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THE IMPACT OF CALORIC AND NON-CALORIC SWEETENER CONSUMPTION ON THE BEHAVIORAL, HORMONAL AND BRAIN RESPONSESTO FOOD

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UNIL | Université de Lausanne

Faculté de biologie
et de médecine

Département de Physiologie

**THE IMPACT OF CALORIC AND NON-CALORIC SWEETENER CONSUMPTION ON
THE BEHAVIORAL, HORMONAL AND BRAIN RESPONSES TO FOOD**

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

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**The impact of caloric and non-caloric sweetener consumption
on the behavioral, hormonal and brain responses to food**

Lausanne, le 17 octobre 2018

pour le Doyen
de la Faculté de biologie et de médecine


Prof. Murielle Bochud

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ABSTRACT (ENGLISH)

Sight is a primary channel conveying information about food, in turn influencing appetite control via homeostatic, hedonic and cognitive factors. Brain responses to visual food cues have been increasingly studied in the past decades. However, the influence of specific dietary factors such as caloric (sugar) and non-caloric sweetener (NNS) consumption on subsequent visual responses to food remains poorly understood. Yet, both sweeteners have been associated to long-term weight gain. The thesis at hand aims at a more integrative view to understand the impact of sugar and NNS consumption on visual food perception and intake behavior, by combining measures of behavioral, physiological and brain responses towards food.

The first exploratory project (study A) investigated changes in behavioral and brain responses to food viewing via a 3-month replacement of sugar-sweetened beverages with NNS-sweetened beverages. We showed intervention-induced modulations in neural activity in response to high-fat, sweet food viewing that were mostly apparent in dorsal prefrontal and precentral cortices, i.e. brain areas associated with inhibitory control and attention. The decrease in activity within the dorsal prefrontal cortex was inversely correlated with changes in body weight, i.e. participants who failed to lose weight also showed decreased activity to palatable food cues in brain areas that have been related to food intake control.

The second project (study B) investigated the acute effects of sucrose- and NNS-beverage consumption, as compared to water, on the subsequent brain responses to food viewing and later spontaneous food intake at an *ad libitum* buffet. Sucrose consumption elicited a differential pattern of neural activity to food viewing as compared to water, and a subsequent decrease in spontaneous food intake. NNS consumption, on the other hand, did not affect food intake, but modified post-prandial brain responses to food viewing, most pronounced in prefrontal areas and the insula, i.e. brain regions that have been associated with food intake control and nutrient-flavor conditioning.

Altogether, the thesis at hand provides insights on the impact of caloric and non-caloric sweetener consumption on the visual perception of tempting food cues. This is of great relevance in our modern environment where visual cues are ubiquitous and guide consumption behavior in daily life. Detailed mechanisms as to how NNS might impact behavior when repeatedly consumed yet need to be investigated in more detail in the future, in particular to disentangle effects driven by NNS-containing foods and beverages as such, as opposed to individuals' expectations related to the consumption of such non-caloric products.

RESUME (FRENCH)

La vision est utilisée comme principal vecteur d'informations lorsqu'un individu est confronté à la nourriture, influençant de ce fait le contrôle de l'appétit par des facteurs homéostatiques, hédoniques et cognitifs. Les réponses cérébrales lors de la perception visuelle de nourriture ont été fortement étudiées dans les dernières décennies. Cependant, l'influence de facteurs alimentaires spécifiques tels que la consommation d'agents sucrants caloriques (les sucres) et non-caloriques (les édulcorants) sur les réponses visuelles ultérieures reste encore peu claire. Les sucres et édulcorants ont pourtant été associés à une prise de poids corporel sur le long terme. Cette thèse a pour but de mieux comprendre l'impact de la consommation de sucres et d'édulcorants sur la perception visuelle de nourriture et sur le comportement alimentaire, en combinant des mesures comportementales, physiologiques et cérébrales.

Le premier projet (étude A) a exploré les changements dans les réponses cérébrales et comportementales à la vision de nourriture induits par un remplacement de la consommation de boissons sucrées par leurs équivalents édulcorés. A la suite de trois mois d'intervention, nous avons mis en évidence des modulations de l'activité neuronale lors de la vision d'aliments sucrés et riches en gras dans des aires cérébrales préfrontales dorsales et précentrales, associées au contrôle inhibiteur et à l'attention. Une diminution d'activité dans l'aire préfrontale dorsale était inversement corrélée au changement de poids corporel, c'est-à-dire que les participants qui n'ont pas perdu de poids ont aussi montré les plus grandes baisses d'activités dans cette aire cérébrale liée au contrôle inhibiteur de la prise alimentaire.

Le deuxième projet (étude B) a étudié les effets aigus d'une consommation de boissons sucrées ou édulcorées, en comparaison à l'eau, sur les réponses cérébrales subséquentes à la vision de nourriture, ainsi que sur le comportement alimentaire lors d'un buffet *ad libitum*. La consommation de sucre, en comparaison à l'eau, a modifié l'activité cérébrale à la vue de nourriture. Ceci était associé à une moindre prise alimentaire lors du buffet. En revanche, la consommation d'édulcorants n'a pas affecté le comportement alimentaire, mais a modifié les réponses cérébrales postprandiales en particulier dans les aires préfrontales ainsi que dans l'insula, des régions associées aux habilités de contrôle de la prise alimentaire et au conditionnement goût-nutriments.

Ensemble, les études réalisées dans le cadre de cette thèse ont fourni des indications sur l'impact d'une consommation de sucres et d'édulcorants sur la perception visuelle de nourriture appétissante. Ceci

est particulièrement important dans notre environnement alimentaire moderne, dans lequel les stimuli visuels de nourriture sont omniprésents et guident notre comportement alimentaire quotidien. Les mécanismes d'action des édulcorants sur notre comportement lorsqu'ils sont consommés de manière répétée restent cependant à étudier de manière plus détaillée, en particulier dans le but de distinguer les effets des édulcorants eux-mêmes des attentes individuelles liées à la consommation de ces produits.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	V
ABSTRACT (ENGLISH)	VII
RESUME (FRENCH)	IX
TABLE OF CONTENTS	XII
LIST OF FIGURES	XIV
LIST OF ABBREVIATIONS	XV
1 GENERAL INTRODUCTION	1
1.1 Food intake regulation and the obesogenic environment.....	1
1.2 Visual cues for prospective food choices	3
1.3 Appetite control, food intake behavior and the brain	4
1.3.1 Central food intake regulation in healthy humans.....	4
1.3.2 Details on key brain areas involved in the regulation of food intake	6
1.3.2.1 <i>Hypothalamus</i>	6
1.3.2.2 <i>Basal ganglia</i>	7
1.3.2.3 <i>Amygdala</i>	7
1.3.2.4 <i>Orbitofrontal cortex</i>	8
1.3.2.5 <i>Anterior cingulate cortex & caudate head</i>	8
1.3.2.6 <i>Dorsal (lateral) prefrontal cortex</i>	9
1.3.2.7 <i>Ventro-lateral prefrontal cortex</i>	9
1.3.2.8 <i>Lateral parietal cortex</i>	10
1.3.2.9 <i>Insula</i>	10
1.3.2.10 <i>Temporal cortices</i>	11
1.3.3 The interplay of gut and brain to modulate responses to foods	11
1.3.4 Central food intake regulation and body weight management.....	13
1.4 Sweetened beverages, food intake and the brain	15
1.4.1 Sugar-sweetened beverage consumption and body weight management	15
1.4.2 Brain responses to sugar and sugar-sweetened beverage intake	16
1.4.3 Non-nutritive sweeteners as substitutes for caloric sweeteners.....	18
1.5 Aim of the thesis at hand	20

2	METHODOLOGY	21
2.1	Image database and online behavioral tasks	21
2.2	EEG methodology: assessment of brain responses to food	23
2.2.1	From EEG recordings to local and global measures of the electric field.....	23
2.2.2	Global Field Power.....	25
2.2.3	Estimation of neural sources	26
2.3	Buffet methodology: assessment of spontaneous food intake	28
3	RESULTS	30
3.1	Study A – ‘BOISSON’	30
3.2	Study B – ‘SUGART’	30
4	GENERAL DISCUSSION	31
4.1	Result summaries of studies A and B	31
4.2	Key areas potentially impacted by longer-term NNS consumption.....	33
4.3	Impact of NNS consumption on ‘bottom-up’ vs. ‘top-down’ mechanisms of food intake regulation – Perspectives	36
4.3.1	‘Bottom-up’ modulations of responses to food by NNS	37
4.3.2	‘Top-down’ modulations of responses to food by NNS	39
4.3.3	Combined future study perspectives	42
4.4	Take-home message.....	43
5	SCIENTIFIC CONTRIBUTIONS AND PARALLEL ACTIVITIES	45
6	REFERENCES	47
7	APPENDICES	A
7.1	Published article for study A – ‘BOISSON’	A
7.2	Published article for study B – ‘SUGART’.....	K

LIST OF FIGURES

Figure 1: Schematic representation of energy balance and factors of influence	1
Figure 2: Brain networks and details on areas involved in the central regulation of food intake upon exposure to a pre-ingestive food cue.....	5
Figure 3: Trial design for the online behavioral tasks	22
Figure 4: Electroencephalographic (EEG) data acquisition and electrical neuroimaging approach	24
Figure 5: Method development for the computation of matrices representing the relative (%) change in neural activity from a 'pre' state to a 'post' state.....	27
Figure 6: Buffet display used in study B	29
Figure 7: Protocol proposal for future combined assessments of 'bottom-up' and 'top-down' effects of NNS consumption.....	43

LIST OF ABBREVIATIONS

AgRP:	Agouti-related peptide
AntCing:	Anterior cingulate cortex
ARC:	Arcuate nucleus
AS:	Artificial sweetener
ASB:	Artificially sweetened beverage
BMI:	Body mass index
CART:	Cocaine- and amphetamine-regulated transcript
D(L)PFC:	Dorsal (lateral) prefrontal cortex
EEG:	Electroencephalography
EMA:	Ecological Momentary Assessment
EPFL:	Ecole Polytechnique Fédérale de Lausanne
ERP:	Event-related potential
fMRI:	Functional magnetic resonance imaging
GABA:	γ -aminobutyric acid
GFP:	Global field power
GIP:	Gastric inhibitory peptide
GLP-1:	Glucagon-like peptide-1
HES-SO:	Haute Ecole de Santé de Suisse Occidentale
HFCS:	High-fructose corn syrup
HF/NSW:	High-fat, non-sweet
HF/SW:	High-fat, sweet
Hypo:	Hypothalamus
Ins:	Insula
IS:	Intense sweetener
ISI:	Inter-stimulus interval
LAURA:	Local autoregressive average
LCS:	Low-calorie sweetener
LF/NSW:	Low-fat, non-sweet
LF/SW:	Low-fat, sweet
MNI:	Montreal Neurological Institute
N1:	Negative voltage occurring around 170ms post-stimulus onset
N2:	Negative voltage occurring around 200-300ms post-stimulus onset

NAcc:	Nucleus accumbens
NCS:	Non-caloric sweetener
NNS:	Non-nutritive sweetener
NPY:	Neuropeptide Y
OFC:	Orbitofrontal cortex
P1:	Positive voltage occurring around 100ms post-stimulus onset
P300:	Positive voltage occurring around 300ms post-stimulus onset
Par:	Parietal cortex
PET:	Positron emission tomography
POMC:	Pro-opiomelanocortin
PYY:	Peptide tyrosine tyrosine
rCBF:	Regional cerebral blood flow
SEM:	Standard error of the mean
SSB:	Sugar-sweetened beverage
UNIL:	University of Lausanne
VEP:	Visual evoked potential
VLPFC:	Ventrolateral prefrontal cortex
VMPFC:	Ventromedial prefrontal cortex
VTA:	Ventral tegmental area

1 GENERAL INTRODUCTION

1.1 Food intake regulation and the obesogenic environment

Food intake is essential for the maintenance of an individual's optimal functioning and survival, and is regulated by the tight interplay of brain and body. To maintain a stable body weight (i.e. energy balance), individuals' energy intake and expenditure must match [1]. Intake comprises of the various energy sources found in the daily diet. The human diet is composed of macro- and micronutrients, that are ingested in solid or liquid form, pertaining various sensory, rewarding and metabolic properties. Energy expenditure, on the other hand, comprises of all expenses necessary for basal metabolic needs, thermogenesis and (un)intended physical activity. The concept of energy balance is illustrated in Figure 1. Several factors influence the overall energy balance, i.e. homeostatic, hedonic and cognitive factors. Whereas homeostatic factors regulate appetite control according to body energy and nutrient needs, hedonic factors tend to favor appetite as a function of food intrinsic rewarding properties, i.e. promoting approach and consumption behavior [2,3]. Cognitive influences, on the other hand, regroup psycho-social, affective aspects and cognitive abilities (e.g. inhibitory control) that also play an important role in humans' daily appetite control. Altogether, these factors interact to elicit sensations of hunger, satiation and satiety [4]. Importantly, they act on regulatory systems upon energy ingestion and expenditure, but also upon exposure to pre-ingestive cues (e.g. visual, odorant or gustatory) to further regulate subsequent behavior [5].

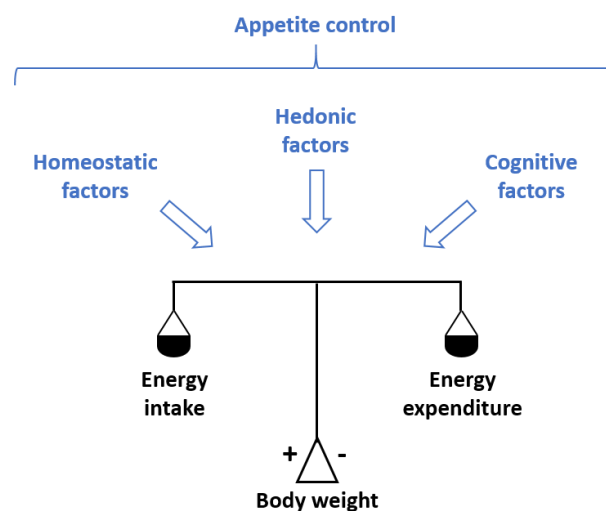


Figure 1: Schematic representation of energy balance and factors of influence. Homeostatic, hedonic, and cognitive factors (in blue) are regrouped under the concept of 'appetite control'.

These homeostatic, hedonic and cognitive mechanisms are essential to adequately guide food intake behavior, and have evolved to optimize food intake and energy storage aiming to ensure survival in times of limited food availability [6]. That is, evolution shaped our food intake regulatory system to be largely responsive to hunger and draining of body energy stores, whereas the system is rather tolerant towards a surplus of energy storage [7]. Nowadays, the availability and proximity of palatable ready-to-eat high-fat and/or high sugar energy-dense foods, the ubiquitous presence of food-related cues (e.g. TV advertising and social media), combined with sedentary lifestyles created a so-called 'obesogenic' environment. Together with human metabolism and food intake regulation shaped by evolutive pressures for survival, this environment facilitates food consumption, in turn favoring a positive energy balance and weight gain on the long run [1,8,9]. Although highest rates of overweight and obesity are attained in the United States, Polynesian islands and some North African countries, Switzerland is not spared by the crisis. In fact, 54.3% and 19.5% of the adult population were considered overweight or obese in 2016, i.e. with body mass indices (BMI) superior to 25 or 30 kg/m², respectively [10]. In addition to effects on the quality of life and social interactions, obesity has serious consequences on health, as it increases the probability of developing cancers, cardiovascular diseases, sleep dysregulation as well as insulin-resistance and type II diabetes, associated with low-grade systemic inflammation [11,12]. Despite the importance of this major public health issue, efficient and multifactorial means and strategies to improve diet quality are still lacking.

Thanks to the development of imaging techniques, appetite control systems have been extensively studied in the last 20-30 years, and have started to provide neural-based mechanistic explanations on the biology of food intake regulation. For example, several neuroimaging studies highlight altered brain responses to pre-ingestive food cues in overweight and obese individuals, paralleling weight change, or indicative of future failure in body weight management [13-15], in favor of dysregulations in extended networks of the food intake regulation system associated with deviant eating behaviors. Yet, the influence of environmental factors such as diet on appetite control, as well as the interplay between body and brain to regulate subsequent behavior remains to be investigated in more details. It is thus of primary importance to perform research aiming at a better understanding of factors impacting dietary food choices and intake behavior, in order to promote healthier consumption in the general population.

In the introduction of my thesis, I first emphasize the importance of visual pre-ingestive food cues for prospective food choices. Second, the central food intake regulation system is described, including details on the brain areas involved and the influence of food motivation states. Third, I highlight brain responses to food cues for body weight management. Finally, I focus on central regulatory mechanisms in the context of caloric and non-nutritive sweetened beverage consumption.

1.2 Visual cues for prospective food choices

Food is multisensory by definition, and pre-ingestive food cues can relate to either of the five senses that humans use for perceiving their environment, i.e. visual, olfactory, gustatory, auditory and somatosensory information. Yet, vision is predominant in humans, and thus a great part of the human food intake behavior is dictated by the eye [16]. Along with the non-human primate and human evolution, the trichromatic vision is thought to have developed to better spot nutritive food items in their natural context, i.e. discriminate colored fruits from green tree leaves, or choose between fresh or ripped fruits [9]. That is, sight is used as a primary channel conveying information about food, and this is well illustrated by the fact that unisensory stimulation of central systems by visual food-related cues is sufficient to trigger profound changes in an individual's brain activity and hormonal signaling in the periphery [5,13,17,18]. It has been shown that the processing of visual food cues temporally occurs in a two-step fashion, i.e. with two peaks of highest neural synchrony in distributed brain areas around 100-150ms and 300ms post-visual cue onset [19], reflecting distinct sensory, cognitive and valuation processes. While sensory perception, attentional filtering and top-down modulation occur rather early during processing, value integration and final decision-making occur later in time (300-400ms post-visual cue onset) [19-23].

Upon repetition of the exposition to a sensory property (i.e. sight) of a particular food item, individuals learn to associate sensory characteristics to physiological consequences, via the so-called 'stimulus-response pairing' [5]. That is, memory formation is strengthened with repeated exposure to food items, as it triggers an automatic retrieval during subsequent exposure of the same cue or related ones. For instance, learning that a particular food category or visual indices towards particular texture leads to the ingestion of a high caloric load will 'pair' the food item with high reward value and automatically trigger strong neural activity in the limbic system upon successive exposure ('wanting' response) [5]. This is likely due to food being a matter of survival, and food items being inherently biologically salient over other visual stimuli such as objects or landscapes, thus triggering automatic reward processing. In line, their visual processing is enhanced over non-food items, both in terms of timing, activity amplitude and number of brain areas recruited. That is, viewing foods elicits stronger and more distributed neural activity within the limbic system, due to stronger hedonic value attributed to the perceived item. For food items, brain responses show an additional bias towards highly palatable items such as high- over low-fat, or high- over low-energy items [24]. Several studies showed that stronger brain responses to the viewing of food items differing in their intrinsic rewarding properties was predictive of prospective food choices and weight status [3,14,25-28]. Regarding the timing of such responses, a study by Toepel and colleagues [19] showed for the first time that the categorization-

and value processing-related activity to high-fat food cue viewing preceded that of responses to low-fat items viewing within early steps of visual processing. Differential responses to high-fat food viewing (as compared to non-food objects) were apparent starting from 90ms post-stimulus onset. By contrast, significant differences in the neural generators underlying the processing of low-fat food cues as compared to non-food objects were apparent from 180ms. Moreover, responses to high-fat food viewing yielded stronger activity within occipito-temporal networks around 170ms post-stimulus onset, as compared to responses to the viewing of low-fat food items [19,29].

Learning mechanisms between sensory properties of food cues conveyed by visual features and rewarding physiological consequences are often used for marketing purposes, i.e. using ubiquitous colorful and attractive visual cues as conditioned cues to trigger a 'wanting' response through repetitive exposure [5,9,16,28]. That is, it is very important to understand the consequences of exposure to visual food cues and perceptive processes, as they are directly linked with food choices and the conditioning of the intake experience.

1.3 Appetite control, food intake behavior and the brain

1.3.1 Central food intake regulation in healthy humans

Food intake behavior is regulated by the interplay between central and peripheral mechanisms, via the so-called 'gut-brain' axis. The central food intake regulation system comprises of two main networks, namely the 'salience' and 'executive function' networks [30], serving to integrate intrinsic rewarding properties of foods, external factors and internal physiological signals to guide behavior [3] (Figure 2).

The salience network comprises of distributed cortical and subcortical areas involved in the processing of internal signals from the body periphery ('homeostatic' regulation) and intrinsic rewarding properties of the perceived food or food-related cues ('hedonic' regulation) [2]. Food intake behavior is regulated to match body energy needs via the so-called 'homeostatic axis', including parts of the brainstem and the hypothalamus, both highly permeable to peripheral gastro-intestinal hormones and somatosensory afferents [7,31]. Parts of the insula are also considered as homeostatic areas, as they comprise the primary gustatory cortex [32].

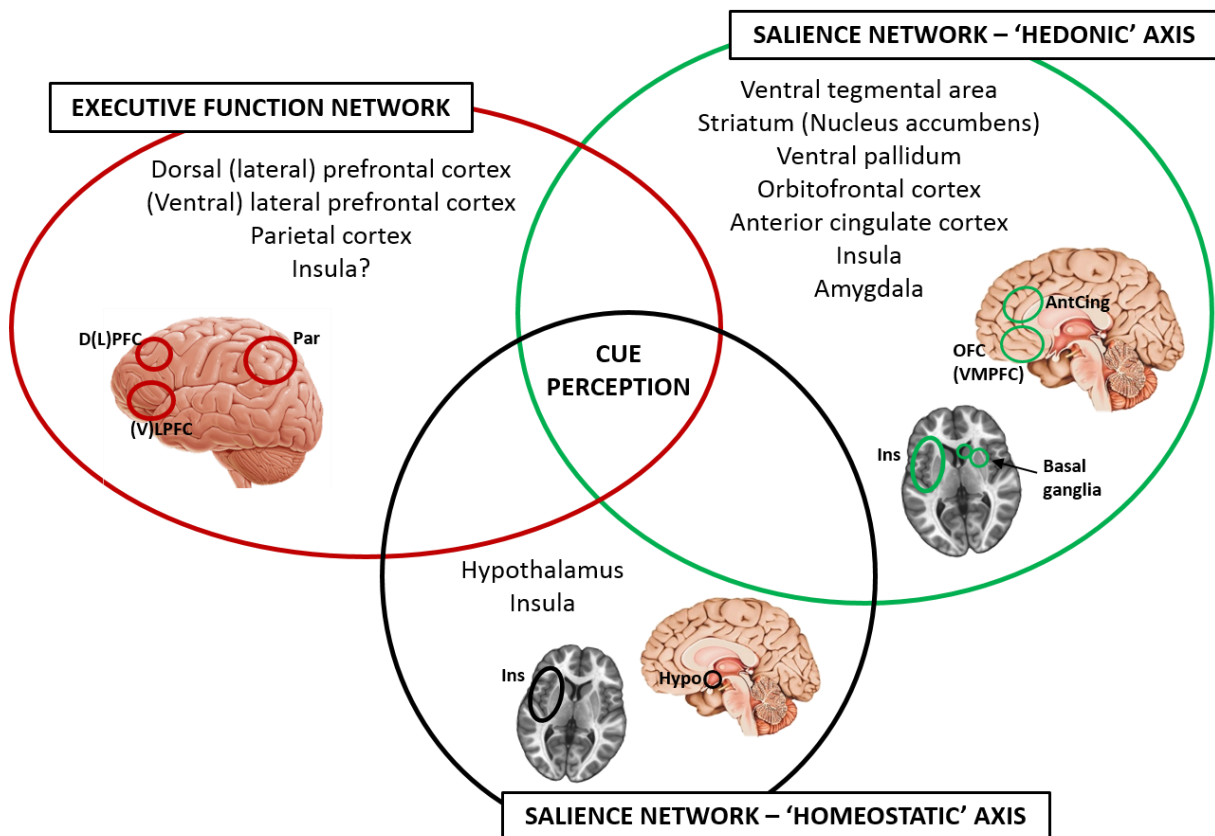


Figure 2: Brain networks and details on areas involved in the central regulation of food intake upon exposure to a pre-ingestive food cue. Brain areas are cited as exemplar areas involved in appetite control, and the listing may not be exhaustive. D(L)PFC: dorsal (lateral) prefrontal cortex. (V)LPFC: (ventral) lateral prefrontal cortex. Par: parietal cortex. Hypo: hypothalamus. Ins: insula. AntCing: anterior cingulate cortex. OFC: orbitofrontal cortex. VMPFC: ventro-medial prefrontal cortex.

The 'hedonic' axis, also called 'reward system', is mostly subserved by corticolimbic areas, and relies on dopamine and opioids as neurotransmitters. As part of this axis, several nuclei of the basal ganglia (i.e. ventral tegmental area, striatum and pallidum) as well as the amygdala, insula and orbitofrontal cortex, are involved in perceiving and processing the intrinsic rewarding properties of visual, odorant, taste, or multisensory food cues. As the food intake regulation system developed in times of limited food availability, drives towards energy-dense palatable foods (mainly in the form of carbohydrates or fat) are favored, since advantageous for survival. It is assumed that high palatability of perceived food-related cues elicits strong activity within this broad network, in turn favoring approach and consumption behavior [4]. Moreover, energy deprivation characterizing the state of hunger relative to satiety (signaled along the 'homeostatic' axis) increases the reward value attributed to food cues ('hedonic' axis) [33]. Thus, there is a high level of cross-talk between hedonic- and homeostatic-related regions, and they are increasingly considered as one comprehensive network rather than separate entities. This network has also been extensively investigated in animal models, thus providing mechanistic explanations on the relative contributions of the aforementioned areas to predict eating behavior [34-38].

The executive function network, on the other hand, mostly comprises of prefrontal and parietal regions of the brain, involved in decision-making, cognitive control and response suppression, together with attentional control and the manipulation of information in working memory [39]. These processes are essential to terminate food intake and elicit satiation and satiety, as well as to cope with the abundance of foods in today's environment [1,40]. For example, executive functions can downregulate reward-directed impulsive behaviors by taking into account longer-term goals [41,42]. Along evolution, this network strongly developed, enabling maturation of higher-level functions that are especially well developed in humans and non-human primates [43]. These functions are highly susceptible to social, (in)attention and cognitive influences, therefore constantly challenged in the present context of abundance of palatable foods [4,40].

Executive functioning and salience evaluation do not only act upon food ingestion, but are also active processes upon exposure to pre-ingestive (visual) food cues, as discussed in the preceding section. The functioning of the various brain areas involved in appetite control will be reviewed in the following chapter with a specific focus on responses to the viewing of food-related cues.

1.3.2 Details on key brain areas involved in the regulation of food intake

1.3.2.1 Hypothalamus

The hypothalamus is one of the main regions for sensing body energy status, and a relay center between the brainstem and other (sub)cortical areas. Several nuclei of the hypothalamus are involved in regulating food intake as a function of body energy needs, the main one being the Arcuate Nucleus (ARC). This nucleus contains two main populations of neurons, either co-expressing Neuropeptide Y (NPY) and Agouti-related peptide (AgRP), or co-expressing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). The first population is orexigenic, promoting hunger feeling and food intake upon activation, whereas POMC/CART neurons are anorexigenic, promoting meal termination and satiety when stimulated [31]. In addition to sensing nervous afferents from the brainstem, the ARC nucleus of the hypothalamus is also highly permeable to peripheral hormones from the adipose tissue, pancreas, and the gastro-intestinal tract, serving to signal hunger or satiety by stimulating either NPY/AgRP-neurons or POMC/CART neurons [7,31,44]. Projections from the hypothalamus to mesocorticolimbic regions modulate the incentive salience attribution to food items via the reward system as a function of the hunger state, i.e. enabling neural cross-talk between homeostatic and hedonic regulation of food intake [45].

