miller 1971).

In an olfactory habituation task designed to assess differences in enantiomer olfactory perception, (Linster et al. 2001) demonstrated that rodents are not able to spontaneously discriminate between the enantiomers of limonene; however, they can do so when odorants are associated with distinct reinforcers (Linster et al. 2002). Other studies have also revealed that rodents require active learning, which likely triggers plasticity in the OB network, to distinguish between two similar sensory inputs during a difficult discrimination task (Alonso et al. 2012).

In my experiments, mice were conditioned to eat an odorized sugary cookie either associated with non-aversive effects (Li+) or with LiCl-induced aversion (Li-). In this feeding context, mice must quickly discriminate whether the cookie is safe to be ingested or whether it represents a potential danger to them.

During the conditioning phase, the palatability and caloric content of these cookies induced feeding in all groups of satiated mice.

Of particular note, on the day of the test, the food intake of fed AgRP-control and AgRP-ablated male and female mice did not decrease in comparison with their corresponding food intake during the conditioning phase, although, both cookies presented on the day of the test were sugar free, indicating that, following the learning phase, the odor of limonene was associated with palatability and gained a hedonic value to induce feeding, no matter the caloric content.

In addition, all groups of satiated mice ate the same amount of odorized cookies ($\approx 1.5\text{g}$) on the day of the test (Figure IV.5I, K), but their food choices were different (Figure IV.5J,L).

After conditioning, the absence of odor preference observed (aversion ratio ≈ 0.5) in fed AgRP-control male mice indicates weak olfactory discrimination capacities during satiation. Meanwhile, when simultaneously presented with both enantiomers, fed AgRP-ablated male

mice exhibited a great preference to the (Li⁺)-odorized cookie previously paired with the non-aversive palatability (aversion ratio < 0.5), indicating that fed AgRP-ablated male mice have high olfactory discrimination capacities.

To summarize, for unknown odorants, olfactory detection and discrimination capacities differed according to the metabolic status (fasted or fed), but this specificity was abolished in AgRP-ablated mice. However, when food-related odorants were used in appetitive or aversive contexts, only discrimination was significantly impacted. However, in all the tests, ablation of AgRP neurons tends to improve olfactory capacities.

In this mouse model, we do find a distinction between neutral and learned odorants. Odor hedonic evaluation (attraction pleasant/aversion unpleasant) is considered the first and most prominent dimension in odor perception (Bontempi, Jacquot, and Brand 2021). According to the work of (Torquet et al. 2014), before conditioning, rodents exhibit no preference between both odorant molecules (enantiomers), suggesting that an aversion ratio of 0.5 could be interpreted in two ways: either the animal is not able to discriminate between the two enantiomers or it is indeed able to discriminate between them but has no preference. However, after conditioning, (Torquet et al. 2014) demonstrates that rodents always exhibit a preference for the odorant previously paired with a positive value when simultaneously presented with both odorants, implying that the animal can discriminate among the two enantiomers after learning. The difference that we observe between the two groups suggests that AgRP-ablated mice generally have better olfactory discrimination abilities than AgRP-control mice, or that the effect of food aversion is stronger in the absence of AgRP neurons, leading to enhanced learning and plasticity. A difference in aversion could align with studies showing that the absence of AgRP neurons reshape reward circuitry. Indeed, the absence of AgRP neurons promotes greater excitability of VTA dopamine neurons (Dietrich et al. 2012) and present exacerbated stress-induced anorexia and increased palatable food intake: palatability drives feeding independent of AgRP neuron activity (Denis et al. 2015). Such a modification could explain why we have different results for carvone⁺, a neutral odorant than for a food-related odorant. Furthermore, (Horio and Liberles 2021) have unveiled the role of NPY projections from the ARC to the PVT in food-odor processing according to the nutritional status. We can hypothesize a rewiring of olfactory circuits involved in food odor learning in the absence of AgRP neurons.

On the other hand, a similar conclusion can be drawn for the different results between males and females, either they have better olfactory capacities or they are more sensitive to aversion. Fed female control mice significantly discriminated between both enantiomers and no significant difference was observed in the olfactory discrimination capacities between fed AgRP-control and fed AgRP-ablated female mice.

Our results showed a sexual dimorphism toward olfaction that is very poorly documented in the literature. Tests on female rodents or in women using the Sniffing Sticks have shown that their performance is slightly better than that of males (Hummel et al. 2023; Baum and Keverne 2002; Doty and Cameron 2009). Sensitivity has been examined for urine (Baum and Keverne 2002), but regarding identification, or discrimination, the overall literature shows no clear differences in odor hedonic judgment between males and females (Bontempi, Jacquot, and Brand 2021). A comprehensive study of this dimorphism was beyond the scope of this thesis but would require further study.

Recent reviews examining published literature have indicated a lack of evidence supporting the idea that female rodents exhibit greater variation than male rodents across various phenotypes (Zeng et al., 2023; Shansky, 2019). Nevertheless, considering the role of reproductive hormones, particularly estrogen as discussed in chapter I of the introduction, in modulating AgRP neuron activity and feeding behaviors, along with the observed sex-dependent differences in innate olfactory performance between male and female groups, we have opted to focus our research efforts on male groups.

2. Modulation of GC activity under different nutritional states

In the context of feeding and energy homeostasis, among all bulbar cell types, we decided to focus our studies on the GC activity, and that's for two main reasons: first, as previously described in chapter II of the introduction, olfactory sensory processing is not a strict one-way feedforward process. Multiple cortical structures project back to the OB, in a top-down centrifugal manner (Matsutani and Yamamoto 2008; Padmanabhan et al. 2019; Zandt et al. 2019), carrying information about the internal state of the body (Palouzier-Paulignan et al. 2012; Debra Ann Fadool and Kolling 2020), hence, modulating intrinsic olfactory processing, mainly at the level of inhibitory interneurons: PGCs and GCs. However, in response to an olfactory sensory cue, each odorant is associated with a specific topographic pattern of activity within the GL of the OB (Miyamichi et al. 2011; Vassar et al. 1994), thereby targeting and recording PGCs is more difficult given the heterogeneity of PGC activity in response to distinct

odorants. On the other hand, GCs constitute the most abundant inhibitory cell type in the OB (Parrish-Aungst et al. 2007) and their central organization and homogeneity facilitates in vivo calcium dynamic recording.

