The brain – gut interaction: Defining the role of the nutrient-induced human brain activation matrix.

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ABSTRACT

Due to the high prevalence of obesity in America (around 35%) and in Europe (above 20%) and its dramatic consequences on human health, research aiming to understand the basic mechanisms that regulate food intake, appetite and body weight is therefore needed.

New evidences suggest that fuel sensing occurs in a number of peripheral cell types, which include specific taste receptors in the gut. These receptors produce a chemical cascade signaling the central nervous system (CNS) for energy balance regulation. At the same time in the CNS specific brain regions directly sense fuel status. An emerging new methodology investigates neural correlates of appetite and satiety, using functional neuroimaging techniques.

In the present work we aim at investigating the brain-gut matrix. First, through a systematic review of the literature, previous studies assessing the effects of nutrients on brain functions were examined to identify a common research methodology and related results.

Afterwards we extensively study the effects of sugars and amino acids on the food-reward system, focusing on brain resting state functional connectivity. Finally, we focus on glucose and fructose effects on cognitive functions, by investigating two of the most common dimensions of cognitive functions such as working memory and response inhibition.

INTRODUCTION

The prevalence of obesity in America is around 35% (Kivimäki *et al*, 2017) and above 20% in the most populated European countries (Blundell *et al*, 2017). Overweight leads to dramatic health consequences (Hruby and Hu, 2015). Research aiming to understand the basic mechanisms that regulate food intake, appetite and body weight is therefore needed.

New evidences suggest that fuel sensing occurs in a number of peripheral cell types, which include specific taste receptors in the gut (Roper and Chaudhari, 2017). These receptors produce a chemical cascade signaling the central nervous system (CNS) for energy balance regulation (Roper and Chaudhari, 2017). At the same time in the CNS specific brain regions directly sense fuel status. There is ample evidence that links levels of glucose to specific populations of neurons in the CNS that are likely to modulate appetite and energy balance (Page *et al*, 2013). Therefore it is nowadays clear that nutrient-activated gut-to-brain signaling pathways play a major role in the control of digestive function, appetite and energy intake. Specifically, the release of a number of signaling peptides from nutrient sensing enteroendocrine cells (EEC), including glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK) signal, the central nervous system (CNS) to the brainstem and hypothalamus, via both the

vagus nerve and the bloodstream, regulating the food-reward networks and regulating satiety and appetite (Chaudhri *et al*, 2008).

Pharmaco-Imaging of nutrients intake.

Every time we eat, nutrients trigger different peptides in our gut, which influence various systems in our body, including the central nervous system (CNS) (Cummings and Overduin, 2007). In parallel, the brain regulates our eating behavior by modulating activations in brain regions controlling appetite, food-reward and body weight (Ahima and Antwi, 2008).

As stated above, neuroscience began to consider these brain–gut interactions as an inter-dependent system, developing a research line aiming at depicting the effects of different nutrients on specific brain areas, which subsequently influence our (eating) behavior (Page *et al*, 2013).

Functional brain imaging techniques have greatly facilitated the investigation of the human brain–gut interaction in the last decades. The effects of nutrients ingestion on the human brain can now be studied by combining the BOLD signal variation (Blood oxygen level dependent, an indirect marker of neuronal activation) together with the measurement of hormones plasma concentration (Aziz, 2012). A pioneering study of Liu (Liu *et al*, 2000a) integrated hormones plasma analyses and Magnetic Resonance Imaging (MRI) examination, demonstrating for the first time a direct link between glucose administration, insulin and glucose plasma level modification and BOLD changes in the hypothalamus and the cingulate cortex. These findings suggest that functional

MRI (fMRI) can depict the effects of nutrients administration to the functional activity of human brain regions involved in appetite and food-reward pathways.

After the first attempt by Liu (Liu *et al*, 2000a), subsequent neuroimaging studies on the brain-gut matrix reported discrepancies in the methodology (Sizonenko *et al*, 2013). This is mainly due to the different nutrients ingested and to the different paradigms used during fMRI examination. A general overview of the brain-gut literature and of the methodologies used in the field is therefore necessary.