1.3.2.2 *Basal ganglia*

The ventral tegmental area (VTA), ventral striatum, nucleus accumbens (NAcc) and globus pallidus are part of the reward system and form the 'basal ganglia', or 'deep-brain reward centers'. The VTA is the origin of dopaminergic neuronal cell bodies, sending axonal projections towards other nuclei, subcortical and cortical structures. These basal nuclei are the basis of the mesolimbic network, or 'reward system.' Reward has been proposed to stem from two processes, 'liking' and 'wanting' [33,46,47]. These aspects are mostly dissociable, as they rely on different mechanisms and neurotransmitters, but they are often experienced together upon exposure to a food stimulus. 'Liking' is the pleasurable experience derived from consuming the food (signaled by opioids, mostly in the ventral pallidum, but also in the NAcc and brainstem), whereas implicit 'wanting' is the motivation to seek the food, i.e. the predominant response to being exposed to food-related cues, mostly signaled by dopaminergic neurons in the VTA and the NAcc [33,48,49]. Thus, when 'wanting' is blocked by dopamine receptor antagonists or by a genetic knock-out of those receptors, animals still experience the pleasure of consumption, but no longer seek rewarding foods. By contrast, when 'wanting' is enhanced by pharmacological means (e.g. with amphetamine agents) or by lesions or genetic manipulations (e.g. hyperdopaminergic mutant mice), animals will work more to seek rewarding food items, even though 'liking' itself is not necessarily enhanced [33,47,50,51]. The terminology 'wanting' is used here in the sense of incentive salience, i.e. basic instinctive urge towards food items. That is, no conscious explicit process and subjective awareness need to be present [46]. The combination of these two aspects of reward experienced upon exposure to cues and food ingestion has been found to improve the storage of the stimulus-response pairing in memory. Over time, an automatic link is thus created between a food-related cue and post-ingestive consequences of this particular food via the reinforcement of learnt associations [52,53]. It is thought that the basal ganglia does not underlie conscious hedonic experience, but rather that this experience is subserved by higher-level cortical centers integrating reward valuation with homeostatic and cognitive signals [3,54].

1.3.2.3 *Amygdala*

The amygdala is involved in the general processing of emotions and reward valuation, as part of the limbic system [55]. It is crucial to encode emotional memory formation, and thus some food-related memories can be associated with emotional aspects and trigger amygdalar activity upon retrieval, i.e. when exposed to food-related cues [56]. For instance, O'Doherty and colleagues [57] showed an increased activity in the amygdala in response to glucose tasting, which positively correlated with pleasantness ratings, likely due to associated positive emotion retrieval.

1.3.2.4 *Orbitofrontal cortex*

The orbitofrontal cortex (OFC) occupies the ventral surface of the prefrontal cortex, behind the eyes. However, attributing functions to this large brain structure and using this general terminology require some caution, as the term 'OFC' commonly refers to only the medial part of the ventral prefrontal cortex (VMPFC), i.e. as opposed to the lateral OFC, which is more often referred to as the ventro-lateral prefrontal cortex (VLPFC). Due to their different anatomical and functional connectivity, the VMPFC (also further referred to as simply 'OFC') is part of the salience network, whereas the VLPFC activity is rather related to the executive function network [30,54,58].

The OFC/VMPFC receives input from the five sensory modalities, and is highly anatomically and functionally connected to other (sub)cortical areas, e.g. NAcc, amygdala, cingulate cortex, insula, hypothalamus, and hippocampus. Thus, this area is considered as a polymodal hub region integrating reward and homeostatic signals, and therefore encoding the 'final' reward value associated with perceived cues [59,60]. Moreover, as the neural activity in the OFC to the viewing of food stimuli has been shown to correlate with subjective pleasantness and motivational ratings [22,61,62], it has been proposed that 'wanting' and 'liking' dimensions of reward are integrated therein, thus giving rise to the conscious perception of hedonic experiences. Not only does the OFC integrate reward aspects of a perceived stimulus, but it also evaluates punishments, by retrieving past affective outcomes associated with similar experiences, longer-term goals and cognitive aspects encoded in more dorsal prefrontal regions. Several studies showed modulations of OFC activity by dorsal prefrontal regions. That is, stronger input from dorsal regions in turn downregulate the neural activity in the OFC, as well as associated reward perception of a given stimulus [20,21,41,63,64]. More globally, the OFC is thus thought to compute the expected reward of perceived stimuli by encoding the cost/benefit balance, and hereby take part in the decision-making process [60,65,66] (also apparent from lesion studies [67,68]).

1.3.2.5 *Anterior cingulate cortex & caudate head*

The anterior cingulate cortex has intense anatomical and functional connectivity with the medial OFC and caudate head (part of the dorsal striatum), as well as with premotor and supplementary motor areas [69-71]. These regions are thought to be necessary 'motor' initiator (caudate head) and intermediate (anterior cingulate cortex) between reward value attribution and motor response output and are thus considered as areas underlying action- or goal-directed behavior [67]. That is, activity in these areas is likely to mediate the translation from valuation to action [72].

1.3.2.6 *Dorsal (lateral) prefrontal cortex*

The dorso-lateral prefrontal cortex (DLPFC) is part of the executive function network and there is great consensus on its predominant role in decision-making and inhibitory control. Seeley and colleagues [30] considered this region as a seed node of the executive function network and highlight its high connectivity with VLPFC and lateral parietal cortices. The DLPFC is activated stronger in response to viewing palatable food images when participants are instructed to focus on controlling their 'wanting' response (i.e. downregulate cravings) [73], and/or when instructed to take into account longer-term goals such as body weight maintenance [74] or health aspects of perceived items [63]. In turn, stronger activation is suggested to downregulate reward and emotional cortical and subcortical structures [75,76] such as the OFC [63,73], and also sensory regions such as occipital visual processing areas [77]. Activity in the DLPFC was found to correlate with individuals' degree of dietary restraint, suggesting a more automatic recruitment of control abilities in participants seeking control over their daily food intake [74,78,79]. Although activated by conscious recruitment of self-control, this region can thus also be more automatically activated and relate to higher cognitive restraint personality traits [74], often in association with activity in the VLPFC [58]. Moreover, several studies indicate that the exertion of cognitive control, as reflected by prefrontal neural activity when exposed to food cues, is predictive of successful weight loss-maintenance [15,78], weight loss induced by a behavioral diet intervention [14,80] or surgical gastric bypass procedure [81].

1.3.2.7 *Ventrolateral prefrontal cortex*

The VLPFC is part of the executive function network and is often found to co-activate with the DLPFC to regulate decision-making by exerting inhibitory control [21,82]. That is, the VLPFC has a role in several functions related to impulse and attentional control, in particular when viewing highly palatable stimuli [83]. Studies using go/no-go paradigms consistently show higher activation of the VLPFC for no-go trials, i.e. when the goal is to retain prepotent motor response [84,85]. This task can also be used to train implicit inhibitory control via strengthening VLPFC activity [86,87]. Stop signal task paradigms performed in patients with VLPFC lesions also highlight the crucial role of the VLPFC in successful impulse retaining [88]. In addition to response suppression, the VLPFC has been found involved in reversal-learning, i.e. adapting behavior to novelty in reward attribution or devaluation of a specific stimulus [83]. That is, this region is not only involved in decision-making regarding high-reward stimuli, but also in the evaluation of costs of punishments [89].

1.3.2.8 *Lateral parietal cortex*

Although relatively few studies so far investigated the specific role of parietal cortices in food cue processing, these regions show a great connectivity with DLPFC and VLPFC, and have been found involved in maintaining sustained attention over relevant items and in efficient working memory, as part of the executive function network [3,30,73]. The precuneus (rostral parietal lobe) also subserve attention towards food cues [90,91].

1.3.2.9 *Insula*

The insula was first considered as a pure sensory area, as it contains the primary gustatory cortex that responds to taste inputs, and integrates oral sensations such as touch and flavor [32,92]. Specific sensory subregions and functionalities in humans remain however debated, e.g. neural correlates of taste-related activity as a function of affective or physiological significance, sensory priming or behavioral tasks performed in parallel [22,93,94].

In addition to its primary role in taste sensing, the insula has been highlighted as the typical cortical region performing interoceptive sensing, i.e. the perception of the internal milieu (visceral awareness) and body energy state through hormonal and nervous peripheral sensing [95,96]. Afferences from the autonomous nervous system project into the insula via the spinal cord, and the insular cortex also sends efferent projections back to the brainstem, as a mechanism to retro-control homeostatic systems [95,96]. This brain region is also highly responsive to hormonal signals from the periphery, as it contains molecular receptors for several gastro-intestinal peptides and adipokines [44], implying a role in sensing and reacting to the internal milieu. Moreover, Critchley and colleagues [97] observed a positive correlation between the reactivity as well as the size of insular grey matter and participants' accuracy in a heartbeat detection task, thus arguing that this region might also be responsible for conscious subjective feelings and emotional experience associated with visceral arousal. In extension to its classical homeostatic and interoceptive role, the insula is functionally connected to the hypothalamus and several regions of the basal ganglia and reward network, especially when the perceived stimulus is potentially nutritive [98]. Further, it has shown reactivity to salient food stimuli in various modalities (visual, olfactory and gustatory) [18,61,99]. Therefore, the insula is considered as a hub relaying information on sensory and hedonic valuation as a function of the body homeostasis [100,101].

However, numerous studies highlight more and more functions of the insula, especially in humans, e.g. pain, empathy, language and working memory tasks [72,102]. The insula has undergone significant expansion throughout the primate evolution, and in particular in humans, and thus several of its features are not easily investigated in animal studies. For example, the primary gustatory cortex is

likely located at the more posterior end of the insular cortex in humans vs. non-human primates, which leaves many other (higher-order) integrative functions associated to the mid-anterior insula [32,103,104]. For example, Singer [105] showed that the insula plays a role in error-based learning and feelings of uncertainty. Located at the intersection between executive function, hedonic and homeostatic networks, the insula is thus also thought to act as a hub region for the integration of information related to control, reward and internal signals, and facilitate the switch between large-scale networks [72]. That is, this region would estimate the salience of perceived items (not only food) in order to efficiently recruit attention and control systems for items with the highest salience, and highlight them for efficient working memory handling, or motor response facilitation via the anterior cingulate cortex (often found to co-activate with the insula). It has also been suggested that the insular cortex captures congruencies between pairs of stimuli that vary in modality, i.e. higher activation when exposed to stimuli properties congruent across modalities, as well as between sensory properties and physiological consequences by nutrient-sensory conditioning [106,107]. As visual areas located in the occipital and lateral inferior parietal lobes anatomically project to the insular cortex, this region would also be able to relay information conveyed by the visual stream and perform nutrient-sensory conditioning relying on visual appearance of stimuli [101]. Stimuli sensed as salient in the insula, as observed by Cornier and colleagues [108,109] in response to food images, would therefore have a facilitated access to working memory and attentional resources.

1.3.2.10 Temporal cortices

Temporal cortices are activated during food categorization, i.e. identification as a particular food category, characteristics or item [24]. Temporal cortices are often found to co-activate with occipital primary and secondary associative areas and are thus related to a network processing visual information as part of the ventral pathway subserving object identification [110]. It has been shown that this categorization activity is more efficient, i.e. happens more quickly in time after visual stimulus onset, for high-fat over low-fat items, and temporal cortices underlie such differences, together with other vision-related areas [19,29].

1.3.3 The interplay of gut and brain to modulate responses to foods

Hunger is a physiological construct promoting food search and consumption. Nervous and hormonal signals for hunger arise from the periphery and convey information to the central regulatory mechanisms about internal experiences and body energy stores. Peripheral hormones carrying information on the body nutritional state, i.e. from adipose tissue or gastro-intestinal tract, are key

players in the regulation of hunger and appetite, via the so-called 'gut-brain' axis [111]. While ghrelin is the only known orexigenic hormone (i.e. promoting hunger and food intake) circulating in the periphery, several circulating hormones promote satiation and satiety, and reduce food intake. These anorexigenic hormones either inform the brain about longer-term energy stores (static information; leptin), or regulate acute and short-term intake (dynamic information; e.g. insulin, Glucagon-Like Peptide-1 (GLP-1) incretin, or peptide-tyrosine-tyrosine (PYY)) [7,44,112,113].

Ghrelin is a peptidic hormone produced and secreted by specialized cells in the stomach during fasting [114]. High plasmatic ghrelin levels can trigger meal initiation and thus ghrelin promotes a positive energy balance [115]. Upon its release by stomach cells, ghrelin crosses the blood-brain-barrier to stimulate NPY/AgRP neurons in the hypothalamus [116]. Molecular receptors for ghrelin have also been found in various regions of the dopaminergic mesolimbic system as well as the amygdala, OFC, and insula, and it has been shown that this orexigenic hormone can enhance food reward valuation by acting directly on the reward-related pathways, e.g. by activating VTA-NAcc dopaminergic neurons [117,118]. Plasma concentrations of ghrelin decrease after eating, i.e. to a greater extent with ingestion of carbohydrates as compared to lipids and proteins.

Insulin is secreted by β -cells of the pancreas when glycemia increases. Insulin is the main hormone responsible for glucose homeostasis, but it also promotes satiety and regulates meal-to-meal intervals [31]. The ARC nucleus of the hypothalamus expresses numerous insulin receptors, upon which insulin up-regulates the activity of POMC/CART anorexigenic neurons, and suppresses firing activity of NPY/AgRP neurons [119,120]. Guthoff and colleagues [121] used intranasal perfusion to directly infuse insulin into the central nervous system and were able to discriminate proper insulin-related effects from those elicited by variations in peripheral glycemia inherent to insulin action when manipulated peripherally. They found that insulin *per se* decreased neural activity to food images within discrete brain regions, i.e. in temporal, vision-related areas (fusiform gyrus), hippocampus and middle frontal cortex, and could thus be involved in the termination of meal intake.

GLP-1 and PYY are both peptidic hormones, secreted by L-cells of the intestine upon nutrient sensing, in proportion to the caloric content of the meal ingested. While GLP-1 secretion is primarily increased by carbohydrate sensing, PYY secretion is mainly stimulated after consumption of fat- and protein-rich meals [122,123]. Both hormones provide satiety signals to the brain by inhibiting NPY neurons in the ARC nucleus of the hypothalamus [124,125]. In humans, a number of reward-related or higher-level brain regions were found to be modulated by peripheral PYY and GLP-1 infusion, in association with decreased food intake and hunger sensations [126-129]. Apart from signaling satiety, GLP-1 also has an incretin effect, thereby contributing to lowering blood glucose by increasing insulin release from pancreatic β -cells.

Leptin, another anorexigenic hormone, is not secreted by the gastro-intestinal tract but by the adipose tissue, and is involved in the signaling of longer-term body energy state [31,130]. Leptin acts on the ARC nucleus of the hypothalamus to downregulate AgRP/NPY-neurons, and enhance POMC/CART-neuron activity. Moreover, leptin receptors are expressed by neurons of the reward system, i.e. this hormone has an impact on the hedonic value attributed to food stimuli. This hedonic value can be modulated by leptin action either directly by dopaminergic neuron inhibition in the VTA, or indirectly by enhancing γ -aminobutyric acid- (GABA-) neuron activity in the hypothalamus, that in turn inhibit dopaminergic neurons in the VTA [131]. Leptin replacement in genetically-deficient individuals has been shown to downregulate neural activity to food viewing in areas associated with the salience network, while upregulating satiety- and control-related neural activity [132].

In light of changes in hormonal signaling occurring with food intake, numerous studies showed increased individuals' drives toward foods, i.e. increased food intake motivation, in the state of hunger relative to satiety. This increase in motivation when hungry, or decrease in drives towards foods when sated, has been highlighted by several neuroimaging studies in humans, both along the salience ('hedonic' and 'homeostatic' axes) and executive function networks. Neural responses upon exposure to food-related cues are stronger in limbic and homeostatic areas in hunger as compared to satiety [93,133-139]. This is particularly apparent for responses to highly palatable food cues, such as high-fat, high-sugar or high-energy foods [140], and when these foods are readily available for consumption [141]. Stronger amplitudes of neural responses to visual food cues have also been observed with electroencephalographic recordings in hungry participants, as compared to sated, around 100 and 300ms post-stimulus onset. These components of the electrical brain responses to food viewing have been associated to attentional- and motivational-related processing, thus also indicating that hungry individuals show greater drives towards food cues [142,143]. In contrast, activity in the executive function network has been found to increase in satiety as compared to hunger [133,139].

In summary, the cerebral regulation of food intake when humans are presented with a food cue (e.g. visual, odorant or multisensory) or when ingesting the food, relies on a fine-tuned balance between body homeostasis conveyed by peripheral signals, intrinsic hedonic properties of the perceived food, and the level of attention and control exerted [3,4].

1.3.4 Central food intake regulation and body weight management

A substantial body of evidence highlights the differential recruitment of central regulatory mechanisms for food intake in normal-weight vs. overweight/obese individuals, individuals at risk for failure as opposed to those succeeding in body weight loss, and longitudinally during weight loss or weight gain.

These alterations in neural activity were observed in hunger and satiety, in response to food ingestion but also when exposed to pre-ingestive (visual) food cues, and impact both the salience and executive function networks [13].

Alterations in the executive control network have been consistently highlighted in overweight and obesity, i.e. decreased activity in prefrontal areas to the viewing of high-calorie foods associated with an impaired cognitive control over food intake and a higher BMI [144,145]. Le and colleagues [146] also reported less increase in post-meal activation in the DLPFC in obese vs. normal-weight men. Along this line, a study by Batterink and colleagues [147] showed a negative correlation between DLPFC activity in response to visual food cues during a go/no-go task and body weight, i.e. participants with highest body weight showed less prefrontal activity and failure in inhibitory control of prepotent motor responses.

In parallel to alterations in brain areas associated with executive functions, several studies highlighted a 'hyperactive' mesocorticolimbic system in response to food cue exposure, i.e. also to visual cues only [13,145]. That is, these studies found stronger activity in the NAcc, VTA, striatum and OFC in overweight and obese participants when exposed to highly palatable food items, as compared with normal-weight counterparts, or correlations between neural activity and weight status [148-151]. A decreased activity in food motivation-related areas from pre- to post-prandial states is observed in normal-weight individuals; yet abnormal sustained neural activity in the sated state was observed for overweight and obese participants [152]. Similar findings were reported when comparing 'obesity-prone' (former obese) to 'obesity-resistant' individuals (long-term stable-weight) after a short-term overfeeding period [108,109]. Sensitivity to reward and impulsivity, as well as other personality traits assessed by questionnaires, have been shown to correlate with stronger activity in the mesolimbic reward system in response to visual food cues relative to aversive or non-food stimuli [25,42,153,154]. This indicates that hyper-reactivity of the salience system to food cue exposure might explain, at least in part, why some individuals are more prone to eat beyond satiety and gain weight over time.

Although a majority of studies showed increased reactivity to visual food cues in overweight and obese relative to normal-weight individuals, some studies found decreased neural activity to food tasting or ingestion in limbic areas, in particular in the dorsal striatum [155-157], or observed decreased dopamine D2 receptor availability in obese individuals as a function of their BMI [158,159]. These findings raised the concern of similarities in mechanisms underlying drug addiction and food intake regulation. Such hypo-activity in response to high-rewarding stimuli (i.e. indicative of habituation and higher tolerance to energy-dense palatable foods) has been termed 'reward deficiency hypothesis', putatively explaining compensatory behaviors in food intake and drug use [160]. It is not clear yet whether hyperactivity of reward-related areas precedes weight gain, and hypo-activity (reward

deficiency) is rather the consequence of habituation, as hypothesized by the 'dynamic vulnerability model of obesity' [161,162]. Alternatively, both alterations could cause weight gain, albeit occurring in different individuals, or in the same individuals but in different limbic regions or stimuli sensing modes [52,156].

Taken together, the imbalance between salience network and executive functions in overweight and obese individuals likely promotes food intake rather due to alterations in valuation (either because of hyper-reactivity of reward systems, or to compensate a deficit) than being a cognitively-driven decision [42,76,163]. Altogether, these findings highlight the need to better understand pre- and post-ingestive food perception in normal-weight, non-clinical populations and how this relates to deleterious eating behaviors, potentially shedding light on the causality of alterations occurring in populations with deviant weight status.

1.4 Sweetened beverages, food intake and the brain

1.4.1 Sugar-sweetened beverage consumption and body weight management

Excess sugar consumption (also termed caloric sweeteners) has increased in parallel to worldwide obesity rates and thus has been widely incriminated as one of the main causes of weight gain and the epidemics of obesity in the last decades, as well as a leading cause for associated metabolic disorders such as type 2 diabetes, cardiovascular diseases and cancers [164,165]. Associating sweetness sensing with a pleasurable experience is innate, as sugar taste pathways have evolved to signal the presence of potentially nutritive items in an environment of limited food availability, and thus predispose us to like sweet taste [32,166,167]. Nowadays however, sugars are added to numerous dietary items as sweetening and conservative agents, taste or texture exhausters, and are thus massively consumed as part of the western-world diet. Added sugars are mostly found in the form of sucrose, a dimer containing one molecule of fructose and one of glucose, or high-fructose corn syrup (HFCS), a mixture of free glucose and fructose molecules in various percentages. A large part of added sugars is consumed through sugar-sweetened beverages (SSBs) [168].

SSBs are of course not the only energy vehicle in the western-world diet, but they have mostly been incriminated as promoting weight gain and metabolic disorders because of several, non-exclusive, reasons. First, in particular the fructose component of sucrose (or HFCS) has been accused of promoting differential hormonal and brain responses, blunted satiety feelings, increased ectopic lipid deposition (e.g. in the liver) and insulin resistance [169-174]. Second, the consumption of SSBs is thought to favor excessive consumption of calories, as some studies showed blunted satiety feeling and incomplete compensatory behavior leading to positive energy balance, as compared to energy

consumed in the solid form [175,176]. Third, sugars are potent hedonic triggers, and these drives toward sweet items might influence our food preferences and daily choices [36]. The body of evidence has long been, and still is, a matter of debate, especially in humans [177-180]. Yet, associations between body weight gain and sugar properties rendered sugars and more particularly SSBs as popular targets for interventions or prevention campaigns aiming at a better body weight management, both in overweight/obese and the general normal-weight population.

1.4.2 Brain responses to sugar and sugar-sweetened beverage intake

Relatively few studies investigated brain responses to sugar ingestion in humans so far. Among them, several investigated the difference in activation upon glucose vs. fructose ingestion [173,181-185], rather than the combined effects of sugars as compared to water on brain responses. On an acute basis, Zald and colleagues [186] used positron emission tomography (PET) to assess regional cerebral blood flow (rCBF) during the tasting of a pleasant sucrose solution, and found an increased rCBF in the OFC and anterior insula. In addition, a study by O'Doherty and colleagues [57] further showed increased activity in the amygdala at the time of tasting sucrose. Since then, several studies have demonstrated strong neural activity in response to sucrose tasting within the salience network [187-189]. Connolly and colleagues [190] showed similar results in the visual modality: they highlighted strong responses to food images, as opposed to non-food images, in the salience network (amygdala, hippocampus, thalamus and anterior insula) subsequent to SSB consumption. Interestingly, Stice and colleagues [191] highlighted differential brain activation in response to the tasting of high-fat vs. high-sugar milkshakes, despite both stimuli being equivalent in their energy load. High-sugar milkshake tasting elicited stronger activation as compared to high-fat milkshake tasting in the insula, the putamen (basal ganglia), thalamus and the rolandic operculum (inferior fronto-parietal junction), indicative of stronger responses related to reward and gustatory-activity at the time of tasting. In contrast, responses to high-fat milkshake tasting were stronger in the caudate, postcentral gyrus, hippocampus and VLPFC, indicating neural responses rather related to oral somatosensory activity encoding fat viscosity. The authors concluded on a potential greater (and quicker) connectivity between gustatory and reward regions, as between somatosensory and reward regions, or on a higher capability of the human brain to detect subtle differences in sugar content as compared to fat content in a tasted stimulus.

Burger and Stice [90] conducted a study mixing both the gustatory and visual modalities on an acute consumption basis, by investigating neural responses to a carbonated soft drink intake, tasting (so called 'anticipated intake'), and to the viewing of product logos. The carbonated soft drink *intake* and

tasting activated the oral somatosensory cortex (postcentral gyrus), insula, inferior lateral occipital cortex, OFC, thalamus, posterior cingulate cortex, midbrain and a range of basal nuclei (striatum, putamen, NAcc and caudate) relative to a tasteless solution. The *viewing* of soft drink logos as compared to other non-food product advertisements activated the lingual gyrus, superior and inferior lateral occipital cortices, postcentral gyrus, posterior insula and putamen, indicative of stronger recruitment of visual- and attention-related areas in response to soft drink-associated cues. In a secondary analysis, this study also assessed modulations in the neural activity to soft drink tasting and product advertisement viewing as a function of individuals' consumer status in a cross-sectional design. Within the neural activity elicited by the soft drink *tasting*, the activity in the VLPFC, associated with impulse retaining and cognitive control, was decreased in frequent soft drink consumers as opposed to non-consumers. By contrast, stronger activity in response to the *viewing* of soft drink logos in the posterior cingulate cortex and precuneus area was found in consumers vs. non-consumers, indicative of stronger attention towards soft-drink related cues that may encourage further intake.

A study by Burger [91] further extended these findings with a longer-term longitudinal trial investigating changes in neural responses to soft drink *intake* and associated logo *viewing* after a 3-week period of repeated daily SSB consumption. This study found decreased striatal (caudate) and anterior cingulate cortex response to soft drink *intake* after the intervention. By contrast, responses to logo *viewing* showed stronger neural activity in the precuneus (parietal), but decreased activity in the temporal lobe and VMPFC after intervention, associated with heightened disinhibition towards the logo. This first longitudinal trial conducted on brain responses to SSB intake in humans implies that adding SSBs to the daily diet of healthy-weight participants leads to brain response adaptations that may help perpetuate consumption of such products. Alterations have also been shown on a longitudinal protocol in animal models highlighting differences in basal brain metabolism within reward-related areas following the consumption of a glucose- or fructose-enriched hypercaloric diet (but not a starch-enriched hypercaloric diet; [162]).

Altogether, these findings show the hedonic potency of sweet taste to elicit strong activity in salience-related brain areas. They also highlight the importance of visual cues associated with soft drink consumption to activate anticipatory reward and 'wanting' responses. Longitudinal and cross-sectional trials further highlight the changes occurring during repeated SSB consumption (heightened attention and decreased impulse control), and place these changes as a causal mechanism for the perpetuation of consumption. Still, the studies did not investigate whether changes in neural responses to the viewing, tasting and intake of such products can be generalized to drives towards other solid food types.