The second reason is that GCs shape the activity of M/TCs. Their dendrites expand through the IPL, MCL, and EPL to connect with multiple bulbar cell types to fine-tune the olfactory message by adapting the olfactory perception to metabolic needs. Reciprocal dendrodendritic synaptic connections between glutamatergic M/TCs and GABAergic GCs allow inhibitory feedback input on the bulbar principal output neurons, the M/TCs (Wellis and Kauer 1993; Rall et al. 1966; Jahr and Nicoll 1982), ultimately resulting in the modulation of the olfactory message conveyed by M/TC axons to the piriform cortex and higher-order cortical structures (Nagayama, Homma, and Imamura 2014).

Therefore, GC activity was recorded in the OB of fed vs. fasted AgRP-control and AgRP-ablated male mice during the first sniff of Chow (food-related odor) and C+ (novel odor). As expected, the GC activity increased in response to every presented odor (Figure IV.6B-I), indicating that the detection of olfactory sensory cues induces an increased GABAergic activity within the OB. This result aligns with previous fiber photometry studies demonstrating that odor-evoked responses in the GCL are excitatory (Sun et al. 2019; Wang et al. 2020). As discussed in Chapter II of the introduction, M/TCs activation by the odorant should result in glutamate release at the level of dendrodendritic synapse with GCs. Glutamate subsequently triggers the depolarization (Ca^{2+} entry) of GC dendrites, inducing GABA release (Wellis and Kauer 1993; Rall et al. 1966; Jahr and Nicoll 1982). Under the hypothesis, the observed large GC stimulation would follow the M/TC stimulation and be shaped by cortical feedback. In response to chow diet odor, GC activation is higher in fasted than in fed condition, for both AgRP-control and AgRP-ablated mice. However, the results were completely different for the presentation of Carvone +, a novel odour with no hedonic component for the mouse. While the metabolic status does not impact GC activity in AgRP-ablated, AgRP-control mice displayed a significantly higher GC activity in response to the presentation of the novel odor C+ in fed condition compared to fasted condition. Intriguingly, as for behavior the impact of AgRP neurons is different for novel (unknown) versus food-related odorant.

The difference observed with the metabolic status aligns with multiple previous studies in which manipulating feeding-related hormonal receptors in the OB modulates olfactory activity, indicating the impact that metabolic hormones have on olfactory processing

(Kuczewski et al. 2014; Savigner et al. 2009; D. A. Fadool et al. 2000; Debra Ann Fadool, Tucker, and Pedarzani 2011; Apelbaum, Perrut, and Chaput 2005; Hardy et al. 2005; Pager 1978). Orexigenic hormones, such as ghrelin, tend to increase olfactory sensitivity, whereas anorexigenic hormones, such as leptin, most likely decrease olfactory sensitivity (Aimé et al. 2007; Cameron, Goldfield, and Doucet 2012; Hanci and Altun 2016; Tucker et al. 2012; A. K. Julliard et al. 2007).

Local inhibitory circuits are thought to shape olfactory information processing in the OB, but it remains unclear how inhibitory neuronal interactions translate into olfactory behavioral performance. The work of Abraham et al. (2010) demonstrates that inhibition of M/TCs via GCs contributes to fast and accurate discrimination of similar odors. However, in a go/no go task, decreased odor-evoked M/TC calcium activity in rodents was associated with reduced olfactory discrimination capacities in response to an i.p. administration of leptin (Sun et al. 2019). On the other hand, the work of Hardy et al. (2005); Apelbaum, Perrut, and Chaput (2005) showed that orexin-induced hyperpolarization of MCs was partly mediated through GABA_A receptors in the OB. And, since GCs express orexin receptors and are GABAergic cells, they were speculated to be involved in this hyperpolarization.

Hence, our data suggest that distinct networks are involved in the processing of food-related odorants. The results for novel unknown odorant processing lead to the hypothesis that hunger or an energy deficit state reduces GC activity, resulting in an increased odor-evoked M/TC neuronal activity, and ultimately a better olfactory performance in fasted control male mice, as in the olfactory behavioral test previously described. AgRP neurons would be involved in the impact of the transition between fed and fasted states at the level of the OB.

3. Limitations of AgRP-ablated mouse model

3.1. AgRP is NOT solely expressed in arcuate AgRP neurons

As described in chapter I of the introduction, extra-hypothalamic AgRP-expressing cells were recently identified in the brainstem, and more specifically in the hindbrain, at the level of the area postrema (AP), the adjacent subpostrema area (SubP), and the commissural nucleus of the solitary tract (cNTS) - collectively referred to as AgRP^{Hind} neurons. In a very recent study, (Bachor et al. 2024) reported that AgRP^{Hind} neurons drive feeding independently of hypothalamic AgRP neurons.

Other recent studies demonstrated the existence of peripheral AgRP-expressing cells, in the pituitary (Liu et al. 2023) and adrenal glands (Gupta et al. 2017), which are also involved in food intake regulation. Chemogenetic inhibition of AgRP-expressing cells of the pituitary gland causes weight loss (Liu et al. 2023). Meanwhile, short-term fasting leads to an increase in adrenal AgRP expression (Gupta et al. 2017). Regardless, we still ignore the mechanisms by which these cells may or may not impact energy homeostasis.

By injecting DT, subcutaneously (1st dose) and intraperitoneally (2nd dose) in AgRP^{DTR/+} mice, we most probably ablated all AgRP-expressing cells/neurons in the ARC but also in extra-hypothalamic locations. Therefore, we cannot exclude that the olfactory behavioral differences observed in AgRP-ablated mice are influenced by other brain regions containing AgRP neurons and are not a direct result of hypothalamic AgRP neuron ablation. However, to our knowledge, no connection between the hindbrain and olfactory structure has been documented in the literature. Since olfaction is dependent on stress, we cannot rule out that modification of the adrenal gland functions could impact olfaction through a modified level of circulating catecholamine (Bombail 2019).