The impact of sugars ingestion on brain functions.

Nutrients ingestion is essential for survival and implies the capacity to adjust food intake in response to changing energy requirements due to environmental demanding tasks (Morton *et al*, 2006). This homeostatic control is regulated by a deep interconnection between cellular, neuronal and behavioural mechanisms that link changes of body fat stores and adaptive adjustments of feeding behaviour to finally comply adaptive tasks.

Sugars operate an important role in metabolic processes and changes were reported at the neural level after their ingestion (Liu *et al*, 2000b). Glucose and fructose, two of the most investigated monosaccharides, have a roughly equal number of calories but are metabolized differently (Luo *et al*, 2015). While glucose stimulates the secretion of insulin, a hormone that signals the brain to increase satiety and to blunt the reward value of food (Figlewicz and Benoit,

2009; Woods *et al*, 1998), fructose is associated to insulin resistance (Aeberli *et al*, 2013). Beside the cellular level, the different metabolism of fructose and glucose may explain also their differential effects on neuronal pathways. As a milestone study of Page has documented (Page *et al*, 2013) investigating neural correlates of appetite, changes in regional cerebral blood flow and increase in functional connectivity after glucose ingestion can be highlighted in regions as the hypothalamus, insula, anterior cingulate, and striatum (appetite and reward regions). Fructose is demonstrated to reduce relative cerebral blood flow (rCBF) and increase functional connectivity in the posterior cingulate cortex, and visual cortex. Ingestion of glucose compared to fructose resulted in a reduction in hypothalamic cerebral blood flow (Luo *et al*, 2015).

Moreover, sugars may also affect brain areas activations involved in complex tasks, as cognitive functions. This is suggested by behavioural results previously documented in studies on humans and animals (Martin and Benton, 1999; Stollery and Christian, 2016; Woodie and Blythe, 2017). Between the different adaptive skills of the human being, in fact, cognitive functions are essential to process the information coming from the reality, requiring resources to support different levels of complex task performance (Kondraske, 2010). They include several domains as attention, working memory and decision-making (Alhola and Polo-Kantola, 2007). While nutrients ingestion can impact our cognitive functions (le Coutre and Schmitt, 2008; Gomez-Pinilla and Hillman, 2013), no previous

studies investigated effects of sugars on brain functions underpinning cognitive processes.

The impact of amino acids ingestion on brain functions.

Amino acids present in several protein-based meals are essential to the brain to function adequately (Lieberman, 1999). The brain uses amino acids, such as tryptophan and tyrosine, to promote the synthesis of various neurotransmitters and neuromodulators, as serotonin, essential for neuronal firing (Laterra *et al*, 1999). In particular, it was demonstrated already in the seventies that mono ammine neurotransmitters are synthesized in the brain from aromatic amino acidy, their precursors and that are present in blood vessels (Daniel *et al*, 1976). Moreover, in highly stressful situations or in pathological conditions, the CNS requirements for amino acids may change and in turn changes in the neurotransmitters synthesis can be detected (Baranyi *et al*, 2016).

In line with these findings, there is evidence that when peripheral concentration of any of the precursors varies, consequences for the brain metabolism, function, and behavior can be observed (Young, 2013).

Amino acids, as L-leucine (Mellinkoff *et al*, 1956; Thimister *et al*, 1996), L-glutamine (Gannon and Nuttall, 2010; Greenfield *et al*, 2009), and L-phenylalanine (Liddle, 2000) modulate appetite in healthy subjects and obese. Previous studies have reported effects on digestive functions (Ballinger and Clark, 1994) and food intake (Colombel *et al*, 1988) after L-tryptophan intake .