1.4.3 Non-nutritive sweeteners as substitutes for caloric sweeteners

For the various reasons mentioned in preceding sections, public health agencies and intervention trials have specifically targeted sugar content in foods and sugar-sweetened beverages in order to improve body weight management, which has led to the massive consumption of sugar-substitutes worldwide, so-called 'non-nutritive sweeteners' (NNS). For instance, aspartame, neotame, saccharin, acesulfam K, sucralose and cyclamate are used in various foods and non-food products such as toothpaste, chewing gums and medications, and are increasingly consumed in the form of artificially sweetened beverages (ASBs; i.e. also called diet soft drinks) [192]. These molecules have different designations, and can be found under the names of sugar substitutes, low-calorie sweeteners (LCS), non-caloric sweeteners (NCS), artificial sweeteners (AS), intense sweeteners (IS) [193]. NNS bind to sweet taste receptors on the tongue (and possibly along the digestive track), and thus elicit the perception of sweet taste [194,195]. Interestingly, their dissociation constant with those receptors is very low (i.e. indicative of a high affinity; [196]), and thus NNS have an intense sweetening power that can be several thousand times higher than that of sucrose [197]. They are non-caloric, either because they cannot be assimilated by the digestive track (e.g. as it is the case for saccharin), or because the quantity used is such that the caloric load is negligible (e.g. for aspartame).

By definition, NNS should help consumers controlling their body weight, as they do not bring excess calories to the diet. Until recently, they have indeed been widely considered as metabolically inert compounds, thus enabling the consumer to enjoy the hedonic properties of sweet taste without consuming extra calories. However, NNS are now also suspected to promote body weight gain, as associations between NNS consumption and weight gain, overweight and obesity prevalence have been observed in epidemiological cohort studies [198-200]. Although reverse causality (i.e. already overweight people choose to consume low-sugar products) might explain results of cohort studies, the problem is still present when investigating the change in BMI together with the change in NNS consumption, or when controlling for BMI at baseline of prospective cohort studies, thereby questioning the positive impact of NNS consumption on body-weight management. Why is this so, since NNS are calorically 'empty' and should therefore lower individuals' overall energy intake? This paradoxical association between NNS consumption and body weight gain led to hypotheses on the putative impact of NNS on sweet taste perception and the regulation of food intake behavior. One of the potential mechanisms by which NNS are accused of impacting behavior is based on the learning abilities of the animal/human food intake regulation system, assuming that it is based on a match between a sweet taste signal and its subsequent caloric input conveyed by gastro-intestinal hormones, vagal afferents or else, and that no adaptive changes have happened over evolution. By eliciting hedonic sweet taste without conveying calories, NNS putatively hinder the food intake regulation

system and lead to inadaptable choices [201]. In rodents, several studies support this hypothesis. Davidson and colleagues [202] showed increased food intake and body adiposity after a prolonged exposure to NNS-containing diet in rats. Wang and colleagues [203] found that sucralose consumption elicited increased food intake via enhancement of a neuronal fasting pathway in *Drosophila*.

In humans however, the impact of NNS on gastrointestinal hormone secretion and food intake behavior remains controversial. Several reviews, meta-analyses of randomized controlled trials and observational cohort studies on the efficiency of NNS to help body weight management indicate that there is currently no scientific evidence that NNS are beneficial for body weight management, nor that they are consistently detrimental [193,204-207]. Neuroimaging studies started quite recently to highlight variations in brain responses to NNS as compared to sugar tasting. A study by Smeets and colleagues [188] found prolonged signal decrease in the hypothalamus following glucose intake as compared to water, but not following aspartame or maltodextrin (polymers of glucose with near-zero sweet taste), indicating that a combination of sweet taste and caloric load is necessary to trigger adaptive responses to SSB intake. Further, Frank and colleagues [187] showed that sucralose tasting activated reward pathways to a lesser extent than sucrose tasting, despite greater connectivity between the insula and deep basal nuclei (striatum, pallidum), thalamus and anterior cingulate cortex in the sucralose condition. The authors concluded on a potential 'unsatisfied' reward system following the consumption of NNS relative to sucrose. Another study by Smeets and colleagues [189] tested the impact of non-caloric vs. caloric orangeade consumption on brain responses to tasting that orangeade. They showed differential impact of consumption with regard to energy content on gustatory-related brain responses, i.e. the non-caloric beverage elicited stronger activity in the amygdala, while the opposite was found for striatal activity. These effects were more pronounced when tasting the stimuli before as compared to after the consumption of the beverage. Overall, the non-caloric beverage tasting led to stronger activity within the VLPFC than the caloric beverage tasting, but this effect was unaffected by the orangeade consumption.

Altogether, these studies show that caloric sweeteners (sugars) are in general more potent than NNS in triggering reward-related activity upon tasting. However, in spite of recent progress in understanding cerebral mechanisms triggered by NNS ingestion, we still do not know how these differences in reward-related processing of sweet tastes modulate subsequent attentional drives towards various types of food, in particular to sweet items. So far, studies have been conducted either on the immediate gustatory effects of NNS on reward-related neural activity, or on behavioral outcomes. However, a link between these two is missing. Are modulations in perceptual responses to NNS subserved by specific brain areas linked to subsequent eating behavior and food choices? These perceptive responses might well be observed when investigating visually tempting food cues

subsequent to NNS ingestion, as a major part of intake decisions in humans are dictated by the eye [5,16]. For this reason, it is essential to better understand if and how NNS modulate visual food perception, as well as the subsequent spontaneous food intake.

1.5 Aim of the thesis at hand

Altogether, the results discussed in section 1.4 highlight the strong need for interdisciplinary studies to better understand how food perception and choices guided by visual food cues are influenced by the consumption of sugars and their non-caloric substitutes. Although the general functioning of the central system regulating food intake is quite well described in the visual modality, studies specifically investigating the effects of sugar or sugar-substitute ingestion, are so far rather restricted to the gustatory modality. The thesis at hand aims at a better understanding of the impact of sugar and sugar-substitute consumption on visual food perception and intake behavior by combining various methodological approaches integrating behavioral, brain and physiological assessments. The thesis consists of two projects:

Study A – ‘BOISSON’ – This exploratory project investigated changes in brain responses to food viewing via a 3-month replacement of SSBs with ASBs, and the associations between diet-induced brain response modulations and changes in food appreciation as well as body weight. The candidate contribution to this study is available in chapter 3.1 and the published article in appendix 7.1.

Study B – ‘SUGART’ – This randomized controlled clinical trial investigated the acute effects of caloric (sucrose) and non-caloric (NNS) beverage consumption on the subsequent brain responses to food viewing and later spontaneous food intake at an *ad libitum* buffet, as well as their interplay with individuals’ hormonal profiles. The candidate contribution to this study is available in chapter 3.2 and the published article in appendix 7.2.

For both studies, electroencephalography (EEG) was used as a neuro-imaging method assessing brain responses to food viewing as a function of study conditions. In study B, an *ad libitum* buffet methodology served to assess spontaneous food intake. Both the general EEG and buffet methodology are explained in chapter 2. A general discussion of published papers and take-home messages are available in chapter 4.

2 METHODOLOGY

In the projects of the thesis at hand, several methodological approaches were combined to investigate brain responses to food viewing as well as their interplay with hormonal signals and food intake behavior measurements. In this chapter, I first provide an overview of the food image database used for studies A and B as well as online behavioral tasks used during the assessments of neural activity to food viewing. In a second step, I describe the electroencephalography (EEG) technique and global electrical neuroimaging approach serving to measure the spatio-temporal brain dynamics to the viewing of foods, as a function of experimental conditions of interest such as food category, nutritive state or beverage consumption. Third, I describe the *ad libitum* buffet setting conducted for study B in order to measure quantitative and qualitative aspects of spontaneous food intake behavior. Further individual details can be found in published manuscripts in appendices 7.1 and 7.2.

2.1 Image database and online behavioral tasks

In the studies conducted for this thesis, visual stimuli were displayed to the volunteer while their EEG was continuously recorded. The image database used for studies A and B consists of color photographs of food and non-food items. All items were placed in a white plate with an identical blue background. All photographs were taken from an identical top-view angle and measured 300 x 300 pixels [19]. In order to control for biases in neural activity due to the perception of low-level visual features, all images were also carefully controlled for luminance and spatial frequency spectra [208]. Non-food object images used for study A consisted of kitchen utensils, and were relevant only for the online behavioral task. In study B, only food images were presented to participants.

The food images presented in studies A and B contained a balanced number of food images from 4 categories based on their fat content and taste quality, i.e. Low-Fat/Non-Sweet (LF/NSW), Low-Fat/Sweet (LF/SW), High-Fat/Non-Sweet (HF/NSW), and High-Fat/Sweet (HF/SW). The rationale for the definition of these categories was that fat content and taste are key dimensions associated with palatability, drives towards foods and overconsumption [47]. The category division in terms of fat content (cut-off threshold set at 10g of fat per 100g of food) was determined using the nutrition database of the United States Department of Agriculture (www.nal.usda.gov/fnic) and the Swiss nutritional database released by the Swiss Federal Office of Health and the Swiss Federal Institute of Technology of Zürich. The fat content of low-fat food items ranged from 0 (e.g. pomegranate) to 5g (e.g. trout) of fat per 100g of food (mean fat content \pm SEM = 0.89 ± 0.13 g), and from 10.68 (e.g. olives)

to 81.10g (e.g. butter) of fat per 100g of food for high-fat items (mean fat content \pm SEM = 27.12 ± 1.39 g). Perceived vs. actual fat content has been assessed in a group of 19 normal-weight participants and was found to be highly correlated [19]. Low-fat and high-fat food items were subdivided based on their taste quality: the perceived sweetness of all items was checked by means of a continuous visual analog scale by 13 normal-weight volunteers, and was found to strongly differ between non-sweet and sweet items ($p < 0.05$; unpublished data). Also, valence and arousal ratings were obtained for each food image by means of 7-point Likert scales, and carefully checked for balance between food categories in order to avoid perception bias.

All EEG recordings were accompanied by an online behavioral task, to engage participants' attention and focus on the visual stimuli. Figure 3 shows an exemplary trial for each study. In study A, participants performed an orthogonal categorization task, i.e. they discriminated food from non-food images by means of button press (Figure 3A). In this study, participants remained uninformed about food subcategorization. Thus, this procedure allowed that volunteers remained naïve towards the main goal of the study, which was to compare variations in brain responses according to the fat content and taste quality of the viewed foods [19].

In study B, participants performed a continuous recognition task [209,210]. Half of the food images presented were repeated after a certain interval, and participants had to keep the images in mind to discriminate initial from repeated encounters by button press (Figure 3B). The number of repetitions and interval lengths between initial and repeated encounters (in number of trials) were carefully controlled, to ensure similarity of task difficulty between food categories, blocks of presentations and nutritional states. For both tasks, inter-stimulus-intervals (ISI) randomly varied between 250 and 750ms, in order to avoid anticipatory responses, and a fixation cross was displayed on the screen to avoid eye movements.

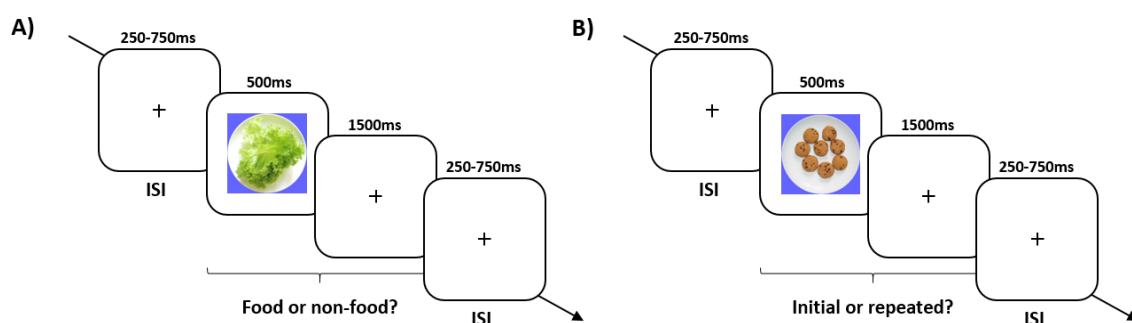


Figure 3: Trial design for the online behavioral tasks. (A) Participants performed a food vs. non-food image categorization task. **(B)** Participants performed a continuous recognition task. Image presentation lasted for 500ms. Participants gave their answer within 2 seconds following image onset. Trial were separated by an Inter-Stimulus-Interval (ISI) varying in duration between 250 and 750ms.

2.2 EEG methodology: assessment of brain responses to food

We used EEG and electrical neuroimaging analyses to assess spatio-temporal brain dynamics to the viewing of food images, while participants performed an online behavioral task. EEG is a technique for recording head-surface neuronal activity used since 1920/1930, mostly for clinical investigations on sleep and epilepsy at the time. In many research domains, EEG is nowadays used as an electrical neuroimaging technique, thanks to high-density electrode montages and powerful algorithms enabling multi-stratified data analyses and the reconstruction of brain generators underlying head-surface electrical activity [211,212]. Most neuroimaging studies in the domain of visual and gustatory food perception are so far performed with functional Magnetic Resonance Imaging (fMRI), relying on the relatively slow coupling between hemodynamics and changes in neural activity. Yet, EEG enables recording of the brain electrical activity from multiple scalp locations with a temporal resolution at the order of the millisecond [213]. Therefore, EEG offers the advantage of discriminating and disentangling distinct early and later steps in sensory and cognitive processing, since it directly measures summations of real-time electrical post-synaptic neuronal activity. Moreover, this low-cost technique is easy to use for studies at bedside, and thus confers practical advantages to the setup of interdisciplinary projects investigating behavioral and physiological parameters in parallel. The approach used here has been successfully applied in several studies of spatio-temporal brain dynamics to visual or auditory stimulations (e.g. [19,209,214,215]).

2.2.1 From EEG recordings to local and global measures of the electric field

In our studies, EEG signal was continuously recorded while visual stimuli were displayed to participants (Figure 4A). Yet, neural responses to one given stimulus (in our case, a food or non-food image) are low in amplitude, as compared to the surrounding electrical noise and activity elicited by face muscle contraction, also recorded by head-surface electrodes. Moreover, the neural response to an individual stimulus is embedded into the stream of ongoing brain activity. Therefore, stimuli have to be repeated several times during EEG recordings in order to increase signal-to-noise ratio.

Following EEG recordings, data are subject to several processing steps. Upon averaging of the repeated stimuli presentations, raw data are filtered and corrected to a pre-stimulus baseline, to dampen the influence of surrounding electrical noise (emitting at specific frequencies), and to correct for drifts in amplitude at baseline within or between participants. Trials are also inspected with a semi-automatic procedure aiming at detecting aberrant electrical activity, i.e. strong amplitude associated with muscular activity such as eye blinking or jaw squeezing, and recalculated to the average reference. The

time-locked waveform obtained at each electrode location by averaging all trials of one condition of interest is called an Event-Related Potential (ERP), or a Visual Evoked Potential (VEP) in the case of visual stimuli (Figure 4A).

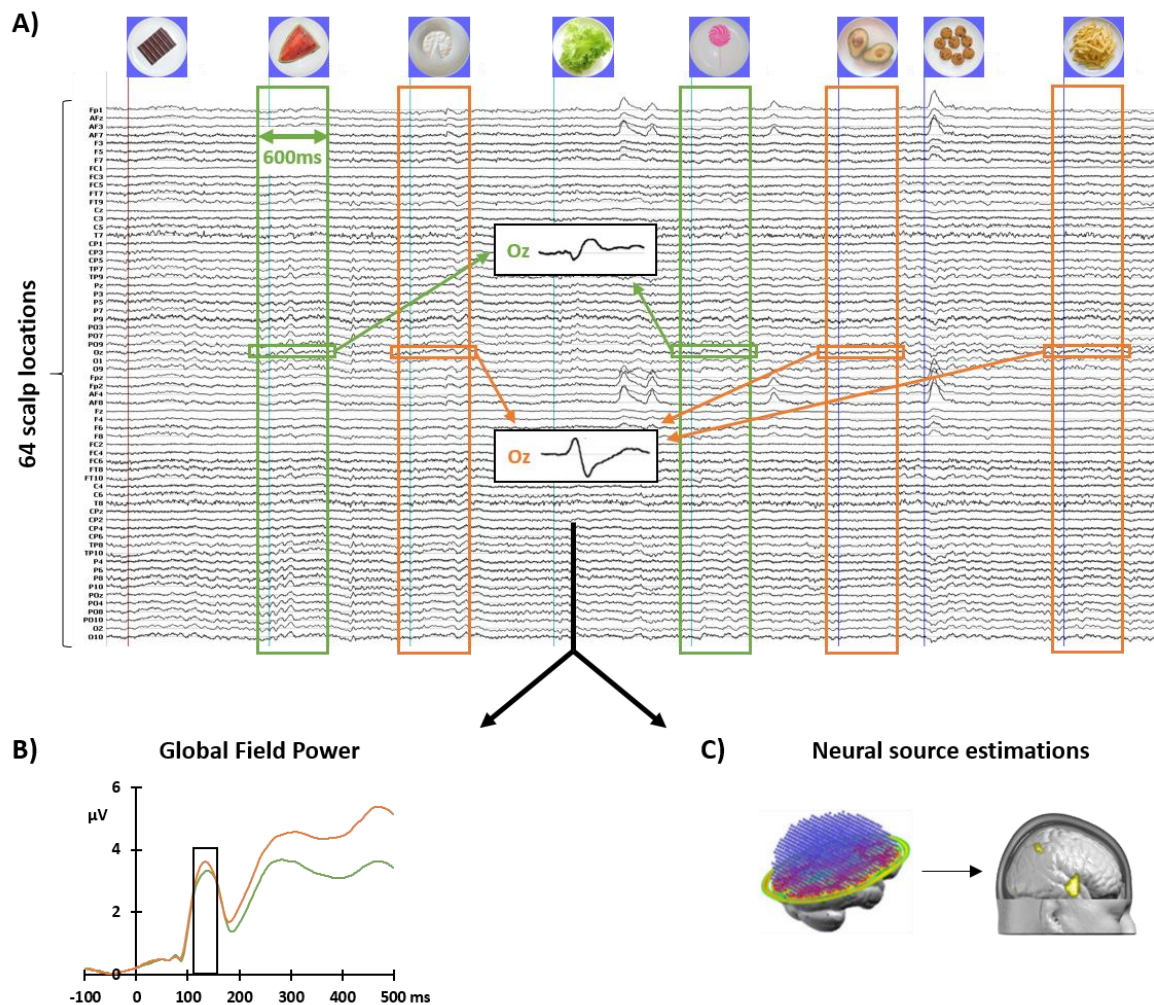


Figure 4: Electroencephalographic (EEG) data acquisition and electrical neuroimaging approach. (A) Display of continuous EEG traces from 64 electrodes (scalp locations) over time. Stimuli (food images) are presented to participants on a screen, with inter-stimulus-intervals varying in duration. The trial averaging procedure to obtain Event-Related Potentials (ERPs) is shown here as an example for one electrode location (Oz), across two different food categories (green and orange peri-stimulus boxes). **(B)** The Global Field Power (GFP) waveform is obtained from the computation of ERPs from all electrode locations over time. Here, exemplar GFP waveforms are shown for the 'green' and the 'orange' food category viewing from (A). A square box indicates a time window of interest determined using the first GFP peak. **(C)** Neural sources can be estimated from the signal recorded at the head-surface using powerful mathematical algorithms called inverse solution models.

ERP waveforms are often named by their components' characteristics, i.e. positive (P) or negative (N) polarity and their latencies post-stimulus onset. In the case of responses to food viewing, as for other biologically salient stimuli, critical components of ERPs are the P1 (around 100ms) and N1 (around 170ms), recorded at lateral temporo-occipital sites, and the P300, recorded from multiple locations on the scalp [216,217]. Whereas the P1 and N1 are thought to rather reflect visual sensory, categorization and attentional processing, the P300 has been involved in several various aspects of cognitive and

emotional processing such as motivation or salience and reward valuation [218]. In addition, the N2 component, recorded from fronto-central sites around 200-300ms, is thought to reflect the level of cognitive control exerted upon exposure to visual food cues [23,219].

Classical analysis approaches of ERP data consist in contrasting amplitudes and latencies of these ERP components to analyze how and when conditions of interest differ. While these techniques have enabled scientists to reveal numerous differences between experimental conditions, they are restricted to locally occurring differences between experimental conditions, and thus cannot provide information on global changes in brain activity, neither disentangle which brain areas underlie the head-surface signal variations. Such 'local' ERP approaches also bear the disadvantage of being subject to bias due to experimenter choice of the electrode location picked for subsequent analyses, and are reference-dependent. That is, the local waveforms (ERPs) recorded at specific electrode locations will vary in amplitude and polarity depending on the location of the reference electrode, e.g. mastoid or average, rendering comparisons between studies using different electrode montages difficult. Yet, the use of high-density electrode montages and software developments have enabled researchers to approach ERP data with more global measures of the electric field, both in terms of signal strength (amplitude) and localization in space (topographic maps and neural source estimates). These global measures have the advantage of being reference-independent, more easily interpretable in terms of neurophysiological mechanisms, and not subject to experimenter's choice bias [211,212,220,221]. Both global measures used in studies A and B of this thesis are described in the following sections.

2.2.2 Global Field Power

The Global Field Power (GFP) is a reference-independent measure of the global strength (i.e. amplitude) of ERPs over the time period peri-stimulus onset (Figure 4B). This measure captures the standard deviation across all electrodes recorded from at a given time point, and is expressed in microvolts (μV). Mathematically, GFP is calculated as the root mean square across the average-referenced electrode values at each time point [211,222]. The GFP gives indications on the amplitude of the signal (or differences in signal amplitude between experimental conditions), but not in terms of spatial localization of the signal. Therefore, the GFP is representative of the amount of synchronized neural activity over time. That is, time periods of stable topography and highest synchrony between neural sources yield strong GFP values, likely indicating 'stable' steps in sensory and cognitive processes. In studies A and B, GFP peaks were used as a means of data reduction, for the definition of time windows of interest serving to investigate the underlying neural source activity [19,223].

2.2.3 Estimation of neural sources

Thanks to the usage of high-density electrode montages, EEG has become a tool for electrical neuroimaging aiming at investigating variations of neural activity in specific brain areas (Figure 4C). The use of neural source reconstruction tools has also enabled many parallels in data interpretation between fMRI and EEG studies. These investigations of differences in neural activity are possible by the use of mathematical algorithms modeling the electrical activity recorded at the head-surface into a 3D estimation of the underlying neural generators. In my thesis, neural sources were estimated with a linear inverse solution model [220,224]. This model takes into account the recorded electrical head-surface signal as a 64-dimension vector (i.e. number of dimensions determined by the number of channels present in the recording system), calculates the putative underlying sources and renders the results on a 3D brain template from the Montreal Neurological Institute (MNI). The result of such computation therefore shows the estimated neural activity (current density values expressed in $\mu\text{A}/\text{mm}^3$) for each node of a X-solution point matrix representing the 3D-gray matter of a realistic head model of the human brain [212,221,225]. In my thesis, we made use of the local autoregressive average (LAURA) distributed linear inversion solution algorithm, and used inverse solution spaces giving an approximate spatial resolution of $6\times 6\times 6\text{ mm}^3$ [224].

In our studies, GFP peaks served to determine time periods of interest following the onset of the food image viewing. In turn, these time periods were used as temporal constraints for the estimation of underlying neural sources. For this purpose, individuals' values recorded at each electrode were first averaged over the time interval, and neural sources were then estimated over time periods. These 3D maps of neural source estimations in response to food image viewing can then be contrasted between experimental conditions to delineate in which brain areas activity differs as a function of the factors of interest.

In studies A and B, we were interested in the changes in brain activity from a 'pre' state to a 'post' state, as a function of the beverage consumed, or the food category viewed. That is, in study A, the 'pre' vs. 'post' states represented individual measures before and after a 3-month diet intervention, whereas in study B, the 'pre' vs. 'post' states represented individual measures in pre- and post-prandial state. Relative changes from before to after an intervention, rather than absolute values in pre- vs. post-intervention state, are frequently assessed for various physiological, behavioral and psychological parameters of interest. This approach enables intra-individual standardization by individuals' baseline values, i.e. a 'control' condition. Such methodology was not available for EEG data, yet of great interest for assessing pre- vs. post-conditions in both studies A and B. In collaboration with Jean-François Knebel and Marie-Laure Notter-Bielser from the Laboratory of Investigative Neurophysiology, I therefore developed a methodology enabling the analyses of relative change in individuals' EEG data

(i.e. in particular for estimations of changes in neural source activity), for crossover experimental designs (Figure 5). This computation of relative changes in brain activity from a 'pre' to a 'post' state consists first in calculating the matrix representative of the difference in neural activity ('post' matrix minus 'pre' matrix). In other words, it calculates the difference in electrical activity at each node of the inverse solution model. In a second step, this difference matrix is divided by the averaged 'pre' matrix presenting the baseline activity. The outcome of this computation are % pre-to-post change for each node of the matrix, indicative of the increment or decrement in neural activity from one state to the other. Please note that for the purpose of computations of neural changes, the difference matrix had to be divided by the baseline activity averaged across all nodes of the inverse solution matrix (i.e. one 'node' representing the whole-brain activity; Figure 5B), in order to avoid fluctuations in statistical variance between neighboring source nodes (Figure 5A).

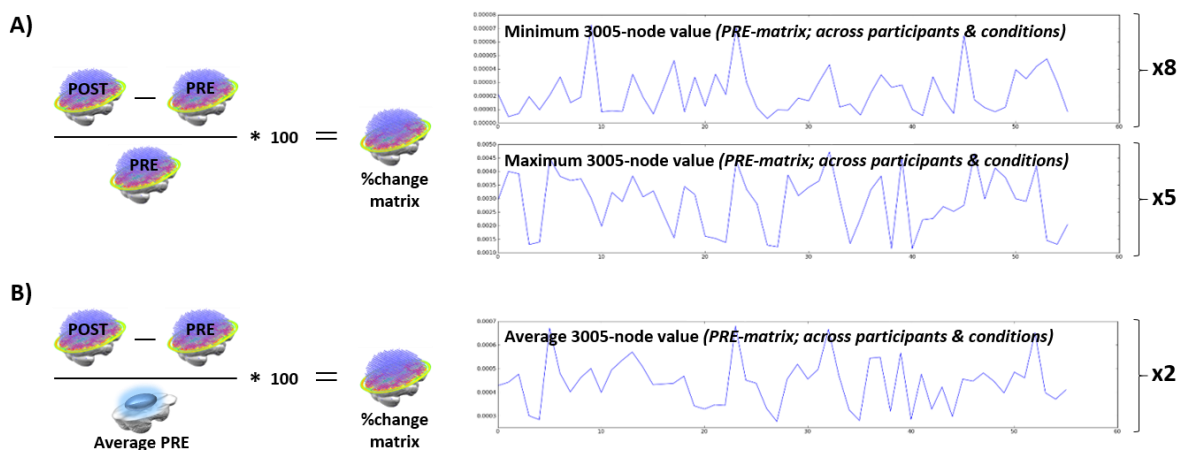


Figure 5: Method development for the computation of matrices representing the relative (%) change in neural activity from a 'pre' state to a 'post' state. A matrix of increment/decrement in neural activity is calculated by first subtracting the 'pre' activity matrix to the 'post' activity matrix, and by secondly dividing the obtained 'difference' matrix by the 'pre' matrix (left panels of (A) and (B)). **(A)** When the %change matrix is computed using a node-by-node division procedure, neighboring nodes can yield increment/decrement values on a wide range. An exemplar distribution of minimal and maximal values (differing by a factor 8 and 5, respectively) is shown on the right. This wide value distribution prevents statistical models to be used for reliable whole-brain contrasts between experimental conditions. **(B)** When the %change matrix is computed using an averaged 'pre' matrix (i.e. all nodes yielding the whole-brain average value), neighboring nodes yield increment/decrement value on a more restricted range. This procedure enables whole-brain statistical models to compute reliable contrasts across neighboring source nodes. The data used for this example are from study A.