3.2. The development of compensatory neuronal networks in response to AgRP neuronal ablation in neonates

First, network-based compensatory mechanisms develop after the ablation of AgRP neurons in neonates (Luquet et al. 2005). AgRP neurocircuitries are not fully formed in mice before day 21 after birth (Nilsson et al. 2005; Bouret, Draper, and Simerly 2004). Thus, neonatal ablation performed before this time point is unlikely to inhibit feeding, since projections arising from AgRP neurons have not yet reached upper feeding structures. However, we still ignore the compensatory neuronal circuits established in response to the early ablation of AgRP neurons.

Second, ablation of AgRP neurons by peripheral administration of DT in adult AgRP^{DTR} mice inhibits food intake, leading to starvation, life-threatening anorexia, and even death (Luquet et al. 2005; Gropp et al. 2005). The work of (Wu, Boyle, and Palmiter 2009) suggested that AgRP neuron ablation leads to the hyperactivation of PBN neurons that play a physiological role in suppressing feeding which ultimately results in severe starvation (Carter et al. 2013).

Therefore, to specifically target AgRP neurons and to avoid any reorganization that might occur during development in the AgRP-ablated mouse model, we decided to employ a chemogenetic approach by stereotaxically injecting a cre-dependent DREADD virus in the ARC of *AgRP-ires-cre* mice. Viral stereotaxic injections were extremely challenging since the ARC is a tiny brain region located at the bottom of the 3rd V and in close vicinity to the median eminence (Langlet et al. 2013; Norsted, Gömüç, and Meister 2008).

4. Stimulating AgRP neurons increases olfactory food-seeking behaviors and food intake while reducing granule cell activity in the OB of AgRP-hM3Dq mice

In order to transiently and exclusively activate arcuate AgRP neurons while avoiding the modulation of circulating hunger and satiety hormonal levels, we used chemogenetic approaches (DREADDs) in fed AgRP-hM3Dq male mice and we compared them to their control cohorts, fed AgRP-mcherry mice. We confirmed previous results showing that stimulation of arcuate AgRP neurons increased food intake in fed AgRP-hM3Dq mice (Krashes et al. 2013; Reed et al. 2023; Fu et al. 2019; Üner et al. 2019).

These mice were able to find the buried cookie in a significantly short time compared to their fed control cohorts, suggesting that CNO-induced activation of arcuate AgRP neurons results in a significantly reduced olfactory detection threshold and an enhanced olfactory food-seeking behavior. This is consistent with the difference between fed and fasted metabolic state (Horio and Liberles 2021; Mortreux et al. 2019). Then, even when circulating hormones correspond to a fed state, the mere activation of AgRP neurons is sufficient to change olfactory behavior from a fed-like to a fasting-like state. We did not test whether AgRP activation can change the blood level of circulating metabolites and nutrients, however, (Üner et al. 2019) have documented that activation of AgRP neurons did not affect glycaemia, a factor that could have modulated olfactory processing (A.-K. Julliard et al. 2017).

Next, we recorded the calcium dynamics of GC activity in response to odorants under CNO-induced AgRP neuronal activation. Here, and for the first time, we provide proof that active arcuate AgRP neurons reduce the activity of GABAergic GCs in the OB of AgRP-hM3Dq mice during the first sniff of different olfactory stimuli (food-related, novel, and social odors). This result aligns with what we've seen previously between fed and fasted AgRP-control male mice in terms of GC activity. In conclusion, we hypothesize that hunger-induced

activation of arcuate AgRP neurons enhances olfactory sensitivity while reducing odor-evoked GC activation in the OB.

Indeed, as previously described in Chapter II of the introduction, the OB receives numerous centrifugal projections. The GCL serves as a target for various glutamatergic and GABAergic inputs arising from cortical structures. Thus, the reduced-odor evoked GC activation in response to different olfactory stimuli under hypothalamic AgRP neuron activation potentially results from either a reduced glutamatergic or an increased GABAergic activation of GCs or even both.

5. Olfactory performance of HFD obese mice

HFD-induced obesity triggers broad dysregulation of hypothalamic hunger AgRP neurons. Indeed, (Beutler et al. 2020) reported that HFD (6 weeks) attenuates the response of AgRP neurons to an array of nutritionally-relevant stimuli including olfactory sensory cues, intragastric nutrients, cholecystokinin and ghrelin. These alterations are specific to dietary fat. In this context, our aim was to assess whether the effect of AgRP activity on the OB was impaired.

Our results show that after 8 weeks of HFD-induced obesity, obese C57/bl6 mice were still able to significantly detect and discriminate odors whether fed or fasted. However, the buried food seeking test showed that the olfactory detection threshold of satiated HFD mice was significantly higher (lower olfactory sensitivity) in comparison with chow mice, indicating that after 8 weeks of HFD, obese mice were slightly hyposmic when compared to control mice. It seems that 8 weeks of DIO was not sufficient to induce major impairment in the olfactory performance of mice.

However, fiber photometry recordings showed that, in response to a novel odor presentation, the modulation of GC activity in the OB of HFD mice between the fed and fasted conditions is blunted. This result appears to be similar to what we previously found in AgRP-ablated mice, eliciting an altered hypothalamic feeding circuit.

Interestingly, however, we found that CNO-induced activation of AgRP neurons in AgRP-hM3Dq-fed obese mice significantly increased food intake. Thus, when the responsiveness to ghrelin is bypassed, AgRP neurons in HFD-fed animals are still fully functional. Activation of AgRP neurons in HFD-fed mice increased olfactory sensitivity by

lowering the olfactory detection threshold, as observed in the buried food seeking test, and showed a massive blunting of GC odor-evoked activity.

6. Synthesis from the different models

Stimulation of AgRP neurons in AgRP-cre mice mimics a fasted state that corroborates with better odorant detection and that is associated with a decrease of inhibition at the level of GCs. This matches the differences observed between fed and fasted mice in AgRP-control mice of the AgRP-DTR model for the neutral odorant. However, our results diverged when food odorant was presented.

Even though GC recordings were not performed during the behavioral tests, we can see that a decrease in GC activity correlates with better olfactory acuity: our recordings showed a lower activity in both fed and fasted conditions for C+ in AgRP-activated mice, a lower activity during AgRP stimulation when mice are better at finding the cookie, as well as in lean and obese mice.