L-Tryptophan is an essential amino acids found in food and is the precursor of serotonin (Young, 2013). As the proportion of carbohydrate relative to protein increases, so does the level of brain serotonin. Consistent with alteration of serotonin, the availability of tryptophan to the brain can alter also behavioral factors such as alertness, level of depression, aggression, and pain sensitivity (Jenkins *et al*, 1987).

L-Tryptophan was demonstrated to have an influence on the activity in prefrontal regions that affect cognitive control and emotion processing (Dantzer *et al*, 2011; Passamonti *et al*, 2012; Seymour *et al*, 2012; Williams *et al*, 2007). In fact, tryptophan depletion is linked to reduced activity in the insula (Krämer *et al*, 2011), that is involved in decision making in potential aggressive situations and to changes in the DMN that may reduce depressive mood (Kunisato *et al*, 2011). However, while studies on tryptophan depletion as regulation of serotonin were extensively investigated, no previous studies from our knowledge investigated the effects of amino acids on food-reward mechanism using neuroimaging techniques. While the effects of sugars on the food-reward system are well documented, the effects of amino acids on brain regions involved in satiety and appetite are unknown.

In the present work we extensively study the effects of sugars and amino acids on the food-reward system. Afterwards, we focus on glucose and fructose effects

on cognitive functions, by investigating two of the most common dimensions of cognitive functions such as working memory and response inhibition.

Aim of the present work and hypothesis.

The present PhD thesis aims at investigating the brain-gut matrix. First, through a systematic review of the literature, previous studies assessing the effects of nutrients on brain functions were examined to identify a common research methodology and related results. On the basis of previous works, we hypothesized that brain areas involved in the food-reward circuit are activated in opposite directions, by gut peptides linked to satiety or to appetite stimulation.

We performed afterwards a set of studies investigating the effects of sugars and amino acids on satiety hormones and Resting state (RS) functional networks involved in appetite regulation. Specifically we focused on the effects of L-Tryptophan and L-Leucine on the human gut-brain system, using a multimodal approach, integrating physiological and neuroimaging data.

Finally, we investigated more specifically the effects of sugars on cognitive functions, discriminating their effects on working memory and response inhibition. In particular, as glucose and fructose follow different metabolic processes at the cellular level, at the brain level sugars and amino acids may also act differentially on regions underpinning different neural functions.

In the present work, a randomized double-blinded cross-over design was used combining the investigation of gut-hormones with multimodal neuroimaging approach (fMRI) after nutrients administration.

The present work is intended to be exploratory aiming at having a global overview of the studies investigating the brain-gut matrix and to set a common ground for future investigations in the field.

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Review article

The impact of gut hormones on the neural circuit of appetite and satiety: A systematic review

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ABSTRACT

The brain–gut-axis is an interdependent system affecting neural functions and controlling our eating behaviour. In recent decades, neuroimaging techniques have facilitated its investigation. We systematically looked into functional and neurochemical brain imaging studies investigating how key molecules such as ghrelin, glucagon-like peptide-1 (GLP-1), peptide tyrosine–tyrosine (PYY), cholecystokinin (CCK), leptin, glucose and insulin influence the function of brain regions regulating appetite and satiety.

Of the 349 studies published before July 2016 identified in the database search, 40 were included (27 on healthy and 13 on obese subjects).

Our systematic review suggests that the plasma level of ghrelin, the gut hormone promoting appetite, is positively correlated with activation in the pre-frontal cortex (PFC), amygdala and insula and negatively correlated with activation in subcortical areas such as the hypothalamus. In contrast, the plasma levels of glucose, insulin, leptin, PYY, GLP-1 affect the same brain regions conversely. Our study integrates previous investigations of the gut-brain matrix during food-intake and homeostatic regulation and may be of use for future meta-analyses of brain-gut interactions.