The methodology developed here has been used, in the context of my thesis, for EEG data of both studies A and B, but also for other experimental protocols conducted at the Laboratory for Investigative Neurophysiology.

2.3 Buffet methodology: assessment of spontaneous food intake

In addition to investigating modulations of brain responses to food viewing, relating these changes to behavioral measures of food liking, intake and ultimately body weight management is a major goal of our studies. In order to assess appetite control and associated food intake, most research performed in laboratory settings relies on indirect markers of food intake behavior, such as subjective hunger, satiation or satiety ratings, appetite-related plasma hormone concentrations (e.g. insulin, ghrelin, GLP-1 or PYY), neural activity to food cues alone, or behavioral parameters collected during a specific food-related behavioral task [226-228]. While these are important to understand mechanisms underlying food choices, they do not assess spontaneous food intake behavior *per se* and are thus limited in their interpretation for result translation to a real setting.

In laboratory settings, spontaneous food intake can be assessed either by single test meal studies. These paradigms serve to assess the influence of variables such as diet intervention on food intake in terms of quantity, and are relatively simple to implement and interpret [229]. However, single test meal studies cannot assess food intake in terms of qualitative aspects (i.e. food choices). That is, participants choose to take a second or third serve of a single given meal until feeling comfortably sated, but do not have the choice of consuming other food or meal types. Sensory-specific satiety, the psycho-physiological phenomenon causing pleasantness ratings to decrease and one to feel sated for a specific food but not for others, can refrain eating and lead to under-estimated food intake in single test meal studies. Indeed, participants may stop eating, yet would have ingested more energy from other sources if exposed to a broader choice, by shifting to other food types differing in taste quality, fat content, or texture [230,231].

Buffet studies, on the other hand, enable to assess both quantitative and qualitative aspects of food intake behavior. By presenting several food items to overcome sensory-specific satiety limitations, buffet studies performed in laboratory settings aim at matching as close as possible the variety of choice in a real-world setting [232-235]. In study B, we therefore introduced an *ad libitum* buffet meal to directly assess spontaneous food intake and choices as a function of the beverage condition.

All buffets were set in the kitchen of the Clinical Research Center of the Lausanne University Hospital (Metabolism, Nutrition and Physical Activity unit), to ensure context similarity and avoid as much as possible any influence of a changing environment. Snack presentation was kept similar between conditions and volunteers, in terms of dishes used (white plates and bowls), dish position on the table, quantities available, and preparation (e.g. vegetables cut into sticks) [232,236,237]. Participants' potential questions were answered in terms of snack identity, but never in terms of nutritional information. In addition to the contextual influences, social interactions also modulate food intake

behavior (e.g. [238]; reviewed in [236]). Therefore, presence and absence of the experimenter with participants was carefully controlled, i.e. participants were accompanied by the experimenter or left alone alternatively for periods of 5 minutes, until indicating by themselves to have finished eating. To avoid further cognitive biases, participants remained uninformed of their intake being measured. Real-life conditions were also matched in terms of day timing, i.e. the buffet was presented to participants around 1.30pm, to serve as their lunch. A display of the buffet as presented to volunteers in study B is shown in Figure 6.



Figure 6: Buffet display used in study B. The ad libitum buffet meal type served to assess participants' spontaneous food intake as a function of the beverage condition (sucrose, NNS, or water).

The buffet contained 12 snacks from 4 food categories. Snacks were served cold, and consisted of items easily found in commercial stores all year round, to ensure similarity of nutritive content and presentation throughout the study duration. The rationale for dividing food categories based on fat content and taste quality was based on the food image database used for EEG recordings (see chapter 2.1). That is, a cut-off threshold was set at 10g of fat per 100g of food for the sorting into Low-Fat vs. High-Fat category. Low- and High-Fat snacks were again subdivided according to taste quality. As for the food image database used for online behavioral tasks, sweet taste, rather than simple sugar content, was used as a subdivision criteria, to ensure a closer match with daily food representations. The *ad libitum* buffet thus consisted of 3 snacks of each of the following food categories: LF/NSW (crackers, natural yoghurt and vegetable sticks), LF/SW (cereals, sugar and fruit salad), HF/NSW (crisps, guacamole and cheese), and HF/SW (biscuits, vanilla cream and chocolate) (Figure 6). In addition, snacks were chosen to ensure as much as possible similarity of texture across categories, i.e. with at least one crispy snack and one semi-liquid snack. Each snack was available in greater quantities than the expected average intake [228]. All snacks were carefully weighed before setting up the buffet, and all leftovers were weighed after consumption.

3 RESULTS

3.1 Study A – ‘BOISSON’

The impact of replacing sugar- by artificially sweetened beverages on brain and behavioral responses to food viewing – An exploratory study

Authors: Camille Crézé, Marie-Laure Notter-Bielsler, Jean-François Knebel, Vanessa Campos, Luc Tappy, Micah M. Murray, Ulrike Toepel.

Accepted in *Appetite*, 2017 Dec 15. To be found in appendix 7.1.

Contribution: The candidate enrolled participants, collected and analyzed the data, developed methodology for EEG-related analyses, and prepared the manuscript for submission.

3.2 Study B – ‘SUGART’

The impact of caloric and non-caloric sweeteners on food intake and brain responses to food: a randomized crossover controlled trial in healthy humans.

Authors: Camille Crézé, Laura Candal, Jérémy Cros, Jean-François Knebel, Kevin Seyssel, Nathalie Stefanoni, Philippe Schneiter, Micah M. Murray, Luc Tappy, Ulrike Toepel.

Accepted in *Nutrients – Special issue “The impact of beverages on ingestive behavior”*, 2018 May 10. To be found in appendix 7.2.

Contribution: The candidate conceived and designed the experiments, attained approval by the Ethics Committee, enrolled participants, collected and analyzed the data and prepared the manuscript for submission.

4 GENERAL DISCUSSION

4.1 Result summaries of studies A and B

Although the knowledge on central regulation of food intake has been increasingly described in the last decades, research investigating the effects of sweetened beverage consumption on brain responses to foods is still rather restricted to studies on gustatory perception and consumption effects. Yet, visual food cues are also of crucial importance in guiding food consumption behavior.

My thesis aimed at providing first insights on the impact of sugar and sugar-substitute consumption on brain responses to visual food cues. Both projects used electrical neuroimaging coupled with behavioral measures to assess the spatio-temporal brain dynamics to food viewing. The projects were conducted in an interdisciplinary setting, further aiming to associate modulations in brain responses to food cues with behavioral measures of food intake behavior and individuals' hormonal profiles.

My first project (study A) explored changes in brain and behavioral (i.e. liking) responses to food viewing occurring in parallel with a 3-month reduction of sugar consumption, via the substitution of caloric sweetened beverages (SSBs) by non-caloric equivalents (ASBs). This diet intervention was conducted in frequent SSB consumers, whose BMI was ranging from normal weight to obese.

Over the 3-month diet intervention, most participants did not lose body weight (and even gained). At the level of brain responses, we showed diet-induced modulations in control and attention-related cortices from pre- to post-intervention. These modulations occurred over time windows consistent with previous literature showing categorization, control, attention and valuation-related processing upon exposure to visual food cues [19-21,223]. Therein, modulations in neural activity from pre- to post-intervention were stronger to high-fat, high-sugar palatable foods. Decreased neural activity from pre- to post-intervention was found in dorsal and lateral prefrontal cortices over early brain response latencies post-food image onset (130-160ms). In contrast, neural activity was enhanced from pre- to post-intervention in response to high-fat, high-sugar food viewing in the precentral gyrus over later latencies (280-310ms post-image onset). The early decrease in activity within the DPFPC, associated with control over intake when exposed to cues, was inversely correlated with changes in body weight, i.e. participants who failed to lose weight also showed strongest decrease in control-related neural activity to palatable food cues. In parallel, we observed an opposite tendency for modulations in behavioral liking ratings of sweet vs. non-sweet foods. Likings for sweet foods tended to decrease,

while ratings for non-sweet foods tended to increase from pre- to post-intervention. These latter results however failed to reach statistical significance.

Altogether, our findings highlight modulations in brain responses to the viewing of palatable energy-dense foods by beverage replacement, likely reflecting impaired control as well as enhanced attention towards tempting food cues. Findings interpretation is yet limited, as spontaneous daily food intake besides the beverage intervention was not reliably monitored. Moreover, the correlation found between modulations in DPFC activity and body weight does not imply causality, nor direction of impact. However, findings do point towards the importance of monitoring and better understanding modulations in central food intake regulation, particularly in control and attention-related brain areas, as these are likely associated with inter-individual variability in food intake choices and body weight management success in the context of nutrition interventions targeting sweetened beverages.

As we were not able to disentangle effects of reducing SSBs from those of adding NNS-sweetened beverages to participants' diet in study A, study B was particularly planned to compare the impact of NNS- vs. sucrose-sweetened beverages (and water) on brain responses to visual food cues and associated food intake behavior. This was investigated on an acute basis (i.e. a one-point beverage consumption), as a randomized crossover controlled trial conducted on normal-weight individuals. Participants' brain responses and hormonal profiles were studied under three conditions, i.e. before and after either consuming water, sucrose-, or NNS-sweetened beverages, always together with a standardized breakfast. Their subsequent spontaneous food intake was assessed by means of an *ad libitum* buffet meal.

Results showed that breakfast intake together with water consumption (the control condition in this study) led to increased post-prandial neural activity to visual food cues within DLPFC and insular cortex over later latencies in cue processing (250-320ms post-image onset). Sucrose consumption also led to increased post-prandial insular activity to food viewing (250-320ms), but suppressed post-prandial modulations in prefrontal cortices. In addition, sucrose consumption led to decreased neural activity to food viewing in the middle temporal cortex (250-320ms). This differential pattern of post-prandial neural activity to food was associated with an elevated glycaemia and insulinemia, and stronger decrease in plasma ghrelin concentrations. These modulations were also associated to subsequently decreased food intake. Altogether, this suggests that an activation of sweet taste receptors coupled to subsequent caloric input leads, as compared to water, to differential recruitment of executive functions and reward valuation when tempting food cues are encountered and regulation of subsequent compensatory behavior. The differential recruitment of brain areas could occur through modulations in appetite-related hormone signaling, but also via nervous afferents or direct receptor

activation. In contrast to sucrose, NNS consumption (i.e. the beverage condition with an incongruence between sensory signaling and physiological properties) did not alter spontaneous food intake when compared to water, but altered postprandial brain responses to visual food cues, most pronounced in prefrontal areas and insular cortex. In particular, NNS consumption led to increased neural activity in the VLPFC over early latencies in food cue processing (120-150ms), and suppressed post-prandial response to food viewing within the insula over later latencies (250-320ms). While VLPFC activity is associated with impulse control when exposed to tempting cues, insular activity is associated with nutrient-flavor conditioning. Modulations observed following NNS consumption could thus reflect an early stage of adaptation to taste-calorie uncoupling, likely impacting behavior with repeated consumption on the long run. Overall, qualitative analyses of food choices at the *ad libitum* buffet did not reveal modulations of participants' food intake pattern as a function of beverage conditions. In other words, only the quantity, but qualitative choices, were different in response to sucrose consumption as compared to both other beverage conditions.

Altogether, the thesis at hand provides insights on the impact of caloric and non-caloric sweetener consumption on the visual perception of tempting food cues. This is of particular relevance in our modern environment where visual cues are ubiquitous and guide consumption behavior in daily life. In addition, the conducted projects complement the existing literature on behavioral and brain responses to food in the gustatory modality regarding potential consequences of NNS consumption by providing insights into key brain regions involved in the pre-ingestive visual regulation of food intake behavior. In the following sections, I first emphasize the importance of findings on key brain areas in light of the current literature. Next, two main lines of study perspectives regarding the longer-term impact of NNS on brain responses to food and intake behavior are discussed, i.e. 'bottom-up' vs 'top-down' effects. Finally, I propose an exemplar protocol for further conducting research investigating these two lines in parallel.

4.2 Key areas potentially impacted by longer-term NNS consumption

NNS consumption in particular impacted the VLPFC, insula and DLPFC as brain regions. The VLPFC functions as part of the executive control network, and activity within this brain area has been associated to impulse retaining (i.e. inhibiting pre-potent responses), and reversal learning in the context of behavioral adjustments for reward valuation, as discussed in the general introduction chapter. In the literature investigating the effects of sugar and NNS beverage consumption on brain

responses to food, increased VLPFC activity has been observed in acute responses to non-caloric sweet taste (sucralose and saccharin), as compared to caloric sweet taste [189,239]. In study B of my thesis, we observed enhanced VLPFC activity to the post-prandial viewing of solid foods. That is, this increased VLPFC activity in response to NNS might not only reflect immediate taste-related processing, but also those of more general features of food-related stimuli. Whether increased VLPFC activity in this context reflects a stronger need for impulse retaining, or reversal learning processes in the light of sweet-taste calorie uncoupling (i.e. mismatch as compared to usually learnt associations) remains to be investigated [83,240]. For example, targeted studies would benefit from behavioral paradigms investigating executive functions along with sensory processing and valuation, such as go/no-go, delayed reward tasks and pleasantness ratings.

By contrast, decreased VLPFC activity in response to food cues has been observed in paradigms investigating repeated consumption of particular food products. Lesser activity of the VLPFC to tasting sugar-sweetened soft drink ('anticipated intake') has been observed in excessive soft drink consumers when compared to non-consumers [90]. In study A, we further showed decreased VLPFC activity to food image viewing when SSBs were replaced by NNS-sweetened equivalents. The decrease in VLPFC activity to food-related cues could therefore be generalizable to repeated consumption of any product, rather than specific to sugar- or artificially-sweetened beverages [91]. In line with this hypothesis, Bruce and colleagues [241] reported decreased VLPFC activity in obese as compared to lean individuals in response to branded food logos (vs. non-food logos). The authors interpreted this finding as increased vulnerability to advertisement, and question whether the observed effects could be extended to food exposure in general. Altogether, previous findings as well as those reported in my thesis rather point to the idea that consumption leads, in a first step, to strong VLPFC activity to cope with impulse retaining (when exposed to tempting cues either via food tasting, or via food image viewing). In a second step, the repeated consumption of NNS-sweetened beverages over time might then induce a 'fading' of VLPFC activity in response to food cues, associated with vulnerability to tempting cues and impaired control over food intake in general.

A second key region showing modulated food responsiveness is the insula. The insular cortex is located at medial surface of the fronto-temporal lobe and presents the interface between the salience valuation and executive function networks. The insula has been shown to serve in various functions, from homeostatic and reward valuation, working memory, to cognitive and attention control over pre-ingestive food cue exposure [72,103]. In light of its role in gustatory processing but also in interoceptive and homeostatic signal integration, the insula has been proposed as a key brain region performing nutrient-flavor conditioning [240]. This is in accordance with its revised role proposed recently, i.e. the insula 'capturing' the salience and biological relevance of perceived stimuli in order to recruit higher-

order cortical areas important for control-, attention- and memory-related functions [72]. In particular in study B of my thesis, we found suppressed post-prandial insular activity in response to visual food cues, following NNS in contrast to sucrose and water consumption. Van der Laan and colleagues [17] proposed in a meta-analysis that the activation of the insular cortex in response to food viewing might relate to memory retrieval of an expected taste. NNS would thus disrupt such learned associations between the perception of food images and the taste expected by dissociating sweet taste from metabolic consequences. In line, two neuroimaging studies have shown a negative correlation between NNS use and insular activity in response to sweet taste [107,239]. These yet used a cross-sectional design, such that longitudinal trials investigating the progressive changes in brain activity occurring along repeated NNS consumption are still needed to validate these hypotheses. The insula is also thought to play a key role in mediating the effects of NNS due to its strong interplay with peripheral hormone sensing [44]. We did not observe differences in post-prandial plasma hormone concentrations (ghrelin, insulin, GLP-1) between water and NNS consumption in study B. Yet, the two beverage conditions forcibly activated sweet taste receptors differently. Thus, the assumed uncoupling between taste and calories under repeated NNS consumption could, at least in part, be related to changes in insular responsiveness to food cues via the sensing of mismatching hormonal- vs. receptor-related information.

Collective modulations in VLPFC and insula responses have been shown by Stewart and colleagues [242], i.e. an effect of reward reinforcement learning and memory retrieval. However, this study did not investigate responses to food cues, but relapsed vs. abstinent patients towards methamphetamine dependence. In particular, the authors reported stronger VLPFC activity combined with attenuated insular (and striatal) activity in response to feedback learning in relapsed methamphetamine dependent vs. abstinent patients, reflecting impairments in associative learning processes. Whether NNS, by their suspected impact on taste-calorie uncoupling, could lead to similar ‘impairments’ in learning and memory processes, likely impacting food intake regulation subsequently, remains to be investigated.

A third key brain region found impacted by the consumption of NNS was the D(L)PFC. In study A, the 3-month replacement of SSBs by NNS-sweetened beverages led to decreased activity within the DPFC in response to the viewing of palatable foods, associated with poor body weight management over the study duration. In study B, drinking water elicited stronger post-prandial activity to visual food cues, whereas NNS (and sucrose) consumption dampened these post-prandial responses. Stronger recruitment of the D(L)PFC when exposed to tempting food cues has consistently been associated with better longer-term body weight management, coping with abundant food offers and choices, and less impulsive behavior towards food [4]. Therefore, one could speculate that decreased or lacking DPFC

activation to food cues observed in both studies here could be associated with poorer food choices when NNS are repeatedly consumed, and difficulties to regulate body weight.

In light of previous findings on brain responses to food tasting or intake, the projects conducted here emphasize the key role of lateral dorsal- and ventral-prefrontal cortices as well as the insular region in the regulation of adequate subsequent food intake behavior. Further acute, cross-sectional and longitudinal trials investigating the impact of sugar and NNS consumption on brain responses to food would also benefit from connectivity analyses between those regions. Such would help to better understand causal relations and the role of the timing of neural modulations. Also, combining the fine temporal resolution from EEG recordings together the spatial resolution of fMRI protocols might provide more precise insights into the direct or indirect connections (e.g. via forebrain reward centers) of those key areas.

4.3 Impact of NNS consumption on ‘bottom-up’ vs. ‘top-down’ mechanisms of food intake regulation – Perspectives

In the preceding sections, I presented results of studies A and B in the light of current literature. Study A highlighted modulations in brain responses to the viewing of palatable foods after a 3-month replacement of SSBs by NNS-sweetened equivalents. In this study, participants consumed the assigned ASBs knowing their identity, and their solid food intake besides the beverage consumption was left *ad libitum* [172]. Modulations observed at the brain level likely reflect a decrease in cognitive control and could be indicative of a compensatory behavior underlying weight loss failure over the duration of the intervention. However, study results do not necessarily imply causality, neither do they inform on possible direction of effects. That is, NNS consumption could have acted on brain responses to food viewing, which in turn impacted participants’ daily food intake habits on the one hand. On the other hand, progressive changes in daily solid food intake habits due to expectations on the benefits of consuming NNS could have driven changes in pre-ingestive food perception (brain responses to food viewing). The first ‘directionality’ would rather indicate ‘bottom-up’ effects of NNS consumption on brain responses and behavior to food, whereas the second ‘directionality’ would rather imply ‘top-down’ modulations by NNS consumption.

In study B, participants were blinded to the beverage they drank (in particular between sucrose and NNS beverages), and therefore only potential ‘bottom-up’ influences of NNS on brain responses and behavior were investigated. In particular, the study was conducted so that participants had no expectations on subsequent effects of NNS consumption. Results indicate that modulations in post-prandial responses to food viewing following NNS consumption were different from those induced by

the consumption of water and sucrose, yet they were not accompanied by decreased immediate subsequent food intake. In the following, I will discuss both potential mechanisms of action and associated research perspectives.

4.3.1 'Bottom-up' modulations of responses to food by NNS

'Bottom-up' modulations on higher-order responses are usually considered to be driven by lower-order signals, such as peripheral signaling from nervous afferents or hormones to the hypothalamus, insula or deep-brain reward centers. 'Bottom-up' actions are also mechanisms by which mesolimbic (reward) or homeostatic signals influence higher-order cortical areas performing decision-making, attention, control or working-memory (reflected mainly by prefrontal and parietal activity) [2]. In the research on NNS consumption effects, these processes would thus typically indicate 'molecular' effects of NNS on brain responses to (pre-ingestive) food perception, i.e. the NNS compound action on brain activity, in turn impacting food perception, choices and intake behavior. A likely target brain area impacted by such progressive dissociations of learned associations (taste-calorie uncoupling) is the insular cortex, as this area is thought to perform nutrient-flavor conditioning [240]. Typically, studies conducted in animal models explore possible 'bottom-up' mechanisms by which NNS might impair longer-term food choices and body weight management [201-203]. In humans, findings from a few studies are in line with this hypothesis. Appleton & Blundell [243] observed a lack of appetite increase in response to sweet taste in non-frequent NNS consumers as compared to frequent NNS consumers. Another study by Rudenga & Small [107] showed a negative correlation between the insula and amygdala responses to sweet taste and participants' habitual NNS consumption. They interpreted these findings as a progressive 'fading' of the response to sweet taste as a function of daily NNS use, indicative of taste-calorie uncoupling processes. Green & Murphy [239] further highlighted differences in caloric and non-caloric sweet taste perception in non-diet soda drinkers, that were suppressed in habitual soda drinkers. This indicated an altered processing of sweet taste as a function of NNS consumption. All of these studies used blinded sugar and NNS consumption, thus forcibly highlighting 'bottom-up' mechanisms of action. In study B of my thesis, results might highlight initial 'bottom-up' action of NNS, i.e. modulations in neural activity to food without behavioral food intake changes at the subsequent buffet. However, it can only be hypothesized that repeated consumption of NNS in this context would also impact food intake behavior and choices on the longer run.

To further delineate 'bottom-up' mechanisms of NNS action, a first line of perspective is therefore to extend the investigation of NNS effects on (visual) food perception and intake behavior to repeated NNS consumption longitudinally, in a strictly controlled context and with blinded beverage

consumption. Since the design of the aforementioned studies was cross-sectional, the current literature is still lacking longitudinal trials investigating progressive modulations in (visual) food perception occurring along with changes in NNS consumption. That is, longitudinal trials further detailing NNS effects on food perception and intake behavior could either assess the impact of adding a certain amount of NNS-sweetened beverages to participants' daily diet, or the impact of stopping NNS-sweetened beverage consumption in frequent consumers. In other words, participants could either be 'trained' to taste-calorie uncoupling, or 're-trained' to taste-calorie congruency. Such studies would benefit from a controlled setting as used for study B, but with a diet intervention similar to the one used for study A (beverage addition or replacement), yet in blinded condition. This would add insights on effects of NNS consumption on neural activity when exposed to tempting food cues and the relationship with intake behavior strictly driven by the NNS product itself. If NNS consumption is indeed affecting food perception and intake behavior on the long-run via 'bottom-up' mechanisms, participants repeatedly consuming NNS in a blinded context would thus eat more and gain body weight, as a function of the degradation of the predictive relationship between sweet taste and calories, likely as a function of attenuated insula responses to food cues. The decrease in insular activity when exposed to visual tempting food cues might in turn be coupled to increases in food intake motivation via cortico-limbic systems, and decreases in valuation-related activity upon tasting and ingestion (e.g. deep-brain reward centers, anterior cingulate cortex and OFC). These processes should not only be investigated on the level of brain responses, but also combined with changes in hormonal or other signaling (nervous, receptor-coupled), and their combined impact on the subsequent intake behavior *per se*.

A recently published trial conducted in humans tested the impact of a repeated longer-term NNS consumption (12-week intervention) on glycemic control, appetite ratings and body weight in a strictly controlled and blinded context [244]. Participants were randomly assigned to consume 0, 350 or 1050 mg of aspartame per day in parallel-arm design. Surprisingly, this trial showed no difference between groups at post-intervention measurements in any appetite-related ratings, body weight and composition, glycemic control, or any of the appetite-related hormones (insulin, leptin, gastric inhibitory peptide (GIP), GLP-1). The authors did not assess brain responses to food exposure (either in the visual or gustatory modality), yet the clinical trial can be considered as testing 'bottom-up' mechanisms, that showed no effect of aspartame on either of the parameters measured. To my knowledge, this is the first and so far only longitudinal trial investigating NNS consumption effects on appetite-related measures in humans. Further studies will need to replicate these findings (also when using other types of NNS), and possibly investigate the concomitant (absence of) changes in food perception at the brain level.

4.3.2 'Top-down' modulations of responses to food by NNS

The sole investigation of 'bottom-up' processes impacted by NNS consumption strongly narrow the potential influences of NNS on food intake behavior, and do not necessarily reflect 'real-world' conditions in which humans consume NNS. That is, humans, in contrast to animals, knowingly consume NNS, with likely additional effects on food perception, intake behavior and *in fine* body weight management. Mattes & Popkin [245] already discussed this issue of 'top-down' influence of NNS consumption on food intake behavior a decade ago (although they did not use this terminology), when writing that "*the purported problem stems from an inappropriate use of NNS rather than an inherent problem with such products*". In other words, NNS could indeed help with body weight management when consumed in the context of a controlled diet program, but it is not certain that they will effectively be consumed that way, and informed use could lead to overcompensation behaviors. That is, the description of 'top-down' mechanisms, i.e. influences from higher-order cortical areas (associated with choice and decision-making) on lower-order processing in sensory, affective, reward and homeostatic areas [246,247] needs to be considered in the 'human' models of NNS consumption. In the research on NNS consumption effects, these processes would predominantly be driven by expectations that consumers have on NNS-containing products [204]. These comprise body weight management strategies, health issues and the need to exert attention on food choices besides NNS-containing products.

In food perception research in general, several studies have explored and assessed differential brain activation to a given set of food images or tastes varying as a function of the attentional focus, e.g. on taste, reward, health properties, or neutral aspects such as color (e.g. [248]). Ohla and colleagues [22] investigated the impact of expectations raised by visual food cues (high- vs. low-calorie items) on the perception of a subsequent neutral taste. The taste, although always identical and neutral, was perceived as more pleasant when preceded by high-calorie as compared to low-calorie food images. This was coupled to a stronger taste-evoked neural activity in the insula and adjacent frontal operculum at an early response latency (~100ms following taste stimulus onset), and in the anterior cingulate cortex, OFC and insula during later latencies (~360ms).