Finally, obesity is characterized by multiple effects throughout the brain including inflammatory processes that impact the olfactory system as well as the hypothalamus. More work would be needed to compare GC activity between lean and obese mice, however, we saw that animals fed with HFD tend to lose the metabolic regulation of the OB. Impairment of metabolic sensing by the arcuate nucleus would then translate into an impaired regulation at the level of the OB. Then, although AgRP neurons display low responsiveness to ghrelin in obese mice (Beutler et al. 2020), CNO-induced activation of AgRP neurons significantly reduces GC activity in response to distinct olfactory cues (food-related, novel and social odors). CNO-induced activation of AgRP neurons potentially rescues the altered GABAergic olfactory processing in the OB observed in obesity.

Perspectives

Our results demonstrate a functional connection between arcuate AgRP neurons and GCs of the OB. However, we still ignore the underlying neuronal network that anatomically connects these two neuronal populations. Retrograde tracing investigations found no direct connectivity between the ARC and the OB. Thus, we could not anatomically consider a monosynaptic pathway between these two structures.

One of the main perspectives of this project is seeking to understand the main source of reduced GC activity under AgRP neuron activation. As stated previously, this reduced activity of GABAergic GCs may be due to reduced glutamatergic or increased GABAergic inputs arriving at the level of GCL. We have two hypothetical circuits:

- Indirect projections (involving two to three synapses) from multiple hypothalamic nuclei, including the ARC, VMH, PVN, and DMH extending toward the OB have been observed (Schneider et al. 2020). Of a particular note, as described in chapter III of the introduction, the OB receives both direct and indirect projections from the LH, with some direct projections originating from distinct LH orexin neurons (Qi, Fadool, and Storace 2023; Peyron et al. 1998; Nambu et al. 1999; Gascuel et al. 2012; Schneider et al. 2020), that receive direct projections from AgRP neurons.
- AgRP neurons indirectly connect with the BF (Stutz et al. 2022) mainly via the LH (Cassidy et al. 2019). Most BF nuclei comprise GABAergic as well as glutamatergic projecting neurons (Gritti et al. 2006; Henny and Jones 2008; Agostinelli, Geerling, and Scammell 2019). Centrifugal inputs from the BF to the OB mainly originate from the HDB (Gielow and Zaborszky 2017; Li et al. 2018; Gracia-Llanes et al. 2010; Záborszky et al. 1986). Interestingly, GABAergic HDB-derived projections strongly innervate the GCL (Böhm, Brunert, and Rothermel 2020; Nunez-Parra et al. 2013; Sanz Diez, Najac, and De Saint Jan 2019). Recent studies showed that optogenetic activation of GABAergic HDB-derived fibers in the OB inhibits spontaneous and weak sensory activity while increasing odor-evoked responses (Böhm, Brunert, and Rothermel 2020). Thus, GABAergic HDB-derived fibers to the OB most likely modulate the OB output via disinhibition of principal cells. Interestingly, disrupting GCs inhibition impairs olfactory sensitivity and affects odor discrimination, indicating the important role of the inhibitory GABAergic afferents in olfactory processing (Nunez-Parra et al. 2013).

In addition, our work shows the impact of hypothalamic AgRP neuron activation on olfactory processing within the OB. It would be interesting to study the impact of AgRP neuron inhibition on GC activity. In my project, using chemogenetic approaches (DREADDs), I tried inhibiting AgRP neurons in fasted mice to assess its impact on olfactory performance. However, inhibiting feeding by solely deactivating AgRP neurons in fasted mice seemed practically hard to achieve. Food intake of fasted mice was not strongly reduced in response to a CNO-induced deactivation of hypothalamic AgRP neurons.

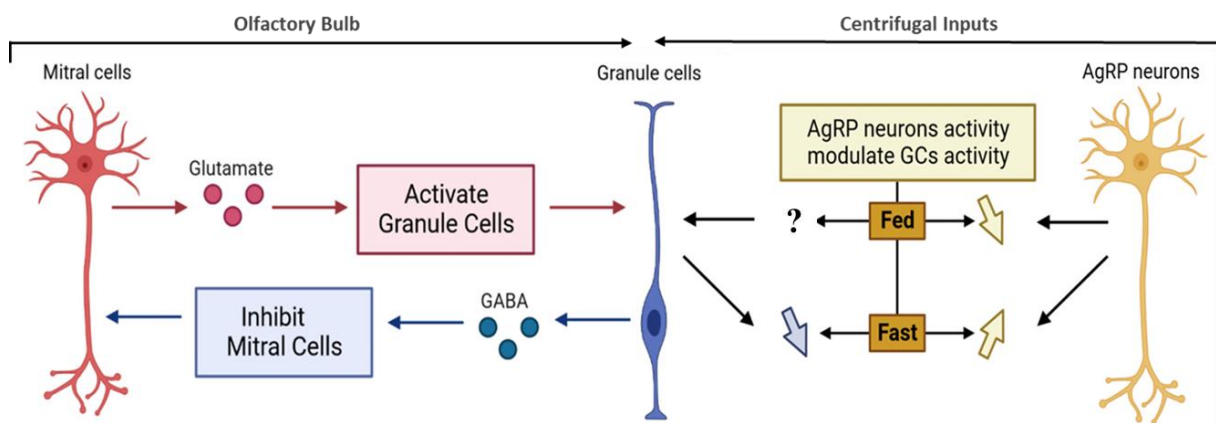


Figure V.1. Hypothesis on the impact of indirect centrifugal input from AgRP neurons on the OB circuit. Both intra-bulbar inputs and centrifugal inputs modulate GC activity. By releasing glutamate, MCs activate GCs. In a quick feedback loop, active GCs release GABA, inhibiting the main OB output neurons, the MCs. On the other hand, GCs also receive numerous direct and indirect centrifugal inputs modulating their activity: AgRP neuron activation was found to reduce the odor-evoked activation of GCs in the OB. Still, a question remains unanswered, under a fed state, how would the reduced AgRP neuronal activity impact GC activity and ultimately odor processing within the OB?