1. Introduction

The brain–gut axis is an interdependent system that affects neural function and controls our eating behaviour through biochemical signalling between the endocrine and nervous system through hormonal peptides in the gastrointestinal tract (Huda et al., 2006; Steinert et al., 2017; Wren and Bloom, 2007). The two main families of gastrointestinal (GI) hormones are a) Appetite stimulators, such as ghrelin, a 28 amino acid peptide that promotes meal initiation by increasing appetite and hunger feelings (Cummings et al., 2001; Kojima et al., 1999), and b) Satiety stimulators, such as the gut hormones glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY3-36) cleaved from PYY1-36, cholecystokinin (CCK) and leptin that signal the brain to decrease hunger and promote meal cessation (Figlewicz, 2003; Woods et al., 1998). Next to these GI hormones, insulin, a pancreatic hormone, as well as insulin regulated glucose, play a major role in human metabolism and eating behaviour (Figlewicz, 2003; Woods et al., 1998).

Neuroimaging techniques have greatly facilitated the investigation of human brain–gut interactions in recent decades. Pioneering studies (Liu et al., 2000) combining functional magnetic resonance imaging (fMRI) with hormonal blood analyses have demonstrated a direct link between changes in plasma concentrations in hormones and modifications in brain regions that are part of the neural circuit of appetite, as identified by Woods et al. (1998). In particular, increased insulin plasma levels are linked to changes in brain activity in the anterior cingulate cortex (ACC), in the orbitofrontal cortex (OFC), in the sensorimotor cortex and in the hypothalamus. On the other hand, it is well established that ghrelin (Malik et al., 2008) acts through the

Abbreviations: ACC, Anterior Cingulate Cortex; ASL, Arterial Spin Labelling; BOLD, Blood Oxygen Level Dependent; CBF, Cerebral Blood Flow; CNS, Central Nervous System; CSF, Cerebrospinal Fluid; dACC, Dorsal Anterior Cingulate Cortex; fMRI, Functional Magnetic Resonance Imaging; OFC, Orbitofrontal Cortex; OGTT, Oral Glucose Tolerance Test; PET, Positron Emission Tomography; PFC, Pre-frontal cortex; rsfMRI, Resting State fMRI; vmPFC, Ventromedial Prefrontal Cortex; vmPFC, Ventromedial Prefrontal Cortex