Another study by Toepel and colleagues [249] investigating the impact of food labeling on visual food perception showed that exposure to labels with positive valence (as opposed to negative and neutral) influenced the subsequent visual perception of high-energy food images, i.e. by a down-regulation of occipital, insular, posterior frontal and anterior cingulate neural sources at ~300ms post-food image onset. The authors interpreted their findings as an impact on cognitive-affective processes, likely

reflecting a decrease in cognitive control and goal-adaptive behavior when exposed to tempting food cues. One study by McClure and colleagues [250] compared anonymous vs. brand-cued delivery of Coke and Pepsi on taste and pleasantness perception. When tasted anonymously, both beverages elicited responses in the OFC that were correlated with pleasantness ratings of the drinks. However, brand knowledge strongly influenced behavioral preference ratings and recruited an additional network composed by the hippocampus, midbrain and DLPFC. Further, Wegman and colleagues [251] tested the impact of food labels on the implicit motivation to obtain rewards. They showed that participants' approach bias in a joystick task was stronger for a beverage labeled as low-calorie (as opposed to the high-calorie labeled version), accompanied with an increased neural response in the sensorimotor cortex. By contrast, the non-preferred beverage labeled as high-calorie elicited stronger neural activity in the insula. This demonstrated implicit biases induced by labeling on the perception of otherwise identical food objects. Interestingly, a study by Ng and colleagues [252] showed that labeling a given milkshake with the mention 'regular' (as compared to the same milkshake labeled as 'low-fat') elicited greater neural activity during tasting and intake in the anterior insula (frontal operculum) and OFC. These results were particularly apparent in obese as compared to normal-weight women, and imply that 'top-down' processing could contribute to weight gain by promoting overeating. Finally, Faulkner and colleagues [253] demonstrated that perceived healthiness of food items led to underestimations of energy density as compared to their standard counterparts, although the so-perceived 'healthy' and 'standard' items were equal in energy density. Moreover, participants selected larger portion sizes of those items perceived as healthier and expressed less anticipated consumption guilt.

Several studies have also tried to directly influence neural activity patterns in executive function network areas in order to support or induce the down-regulation of 'urges' elicited by salient food cues. This was done by using techniques such as reappraisal and devaluation training via motor response inhibition [254,255]. For instance, Hare and colleagues [63] used exogenous cues to direct participants' attention to health aspects of visually perceived foods, and highlighted the role of self-control (reflected by increased DLPFC activity) in modulating food valuation (reflected by OFC activity). Another study by Werthmann and colleagues [256] tested the effects of inducing different mindsets (i.e. healthy vs. palatable), in turn influencing attentional biases towards high-calorie food during a visual probe task. In their experiment, participants were instructed to focus on health consequences or pleasure elicited by the prospective consumption of the viewed food items. A 'healthy' mindset attenuated attentional biases towards high-calorie foods, but only for participants with a high eating restraint score, highlighting the interaction between trait and mindset differences.

Altogether, previous research has highlighted ‘top-down’ modulations of brain responses to food cues as a function of contextual information and expectations. Such line of research is still largely missing in the context of NNS consumption, whose impact might not only arise from the product itself, but also be driven by the context and aim of consumption. As compared to ‘bottom-up’ mechanisms, the direction of ‘top-down’ effects would thus be opposite. That is, rather than being directly deleterious itself, NNS consumption would be indicative of misled expectations on their consumption and individuals’ general detrimental food habits, and therefore be associated to body weight-related outcomes in epidemiological studies (e.g. [200]) despite NNS molecules having no direct physiological effect *per se* [245]. Most likely, ‘top-down’ effects of NNS consumption on brain responses to (visual) food perception should be observed within dorsal and lateral prefrontal cortices, and be associated with altered processes in the control over food intake. In order to further delineate ‘top-down’ processes on food intake behavior as a function of NNS consumption, inducing maladaptive food choices (ultimately leading to longer-term weight gain), then further research likely needs to compare blinded vs. non-blinded NNS consumption.

A second line of perspective for projects conducted in my thesis is therefore to delineate which part of the human food perception and food intake behavior is impacted by so-called ‘bottom-up’ effects of NNS *per se*, as compared to which part can be ‘manipulated’ by expectations. To do so, identical outcomes (e.g. spontaneous food intake, brain responses to food viewing and tasting) should be assessed when individuals consume NNS with expectations, as compared to without. Expectations could be induced simply by telling participants which beverage they are consuming, but also by providing them with erroneous information, e.g. sucrose consumption with ‘NNS’ label, and inversely. The rationale behind such protocols is that non-blinded NNS consumption (or even mislabeled sucrose consumption) would attenuate control processes towards tempting food cues, and be reflected by lower DLPFC and VLPFC, possibly associated with increased attention reflected by parietal activity. In turn, these alterations would be associated with subsequent higher caloric intake and body weight gain on the longer run. Various online behavioral tasks should be investigated in parallel and contrasted to each other, e.g. cognitive control and impulsivity tasks, attention tasks, as well as reward-related tasks, to ensure validation of the proposed ‘top-down’ mechanisms of action in the context of NNS consumption [4,42]. The projects conducted in my thesis provide first evidence that prefrontal control mechanisms are impacted by NNS consumption, also at early latencies of food cue processing usually reflecting ‘top-down’ regulatory mechanisms [20]. In particular study A, investigating NNS consumption in a non-blinded context, highlighted the likely role of DPFC activity in the regulation of subsequent responses to tempting food cues, in turn important for managing body weight on a longer-term duration.

4.3.3 Combined future study perspectives

Notwithstanding, 'bottom-up' and 'top-down' processes are not necessarily exclusive, i.e. both could explain part of the reason why NNS consumption has been repeatedly associated with higher BMI and long-term weight gain [198,200]. Animal models are of great help when investigating 'bottom-up' pathways and mechanistic effects of NNS consumption. Yet, some cognitive functions occurring in humans (i.e. rather involved in 'top-down effects') cannot be translated from animals, e.g. control mechanisms in animals are strictly metabolic, not cognitive. This might explain the stronger consensus on the deleterious impact of NNS consumption in the animal literature, although precise mechanisms are still poorly understood. In humans, both 'bottom-up' and 'top-down' mechanisms likely play a role, but so far have not been contrasted or investigated in parallel. Such multi-causality of NNS consumption effects could explain discrepancies in the current literature in human research, especially between epidemiological cohort studies and lab-controlled experimental studies. Studies A and B provide some initial evidence for both 'bottom-up' and 'top-down' directions of effects. However, study B (more representative of 'bottom-up' effects), was conducted to only investigate acute effects, preventing conclusions on the impact of a longer-term repeated consumption. In addition, the only trial so far investigating longitudinal blinded NNS consumption in humans showed no effect [244].

These issues raised here do not mean that 'bottom-up' mechanistic explanations do not play a role in potential deleterious impact of NNS consumption on appetite regulation and body weight management, but rather that 'top-down' mechanisms might override 'bottom-up' effects in the context of the modern human eating environment [257]. That is, further studies are needed that investigate blinded vs. non-blinded NNS consumption, first on a short-term, then on a longer-term period. In this context, I would propose to assess food perception (sweet taste and/or visual food cues), spontaneous food intake behavior and appetite-related feelings before and after an intervention consisting in the consumption of sucrose- or NNS-sweetened beverages, coupled to either correct or misleading information on the beverage identity. This trial would therefore consist of a 4-arms design, i.e. consumption of sucrose-sweetened beverage with the correct 'sucrose' or the misleading 'NNS' information, and consumption of NNS with the correct 'NNS' or misleading 'sucrose' information (Figure 7). By contrasting the 4 arms in a 2x2 design, the trial would assess a) the main effect of the product (sucrose vs. NNS consumption) representing 'bottom-up' effects, b) the main effect of consumers' expectations representing 'top-down' effects (correct vs. misleading product identity information), and c) possible interaction between both pathways, on gustatory and visual food perception, as well as on the spontaneous food choices and intake behavior.

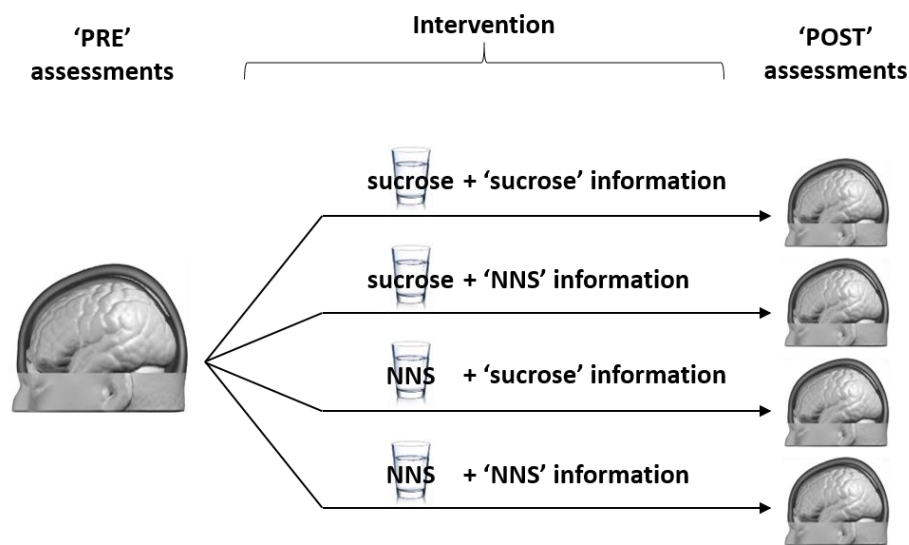


Figure 7: Protocol proposal for future combined assessments of 'bottom-up' and 'top-down' effects of NNS consumption. 'PRE' and 'POST' assessments may include gustatory and visual food perception, as well as spontaneous food intake, hormonal profiles and appetite-related subjective ratings. The intervention may consist of a one-point consumption, or a repeated consumption over a longer period.

The intervention could either be a one-point beverage consumption, enabling to assess acute effects on the aforementioned outcomes (as in study B), or a longer-term consumption, enabling to assess the impact of a repeated consumption (as in study A). In the case of a trial investigating repeated, longer-term consumption, food intake should be monitored during the intervention, to investigate whether or not there is a risk for (over)compensatory behavior and weight gain (and if so, as a function of product identity or expectations). So far, the best way to assess food intake behavior in free-living conditions is via Ecological Momentary Assessment (EMA), i.e. with smartphone applications [79,258]. Moreover, human cognitive studies often bear a high inter-individual response variability, potentially due to the presence of 'responders' and 'non-responders' to NNS consumption in the population. This point is especially interesting for the study of 'top-down' mechanisms of action, where future research should also model predictions based on data, i.e. investigate to which extent individuals' expectations can predict later food perception, spontaneous intake behavior and possibly (over)compensatory behavior over the intervention duration.

4.4 Take-home message

The projects conducted in my thesis aimed at providing insights on the impact of caloric and non-caloric sweetener consumption on the behavioral and brain responses to food in the visual modality. So far, research on the impact of sucrose and NNS consumption, is rather restricted to the gustatory modality investigating brain responses to sweet taste. Yet, visual food cues are ubiquitous in the

modern environment and are an important trigger of food choices and intake. Therefore, the projects conducted in my thesis are among the first to investigate the impact of NNS consumption on brain responses to food viewing and subsequent food intake behavior, and the first ones to investigate generalizability of visual responses to other solid food cues.

In the first study, I showed that replacing sugar-sweetened by NNS-sweetened beverages resulted in modulations in brain activity to the viewing of palatable high-fat, sweet foods, likely reflecting a decrease in control over intake associated with failure in body weight management. These modulations were particularly apparent in dorsal and lateral prefrontal cortex. In the second study, the blinded consumption of a NNS-sweetened beverage did not affect subsequent spontaneous food intake behavior nor post-prandial plasma concentrations of appetite-related hormones as compared to water. Yet, the drink consumption yielded differential post-prandial brain responses to the viewing of solid foods, particularly apparent in the insular and ventrolateral prefrontal cortex. These modulations in responses could reflect an initial phase in taste-calorie uncoupling likely indicating that repeated NNS consumption further impacts subsequent food intake. Altogether, these projects show that consuming NNS do impact brain responses to food viewing, thus warranting interests in further longitudinal trials studying associations and causality between brain responses to visual food cues and intake behavior in the context of sweetened beverage consumption. Mechanisms by which NNS might impact longer-term appetite control and food intake behavior yet remain to be investigated in more details [204,245]. Future research should in particular disentangle 'bottom-up' and 'top-down' action pathways, i.e. investigate blinded vs. non-blinded consumption of sweetened products, for which the nutrient and energy content do not necessarily match consumers' expectations.

5 SCIENTIFIC CONTRIBUTIONS AND PARALLEL ACTIVITIES

Collaborations for scientific publications

* denotes equal authors' contribution

- Bielser ML*, **Crézé C***, Murray MM, Toepel U. (2016) Does my brain want what my eyes like? – How food liking and choice influence spatio-temporal brain dynamics of food viewing. *Brain and Cognition*, 110: 64-73 ('FoodDec' study).
- Seyssel K, Cros J, **Crézé C**, Tappy L. (In press) Metabolic risks associated with fructose consumption: established evidence and persistent hypotheses. *Médecine des maladies Métaboliques*.
- Cros J, Bidlingmeyer L, Rosset R, Seyssel K, **Crézé C**, Beyene S, Jegatheesan P, Candal L, Campos V, Schneiter P, Tappy L. (In preparation) The impact of caloric and non-caloric sweeteners on stress responses: a randomized controlled trial in healthy young women.
- Francey C, Cros J, Rosset R, **Crézé C**, Rey V, Stefanoni N, Schneiter P, Tappy L, Seyssel K. (In preparation) A non-negligible amount of fructose is able to escape first-pass splanchnic extraction: an observational study in humans using a two tracers method.

Conferences and presentations

- Swiss Winter Conference on Ingestive Behavior, St-Moritz, Switzerland, February 2015 ('Boisson' study – Oral presentation);
- Experimental Biology, Annual meeting of the American Society for Nutrition, Boston MA, USA, March-April 2015 ('Boisson' study – Poster presentation);
- World Obesity Hot Topic Conference – Dietary sugars, obesity and metabolic disease risk, Berlin, Germany, June 2015 ('Boisson' study – Oral presentation – *Travel Scholarship*);
- Lemanic Neuroscience Annual Meeting, Les Diablerets, Switzerland, August 2015 ('FoodDec' study – Poster presentation).
- Fribourg Obesity Research Conference, Fribourg, Switzerland, September 2015 ('Boisson' study – Poster presentation).
- Progress report seminar, Department of Physiology, UNIL, Lausanne, Switzerland, October 2015 ('Boisson' study – Oral presentation);
- Progress report seminar, Department of Physiology, UNIL, Lausanne, Switzerland, November 2017 ('SugArt' study – Oral presentation);
- European Congress on Obesity, Vienna, May 2018 ('SugArt' study – Poster presentation).

Student supervision and teaching

- Supervision of Ms Laura Candal, Master thesis in Medical Biology (University of Lausanne (UNIL); directed by Prof. Luc Tappy; April 2016 – January 2017). She worked on physiological and behavioral data from the ‘SugArt’ study and successfully defended her work in January 2017.
- Supervision of Ms Sara Pekovic (Faculty of Medicine, University of Novi Sad, Serbia), 2-month summer school (UNIL & Ecole Polytechnique Fédérale de Lausanne (EPFL); directed by Dr. Ulrike Toepel; July – August 2016). Her internship aimed at learning EEG data acquisition and analyses.
- Teaching of practical courses in physiology (“Exercise efficiency” and “Spirometry”), 1st year Bachelor Sport Science students and 2nd year Bachelor Medical students (UNIL; Course by Dr. Philippe Schneiter; 2016-2018).
- Expertise for oral examinations in physiology, 1st year Bachelor Sport Science students (UNIL; Course by Dr. Philippe Schneiter; 2016-2018).
- Teaching of ‘Neurobiology of eating behavior’ seminar, 1st year Master in Nutrition and Dietetics (Haute Ecole de Santé de Suisse Occidentale (HES-SO); 2018)

Scientific communication & other projects

- Instructor for the following scientific communication events:
 - *Exhibit “L’oeil nu”, Espace des Inventions, Lausanne, 2014-2015*
 - *Workshop « Les cinq sens », TecDays at Carrouge & Neuchatel Colleges, 2015-2016*
 - *Workshop « Le Cerveau dans tous ses états », Le Noirmont, 2016*
 - *Workshop « Les Méandres de la Mémoire », Open Doors at EPFL, 2016*
- PhD student representative; Council of the Department of Physiology (UNIL; 2016-2018)
- Member of the BioScience Network Lausanne (UNIL & EPFL)
 - *Board member (Association Coordinator; January 2017 – January 2018)*
 - *Event organizer for FameLab Switzerland (editions 2017 & 2018)*
 - *Staff for the Life Science Career Day (UNIL; editions 2017 & 2018)*

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7 APPENDICES

7.1 Published article for study A – ‘BOISSON’

The impact of replacing sugar- by artificially sweetened beverages on brain and behavioral responses to food viewing – An exploratory study

Camille Crézé, Marie-Laure Notter-Bielsler, Jean-François Knebel, Vanessa Campos,
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The impact of replacing sugar- by artificially-sweetened beverages on brain and behavioral responses to food viewing – An exploratory study

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ABSTRACT

Several studies indicate that the outcome of nutritional and lifestyle interventions can be linked to brain 'signatures' in terms of neural reactivity to food cues. However, 'dieting' is often considered in a rather broad sense, and no study so far investigated modulations in brain responses to food cues occurring over an intervention specifically aiming to reduce sugar intake. We studied neural activity and liking in response to visual food cues in 14 intensive consumers of sugar-sweetened beverages before and after a 3-month replacement period by artificially-sweetened equivalents. Each time, participants were presented with images of solid foods differing in fat content and taste quality while high-density electroencephalography was recorded. Contrary to our hypotheses, there was no significant weight loss over the intervention period and no changes were observed in food liking or in neural activity in regions subserving salience and reward attribution. However, neural activity in response to high-fat, sweet foods was significantly reduced from pre-to post-intervention in prefrontal regions often linked to impulse control. This decrease in activity was associated with weight loss failure, suggesting an impairment in individuals' ability to exert control and adjust their solid food intake over the intervention period. Our findings highlight the need to implement multidisciplinary approaches when aiming to help individuals lose body weight.

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1. Introduction

Pre-ingestive responses to food viewing are of particular importance as visual features of foods (e.g. perceived caloric load by macronutrient identification) become conditioning stimuli able to trigger food wanting or control over food intake (Berridge, 2009; Dagher, 2012). In healthy individuals, the exposure to food cues (e.g. visual) triggers complex brain processes, i.e. categorizing what

is being perceived, integrating the salience of external food cues with internal metabolic needs, and evaluating the physiological adequacy to guide food intake (Van der Laan, De Ridder, Viergever, & Smeets, 2011). The pre-ingestive integrative treatment of food-related information is essential to promote need-adequate intake behaviors, and relies, among other things, on homeostatic and reward areas (hypothalamus, insula and the limbic system; Suzuki, Simpson, Minnion, Shillito, & Bloom, 2010). However, this regulatory system also requires inputs from brain areas involved in attentional control and decision-making processes (i.e. parietal and dorsal prefrontal areas as part of the executive function network; Seeley et al., 2007) to counterbalance the salient properties inherent to palatable foods and prevent food intake beyond homeostatic needs.

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Abbreviations		LF/NSW	Low-Fat/Non-Sweet
ASB	Artificially sweetened beverage	LF/SW	Low-Fat/Sweet
BMI	Body mass index	LPFC	Lateral prefrontal cortex
DPFC	Dorsal prefrontal cortex	MNI	Montreal Institute template brain
EEG	Electroencephalography	NNS	Non-nutritive sweeteners
GFP	Global field power	PreCG	Pre-central gyrus
HF/NSW	High-Fat/Non-Sweet	ROI	Region of interest
HF/SW	High-Fat/Sweet	SSB	Sugar-sweetened beverage
IPL	Inferior parietal lobe	TMS	Transcranial magnetic stimulation
ITI	Inter-trial interval	TW	Time window
LAURA	Local autoregressive average	VEP	Visual evoked potential

Increasing evidence suggests that weight gain may be a 'brain disorder' in which pre-ingestive homeostatic and control mechanisms, involved in the regulation of food intake according to body energy needs, are overridden by hedonic drives towards abundant palatable energy-dense foods (Berthoud, 2011; Morris, Beilharz, Maniam, Reichelt, & Westbrook, 2015). In overweight/obese individuals, neural activity in the above-mentioned brain areas in reaction to food cues have been shown to be altered in comparison to normal-weight individuals, and may account for an over-consumption of palatable energy-dense foods (see Garcia-Garcia et al., 2013; Martin & Davidson, 2014; Pursey et al., 2014 for reviews). In support of this assumption, several functional neuroimaging studies have identified changes in brain responsiveness to food cues due to longitudinal nutrition and lifestyle interventions. For instance, Murdaugh, Cox, and Cook Weller (2012) showed that hyper-reactivity to high-calorie food images in brain areas involved in reward valuation was predictive of individuals' short- and longer-term failure in a weight-loss program. Similar findings were reported by Weygandt and colleagues regarding impulse control mechanisms, i.e. greater neural activity to food viewing in dorso-lateral prefrontal brain region involved in cognitive control was associated with subsequent weight loss (2013) and prevention of weight regain (2015).

These studies indicate that the outcome of nutritional and lifestyle interventions can be linked to brain 'signatures', both from the homeostatic-salience and the executive function networks. However, these studies considered 'dieting' in a rather broad sense, and no study so far investigated modulations in brain responses to food cues in the context of a nutritional intervention specifically aiming to reduce sugar consumption. Yet, the consumption of sugar, more specifically of sugar-sweetened beverages (SSBs), has been associated with the high prevalence of obesity worldwide, and reduction of SSB consumption has become a prime target for body weight control interventions and policies (Bray, Nielsen, & Popkin, 2004; Popkin & Nielsen, 2003; SACN report, 2015; Vartanian, Schwartz, & Brownell, 2007). SSBs have been proposed to affect body weight through a variety of mechanisms (Dimeglio & Mattes, 2000; Malik, Schultze, & Hu, 2006); but how a reduction in sugar intake impact hedonic drives and impulse control to foods remains poorly understood.

The goal of our study was to investigate changes in behavioral and brain responses to visual solid food cues occurring over a 3-month intervention targeting sugar consumption, i.e. the replacement of SSBs by artificially sweetened equivalents. In parallel with expected individuals' weight loss, we hypothesized that neural activity to food viewing would increase from pre-to post-intervention in brain regions associated with control over food intake. Moreover, we expected a decrease in neural activity in brain

regions associated with salience and reward attribution, together with decreased visual like ratings (i.e. behavioral responses). Both brain and behavioral outcomes would be most pronounced in response to high-calorie sweet food viewing as compared to other food types. Spatio-temporal brain response modulations to the viewing of food types differing in their fat content and taste quality were therefore investigated before and after a 3-month intervention period using high-density electroencephalography (EEG) and electrical neuroimaging analyses (Bielser, Creze, Murray, & Toepel, 2016; Toepel, Knebel, Hudry, Coutre, & Murray, 2009). In parallel, behavioral ratings of visual food appreciation served to assess intervention-induced modulations in food liking.

2. Material and methods

2.1. Participants

Fourteen healthy volunteers (6 women) were recruited for this study, i.e. intensive consumers of SSBs on a daily basis (between 2 and 6 cans of 33 cl of soft drinks, corresponding to 70–210g of added sugar per day). The volunteers were a subsample of participants of a clinical intervention conducted at the Department of Physiology of the University of Lausanne targeting metabolic changes (Campos et al., 2015; 2017). Their age ranged from 18 to 40 years (mean age \pm SEM = 27.1 \pm 1.6), and body mass indices (BMI) from 21.2 to 35.4 kg/m² (mean BMI \pm SEM = 28.3 \pm 1.3 kg/m²). All volunteers had normal or corrected-to-normal vision. None of the participants had current or prior diabetes, cardiovascular, kidney, hepatic, neurological or psychiatric disease. Further exclusion criteria were particular diets (e.g. vegetarianism), exercising for more than 3 h per week and/or walking more than 1 h per day, having gained or lost more than 4 kg in body weight during the last 12 months, current medication or drug-taking, and consuming more than 10g of alcohol per day. Women were excluded when pregnant or when having a desire for pregnancy. All volunteers were informed about the procedures and signed a written consent previously approved by the Ethics Committee of the Canton of Vaud.

2.2. General procedure

Overall, the study lasted 16 weeks and comprised of two sessions of behavioral assessments and EEG recordings (Fig. 1A). First, all participants underwent a 4-week run-in period where they were asked to consume a regular amount of sugar-sweetened beverages per day, in line with their habitual consumption. Second, they underwent a 12-week intervention period. Over this period, participants had to continue drinking the same amount of soft drinks per day as during the run-in period, but the

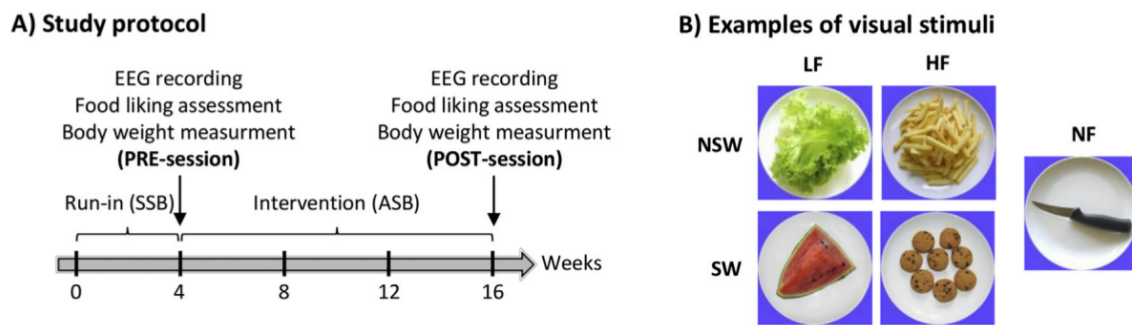


Fig. 1. (A) Timeline of overall experiment conducted over 16 weeks. Electroencephalography (EEG) recordings, food like ratings and body weight measurements were performed before (PRE-session) and after (POST-session) the intervention. (B) Exemplar stimuli shown during EEG recordings and food like rating task. Food image categories differed with respect to fat content and taste quality of the displayed foods. SSB: sugar-sweetened beverage. ASB: artificially sweetened beverage. LF: low-fat. HF: high-fat. NSW: non-sweet. SW: sweet. NF: task-relevant non-food images.

commercially available artificially sweetened equivalent (artificially sweetened beverage; ASB) of their usual beverage. That is, volunteers consumed a mix of aspartame, cyclamate, acesulfam K and sucralose. Over the 16 weeks, volunteers were asked to drink only beverages distributed to them by the Department of Physiology, University of Lausanne. Their food and non-caloric beverage intakes were otherwise left *ad libitum*. Compliance to the intervention was monitored by counting the number of returned soda cans as well as from urine samples pre-, mid-, and post-intervention (Campos et al., 2015).

In the end of the 4-week run-in period and in the end of the 12-week intervention period (i.e. PRE- and POST-intervention session respectively), participants reported to the laboratory for the assessment of behavioral and brain responses to food viewing, by means of visual appreciation ratings and EEG recordings. Participants were instructed and reported to have eaten a normal breakfast or lunch, and assessments took place 2–3 h after the last food intake. Before and after each of the EEG recording session, participants rated their hunger level by means of a visual analog scale anchored to 0–100%. Differences in hunger across the EEG session were analyzed using a paired student t-test (two-tailed). Additionally, participants' pre-prandial body weight had been measured PRE- and POST-intervention (Campos et al., 2015), and changes from PRE- to POST-intervention sessions were investigated using a paired student t-test (two-tailed).