Furthermore, one of the most prominent perspectives is to record MCs activity in response to the modulation of AgRP neuron activity. In the present study, we assessed the modulation of GC activity in response to hypothalamic AgRP neuron activation but it would be of a great interest to study the impact of AgRP neuron activity on MC activity given that MCs represent the main output olfactory neurons of the OB. A previous study reported that Thy1 promoter is specifically expressed in M/TCs of the OB (Arenkiel et al. 2007). By injecting the AAV-DIO-GCaMP6s virus and implanting an optic fiber into the OB of Thy1-cre mice, we would be able to record the odor-evoked response of MCs (Sun et al. 2019) in awake fed vs. fasted mice.

Finally, although modulating metabolic hormonal receptors at the level of the OB modulates olfaction indicating an impact of satiety and hunger hormones on olfaction, it would be interesting to study the direct impact of hormones and nutrients on the activity of the OB. Most studies pointing out hormonal modifications of olfactory performance are based on peripheral administration of hormones with no clear evidence of increased hormonal access to the OB.

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ANNEXES

Annex 1 : Statistics

Statistics of Figure 1								
Figure panels	n	Statistical analysis	F-value	p-value	multiple comparisons	adjusted p value		
Fig1A	Body weight	AgRP-ctl n=14 AgRP-abl n=23	Unpaired t-test		p=0,7448			
Fig1B	Food intake / BW males	AgRP-ctl n=14 AgRP-abl n=23	2-Way ANOVA	Interaction	F (1, 35) = 27,42	p<0.0001	ctl sal vs. ctl ghr abl sal vs. abl ghr ctl sal vs. abl sal ctl ghr vs. abl ghr	<0,0001 0,7972 0,0057 <0,0001
				Treatment	F (1, 35) = 24,18	p<0.0001		
				Group	F (1, 35) = 28,40	p<0.0001		
Fig1C	fat mass, males	AgRP-ctl n=7 AgRP-abl n=13	2-Way ANOVA	Interaction	F (9, 162) = 1,988	p=0,0438	AgRP-ctr - AgRP-abl	8 0,0163
				time	F (1,863, 33,54) = 3,668	p=0,0391	9 0,0514	
				Group	F (1, 18) = 6,989	p=0,0165	10 0,464	
	lean mass, males	AgRP-ctl n=7 AgRP-abl n=13	2-Way ANOVA	Interaction	F (9, 162) = 3,637	p=0,0004	11 0,163	
				time	F (1,363, 24,53) = 8,402	p=0,0042	12 0,0238	
				Group	F (1, 18) = 9,761	p=0,0059	14 0,0823	
						15 0,0083		
						17 0,145		
						19 0,9712		
						20 >0,9999		
Fig1D	Body weight	AgRP-ctl n=11 AgRP-abl n=12	Unpaired t-test		p=0,0226			
Fig1E	Food intake / BW females	AgRP-ctl n=11 AgRP-abl n=12	2-Way ANOVA	Interaction	F (1, 21) = 5,974	P=0,0234	ctl sal vs. ctl ghr abl sal vs. abl ghr ctl sal vs. abl sal ctl ghr vs. abl ghr	0,0002 0,2733 0,0038 <0,0001
				Treatment	F (1, 21) = 16,00	P=0,0006		
				Group	F (1, 21) = 40,70	F (1, 21) = 40,70		
Fig1G	Western blot	AgRP-ctl n=3 AgRP-abl n=3	Unpaired t-test		p=0,0053			

Statistics of Figure 3							
Figure panels	n	Statistical analysis	F-value	p-value	multiple comparisons	adjusted p value	
Fig3B	MO Sniffing duration	AgRP-ctl n=18 AgRP-abl n=19	Paired t-test	ctl Fasted vs. ctl Fed	0,1032		
				abl Fasted vs. abl Fed	0,8728		
			Unpaired t-test	ctl Fasted vs. abl Fasted	0,8041		
				ctl Fed vs. abl Fed	0,2498		
Fig3C	Hab/deshab males	AgRP-ctl fasted n=18 AgRP-ctl fed n=18	2-Way ANOVA	Interaction	F (8, 272) = 3,721	P=0,0004	A1 AgRP-ctl Fasted (n=18) vs. AgRP-ctl Fed (n=18) <0,0001
				Stimulus	F (5,753, 195,6) = 8,018	p<0.0001	A2 AgRP-ctl Fasted (n=18) vs. AgRP-ctl Fed (n=18) 0,0253
				Group	F (1, 34) = 10,99	P=0,0022	A3AgRP-ctl Fasted (n=18) vs. AgRP-ctl Fed (n=18) 0,0351
							B1 AgRP-ctl Fasted (n=18) vs. AgRP-ctl Fed (n=18) 0,0033
							B2 AgRP-ctl Fasted (n=18) vs. AgRP-ctl Fed (n=18) 0,2324
							B3 AgRP-ctl Fasted (n=18) vs. AgRP-ctl Fed (n=18) 0,0655
Fig3D	MO3-A1	AgRP-ctl fasted n=18 AgRP-ctl fed n=18	Paired t-test	A1 fasted MO3 fasted	p<0,0001		
				A1 fed MO3 fed	p=0,0275		
Fig3E	A3-B1	AgRP-ctl fasted n=18 AgRP-ctl fed n=18	Paired t-test	B1 fasted A3 fasted	p=0,0042		
				B1 fed A3 fed	p=0,0205		
Fig3F	MO3-A1	AgRP-ctl fasted n=18 AgRP-ctl fed n=18	Paired t-test		p<0,0001		
Fig3G	A3-B1	AgRP-ctl fasted n=18 AgRP-ctl fed n=18	Paired t-test		p=0,0039		
Fig3H	Hab/deshab males	AgRP-abl fasted n=19 AgRP-abl fed n=19	2-Way ANOVA	Interaction	F (8, 288) = 0,4656	P=0,8798	A1 AgRP-ctl Fasted (n=19) vs. AgRP-ctl Fed (n=19) 0,8376
				Stimulus	F (6,146, 221,3) = 15,48	p<0.0001	A2 AgRP-ctl Fasted (n=19) vs. AgRP-ctl Fed (n=19) 0,2946
				Group	F (1, 36) = 1,961	P=0,1699	A3 AgRP-ctl Fasted (n=19) vs. AgRP-ctl Fed (n=19) 0,8213
							B1 AgRP-ctl Fasted (n=19) vs. AgRP-ctl Fed (n=19) 0,2049
							B2 AgRP-ctl Fasted (n=19) vs. AgRP-ctl Fed (n=19) 0,4821
							B3 AgRP-ctl Fasted (n=19) vs. AgRP-ctl Fed (n=19) 0,3754
Fig3I	MO3-A1	AgRP-abl fasted n=19 AgRP-abl fed n=19	Paired t-test	A1 fasted MO3 fasted	p<0,0001		
				A1 fed MO3 fed	p<0,0001		
Fig3J	A3-B1	AgRP-abl fasted n=19 AgRP-abl fed n=19	Paired t-test	B1 fasted A3 fasted	p=0,0002		
				B1 fed A3 fed	p=0,0010		
Fig3K	MO3-A1	AgRP-abl fasted n=19 AgRP-abl fed n=19	Paired t-test		p=0,8024		
Fig3L	A3-B1	AgRP-abl fasted n=19 AgRP-abl fed n=19	Paired t-test		p=0,2289		