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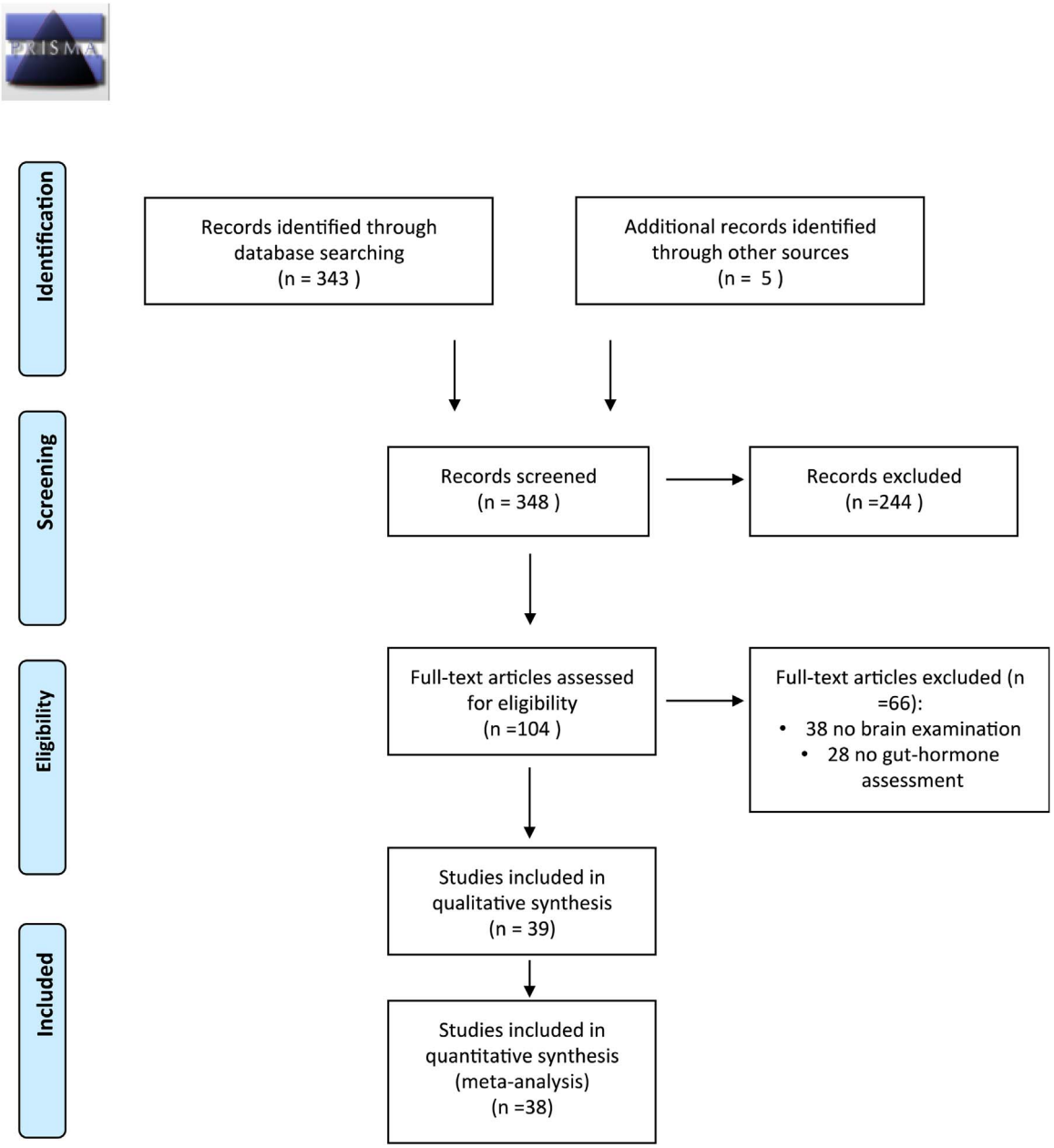

Fig. 1. Flowchart of the selection procedure.

hypothalamus to influence several brain regions involved in the food-reward pathway, including the ventral tegmental area (VTA), nucleus accumbens, amygdala, and hippocampus (Abizaid et al., 2006; Diano et al., 2006; Nakazato et al., 2001). These findings suggest that different gut peptides divergently modulate brain activation in the neural circuit controlling appetite and thereby regulate our prospective eating behaviour.

However, studies often report inconsistent findings making a general interpretation difficult. There are different reasons for the discrepancies: study designs have been variable with different nutrients ingested (stimulating different gut peptides) and different paradigms have been used during fMRI examination.

A general overview of the different studies and of the methodologies used in the field is therefore necessary.

In the present study, we systematically reviewed functional and neurochemical brain imaging studies investigating how the main gut peptides (ghrelin, PYY3-36, leptin, GLP-1 and CCK), insulin and glucose influence activation in brain regions regulating appetite and satiety in healthy and obese subjects. On the basis of the findings of these studies, we hypothesised that the brain areas involved in the food-reward circuit, such as the anterior cingulate cortex (ACC), the insula and the hypothalamus, are activated in opposite directions, by gut peptides linked to satiety or to appetite stimulation.

2. Methods

To ensure high quality reporting, PRISMA guidelines for systematic reviews were followed (Moher et al., 2015).

2.1. Search strategy

An electronic search was performed using the PubMed database. The following search terms were used: ((ghrelin OR glucose OR insulin OR peptide YY OR leptin OR GLP-1 OR cholecystokinin) AND (appetite OR satiety)) AND (mri OR fmri OR pet OR spect OR imaging OR neuroimaging). All studies published before July 2016 were included,

without any language restriction. Additionally, the reference lists of all included studies identified in the database search were manually screened for relevant studies.