2.3. Visual stimuli and online behavioral task

In each EEG recording session, color photographs either containing food (360 items) or non-food objects (180 items) were shown to participants on a computer screen. Four types of food were presented, differing in fat content and in taste quality, i.e. Low-Fat/Non-Sweet (LF/NSW), Low-Fat/Sweet (LF/SW), High-Fat/Non-Sweet (HF/NSW) and High-Fat/Sweet (HF/SW) (Fig. 1B). The fat content of low-fat foods ranged from 0 to 5g of fat per 100g (mean fat content \pm SEM = 0.89 ± 0.13 g), and from 10.68 to 81.10g of fat per 100g for high-fat foods (mean fat content \pm SEM = 27.12 ± 1.39 g). Non-food pictures consisted of kitchen utensils and were relevant for the online behavioral task only (see below). Pictures were controlled for low-level visual features (Knebel, Toepel, Hudry, Le Coultre, & Murray, 2008).

Data recordings took place in a sound attenuated booth and images were presented centrally on a 19" computer screen for 500ms each, in 6 consecutive blocks lasting 3–4 min. Each block contained pictures of food and non-food items in a pseudo-randomized order controlled by the E-prime software (Psychology Software Tools, Inc. Pittsburgh, USA). Participants were asked to categorize food from non-food pictures via button-press, thus

remaining uninformed about the various food categories viewed (Toepel et al., 2009). They were instructed to perform as quick and accurate as possible. Following the response, the Inter-Trial-Interval (ITI) randomly varied between 250 and 750ms to avoid anticipatory responses. During the ITI, a fixation cross was centrally displayed on screen to avoid eye movements.

2.4. EEG acquisition and preprocessing

Continuous EEG was recorded while participants viewed images and performed the categorization task. EEG was acquired at a sampling rate of 512 Hz using a 64-channel Biosemi ActiveTwo system, referenced to a CMS-DRL ground (see http://www.biosemi.com/pics/zero_ref1_big.gif for a detailed diagram of this circuitry). All pre-processing analyses were performed using the CarTool software (<https://sites.google.com/site/fbmlab/cartool>). Visual evoked potentials (VEPs) were computed over the period from -98ms to +488ms peri-stimulus epoch for each image. During single subject averaging, EEG epochs were cleaned from artifacts with a semi-automatic procedure using a 80 μ V rejection criterion and visual trial-by-trial inspection. Epochs containing eye blinks or other motor artifacts were manually removed. During averaging, data was band-pass filtered at 0.1–40Hz (plus at 50Hz for smoothing edges). First, VEPs were averaged for each single subject, food category (LF/NSW, LF/SW, HF/NSW and HF/SW) and recording session (PRE- and POST-intervention). Electrodes with artifactual signals were then interpolated (Perrin, Pernier, Bertrand, Giard, & Echallier, 1987). In a second step, group-average VEPs were calculated for each food category and session, while baseline-correcting over the pre-stimulus period and recalculating the VEPs to an average reference (Murray, Brunet, & Michel, 2008).

2.5. EEG analyses and source estimations

In order to determine time windows of interest for analyzing intervention-induced changes in brain responses to food viewing, we identified peaks in the Global Field Power (GFP) in the group-average responses and validated their timing in the single-subject responses. The GFP is a reference-independent measure of the global strength (i.e. amplitude) of VEPs over the electric field over time. Mathematically, it is calculated as the standard deviation of the electric field amplitude across all electrodes at a given time point. GFP peaks are representative of maximally synchronized neural activity underlying cognitive processes within a given condition (Lehmann & Skrandies, 1980; Michel & Murray, 2012; Murray et al., 2008). Thus, periods of maximal GFP served as a rationale for the further investigation of intervention-induced changes in neural source estimates (Toepel et al., 2009; 2015).

Over the time windows of interest, estimations of neural source activity based on the head-surface recorded VEPs served to determine brain regions showing intervention-induced modulations as a function of the viewed food category. For this purpose, the neural activity was analyzed over time windows of interest using a local autoregressive average (LAURA) distributed linear inverse solution (Michel et al., 2004). That is, mean amplitudes of activity were calculated for each of the 3005 solution points of an inverse solution matrix based on a realistic 3D head model (resolution of $6 \times 6 \times 6 \text{ mm}^3$) over each time window of interest, as in former studies (Bielser et al., 2016; Lietti, Murray, Hudry, Le Coutre, & Toepel, 2012; Toepel et al., 2009; 2014; 2015). The output of the algorithm is one scalar value ($\mu\text{A}/\text{mm}^3$) per solution point per food viewing condition and time window. As the goal of our study was to investigate the effects of a diet intervention on the spatio-temporal brain dynamics to food viewing, we focused the analyses on the relative change in neural signal from PRE-to POST-intervention recording sessions. For this purpose, the PRE-intervention neural activity strength (in $\mu\text{A}/\text{mm}^3$) at each node of the solution point matrix was first subtracted from the POST-intervention signal in each participant and for each food category viewed. The difference values obtained for each source node were then multiplied by 100 and divided by the mean PRE-intervention activity across all nodes of the solution point matrix in each individual and each food category viewed. This approach accounts for inter-subject variability in neural activity at baseline, since relative (% change) and not absolute values entered the analyses.

For each time window of interest, statistical analyses first comprised of whole-brain repeated measure ANOVAs with the within-subject factors of fat content (i.e. Low-Fat vs. High-Fat foods) and taste quality (i.e. non-Sweet vs. Sweet foods), computed on the % change in signal from PRE-to POST-intervention sessions on each node of the solution point matrix. Only regions showing a significant interaction between fat content and taste quality (extending the cluster size criterion of >10 neighbors) were considered for post-hoc region-of-interest (ROI) analyses (Toepel et al., 2009). Results were rendered on the Montreal Institute template brain (MNI) and Talairach coordinates of the area showing the maximal statistical difference between conditions are given (Talairach & Tournoux, 1988).

In each ROI showing a significant interaction, neural activity of the source node revealing maximal statistical differences (plus its 6 immediate neighbors) was extracted and averaged in each individual's data for each food category viewed. These results are visualized as bar plots, indicating pre-to-post increases or decreases in food viewing neural activity. Post-hoc paired t-tests (two-tailed) were conducted on the % change in signal from PRE-to POST-intervention to investigate how fat content and taste quality of the viewed foods relates to changes in activity. Furthermore, orthogonal one-sample t-tests (two-tailed) assessed, within each ROI and each food category, whether the % change in signal significantly differed from baseline (i.e. PRE-intervention activity). Overall, only results with $p \leq .05$ were considered as significant. Effect sizes (i.e. Cohen's d values) are reported for all significant results and trends. All analyses were conducted using customized Python scripts and the software tools R and STEN (Sten toolbox programmed by Jean-François Knebel from the Laboratory for Investigative Neurophysiology, CHUV and UNIL, Lausanne).

2.6. Post-EEG assessment of visual food liking

Following each EEG recording session, participants rated their appreciation of each food image offline to test for intervention-induced modulations in liking of solid foods. All 360 food images were randomly presented in three blocks of 120 pictures.

Participants were asked to rate how much they liked each viewed food on a 5-point Likert scale (1-dislike, 5-strongly liked) by button press. Pictures were displayed centrally on the same 19" computer screen, controlled by the E-prime software. For statistical analyses of these behavioral data, a mean 'liking' value was calculated for each food category, i.e. LF/NSW, LF/SW, HF/NSW and HF/SW, for each participant and session. In line with the goal of our study, we focused on the relative change in liking from PRE-to POST-intervention sessions in %. One-sample t-tests served to investigate whether the % change in liking from PRE-to POST-intervention session significantly differed from baseline (i.e. PRE-intervention session) for each food category. Overall, only results with $p \leq .05$ were considered as significant. All statistical analyses were conducted using the R software.

2.7. Associations between PRE-to POST-session changes in body weight, food liking and neural responses

Spearman correlation analyses tested associations between changes in neural activity induced by SSB replacement and changes in body weight as well as in food liking. Only the % change in signal in brain regions showing significant modulations in neural activity from PRE-to POST-intervention sessions entered these additional analyses. Spearman rho values are reported only when significant ($p \leq .05$). All analyses were conducted using the R software.

3. Results

3.1. Hunger level and changes in visual food liking

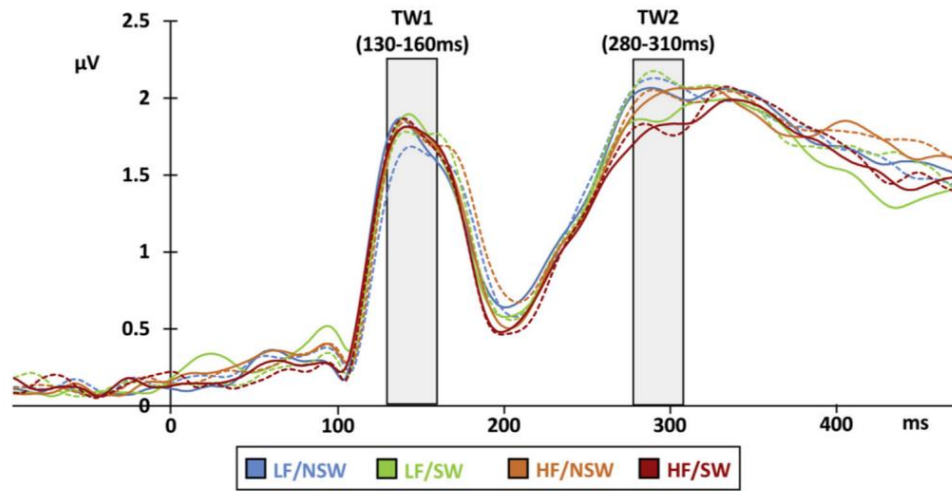
Average hunger ratings across each EEG recording session were 45.6% (SEM \pm 7.6) in PRE-intervention session, and 49.9 (SEM \pm 6.6) in POST-intervention session, and showed no significant difference between sessions. Relative changes (in %) in the appreciation of solid foods (food like ratings) were +2.8% (SEM \pm 3.2), -2.8% (SEM \pm 2.7), +3.5% (SEM \pm 2.2) and -5.0% (SEM \pm 3.5) for LF/NSW, LF/SW, HF/NSW and HF/SW food categories, respectively. Orthogonal one-sample t-tests showed no significant changes in appreciation from baseline (i.e. PRE-intervention session) for either food category.

3.2. Changes in neural source activity to the viewing of solid foods from PRE-to POST-intervention

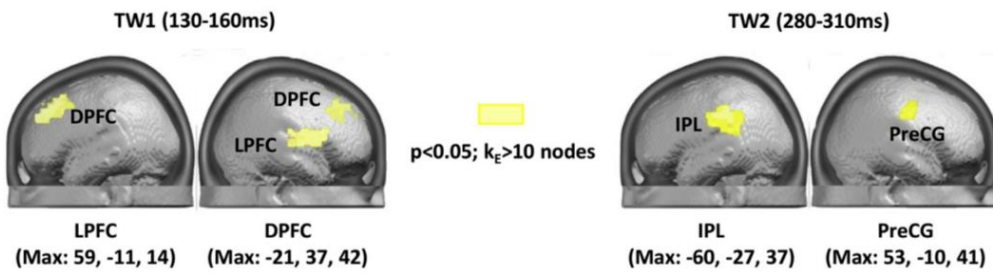
Fig. 2A shows the GFP waveforms for all food viewing conditions over time and highlights GFP peaks, i.e. time windows of interest for further analyses. A first peak was identified between 130 and 160ms after food image onset, and a second peak between 280 and 310ms after food image onset.

Over the first time period of interest (130-160ms post-image onset), whole-brain analyses revealed interactions of fat content \times taste quality on PRE-to POST-intervention % change in neural activity to food viewing in the right lateral prefrontal cortex (LPFC; Max: $x = 59, y = -11, z = 14$) and in the medial dorsal prefrontal cortex (DPFC; Max: $x = -21, y = 37, z = 42$) (Fig. 2B, left panel). That is, SSB substitution differentially influenced neural activity to food viewing in these areas as a function of fat content and taste quality of the viewed foods. Modulations in neural source activity by SSB intervention in these regions of interest were further assessed by post-hoc tests. In the LPFC as well as in the DPFC, one sample t-tests for each food category (vs. baseline PRE-intervention session) showed that specifically the activity to viewing HF/SW food images was significantly lower after the SSB intervention (LPFC: $t_{13} = -2.23$; $p < .05$; Cohen's $d = -0.60$; DPFC: $t_{13} = -2.37$; $p < .05$; Cohen's $d = -0.63$). Between-category differences became apparent

A) GFP over food viewing periods



B) Brain regions revealing an interaction of Fat content and Taste quality



C) Changes in neural activity in regions of interest

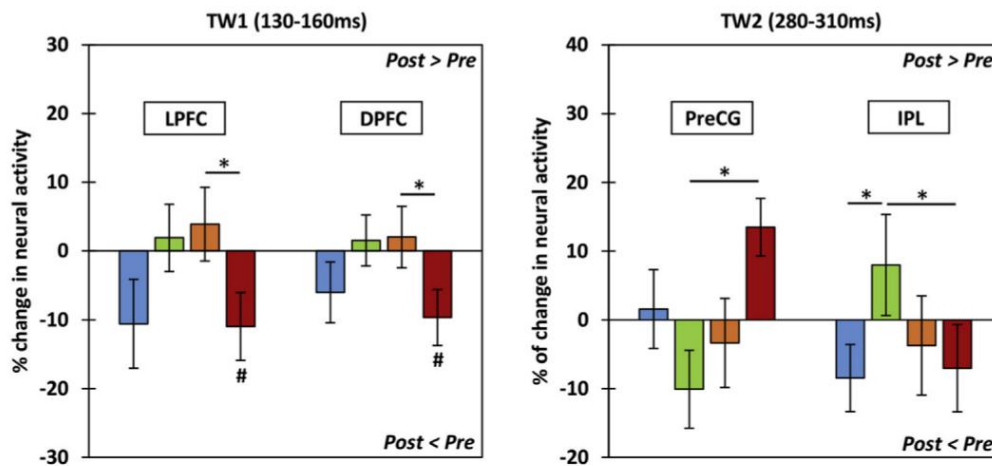


Fig. 2. (A) Group average Global Field Power (GFP) waveform for the 4 food categories over the peri-stimulus period (-98 to +488ms from image onset). Solid lines indicate GFP during the PRE-session, and dotted lines show GFP during the POST-session. Grey boxes highlight time windows (TW) of interest for subsequent analyses. (B) Visualization of brain regions showing an interaction of fat content x taste quality in the whole brain analyses of changes in estimated neural activity from PRE-to POST-intervention session. Talairach coordinates (x, y, z) indicate the position of the source node showing maximal statistical differences. (C) Results of post-hoc analyses on changes in neural activity in each region of interest. Bar plots detail the direction of pre-to-post changes in each region of (B), and for each food category viewed. Data are shown as mean % change ± SEM. *: $p < .05$ for paired t -tests on between-food category responses. #: $p < .05$ for one-sample t -tests on responses to each food category against baseline (PRE-intervention session). LPFC: lateral prefrontal cortex. DPFC: dorsal prefrontal cortex. IPL: inferior parietal lobe. PreCG: pre-central gyrus. LF/NSW: Low-Fat/Non-Sweet. LF/SW: Low-Fat/Sweet. HF/NSW: High-Fat/Non-Sweet. HF/SW: High-Fat/Sweet.

between HF/NSW and HF/SW foods (LPFC: $t_{13} = 2.18$; $p < .05$; Cohen's $d = 0.73$ and DPFC: $t_{13} = 2.18$; $p < .05$; Cohen's $d = 0.70$) (Fig. 2C, left panel). Trends towards differences were observed between LF/NSW and HF/NSW foods ($t_{13} = -1.97$; $p = .07$; Cohen's

$d = -0.63$) in the LPFC, as well as between LF/SW and HF/SW foods ($t_{13} = 2.14$; $p = .05$; Cohen's $d = 0.73$) in the DPFC region.

Over the second time window of interest (280-310ms post-image onset), an interaction of fat content x taste quality was found for

pre-to-post neural activity changes in the left inferior parietal lobe (IPL; Max: $x = -60, y = -27, z = 37$) and in the right pre-central gyrus (PreCG; Max: $x = 53, y = -10, z = 41$) (Fig. 2B, right panel). One sample t-tests considering changes with respect to baseline (i.e. PRE-intervention session) showed that in particular PreCG activity in response to HF/SW food viewing tended to be increased after SSB intervention ($t_{13} = 2.13; p = .05$; Cohen's $d = 0.57$). Further, significant between-category differences in intervention-induced modulations were observed between both sweet foods categories (i.e. LF/SW and HF/SW; $t_{13} = -2.47; p < .05$; Cohen's $d = -0.84$), and trends towards differences between HF/NSW and HF/SW foods ($t_{13} = -2.06; p = .06$; Cohen's $d = -0.64$). In the IPL, the responses to LF/SW foods differed from those to LF/NSW foods ($t_{13} = -2.93; p < .05$; Cohen's $d = -0.73$) and to HF/SW foods ($t_{13} = 2.28; p < .05$; Cohen's $d = 0.76$) (Fig. 2C, right panel).

3.3. Association between changes in neural source activity to food viewing, body weight and food liking

To complement primary findings on brain responses, we investigated associations between PRE-to POST-intervention changes in neural activity to food viewing, and changes in body weight as well as in food liking using correlation analyses. Participants' body weight at baseline (i.e. PRE-intervention session) ranged from 67 to 120 kg, corresponding to BMIs ranging from 21.2 to 35.4 kg/m². Changes in body weight from PRE-to POST-intervention sessions ranged from a loss of 3 kg (-3.3% of initial body weight) to a gain of 5 kg (+6.3% of initial body weight).

These changes in body weight were negatively associated with changes in DPFC activity when HF/SW foods had been viewed ($\rho_{12} = -0.67; p < .01$). That is, participants who showed higher gains of body weight also showed greater pre-to post-intervention decreases in neural activity when viewing the solid HF/SW foods (Fig. 3). No associations between changes in food liking and changes in neural activity were found.

4. Discussion

Our study investigated the effects of a replacement of sugar-sweetened beverages (SSBs) by artificially-sweetened equivalents on brain and behavioral responses to food viewing in intensive SSB consumers. Electrical neuroimaging served to delineate neural sources whose activity was modulated when viewing solid food after the SSB replacement. Although a few EEG studies have been conducted on differential brain responses over nutritional and lifestyle interventions (Murdaugh et al., 2012; Nock, Dimitropoulos, Tkach, Frasure, & Von Gruenigen, 2012; Weygandt et al., 2013;

2015), this study is, to our knowledge, the first one to investigate changes in the spatio-temporal dynamics to food viewing following a sugar-targeting nutritional intervention. The timing of these modulations converges with previous findings (Harris, Hare, & Rangel, 2013; Toepel et al., 2009). Contrary to our hypotheses, the weight loss over the intervention period was not significant and no pre-to-post changes were observed neither in visual food liking nor in brain responses to palatable food viewing in regions subserving salience and reward attribution. However, neural activity in response to the viewing of high-fat, sweet foods was significantly reduced from pre-to post-intervention in brain regions associated with impulse control. This decrease in activity was associated with weight loss failure, suggesting an impairment in individuals' ability to exert control and adjust their solid food intake during the intervention period.

Decrease in prefrontal activity to palatable food viewing and impairment in cognitive control – Predominantly, a decrease in neural source activity pre-to-post intervention, specifically when high-fat sweet foods were viewed, were found in prefrontal regions. Dorsal prefrontal regions are associated with executive functions such as cognitive control or planning, both in the neurobehavioral science of appetite and object perception in general (Dagher, 2012; Miller, 2000). In particular, dorsal prefrontal functioning is linked to the ability to exert self-control over food intake upon exposure to appetitive food cues (Hare, Camerer, & Rangel, 2009). For example, Del Parigi and colleagues (2007) showed stronger dorsal prefrontal activity after meal intake in cognitively restrained eaters as compared to non-restrained individuals, suggesting higher cognitive control, or higher need for control, in restrained eaters (Heatherton & Wagner, 2011). Several similar observations are reported in the review of Rooke et al. (2008), who highlight the importance of implicit 'impulse control' for the prevention of substance abuse. In agreement, Batterink, Yokum, and Stice (2010) showed a negative association between BMI and prefrontal brain activity (both in dorsal and ventro-lateral regions) when adolescent participants were required to inhibit responses to palatable food stimuli. A causal link between the dorsolateral prefrontal cortex and the modulation of valuation processes when viewing palatable foods was established by Camus et al. (2009). They used transcranial magnetic stimulation (TMS) to evince the impact of dorsolateral prefrontal cortex on ventral frontal regions at the time of decision-making. Additional ventral and lateral regions of the PFC were found to be involved in cognitive control exertion. For example, Hollman et al., (2012) showed that increases in inferior frontal gyrus activity during food response inhibition positively correlated with dietary restraint. Furthermore, Cools, Clark, Owen, and Robbins (2002) showed that ventro-lateral prefrontal cortices

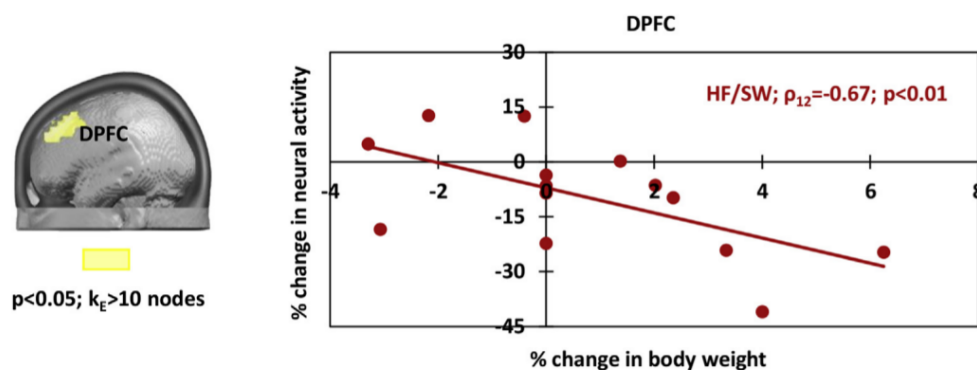


Fig. 3. A negative correlation between changes in body weight and in neural activity to HF/SW food viewing from PRE-to POST-intervention session was obtained in the DPFC over TW1. DPFC: dorsal prefrontal cortex. HF/SW: High-Fat/Sweet.

promote behavioral inhibition and adaptations. Taken together, these studies highlighted the importance of prefrontal brain areas in control exertion over drives towards palatable foods.

Although few studies have so far investigated longitudinal changes occurring along with nutritional and lifestyle interventions, an increase in dorsal prefrontal activity in response to the viewing of palatable foods has been consistently associated with greater success in weight loss and prevention of weight (re)gain (Bruce et al., 2011; Murdaugh et al., 2012; Nock et al., 2012; Weygandt et al., 2013; 2015). A study by McCaffery and colleagues (2009) also showed stronger frontal neural activity to high-calorie food image viewing in successful weight-loss maintainers (as defined by lifetime weight history) as compared to their normal-weight and obese counterparts. While an increase in activity in prefrontal regions in response to food cues corroborates with being able to exert cognitive control and lose weight, our results revealed decreased activity in the prefrontal cortex to high-fat sweet foods after the intervention, these changes being associated with weight loss failure (or even weight gain). This failure in weight loss might thus be related to participants' inability to exert cognitive control when faced with highly palatable food items during the diet intervention period, resulting in the increased consumption of such items. Yet, since beverage consumption was not blinded, an alternative explanation of our finding can also be that when consuming the ASB counterpart of their preferred SSB, participants became more inclined to consume more calories from solid foods, i.e. exerting less control over their food intake and preventing weight loss. Since the relation between DPFC activity changes and body weight was only attested via a correlational measure in the present study, future studies are needed to further delineate the general or additional, respectively, impact of non-nutritive sweetener consumption on pre-ingestive brain responses to solid foods cues.

Also, we found intervention-induced modulations as a function of the viewed food category within inferior parietal and precentral gyrus. Neural activity in these regions has been related to attentional (Karhunen, Lappalainen, Vanninen, Kuikka, & Uusitupa, 1997) and decision-making processes in the light of subsequent food choices (Kable & Glimcher, 2009). Decreased responses to high-fat and sweet foods during early visual responses have been associated with blunted control mechanisms hindering the down-regulation of attention towards food cues (Harris et al., 2013), in turn increasing motivation toward food intake (Hume, Howells, Rauch, Kroff, & Lambert, 2015). That is, an early decrease in prefrontal cortex activity (as described above) could promote a later increase in attention towards palatable foods. In our study, such pattern can be observed in the precentral gyrus responses. Greater activity in precentral regions has also been found in obese individuals as compared to normal-weight controls and successful weight loss maintainers, which might reflect greater attention towards palatable foods (McCaffery et al., 2009). Altogether, our results likely point out that an imbalance between the exertion of cognitive control and attention towards palatable food cues could promote intake of solid foods over the SSB replacement period due to 'reflexive' eating instead of being a cognitively-driven decision ('reflective'), resulting in weight loss failure (Alonso-Alonso & Pascual-Leone, 2007). Future intervention studies might benefit from a monitoring of daily food choices in parallel to modulations in brain responses to food cues to assess absolute changes in intake behavior and experimental paradigms such as 'go/no-go' tasks specifically investigating impulse control (Carbine et al., 2017) to further corroborate this assumption.

Modulations in food liking and reward valuation processes –

Regarding food appreciation ratings (liking of food images), no significant changes were found with respect to baseline pre-intervention for either food category. A similar absence of changes

in 'reward' value attribution (assessed both with behavioral tasks and functional magnetic resonance imaging) was observed by Griffioen-Roose et al., (2013), who repeatedly exposed participants to ASBs or SSBs. Paralleling behavioral results, no changes were observed from pre- to post-intervention session in brain activity to food viewing in areas associated with reward and salience attribution. We would yet remind the reader that the online image categorization task performed by participants during EEG recordings (as in Toepel et al., 2009) does not directly assess reward processing.

Some additional limitations regarding the interpretation of our study results should be mentioned. Our design did not include a control group, i.e. participants continuing to consume SSBs over the 3-month intervention period. For this reason, we cannot be certain whether the observed liking and brain responses are specific to the SSB replacement. This protocol was a nested, observational study, and no statistical power analysis was done beforehand. Thus, and as discussed beforehand, it is possible that the sample was too small to detect some differences. Most important is, however, that the current protocol cannot distinguish effects induced by adding artificially-sweetened beverages (ASBs) from those of SSBs removal over the intervention period. In other words, the modulations we observed could well be due to the forced introduction of ASBs to participants' diet, and not necessarily to the cut in SSB consumption. Whether ASB consumption changes the perception of sweet taste has only recently begun to be studied. There is some evidence that chronic ASB consumption might change the way the brain associates sweet taste to high caloric intake, and promotes modulations in intake behavior (Davidson, Martin, Clark, & Swithers, 2011; Frank et al., 2008; Green & Murphy, 2012; Pepino & Bourne, 2011), but results are still not conclusive (Bruyere et al., 2015; Harvey-Anderson, Foreyt, Sigman-Grant, & Allison, 2012; Mattes & Popkin, 2009). Most of these studies used taste stimuli, so that to our knowledge, our study is the first to investigate changes in solid food perception by SSB replacement. Future projects yet also need to explore such changes induced by ASB consumption when novel to the diet.