Statistics of Figure 4								
Figure panels		n	Statistical analysis		F-value	p-value	multiple comparisons	adjusted p value
Fig4A	MO Sniffing duration	AgRP-ctl n=16 AgRP-abl n=20	Paired t-test		ctl Fasted vs. ctl Fed	p=0,2582		
			Unpaired t-test		abl Fasted vs. abl Fed	p=0,0783		
					ctl Fasted vs. abl Fasted	p=0,4054		
					ctl Fed vs. abl Fed	p=0,3118		
Fig4B	Hab/deshab females	AgRP-ctl fasted n=16 AgRP-ctl fed n=16	2-Way ANOVA	Interaction	F (8, 237) = 2,914	p=0,0040	A1 AgRP-ctl Fasted (n=16) vs. AgRP-ctl Fed (n=16)	0,2916
				Stimulus	F (6,018, 178,3) = 3,639	p=0,002	A2 AgRP-ctl Fasted (n=16) vs. AgRP-ctl Fed (n=16)	0,0329
				Group	F (1, 30) = 10,97	p=0,0024	A3AgRP-ctl Fasted (n=16) vs. AgRP-ctl Fed (n=16)	0,0471
							B1 AgRP-ctl Fasted (n=16) vs. AgRP-ctl Fed (n=16)	0,0181
							B2 AgRP-ctl Fasted (n=16) vs. AgRP-ctl Fed (n=16)	0,0011
							B3 AgRP-ctl Fasted (n=16) vs. AgRP-ctl Fed (n=16)	0,2828
Fig4C	MO3-A1	AgRP-ctl fasted n=16 AgRP-ctl fed n=16	Paired t-test		A1 fasted MO3 fasted	p=0,0128		
					A1 fed MO3 fed	p=0,0563		
Fig4D	A3-B1	AgRP-ctl fasted n=16 AgRP-ctl fed n=16	Paired t-test		B1 fasted A3 fasted	p=0,0531		
					B1 fed A3 fed	p=0,3392		
Fig4E	MO3-A1	AgRP-ctl fasted n=16 AgRP-ctl fed n=16	Paired t-test		/		0,2044	
Fig4F	A3-B1	AgRP-ctl fasted n=16 AgRP-ctl fed n=16	Paired t-test		/		0,0397	
Fig4G	Hab/deshab males	AgRP-abl fasted n=20 AgRP-abl fed n=20	2-Way ANOVA	Interaction	F (8, 304) = 1,785	P=0,0794	A1 AgRP-ctl Fasted (n=20) vs. AgRP-ctl Fed (n=20)	0,0579
				Stimulus	F (6,548, 248,8) = 8,625	P<0,0001	A2 AgRP-ctl Fasted (n=20) vs. AgRP-ctl Fed (n=20)	0,5687
				Group	F (1, 38) = 0,9880	P=0,3265	A3 AgRP-ctl Fasted (n=20) vs. AgRP-ctl Fed (n=20)	0,502
							B1 AgRP-ctl Fasted (n=20) vs. AgRP-ctl Fed (n=20)	0,0808
							B2 AgRP-ctl Fasted (n=20) vs. AgRP-ctl Fed (n=20)	0,2317
							B3 AgRP-ctl Fasted (n=20) vs. AgRP-ctl Fed (n=20)	0,2991
Fig4H	MO3-A1	AgRP-abl fasted n=20 AgRP-abl fed n=20	Paired t-test		A1 fasted MO3 fasted	p=0,0074		
					A1 fed MO3 fed	p<0,0001		
Fig4I	A3-B1	AgRP-abl fasted n=20 AgRP-abl fed n=20	Paired t-test		B1 fasted A3 fasted	p=0,3882		
					B1 fed A3 fed	p=0,0004		
Fig4J	MO3-A1	AgRP-abl fasted n=20 AgRP-abl fed n=20	Paired t-test		/		0,043	
Fig4K	A3-B1	AgRP-abl fasted n=20 AgRP-abl fed n=20	Paired t-test		/		0,0732	