2.2. Selection criteria and study selection

The review included original publications in peer reviewed journals, observational or interventional study designs and applications of functional or neurochemical neuroimaging techniques. All the included articles used a randomised double-blinded placebo-controlled design. Based on previous studies and on the existing literature (Huda et al., 2006; Jenkins et al., 1987; Wren and Bloom, 2007), one gut peptide regulating appetite (ghrelin) and four regulating satiety (peptide YY, leptin, GLP-1, CCK), as well as insulin and glucose, were investigated. The current review focuses on how changes in plasma concentration of gut hormones result in modifications of brain functions regulating appetite and satiety.

After inspection for duplicates, the titles and abstracts of all records were reviewed. Publications that clearly did not meet inclusion criteria were excluded. The decision for inclusion or exclusion of the remaining publications was made on the basis of a review of the full texts. The whole process was independently conducted by two reviewers (DZ, SB). In case of disagreement, reviewers discussed their reasons for initial inclusion and exclusion. If consensus was not reached, a third reviewer (AS) was included.

2.3. Recorded variables, data extraction and analysis

The recorded variables for each article included in the review were: authors and year of publication, study design, assessed peptides, administered substance, amount of nutrient received, modality of administration, imaging method, number of healthy subjects, number of obese subjects, gender distribution, age, Body max index (BMI), brain region investigated, analysed brain regions, statistical thresholds and main findings. If overlaps between subjects were suspected but the original publications did not contain information on that topic, we contacted the authors and included the obtained data in the review.

3. Results

3.1. Identified studies

Of 343 publications found in the PubMed database and 6 articles identified in the reference lists, 40 articles were included in this review.

244 publications did not meet the inclusion criteria (e.g. animal models, case reports, review articles, pathological conditions) and were thus excluded. 66 studies were excluded since they investigated appetite without including any brain examination or hormone administration.

A flowchart of the selection procedure, with the included and excluded studies, is shown in Fig. 1.

3.2. Study characteristics

Of the 40 included articles, 17 studies used fMRI with a “food-cue paradigm” (van Bloemendaal et al., 2014; De Silva et al., 2011; Douglas et al., 2015; Goldstone et al., 2014; Grosshans et al., 2012 Heni et al., 2014, 2015, p. 201; Hinkle et al., 2013; Karra et al., 2013; Kroemer et al., 2013a,b, 2015; Leidy et al., 2013; Malik et al., 2008; Page et al., 2011; Rosenbaum et al., 2008; Wallner-Liebmann et al., 2010), eleven an “on-off treatment related block design” (Batterham et al., 2007; Eldeghaidy et al., 2016; Jones et al., 2012; Lassman et al., 2010; Li et al., 2012; Little et al., 2014; Liu et al., 2000; Purnell et al., 2011; Spetter et al., 2014; Sun et al., 2014), five a resting state fMRI (rsfMRI) paradigm (Jastreboff et al., 2016; Page et al., 2013; Wölnerhanssen et al., 2015; Wright et al., 2016; Zhang et al., 2015) and five studies an fMRI-ASL (arterial spin labelling) sequence (two studies used both rsfMRI and ASL) (Jastreboff et al., 2016; Lennerz et al., 2013; Page et al., 2009, 2013; Schilling et al., 2014). Four were neurochemical imaging studies using positron emission tomography (PET) (Gautier et al., 2000; Pannacciulli et al., 2007; Savage et al., 2014; Tataranni et al., 1999).