In conclusion, our study for the first time explored modulations in spatio-temporal brain dynamics to food viewing in the context of a nutritional intervention targeting to reduce sugar intake. Our data provide a valuable starting point for emphasizing the importance of investigating brain responses to food cues occurring in parallel with nutritional intervention, and suggest that a decreased exertion of cognitive control when exposed to palatable food cues, together with functional alterations in brain areas supporting attention, are associated with compensatory intake behaviors and weight loss failure. The absence of other changes such as modulations in liking and in brain areas related to the reward valuation of foods (although potentially explained by the small sample size) further questions the efficiency and relevance of SSB replacement as a sole nutritional intervention. Our study thus highlights the need for implementing multidisciplinary approaches, e.g. providing behavioral training of self-control in daily food intake to dieters (Houben & Jansen, 2011), to render nutritional interventions aiming to decrease body weight successful.

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Conflicts of interest

The authors declare no conflict of interest.

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7.2 Published article for study B – ‘SUGART’

The impact of caloric and non-caloric sweeteners on food intake and brain responses to food: a randomized crossover controlled trial in healthy humans

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Article

The Impact of Caloric and Non-Caloric Sweeteners on Food Intake and Brain Responses to Food: A Randomized Crossover Controlled Trial in Healthy Humans

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Abstract: Whether non-nutritive sweetener (NNS) consumption impacts food intake behavior in humans is still unclear. Discrepant sensory and metabolic signals are proposed to mislead brain regulatory centers, in turn promoting maladaptive food choices favoring weight gain. We aimed to assess whether ingestion of sucrose- and NNS-sweetened drinks would differently alter brain responses to food viewing and food intake. Eighteen normal-weight men were studied in a fasted condition and after consumption of a standardized meal accompanied by either a NNS-sweetened (NNS), or a sucrose-sweetened (SUC) drink, or water (WAT). Their brain responses to visual food cues were assessed by means of electroencephalography (EEG) before and 45 min after meal ingestion. Four hours after meal ingestion, spontaneous food intake was monitored during an ad libitum buffet. With WAT, meal intake led to increased neural activity in the dorsal prefrontal cortex and the insula, areas linked to cognitive control and interoception. With SUC, neural activity in the insula increased as well, but decreased in temporal regions linked to food categorization, and remained unchanged in dorsal prefrontal areas. The latter modulations were associated with a significantly lower total energy intake at buffet (mean kcal \pm SEM; 791 \pm 62) as compared to WAT (942 \pm 71) and NNS (917 \pm 70). In contrast to WAT and SUC, NNS consumption did not impact activity in the insula, but led to increased neural activity in ventrolateral prefrontal regions linked to the inhibition of reward. Total energy intake at the buffet was not significantly different between WAT and NNS. Our findings highlight the differential impact of caloric and non-caloric sweeteners on subsequent brain responses to visual food cues and energy intake. These variations may reflect an initial stage of adaptation to taste-calorie uncoupling, and could be indicative of longer-term consequences of repeated NNS consumption on food intake behavior.

Keywords: electroencephalography; non-nutritive sweeteners; sweet taste; visual food cues; food intake; ad libitum buffet

1. Introduction

Excess sugar consumption, in particular in the form of sugar-sweetened beverages (SSBs), has been repeatedly identified as a major factor contributing to weight gain, overweight and obesity prevalence as well as associated metabolic disorders [1–3]. In order to fight increasing rates of overweight and obesity and help body weight management, many non-caloric molecules with a high sweetening power, called non-caloric or non-nutritive sweeteners (NNS), were developed and introduced into our daily diet. That is, hedonic properties of sweet taste can be enjoyed without consuming excess liquid calories. Yet, epidemiological studies have shown a link between NNS consumption and an increased prevalence of overweight and obesity risk on the long-run [4–6]. Although risks of reverse causality cannot be excluded when interpreting the results of observational cohort studies, one other reason for such a link could be functional properties of the central food intake regulation system discussed below.

In healthy humans, food intake is regulated by a fine-tuned balance between drives towards palatable items ('hedonic' processing and reward valuation), physiological needs ('homeostatic' processing), and inhibitory control. While inhibitory control is supported by brain areas of the executive function network, i.e., dorsal, ventrolateral prefrontal and parietal regions [7], reward valuation is assisted by cortico-limbic networks comprising basal ganglia, the anterior cingulate and orbitofrontal cortices [8]. Reward is integrated with homeostatic signals in the hypothalamus and insula. Gastro-intestinal hormones secreted before (e.g., ghrelin) and after a meal (e.g., insulin), nervous afferents as well as sweet taste receptor activation in the mouth (and possibly along the digestive track) all provide feedbacks to the brain and thereby inform these networks on physiological states and consequences of food ingestion [9–11]. This regulatory system likely evolved with a caloric value assigned to sweet taste, as naturally occurring sweeteners generally contain about 4 kcal/g. Due to their sweet but non-caloric properties NNS might thus provide erroneous information to the food intake regulatory system, potentially inducing maladaptive food choices as compensatory mechanism [12,13].

In animals, findings support this hypothesis by showing detrimental effects of NNS consumption on food intake behavior and increased adiposity and body weight longitudinally [14,15]. So far, the impact of NNS consumption on food choices and body weight control in humans remains controversial, showing either beneficial, detrimental, or no effects [16–19]. From a neurophysiological perspective, some studies have shown differences in the cerebral processing of taste information between NNS and sucrose stimuli of equal sweetness intensity, highlighting the capacity of the human brain to readily discriminate between caloric and non-caloric sweet taste [20–22]. However, these studies assessed immediate gustatory responses and neural activity, but not subsequent food cravings and intake of excess solid calories.

Neuroimaging studies in humans have further shown that food cravings and choices often result from exposure to visual food cues [23,24]. However, only one study, to our knowledge, has investigated brain response modulations to food cue exposure following NNS consumption in humans, i.e., in the context of a 3-month replacement of sucrose-sweetened beverages by artificially sweetened equivalents [25]. This former study of our group highlighted the potential implication of central cognitive control mechanisms in weight loss failure. However, that study did not directly compare the impact of consuming caloric vs. non-caloric sweeteners on responses. Further, participants had not been blinded to the type of beverage they consumed. Thus, how NNS influence subsequent drives towards certain types of foods (in particular sweet foods) and how this relates to modulations in food intake behavior remains largely unknown.

The goal of our current study was thus to investigate whether, as compared to water consumption, activation of sweet taste receptors with NNS or with sucrose, as part of a standardized meal, exert different acute effects on (a) postprandial brain responses to food viewing, (b) postprandial gastro-intestinal hormone secretion known to impact hunger and satiety feelings and (c) subsequent food intake behavior, both in terms of quantity and quality of choices (ad libitum buffet).

2. Materials and Methods

2.1. Participants

Eighteen healthy, normal-weight men were recruited. All volunteers used to drink on average ≥ 3 cans of 33 cL of SSBs per week. None of the participants had current, prior, or family history of diabetes, cardiovascular, kidney, hepatic, neurological or psychiatric disease. Further exclusion criteria were color blindness, particular diets (e.g., vegetarianism), any food intolerance or allergy, arterial blood pressure $> 140/90$ mmHg at rest, exercising for more than 3 h per week, current medication, drug-taking or smoking habits, and consuming more than 10 g of alcohol per day. Only infrequent consumers of NNS-sweetened beverages (≤ 1 can of 33 cL per week) were included. All volunteers were weight-stable, right-handed according to the Edinburgh Handedness Inventory [26], and had normal or corrected-to-normal vision. To ensure medical safety, volunteers were not included when hemoglobin and ferritin levels were below 13.5 g/L and 50 $\mu\text{g/L}$ respectively, when weighing less than 50 kg, and when having donated blood or participated to another clinical trial in the prior three months.

Recruitment was done by means of advertising at local university campuses. Recruitment, screening, and follow-up of participants is shown in Figure 1A. Potential participants were first screened by email and then invited to a screening visit. Nineteen volunteers met all eligibility criteria and were enrolled in the study. One participant had to be excluded due to medical discomfort during the first test day; so that 18 volunteers completed the entire protocol.

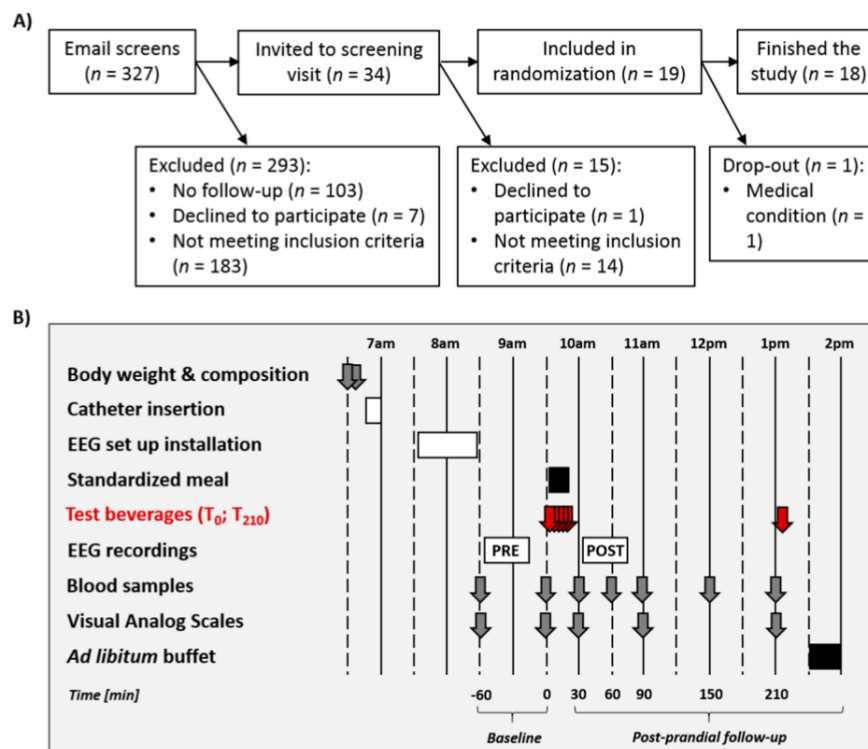


Figure 1. (A) Flow diagram for study participants' eligibility, enrollment, and follow-up. (B) Detailed protocol of the in-center test days. Identical procedures were used on the three test days. Test beverages (WAT, SUC, NNS) were ingested at $T = 0$ min (350 mL; five 70 mL-glasses every 5 min) and at $T = 210$ min (one 200 mL glass). EEG: electroencephalographic recording session.

2.2. General Procedure

The study consisted of three in-center test days for each volunteer, on which one of the three beverage conditions (i.e., Water, Sucrose, and NNS consumption, further referred to as WAT, SUC, and

NNS) was tested in a randomized crossover controlled design. The beverage conditions were separated by a wash-out period of three weeks. Each test day was preceded by a 5-day nutritional and lifestyle recommendation period followed by a 2-day run-in period. During this run-in period, volunteers received a controlled weight-maintenance diet (55% carbohydrates (including 10% as sugars), 30% lipids and 15% proteins) calculated from the Harris–Benedict equation with a physical activity factor set at 1.5. Participants were instructed to consume all meals and snacks at specified times of the day (7 a.m., 10 a.m., 12 a.m., 3 p.m., 7 p.m.), and to drink only water.

Detailed procedures for the in-center test days are provided in Figure 1B. On each test day, volunteers reported to the Metabolism, Nutrition, and Physical Activity unit from the Clinical Research Center of the Lausanne University Hospital at 6.30 a.m. They were fasting since 10 p.m. the evening before the test. They were asked to void, and body weight was measured thereafter (Seca 708, Seca GmbH, Hamburg, Germany). Body composition was assessed using bio-electrical impedancemetry (Biacorpus, Medical Healthcare GmbH, Germany). Each volunteer was then placed in a bed, and a catheter was inserted into an antecubital vein of the left forearm to allow for repeated blood collection throughout the test day. The venous path was kept open with a slow perfusion of saline solution (NaCl 0.9%). Two blood samples were collected in fasted state at $T = -60$ and 0 min before standardized meal and beverage ingestion. At those time points, participants were also asked to fill visual analog scales (VAS; 0–100) for hunger, thirst, and satiety levels as well as a Likert scale (LS; 1–9) for taste cravings. Each volunteer was then accompanied to a light-proof room where the electroencephalographic (EEG) recording system was installed. A cap with 64 active electrodes was placed on the participants' head and prepared so that electrical impedance between electrical sensor and scalp were kept below 40 k Ω . A first EEG recording took place between $T = -60$ and 0 min (i.e., further referred to as the pre-prandial recording session), while participants completed an online continuous recognition task. The EEG recording procedure, as well as visual stimuli and task used are detailed below (Section 2.8). At $T = 0$ min, a standardized meal and the 350 mL test drink (T_0 -beverage; WAT, SUC, or NNS) were given to each volunteer. Five blood samples were collected over the post-prandial period, at $T = 30, 60, 90, 150$ and 210 min after meal and beverage consumption had started. At $T = 30, 60$ and 210 min, participants also filled VAS and LS for hunger, thirst, satiety, and taste cravings. A second EEG recording took place 45 min after meal and beverage ingestion (i.e., further referred to as the post-prandial recording session), that followed the same procedure as the pre-prandial session. In order to avoid ceiling effects and further strengthen the impact of beverage type on spontaneous food intake, participants received a 200 mL pre-buffet drink 210 min after the meal ingestion (T_{210} -beverage; SUC, WAT or NNS), its composition repeating the beverage condition. This drink served as a preload for further quantitative and qualitative assessments of spontaneous food intake at an ad libitum buffet taking place 20 min after the preload ingestion.

The primary study outcomes were pre- to post-prandial changes in brain responses to food viewing across beverage conditions. All behavioral and physiological parameters were considered as secondary outcomes. Study sample size was determined by assuming the same effect size on the spatio-temporal brain dynamics as in [25] ($1 - \beta$: 80%; α : 5%). The randomization sequence of treatment allocation was determined before the start of recruitment by random generation of blocks using the R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). To ensure double-blinding of both participants and experimenters to the beverage condition, a third person (J.C.) prepared the beverages. The experimental protocol was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of the Canton de Vaud in September 2015 (protocol number 353/15). The protocol is registered in the international and national registers for clinical trials (clinicaltrials.gov: NCT02853773 and kofam.ch: SNCTP000001882). Participants were enrolled between February 2016 and April 2017. All test days were performed between March 2016 and July 2017. All experimental visits took place in the Metabolism, Nutrition and Physical Activity unit from the Center for Clinical Research of the Lausanne University Hospital. All volunteers were informed about the procedures during the screening visit and signed a written consent.

2.3. Meal and Test Beverage Composition

The T₀-beverage consisted of five 70 mL-glasses (i.e., 350 mL in total) corresponding to one of the three beverage conditions (WAT, SUC, NNS). Beverage composition was based on commercialized concentrations, as determined by Ordoñez and colleagues [27]. The SUC T₀-beverage provided 149 kcal (37.1 g of sucrose). The WAT and NNS T₀-beverages provided 0 kcal, with 137.2 mg cyclamate, 63.35 mg acesulfame K and 40.6 mg aspartame for the NNS. The standardized meal provided at T = 0 min was identical for all three beverage conditions. It corresponded to 30% of the estimated individual 24-h energy requirements calculated from the Harris–Benedict equation, and was low in sugars and sweet taste (55% carbohydrates (2% sugars), 30% lipids and 15% proteins). Participants were asked to drink one 70 mL-glass every five minutes and consumed the provided meal in-between, i.e., starting and ending the meal with the consumption of a 70 mL-glass. To maximize the sweet taste receptor stimulation by sucrose or NNS, each mouthful of liquid was to be kept in the mouth for ten seconds before swallowing. The T₂₁₀-beverage was of the same composition as the T₀-beverages. The SUC T₂₁₀-beverage thus provided 85 kcal (21.2 g of sucrose). The WAT and NNS T₂₁₀-beverages provided 0 kcal, with 78.4 mg cyclamate, 36.2 mg acesulfame K and 23.2 mg aspartame for the NNS. All beverages were provided at room temperature.

2.4. Qualitative and Quantitative Assessments of Spontaneous Food Intake

The ad libitum buffet presented at the end of the test day comprised 12 snacks, subdivided into 4 categories (3 snacks each) based on the fat content and taste quality of the foods provided, i.e., Low Fat/Non-Sweet (LF/NSW), Low Fat/Sweet (LF/SW), High Fat/Non-Sweet (HF/NSW), and High Fat/Sweet (HF/SW). The threshold for Low-Fat/High-Fat and Non-Sweet/Sweet subdivisions was set at 10 g of lipids/sugars per 100 g of food based on the nutritional information available on the packaging. The textures were matched as much as possible between food categories. The presentation context was kept as identical as possible between all beverage conditions, and always took place in the kitchen of the Clinical Research Center. Snacks were consistently prepared by the same experimenters (C.C. and L.C.), weighed and presented following the same protocol, and served on identical white dishes. Each snack was available in larger quantity than the expected average intake. The environment was kept neutral (e.g., no visible food packaging and no particular odor), and each participant was either accompanied by the experimenter or left alone for periods of five minutes. Participants remained uninformed of their food intake being measured, and were told to eat until feeling comfortably sated. Questions regarding food type were answered, but no nutritional information was given. Water was provided ad libitum with the snacks. All snack leftovers were carefully weighed after consumption. Total energy intake and energy intake per food category was calculated based on the nutritional information available on the packaging.

2.5. Analytical Procedures for Plasma Samples

Plasma was separated from blood cells immediately after sampling by centrifugation during 10 min at 4 °C and 3500 rotations per minute. Aliquots of plasma were stored at −20 °C until analysis. Plasma glucose concentrations were measured by enzymatic methods (Randox Laboratories Ltd., Crumlin, UK). Plasma insulin and ghrelin concentrations were determined by radioimmunoassay (Merck Millipore Merck KGaA, Darmstadt, Germany).

2.6. Behavioral Ratings

VAS for hunger, thirst, and satiety consisted of 15-cm long lines with '0' and '100' anchored to the left and right side, respectively, presented with the written indication "Please indicate how hungry/thirsty/satiated you are at present by drawing a point on the line below". Individuals' responses were converted to % of the scale maximum. Taste cravings were assessed with a 9-point LS with 'salty' and

'sweet' anchored to the left and right side, presented with the written indication "Please indicate how much you crave for a rather salty or sweet food item at present by ticking the correct box on the scale below".

2.7. Statistical Analyses of Food Intake, Behavioral Ratings and Plasmatic Parameters

Data distribution was controlled for normality and homoscedasticity using the Shapiro–Wilk and Bartlett tests, respectively. Data that were not normally distributed were transformed using the BoxCox algorithm. All anthropometric parameters (body weight, body mass index (BMI), and body composition), plasma concentrations and behavioral parameters (VAS and LS ratings) were tested for differences between beverage condition baselines with a one-way repeated-measure ANOVA including the independent within-subject factor of Beverage (three levels: WAT, SUC, and NNS). The impact of beverage type on food intake at the ad libitum buffet (total energy intake) was investigated first by a one-way repeated-measure ANOVA with the within-subject factor of Beverage. In a second step, the impact of the beverage type was detailed for food categories ingested by a two-way repeated-measure ANOVA with the within-subject factors of Beverage and Food category (four levels: LF/NSW, LF/SW, HF/NSW and HF/SW). Whenever a significant main effect of Beverage or an interaction Beverage \times Food category was found, post-hoc paired *t*-tests (two-tailed) were conducted between Beverage conditions and/or Food categories. The effect of beverage type on the kinetics of plasma concentrations and behavioral scales was assessed using two-way repeated-measure ANOVAs with the within-subject factors of Beverage and Time. Whenever an interaction Beverage \times Time was found, post-hoc paired *t*-tests (two-tailed) were conducted between Beverage conditions at each time point. Further, one-way repeated-measure ANOVAs with the within-subject factor of Beverage were conducted on plasma concentrations and behavioral scales at $T = 210$ min, irrespective of the kinetic results, to investigate the pre-buffet state. Whenever a main effect of Beverage was found, post-hoc paired *t*-tests (two-tailed) were conducted between beverage conditions. All data are expressed as mean \pm SEM (standard error of mean). All analyses were performed with R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria), and *p*-values ≤ 0.05 were considered as significant.

2.8. Electroencephalography (EEG) Stimuli Presentation Procedure, EEG Acquisition and Preprocessing

During both EEG recording sessions, color photographs showing foods that differed in fat content and in taste quality were presented to participants. This image database (240 items) has been used in several studies investigating food perception [25,28–30] and pictures were controlled for low-level visual features [31]. Stimulus presentation took place in a light-proof room, using the E-prime 2 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Images were presented centrally for 500 ms each on a 19" computer screen, in 3 consecutive blocks lasting 5 min. Each block contained 120 items, i.e., 80 initial encounters and 40 repeated items. Participants were asked to categorize initial from repeated image encounters via button-press. This behavioral task served to ensure participants' attention to food images, and they were instructed to perform as quickly and accurately as possible. Following participants' button press, the Inter-Trial-Interval (ITI) randomly varied between 250 and 750 ms to avoid anticipatory responses. During the ITI, a fixation cross was centrally displayed on screen to avoid eye movements. Number of trials between initial and repeated items were controlled across blocks and recording sessions to ensure similar difficulty of the recognition task.

Continuous EEG was recorded while participants viewed the images and performed the online recognition task. EEG was acquired at a sampling rate of 500 Hz using a 64-channel BrainProducts ActiCAP system. Details of the electrode montage can be found on the BrainProducts website (http://www.brainproducts.com/products_apps.php). All pre-processing analyses were performed using the CarTool software version 3.51 (2268) (<https://sites.google.com/site/fbmlab/cartool>). Only the responses to initially encountered images were used to compute visual evoked potentials (VEPs). VEPs were computed over the period from -100 ms to $+500$ ms peri-stimulus epoch for each image. During single subject averaging, EEG epochs were cleaned from artifacts with a semi-automatic procedure

using a 80 μV rejection criterion and visual trial-by-trial inspection. Epochs containing eye blinks or other motor artifacts were manually removed. During averaging, baseline correction was applied on the peri-stimulus period (i.e., -100 ms to $+500$ ms), and data was band-pass filtered at 0.1–30 Hz. An additional notch filter of 50 Hz was applied. First, VEPs were averaged for each single subject, recording session (Pre- and Post-prandial) and beverage condition (WAT, SUC, and NNS). Electrodes with artefactual signals were then interpolated [32]. In a second step, group-average VEPs were calculated for each session and beverage condition, while baseline-correcting over the pre-stimulus period and recalculating the VEPs to an average reference [33].

2.9. EEG Analyses and Estimations of Neural Source Activity

Time windows of interest in brain responses to food viewing in the SUC, WAT, and NNS conditions were determined around GFP peaks in the group-average Global Field Power (GFP) waveform. The GFP is a reference-independent measure of the global amplitude of the electric field (VEPs), i.e., calculated as the standard deviation of the electric field amplitude across all 64 electrodes at a given time point [34]. These peak periods in the GFP represent the time windows of highest synchronized neural activity underlying distinct steps in sensory and cognitive processes, and thus served as a rationale for further investigation of beverage type-induced modulations in source activity [25,35]. The center and width of each time windows of interest were determined by the average peak timing and the standard deviation across participants' individual GFP peaks, across sessions and beverage conditions.

Over each time window of interest, we estimated the neural source activity based on the head-surface recorded VEPs using a local autoregressive average (LAURA) distributed a linear inverse solution [36]. That is, mean amplitudes of neural activity were calculated for each of the 4350 solution points of an inverse solution matrix based on a realistic 3D head model. The output of this algorithm is one scalar value ($\mu\text{A}/\text{mm}^3$) per solution point, per viewing condition and time window. As the goal of our study was to investigate the differential effects of three beverages on the meal-induced modulations in brain responses to food viewing, we focused our analyses on the relative change in neural activity from pre- to post-prandial recording sessions [25].

For each time window of interest, statistical analyses first comprised of whole-brain repeated-measure one-way ANOVAs with the within-subject factor of Beverage, computed on the % neural activity change from pre- to post-prandial session on each node of the 4350 solution point matrix. Only regions showing a significant main effect of Beverage in a cluster of ≥ 10 neighboring nodes were considered for post-hoc region-of-interest (ROI) analyses. Results of these analyses were rendered on the Montreal Institute template brain (MNI) and Talairach coordinates of the node showing the maximal statistical difference between beverage conditions are given for each statistically determined brain region. In each ROI showing a significant main effect of Beverage, neural activity of the source node revealing maximal statistical differences plus the 6 neighboring nodes was extracted and averaged in each individuals' data, for each beverage condition. These results are visualized as bar plots, indicating pre-to-post changes in neural activity to food viewing. Statistical outliers ($<$ or $>$ 3 standard deviations from the mean) were removed from further analyses. Post-hoc paired *t*-tests (two-tailed) were then conducted on the respective pre-to-post change in neural activity (in %) between beverage conditions. Additionally, orthogonal one-sample *t*-tests (two-tailed) assessed, within each ROI and in each beverage condition, whether the relative pre-to-post % change in signal significantly differed from baseline (i.e., pre-prandial activity; [25]). Overall, only results with $p \leq 0.05$ were considered significant. All analyses were conducted using customized Python scripts, the software R version 3.3.1, and the STEN toolbox version 2.0 developed by Jean-François Knebel and Michael Notter (<http://doi.org/10.5281/zenodo.1164038>).

3. Results

Participants' body weight, BMI, body composition, plasma concentrations of glucose, insulin and ghrelin and behavioral ratings for hunger, thirst, satiety, and taste cravings are shown in Table 1 for

each beverage condition. None of the parameters showed differences between beverage conditions at baseline (all $p = ns$).

Table 1. Study participants' anthropometric, metabolic, and behavioral characteristics at baseline.

	Beverage Condition			One-Way ANOVA	
	WAT	SUC	NNS	F-Value	p-Value
Body weight [kg]	66.6 ± 1.2	66.5 ± 1.2	66.5 ± 1.1	0.39	0.68
BMI [kg/m ²]	21.4 ± 0.4	21.4 ± 0.4	21.3 ± 0.4	0.12	0.89
Body fat mass [kg]	12.3 ± 1.2	12.2 ± 0.9	12.2 ± 1.1	0.04	0.97
Plasma glucose [mmol/L]	4.9 ± 0.1	4.8 ± 0.1	4.9 ± 0.1	1.23	0.31
Plasma insulin [μU/mL]	10.3 ± 0.7	9.8 ± 0.5	9.5 ± 0.7	2.33	0.11
Plasma ghrelin [pg/mL]	984.8 ± 94.1	925.7 ± 70.4	963.3 ± 94.1	0.90	0.41
Hunger level [%]	71.4 ± 5.1	73.3 ± 4.4	72.6 ± 4.7	0.12	0.89
Thirst level [%]	55.7 ± 5.5	67.1 ± 3.8	61.5 ± 5.1	2.38	0.11
Satiety level [%]	21.7 ± 4.2	23.6 ± 4.8	20.4 ± 3.4	0.25	0.78
Taste cravings [1–9]	5.4 ± 0.4	5.6 ± 0.5	5.8 ± 0.5	0.30	0.75

Data are expressed as mean ± SEM (standard error of mean). WAT, SUC, NNS: Water-, Sucrose-, NNS-beverage conditions. BMI: body mass index.