Statistics of Figure 5						
Figure panels	n	Statistical analysis	F-value	p-value	multiple comparisons	adjusted p value
Fig5B	Buried food test Fed AgRP-ctl n=10 AgRP-abl n=12	Unpaired t-test	/	p=0,3628		
Fig5C	Buried food test Fasted AgRP-ctl n=6 AgRP-abl n=9	Unpaired t-test	/	p=0,1270		
Fig5E	Total Food intake AgRP-ctl n=7	Repeated measures one-way ANOVA		p<0,0001	Tukey's multiple comparisons test D1 vs. D2 D1 vs. D3 D1 vs. D4 D1 vs. D5 D2 vs. D3 D2 vs. D4 D2 vs. D5 D3 vs. D4 D3 vs. D5 D4 vs. D5	0,0003 0,0004 0,0016 <0,0001 0,01 0,0352 0,0043 0,8469 0,0558 0,0887
Fig5F	Total Food intake AgRP-abl n=13	Repeated measures one-way ANOVA		p<0,0001	Tukey's multiple comparisons test D1 vs. D2 D1 vs. D3 D1 vs. D4 D1 vs. D5 D2 vs. D3 D2 vs. D4 D2 vs. D5 D3 vs. D4 D3 vs. D5 D4 vs. D5	<0,0001 <0,0001 <0,0001 <0,0001 0,2411 0,0001 0,001 0,0034 0,0015 0,4136
Fig5G	Total Food intake AgRP-ctl n=12	Repeated measures one-way ANOVA		p<0,0001	Tukey's multiple comparisons test D1 vs. D2 D1 vs. D3 D1 vs. D4 D1 vs. D5 D2 vs. D3 D2 vs. D4 D2 vs. D5 D3 vs. D4 D3 vs. D5 D4 vs. D5	0,0033 <0,0001 <0,0001 <0,0001 0,0001 <0,0001 0,0039 0,1692 0,1656 0,938
Fig5I	Total Food intake AgRP-abl n=12	Repeated measures one-way ANOVA		p<0,0001	Tukey's multiple comparisons test D1 vs. D2 D1 vs. D3 D1 vs. D4 D1 vs. D5 D2 vs. D3 D2 vs. D4 D2 vs. D5 D3 vs. D4 D3 vs. D5 D4 vs. D5	0,0003 <0,0001 <0,0001 <0,0001 0,2588 0,013 0,0044 0,0132 0,0022 0,5017
Fig5I	Total FI/BW AgRP-ctl n=14 AgRP-abl n=23	Unpaired t-test	/	p=0,6601		
Fig5J	Aversion ratio AgRP-ctl n=14 AgRP-abl n=23		/			
Fig5K	Total FI/BW AgRP-ctl n=14 AgRP-abl n=23	Unpaired t-test	/	p=0,32338		
Fig5L	Aversion ratio AgRP-ctl n=14 AgRP-abl n=23		/			

Statistics of Figure 6							
Figure panels		n	Statistical analysis	F-value	p-value	multiple comparisons	adjusted p value
Fig6C	AUC control chow fasted/fed	ctrl fast n=10 ctrl fed n=10	Paired t-test	/	p=0,0452		
Fig6E	AUC ablated chow fasted/fed	ablated fasted n=7 ablated fed n=7	Paired t-test	/	p=0,0101		
Fig6G	AUC control C+ fasted/fed	ctrl fast n=6 ctrl fed n=6	Paired t-test	/	p=0,0215		
Fig6I	AUC ablated C+ fasted/fed	ablated fasted n=6 ablated fed n=6	Paired t-test	/	p=0,7533		

Statistics of Figure 7								
Figure panels		n	Statistical analysis		F-value	p-value	multiple comparisons	adjusted p value
Fig7C	Food intake / BW females	mCherry n=13 Dq n=9	2-Way ANOVA	Interaction	F (1, 17) = 84,89	P<0,0001	sal mcherry vs. Dq	0,0196
				Treatment	F (1, 17) = 79,34		cno mcherry vs. Dq	<0,0001
				Group	F (1, 17) = 53,60		mcherry sal vs. cno	0,8163
Fig7D	Food buried test	mCherry n=13 Dq n=9	Unpaired t-test			p<0,0001	Dq sal vs. cno	<0,0001
Fig7F	Ca2+ chow	mCherry n=13 Dq n=9	Unpaired t-test			p=0,0287		
Fig7H	Ca2+ cumin	mCherry n=13 Dq n=9	Unpaired t-test			p=0,0223		
Fig7J	Ca2+ urine	mCherry n=13 Dq n=9	Unpaired t-test			p=0,0026		

Statistics of Figure 8								
Figure panels		n	Statistical analysis		F-value	p-value	multiple comparisons	adjusted p value
Fig8A	Body weight	chow n=8 HFD n=8	Paired t-test			p<0,0001		
Fig8B	% fat mass	chow n=8 HFD n=8	Paired t-test			p<0,0001		
Fig8C	Buried food test	chow n=8 HFD n=8	2-Way ANOVA	Interaction	F (1, 23) = 0,8340	P=0,3706	chow Fasted vs. chow Fed chow Fasted vs. HFD Fasted chow Fed vs. HFD Fed HFD Fasted vs. HFD Fed	0,0429 0,1075 0,011 0,0017
				metabolic state	F (1, 23) = 6,627	P=0,0170		
				Group	F (1, 23) = 16,03	P=0,0006		
Fig8D	MO Sniffing duration	chow n=8 HFD n=8	Paired t-test		chow Fasted vs. chow Fed chow Fasted vs. HFD Fasted	0,9821 0,771		
			Unpaired t-test		chow Fed vs. HFD Fed HFD Fasted vs. HFD Fed	0,9375 0,6662		
Fig8E	Hab/deshab	chow n=8	2-Way ANOVA	Interaction	F (8, 104) = 1,781	P=0,0890		
				Stimulus	F (4, 855, 63, 12) = 3,767	P=0,0051		
				Group	F (1, 13) = 2,317	P=0,1519		
Fig8F	MO3-A1	chow n=8	Paired t-test		A1 fasted MO3 fasted A1 fed MO3 fed	0,0045 0,4699		
Fig8G	A3-B1	chow n=8	Paired t-test		B1 fasted A3 fasted B1 fed A3 fed	0,0412 0,3929		
Fig8H	MO3-A1	chow n=8	Paired t-test			0,0349		
Fig8I	A3-B1	chow n=8	Paired t-test			0,056		
Fig8J	Hab/deshab	HFD n=8	2-Way ANOVA	Interaction	F (8, 112) = 0,9823	P=0,4536		
				Stimulus	F (3, 765, 52, 72) = 3,390	P=0,0170		
				Group	F (1, 14) = 0,4416	P=0,5171		
Fig8K	MO3-A1	HFD n=8	Paired t-test		A1 fasted MO3 fasted A1 fed MO3 fed	0,0093 0,1678		
Fig8L	A3-B1	HFD n=8	Paired t-test		B1 fasted A3 fasted B1 fed A3 fed	0,0025 0,0001		
Fig8M	MO3-A1	HFD n=8	Paired t-test			0,1851		
Fig8N	A3-B1	HFD n=8	Paired t-test			0,9941		

Statistics of Figure 9					
	Figure panels	n	Statistical analysis	F-value	p-value
Fig9B	AUC chow	chow fast n=6 chow fed n=6	Paired t-test		p=0,0014
Fig9D	AUC HFS	HFD fasted n=6 HFD fed n=6	Paired t-test		p=0,3780

Statistics of Figure 10							
Figure panels		n	Statistical analysis	F-value	p-value	multiple comparisons	adjusted p value
Fig10A	Body weight	chow n=8 HFD n=8	Unpaired t-test		p<0,0001		
	% fat mass	chow n=8 HFD n=8	Unpaired t-test		p<0,0001		
Fig10B	Food intake / BW	mCherry n=13 Dq n=9	Paired t-test		p=0,0002		
Fig10C	Food buried test	mCherry n=13 Dq n=9	Paired t-test		p=0,0159		
Fig10E	Ca2+ chow	mCherry n=13 Dq n=9	Paired t-test		p=0,0195		
Fig10G	Ca2+ HFD	mCherry n=13 Dq n=9	Paired t-test		p=0,0195		
Fig10I	Ca2+ C+	mCherry n=13 Dq n=9	Paired t-test		0,0534		
Fig10K	Ca2+ urine	mCherry n=13 Dq n=9	Paired t-test		0,7556		

Annex 2

COMMUNICATIONS

1. **Faour M.**, Castel J., Luquet S., Martin C. Role of the Hypothalamus in Odor Processing. **Society for the Study of Ingestive Behavior (SSIB)**, Portland, Oregon, USA, July 2023. Poster presentation.
2. **Faour M.**, Castel J., Luquet S., Martin C. Role of the Hypothalamus in Odor Processing. **Journée scientifique 2023– UFR SDV**, Université Paris Cité, Paris, France, July 2023. Oral Presentation.
3. **Faour M.**, Castel J., Luquet S., Martin C. Role of the Hypothalamus in Odor Processing. **Kickoff meeting of the new Institute for Neuroscience and Cognition (INC)**, Hôpital Necker, Paris, France, June 2023. Poster presentation.
4. **Faour M.**, Castel J., Luquet S., Martin C. Role of the Hypothalamus in Odor Processing. **SNE (Société de Neuroendocrinologie) – Young Researchers Webinar**, online, Paris, France, June 2023. Oral Presentation.
5. **Faour M.**, Castel J., Luquet S., Martin C. Role of the Hypothalamus in Odor Processing. **NeuroFrance 2023**, Lyon, France, May 2023. Poster communication.
6. **Faour M.**, Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **Le Vendredi 13 de l'INC (Institut Neurosciences Cognition)**, Paris, France, January 2023. Three minutes' oral communication.
7. **Faour M.**, Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **Scientific Meeting Graduate School METOD**, Paris, France, December 2022. Oral communication.
8. **Faour M.**, Martin C. Food Intake and Olfaction. **Fête des sciences 2022**, BFA, Paris, France, October 2022. Oral communication.
9. **Faour M.**, Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **FENS Forum 2022 - Federation of European Neuroscience Societies**, Paris, France, July 2022. Poster communication.

10. Faour M., Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **Journée scientifique 2022 – UFR Sciences du Vivant**, Université Paris Cité, France, July 2022. Poster communication. *Award: Best Poster Presentation.*

11. Faour M., Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **Journée Scientifique BFA – Ma thèse en 3 minutes**, Université Paris Cité, France, June 2022. Three minutes' oral communication. *Award: Best 3 Minutes Oral Presentation (second place).*

12. Faour M., Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **UChicago Collaborators Meeting, L. Kay Lab (CNRS-UChicago)**, Chicago, USA, May 2022. Oral communication and 2 weeks' collaboration program at Leslie Kay's Lab (University of Chicago).

13. Faour M., Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **44th annual meeting - Association for Chemoreception Sciences - AChemS 2022**, Bonita Springs, Florida, USA, April 2022. Poster communication.

14. Faour M., Etner C. Obesity and Olfaction. **La Semaine du Cerveau 2022**, Lycée Victor Duruy, Paris, France, March 2022. Oral communication.

15. Faour M., Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **Forum de l'ED BioSPC**, Paxton Resort, France, October 2021. Poster communication. *Award: Best Poster Presentation (second place).*

16. Faour M., Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **Joint Congress of Neuroendocrinology: British Society of Neuroendocrinology & Société de Neuroendocrinologie (BSN-SNE)**, online congress, September 2021. E-Poster communication.

17. Faour M., Castel J., Luquet S., Martin C. Obesity and Neural Networks Involved in Olfaction – Food Intake Interactions: Role of the Hypothalamus in Odor Processing. **NeuroFrance Meeting 2021**, online meeting, May 2021. E-Poster communication.

18. Faour M., Li H., Martin C., Kay L. Role of the Trigeminal System in Odor Perception. **My thesis in 180 secs, CNRS-UChicago Partnership Online Event**, online event, April 2021. Three minutes' oral communication.

**** These international meetings and conferences allowed very enriching discussions with the most prominent scientists in the field and opened great opportunities for new collaborations.*

Annex 3

PUBLICATIONS

Faour, M., Magnan, C., Gurden, H., Martin, C., 2022. Olfaction in the context of obesity and diabetes: Insights from animal models to humans. Neuropharmacology 206, 108923. <https://doi.org/10.1016/j.neuropharm.2021.108923>

Vivot, K., Meszaros, G., Pangou, E., Zhang, Z., Qu, M., Erbs, E., Yeghiazaryan, G., Quiñones, M., Grandgirard, E., Schneider, A., Clauss-Creusot, E., Charlet, A., **Faour, M.**, Martin, C., Berditchevski, F., Sumara, I., Luquet, S., Kloppenburg, P., Nogueiras, R., Ricci, R., 2023. CaMK1D signalling in AgRP neurons promotes ghrelin-mediated food intake. Nat Metab. <https://doi.org/10.1038/s42255-023-00814-x>

Others Activities:

- **CBEA** (Comité du Bien Etre Animal) **member** at IJM (Institut Jacques Monod), France.
- **Animal Facility Mission** 2021-2023 (2 years), Université Paris Cité, Paris, France.