3.1. Spontaneous Food Intake at the Ad Libitum Buffet

Total energy intake and energy intake by food category at the ad libitum buffet are shown in Table 2. Regarding total energy intake, we observed a main effect of Beverage ($F_{2,17} = 3.62$; $p < 0.05$), i.e., participants on average ingested significantly less energy in SUC than in WAT ($\Delta = -151 \pm 59$ kcal; $t_{17} = -2.58$; $p < 0.05$) and NNS ($\Delta = -126 \pm 56$ kcal; $t_{17} = -2.26$; $p < 0.05$). However, no significant difference was observed between WAT and NNS ($\Delta = 25 \pm 66$ kcal; $t_{17} = 0.38$; $p = ns$). Further analyses on energy intake segregated by food categories did not show an interaction between Beverage × Food category ($F_{2,17} = 0.54$; $p = ns$), i.e., participants did not modify their food choice pattern as a function of the beverage type ingested.

Table 2. Spontaneous food intake at the ad libitum buffet.

	Beverage Condition		
	WAT	SUC	NNS
Total energy intake [kcal]	942 ± 71	791 ± 62 ^{a,b}	917 ± 70
Energy intake from LF/NSW foods [kcal]	142 ± 28	141 ± 29	167 ± 37
Energy intake from LF/SW foods [kcal]	77 ± 22	62 ± 15	78 ± 20
Energy intake from HF/NSW foods [kcal]	515 ± 74	428 ± 50	449 ± 56
Energy intake from HF/SW foods [kcal]	209 ± 36	161 ± 24	224 ± 35

Data are expressed as mean ± SEM. WAT, SUC, NNS: Water-, Sucrose-, NNS-beverage conditions. LF, HF: low-, high-fat. NSW, SW: Non-sweet, sweet. ^a $p < 0.05$ for post-hoc paired t -test (two-tailed) between SUC and WAT.

^b $p < 0.05$ for post-hoc paired t -test (two-tailed) between SUC and NNS.

3.2. Plasma Concentrations of Metabolites and Gastro-Intestinal Hormones

Plasma concentrations of glucose, insulin, and ghrelin in response to meal and test beverage ingestion are shown in Figure 2. The main effect of Beverage was significant for insulin (Figure 2B; $F_{2,17} = 8.29$; $p < 0.05$; i.e., plasma insulin yielded overall higher values in SUC as compared with both WAT and NNS) and ghrelin (Figure 2C; $F_{2,15} = 4.56$; $p < 0.05$; i.e., plasma ghrelin yielded overall lower values in SUC as compared with both WAT and NNS), but not for plasma glucose (Figure 2A; $F_{2,17} = 0.15$; $p = ns$). More importantly, a significant interaction between Beverage × Time was observed for all parameters (glucose: $F_{2,17} = 2.83$; insulin: $F_{2,17} = 5.31$; ghrelin: $F_{2,15} = 2.41$; all $p < 0.05$). Plasma glucose and insulin concentrations were significantly higher in SUC at $T = 30$ min (glucose: $t_{17} = -3.77$ and $t_{17} = -2.46$; insulin: $t_{17} = -3.94$ and $t_{17} = -5.11$; all $p < 0.05$) as compared to WAT and NNS, respectively. Plasma ghrelin concentration, on the other hand, was significantly lower in SUC, as compared to WAT; this difference being significant at $T = 30$ min ($t_{15} = -2.77$; $p < 0.05$) and

$T = 60$ min ($t_{15} = -2.48$; $p < 0.05$). No differences were observed between WAT and NNS for any of the parameter kinetics.

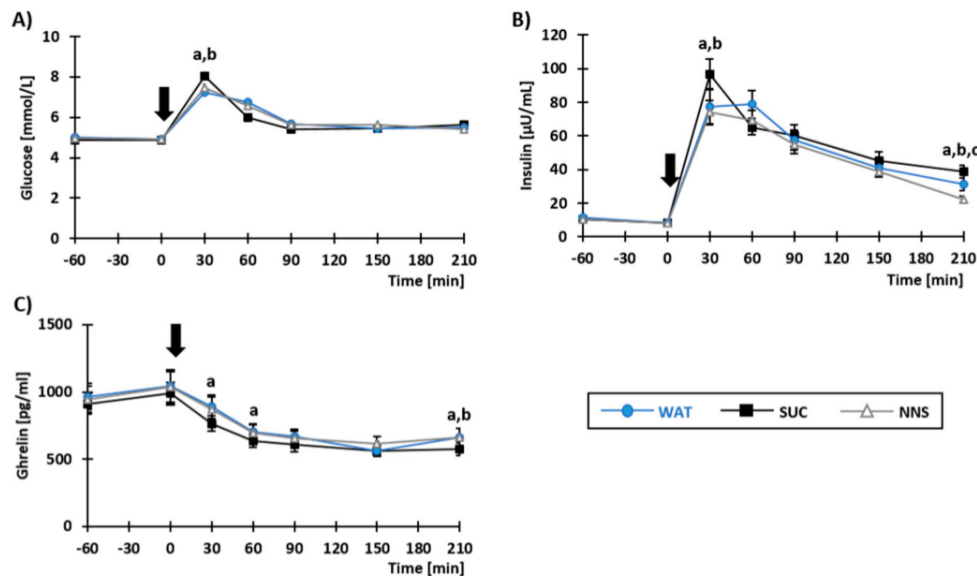


Figure 2. Plasma concentrations of glucose (A), insulin (B), and ghrelin (C) in response to beverage and concomitant meal ingestion at $T = 0$ min, indicated by a black arrow. Data are presented as mean \pm SEM. ^{a,b,c}: $p < 0.05$ for post-hoc paired t -tests (two-tailed), respectively between SUC-WAT, SUC-NNS, and WAT-NNS. WAT, SUC, NNS: Water-, Sucrose-, NNS-beverage conditions.

Before the buffet ($T = 210$ min), plasma glucose concentrations were similar in all beverage conditions ($F_{2,17} = 0.70$; $p = \text{ns}$). By contrast, a significant main effect of Beverage was observed for plasma insulin ($F_{2,17} = 13.04$; $p < 0.05$) and ghrelin concentrations ($F_{2,16} = 9.44$; $p < 0.05$). Plasma insulin concentration was most elevated in SUC ($t_{17} = 2.55$ and $t_{17} = 4.77$; both $p < 0.05$ against WAT and NNS, respectively), and the lowest in NNS ($t_{17} = 2.74$; $p < 0.05$ against WAT). Plasma ghrelin concentration was lower in SUC as compared with WAT ($t_{17} = 2.88$; $p < 0.05$) and NNS ($t_{17} = 3.89$; $p < 0.05$), but there was no difference between WAT and NNS ($t_{17} = 0.81$; $p = \text{ns}$).

3.3. Results of Behavioral Ratings

No main effect of Beverage nor interaction between Beverage \times Time were observed for any of the parameter kinetics (Supplementary Figure S1A–D for hunger, satiety, thirst ratings and taste cravings).

Before the buffet ($T = 210$ min), a significant main effect of Beverage was found on hunger ratings ($F_{2,17} = 5.68$; $p < 0.05$). Hunger ratings were lower in SUC as compared with WAT ($t_{17} = -2.71$; $p < 0.05$) and NNS ($t_{17} = -2.66$; $p < 0.05$). No difference in hunger ratings was found between WAT and NNS ($t_{17} = 0.28$; $p = \text{ns}$). No significant main effect of Beverage was found for thirst ratings ($F_{2,17} = 0.07$), satiety ratings ($F_{2,17} = 1.10$) and taste cravings ($F_{2,17} = 0.37$; all $p = \text{ns}$).

3.4. Pre- to Post-Prandial Changes in Neural Source Activity To Food Viewing

Two time periods of interest were defined around the peaks of the group-average GFP waveform. A first period of interest ranged from 120 to 150 ms, and a second period of interest ranged from 250 to 320 ms after image onset (Figure 3A).

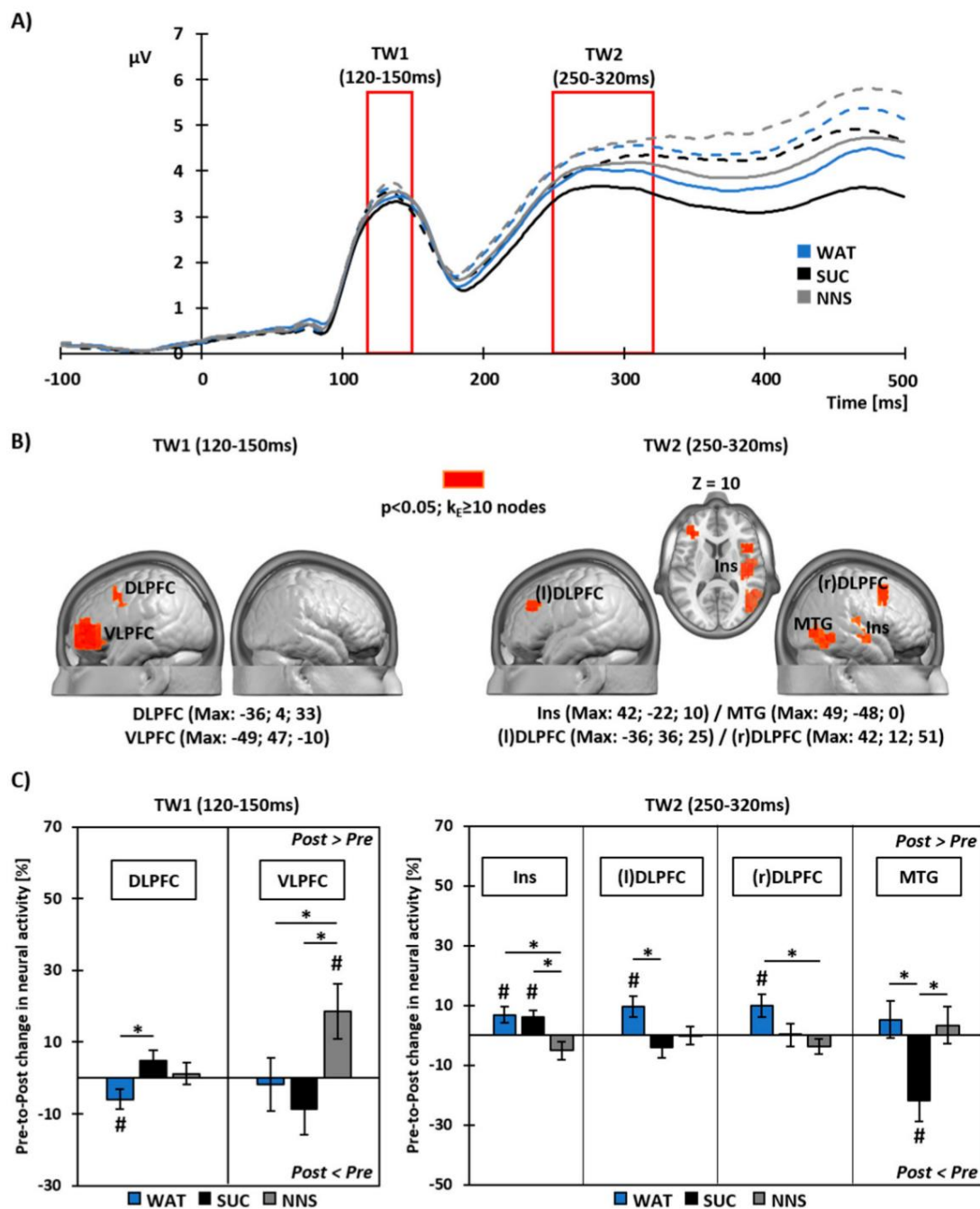


Figure 3. (A) Group-average Global Field Power (GFP) waveform over the peri-stimulus period (-100 to +500 ms from image onset). Red borders indicate the time windows of interest (TW) for subsequent neural source analyses. Solid lines illustrate the GFP during the pre-prandial recording session and dotted lines show GFP during the post-prandial recording session. (B) Visualization of brain regions showing a main effect of Beverage in the whole brain analyses on pre- to post-prandial changes in neural activity. Talairach coordinates (x, y, z) indicate the position of the source node showing maximal statistical differences. (C) Results of post-hoc analyses on changes in neural activity within each region of interest. Bar plots detail the direction of changes in each region of (B) and for each Beverage condition. Data are shown as mean (\pm SEM). *: $p < 0.05$ for paired t -tests (two-tailed) between Beverage conditions. #: $p < 0.05$ for one-sample t -tests vs baseline (pre-prandial). VLPFC: ventrolateral prefrontal cortex. Ins: insula. (l)- & (r)DLPFC: left & right dorsolateral prefrontal cortex. MTG: Middle Temporal Gyrus. WAT, SUC, NNS: Water-, Sucrose-, NNS-beverage conditions.

Over the first time window of interest (TW1: 120–150 ms post-image onset), whole brain analyses revealed a main effect of Beverage on the pre- to post-prandial % change in neural activity in the left dorsolateral prefrontal cortex (DLPFC; Max: $x = -36, y = 4, z = 33$) and in the left ventrolateral prefrontal cortex (VLPFC; Max: $x = -49, y = 47, z = -10$). That is, the beverage type differentially modulated the neural activity to food viewing within these brain areas (Figure 3B, left panel).

With WAT, meal intake led to decreased neural activity within the DLPFC ($t_{16} = -2.16; p < 0.05$ for *t*-test against baseline), but did not impact neural activity within the VLPFC ($t_{17} = -0.25; p = \text{ns}$ for *t*-test against baseline) (Figure 3C, left panel). Unlike WAT, there was no modulation in the neural response within the DLPFC with SUC ($t_{16} = 1.66; p = \text{ns}$ for *t*-test against baseline; $t_{16} = 3.31; p < 0.05$ for paired *t*-test between WAT and SUC responses). Like WAT however, SUC did not lead to modulated neural activity within the VLPFC ($t_{17} = -1.22; p = \text{ns}$ for *t*-test against baseline). NNS also did not impact neural activity within the DLPFC ($t_{16} = 0.39; p = \text{ns}$). Yet, in contrast to WAT and SUC, NNS led to increased neural activity within the VLPFC ($t_{17} = 2.42; p < 0.05$ for *t*-test against baseline; $t_{17} = -3.20$ and $t_{17} = -4.19$; both $p < 0.05$ for paired *t*-tests between NNS-WAT and NNS-SUC, respectively).

Over the second time window of interest (250–320 ms post-image onset), a main effect of Beverage on the pre- to post-prandial % change in neural activity was observed in the right insula (Ins; Max: $x = 42, y = -22, z = 10$), in the left (l) and right (r) DLPFC ((l)DLPFC Max: $x = -36, y = 36, z = 25$ and (r)DLPFC Max: $x = 42, y = 12, z = 51$), and in the right middle temporal gyrus (MTG; Max: $x = 49, y = -48, z = 0$) (Figure 3B, right panel).

With WAT, meal intake led to increased neural activity within the insula ($t_{17} = 2.55; p < 0.05$), (l)DLPFC ($t_{17} = 2.82; p < 0.05$) and (r)DLPFC ($t_{16} = 2.60; p < 0.05$), but did not impact neural activity within the MTG ($t_{16} = 0.85; p = \text{ns}$; all *t*-tests against baseline) (Figure 3C, right panel). Like WAT, SUC also led to increased neural activity within the insula ($t_{17} = 2.48; p < 0.05$ for *t*-test against baseline). Unlike WAT however, there were no pre-to-post changes in neural activity in SUC in the (l)DLPFC ($t_{17} = -1.09; p = \text{ns}$ for *t*-test against baseline; $t_{17} = -4.94; p < 0.05$ for paired *t*-test between SUC and WAT) and the (r)DLPFC ($t_{16} = 0.04; p = \text{ns}$ for *t*-test against baseline). In addition, SUC led to decreased neural activity within the MTG ($t_{16} = -3.21; p < 0.05$ for *t*-test against baseline; $t_{16} = -3.74; p < 0.05$ for paired *t*-test between SUC and WAT). In contrast to WAT and SUC, NNS did not impact neural activity within the insula ($t_{17} = -1.72; p = \text{ns}$ for *t*-test against baseline; $t_{17} = 3.11$ and $t_{17} = 2.86$; both $p < 0.05$ for paired *t*-tests between NNS-WAT and NNS-SUC, respectively). Like in SUC, there were no pre-to-post changes in neural activity in NNS within the (l)DLPFC ($t_{17} = -0.01; p = \text{ns}$ for *t*-test against baseline) and the (r)DLPFC ($t_{16} = -1.49; p = \text{ns}$ for *t*-test against baseline; $t_{16} = 2.56; p < 0.05$ for paired *t*-test between NNS and WAT). Like WAT, but unlike SUC, NNS consumption did not impact neural activity within the MTG ($t_{16} = 0.55; p = \text{ns}$ for *t*-test against baseline; $t_{16} = -2.46; p < 0.05$ for paired *t*-test between NNS and SUC).

4. Discussion

Our study aimed at investigating the acute impact of consuming caloric (sucrose) and non-caloric sweeteners (NNS), as compared to water, on the subsequent brain responses to visual food cues and spontaneous food intake behavior. As expected, we found neurophysiological and physiological markers of satiety following the ingestion of the standardized meal with water. We further observed that sucrose consumption impacted the responses in brain areas associated with cognitive control (prefrontal cortices) and food categorization (temporal cortices), and led to decreased subsequent food intake, indicating an adequate compensatory behavior. In contrast, NNS consumption did not alter spontaneous food intake when compared to water, but altered postprandial brain responses to visual food cues, most pronounced in prefrontal areas and in the insula.

4.1. Brain Responses to Food Viewing Following Water or Sucrose Consumption

Meal ingestion combined with water (i.e., control beverage condition lacking sweet taste and caloric load) impacted brain responses to visual food cues in bilateral dorsal prefrontal areas and

in the right insula. The neural activity in dorsal prefrontal areas has long been linked with the capacity to exert cognitive control over food intake when exposed to palatable food cues, as part of the executive function network. Tataranni and colleagues [37] were the first to highlight differences in brain responses between hunger and satiety beyond hypothalamic areas using functional neuroimaging, and found increased neural activity in the dorsolateral prefrontal cortex. Since then, many other studies have found dorsolateral prefrontal regions to be involved in top-down cognitive control over food intake [38–40]. A study of Camus and colleagues [41] could even attest causality in the role of dorsolateral prefrontal regions in control and decision-making using transcranial magnetic stimulation. Using the high temporal resolution of EEG, Harris and colleagues [42] were able to provide further insights on the dual role of DLPFC in cognitive control, showing that early response modulations (around 150 ms post-stimulus onset) were associated with top-down filtering of sensory input, whereas later ones (from 450 ms post-stimulus onset) were associated with reward value modulation. In our study, we observed decreased activity in the left dorsal prefrontal region over an early time window following food viewing (120–150 ms) and increased activity in the bilateral dorsal prefrontal region over later timing (250–320 ms). Our findings thus likely reflect elevated cognitive control following meal ingestion, which is rather due to value integration than to response modulation by the sensory input per se.

We also observed increases in neural activity in the insula following meal ingestion accompanied by water. Insular responses to visual food cues have consistently been associated with interoception, i.e., awareness of bodily energy states. Also, the insula does contain molecular receptors for several gastro-intestinal hormones relaying this peripheral information to central nervous responses [11,43,44]. Furthermore, other studies have found increased insular activity subsequent to PYY infusion mimicking satiety [45], to mouth rinsing with a glucose drink mimicking food intake anticipation [46], but also in response to calorie ingestion as such [20,47]. The insula, being a hub between salience, homeostatic and control networks, is generally involved in signal integration, and thought to perform flavor-nutrient conditioning, too [48]. In accordance with these findings, we also found a higher postprandial insular activity following sucrose ingestion. Altogether, increases in insular activity both in the sucrose and water conditions thus likely reflect the adequate adaptation of participants' responses as a function of the beverage consumed when taste properties and caloric load were congruent.

Sucrose drinking (i.e., the beverage condition combining sweet taste and caloric load) elicited partially different modulations in brain responses to visual food cues as compared to meal ingestion with water. In particular, the postprandial response to visual food cues in cognitive control related areas was found blunted in the sucrose condition. Sucrose consumption also led to markedly decreased neural activity in the middle temporal lobe. This brain area is involved in the categorization and optimization of visual stimulus processing by attention [49], and is generally more active when participants are exposed to palatable food over neutral stimuli [50], as well as when responses to food are compared between hunger and satiety [24,51]. These differential patterns of brain responses to food cues following sucrose *vs.* water ingestion likely show that when sweet taste is coupled to a caloric load, brain responses shift from a rather reflective (usually involving prefrontal brain areas) to a more reflexive processing of food cues [52].

4.2. Brain Responses to Food Viewing Following NNS Consumption

NNS consumption (i.e., the beverage condition with discrepant sweet taste and caloric load) also yielded differential neural activity in response to subsequent exposure to visual food cues. In contrast to the ingestion of the meal with water or a sucrose drink, we observed early enhanced ventrolateral prefrontal cortex activity (120–150 ms after food image onset), but no changes in insular activity to food viewing over the later time window of interest (250–320 ms).

Increases in neural activity following NNS tasting in ventrolateral prefrontal cortices has been highlighted in gustatory processing when neural responses were assessed at the time of or immediately after tasting. For instance, Smeets and colleagues [53] have shown greater activation of the ventrolateral

prefrontal cortex directly after the ingestion of an artificially sweetened beverage as compared to sucrose. Ventral prefrontal regions have been widely associated with hedonic integration and reward valuation of (visually) perceived stimuli, including food cues [54–56]. However, these functions were mostly attributed to medial parts of the ventral prefrontal cortex, whereas we show enhanced activity within the ventrolateral prefrontal cortex in response to visual cues following NNS consumption. Ventrolateral regions of the prefrontal cortex are part of the executive function circuitry, supporting decision-making adjustments, in particular related to motor response inhibition when exposed to cues associated with high reward, as well as targeting attention to behaviorally significant stimuli (reviewed in [57]). Thus, this area is proposed to be responsible for altering behavior as a function of estimated changes in the reward value of (viewed) stimuli. Our results show increased neural activity to visual food cues within the ventrolateral prefrontal area, likely related to greater (need for) impulse retaining and control over anticipated food intake. Although no study so far investigated the impact of NNS consumption on brain responses to food cues longitudinally, research in the gustatory modality showed differences in neural activation to NNS tasting between non-diet soda drinkers and frequent consumers of diet soda [58]. The study of Green & Murphy found increased responses to saccharin vs. sucrose in the ventrolateral prefrontal cortex in non-diet soda drinkers, whereas this difference was absent in frequent diet soda drinkers. These findings were interpreted as reflecting ‘fading’ neural activity in this region with repeated consumption of NNS, impacting impulse control over time. In line, we previously found decreased activity in the more posterior part of the ventrolateral prefrontal cortex following a 3-month replacement of SSBs by non-calorically sweetened equivalents [25]. Our current results thus provide additional evidence as to a target region for future longitudinal studies on the longer-term impact of sweet taste stimulation by NNS, and on responses to tempting visual food cues following NNS ingestion.

With NNS consumption, on the other hand, we did not observe pre-to postprandial changes in insular activity. This suggests that congruent caloric and taste signaling is required to elicit adequate response adaptation to food cues, and that incongruences between taste information and caloric load may impair nutrient-flavor conditioning [48]. Rudenga and Small [59] have further shown that the neural response to sucrose tasting in the insula (and also in the amygdala) decreased as a function of NNS consumption habits of participants, implying that this region might be more vulnerable to chronic dissociations between sweet taste signaling and metabolic consequences.

4.3. Integration of Postprandial Brain Responses to Food Viewing with Gastro-Intestinal Hormone Secretion and Food Intake Behavior

Sucrose drinking during meal ingestion, as compared to water, led to subsequent decreased food intake at the ad libitum buffet indicative of a compensatory food intake behavior. In parallel, we observed elevated plasma concentrations of insulin (an anorexigenic hormone) and decreased plasma concentrations of ghrelin (an orexigenic hormone), likely promoting some of the brain response alterations. Thus, the effects of sucrose intake may be related to hormonal signaling and/or to sweet taste receptor activation coupled with other peripheral satiety signals (e.g., vagal afferents). Yet, whether the observed differences are sucrose-specific effects or more general ones driven by an extra caloric load cannot be concluded from our current study, as there was no condition with a caloric load from another nutrient source (e.g., maltodextrin or fat).

NNS consumption did not lead to pronounced modulations of glucose, insulin, and ghrelin concentrations, nor to higher caloric consumption or variation of the food choice pattern at the ad libitum buffet. Thus, the observed changes in brain activity to food viewing post-meal and food intake pattern cannot be attributed solely to differential signaling of gastro-intestinal mediators. Although we did not measure other anorexigenic hormones such as leptin or PYY, the observed differences in brain responses between the water and NNS condition are congruent with the idea that discrepant information between sweet taste receptor activation and gastrointestinal hormone signaling leads to changes in brain response patterns [12].

4.4. Limitations

Several limitations of our work need to be considered. First, the study design likely pronounces the impact of the meal ingestion stronger than the impact of the test beverage. However, we aimed at designing this study with the highest ecological validity, i.e., having volunteers consuming standard amounts of beverages concomitant with a meal (quantity close to a commercially available can size). For this reason, we cannot exclude that the design was not sensitive enough to detect all secondary outcome differences, especially between the water and NNS conditions, and in terms of qualitative analyses on food choice patterns. Second, while we used a double-blinded design, participants could still detect the absence of sweet taste in the water condition, as opposed to both sweet taste conditions. Thus, some differences in brain response patterns might have arisen from these perceptual properties [60]. Finally, using electroencephalographic recording and electrical neuroimaging analyses, we are not able to detect deeper activity changes, e.g., in the basal ganglia (dopaminergic origin of the reward system), that might occur together with response modulations in cortices associated with higher-level functions.

5. Conclusions

To our knowledge, this is the first study to assess the impact of NNS consumption on neural activity to food viewing, and the relationship with food intake behavior. We did not observe an acute effect of NNS consumption on immediate food intake in humans who are not frequently drinking NNS beverages. Yet, we observed imminent changes in brain response patterns in brain areas that are key players in food intake regulation. The responsiveness of these brain areas to sweet taste has been shown to ‘fade’ as a function of longer-term NNS consumption [58,59]. Thus, it remains to be investigated whether such longer-term brain response alterations can also be observed to visual food cues, often mediating pre-ingestive food choices. Given such longer-term alterations, the brain response modulations observed under the NNS condition in our study might reflect an initial stage of adaptation to taste-calorie uncoupling, possibly indicating that longer-term alterations of food intake regulation (via responses to tempting visual cues) take place when NNS are repeatedly consumed over time. Our study thus provides first insights linking neuroimaging research in the gustatory modality and behavioral research on the impact of non-caloric sweetener consumption on food intake, by investigating the neural correlates of drives towards visually conveyed food cues.

Supplementary Materials: The following materials are available online at <http://www.mdpi.com/2072-6643/10/5/615/s1>. Figure S1: Behavioral ratings for hunger, satiety, thirst, and taste cravings in response to drink and concomitant meal ingestion.

Author Contributions: C.C., L.T. and U.T. conceived and designed the experiments; C.C. and L.C. enrolled participants; J.C. generated and assigned participants to allocation sequence, and carried out beverage preparation; C.C. collected the electroencephalographic data. C.C., L.C., J.C., K.S., P.S. and U.T. performed the metabolic tests; C.C., L.C., N.S. and J.-F.K. analyzed the data; M.M.M. and J.-F.K. contributed with materials and analysis tools; C.C., L.T. and U.T. wrote the manuscript. All authors had final approval of the submitted version.

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