All included studies were published between 2007 and 2016. 13 studies investigated the effect of the appetite-stimulating hormone ghrelin (Batterham et al., 2007; Goldstone et al., 2014; Jastreboff et al., 2016; Jones et al., 2012; Kroemer et al., 2013a,b, 2015; Leidy et al., 2013; Li et al., 2012; Malik et al., 2008; Savage et al., 2014; Sun et al., 2014, 2015), while 30 studies investigated the impact of satiety-inducing hormones and glucose (Batterham et al., 2007; van Bloemendaal et al., 2014; De Silva et al., 2011; Douglas et al., 2015; Eldeghaidy et al., 2016; Gautier et al., 2000; Grosshans et al., 2012; Heni et al., 2014, 2015; Hinkle et al., 2013; Jastreboff et al., 2016; Kroemer et al., 2013b, 2015; Leidy et al., 2013; Lennerz et al., 2013; Li et al., 2012; Liu et al., 2000; Page et al., 2009, 2011, 2013; Pannacciulli et al., 2007; Purnell et al., 2011; Rosenbaum et al., 2008; Schilling et al., 2014; Spetter et al., 2014; Tataranni et al., 1999; Wallner-Liebmann et al., 2010; Wölnerhanssen et al., 2015; Wright et al., 2016; Zhang et al., 2015). Eight studies focused on glucose (Gautier et al., 2000; Heni et al., 2014; Lennerz et al., 2013; 2009, Page et al., 2011; Purnell et al., 2011; Wallner-Liebmann et al., 2010; Wright et al., 2016), 15 on insulin (van Bloemendaal et al., 2014; Gautier et al., 2000; Heni et al., 2014; Jastreboff et al., 2016; Kroemer et al., 2013a; Lennerz et al., 2013; Li et al., 2012; Liu et al., 2000; Page et al., 2009, 2013; Schilling et al., 2014; Tataranni et al., 1999; Wallner-Liebmann et al., 2010; Wölnerhanssen et al., 2015; Zhang et al., 2015), four on peptide YY (Batterham et al., 2007; De Silva et al., 2011; Douglas et al., 2015; Leidy et al., 2013), five on leptin (Grosshans et al., 2012; Hinkle et al., 2013; Jastreboff et al., 2016; Kroemer et al., 2015; Rosenbaum et al., 2008), five on GLP-1 (van Bloemendaal et al., 2014; Douglas et al., 2015; Heni et al., 2015, p. 2; Li et al., 2012; Pannacciulli et al., 2007) and four on CCK (Eldeghaidy et al., 2016; Lassman et al., 2010; Li et al., 2012; Little et al., 2014).

To assess brain changes associated with these gut peptides, a broad variety of nutrients with extensive differences in protein load were administered. In 16 studies, subjects directly received the target nutrient (such as glucose) (Batterham et al., 2007; De Silva et al., 2011; Eldeghaidy et al., 2016; Heni et al., 2014, 2015; Hinkle et al., 2013; Jones et al., 2012; Kroemer et al., 2013a,b; Little et al., 2014; Malik et al., 2008; Page et al., 2009, 2011, 2013; Rosenbaum et al., 2008; Schilling et al., 2014), while in 24 studies subjects consumed standardised meals (containing for instance: fibres, soy or chocolate milkshake) with different amounts of protein (van Bloemendaal et al., 2014; Douglas et al., 2015; Gautier et al., 2000; Goldstone et al., 2014; Grosshans et al., 2012; Jastreboff et al., 2016; Karra et al., 2013; Kroemer et al., 2015; Lassman et al., 2010; Leidy et al., 2013; Lennerz et al., 2013; Li et al., 2012; Liu et al., 2000; Pannacciulli et al., 2007; Purnell et al., 2011; Savage et al., 2014; Schilling et al., 2014; Spetter et al., 2014; Sun et al., 2014, 2015; Tataranni et al., 1999; Wallner-Liebmann et al., 2010; Wright et al., 2016; Zhang et al., 2015).

As regards the modality of administration, 12 studies used an intravenous canula (Figlewicz, 2003; Goldstone et al., 2014; Grosshans et al., 2012; Heni et al., 2014; Hinkle et al., 2013; Karra et al., 2013; Kojima et al., 1999; Liu et al., 2000; Nakazato et al., 2001; Spetter et al., 2014; Sun et al., 2015), in 22 studies the substances were ingested orally (Douglas et al., 2015; Eldeghaidy et al., 2016; Gautier et al., 2000; Heni et al., 2014, 2015; Jastreboff et al., 2016; Karra et al., 2013; Kroemer et al., 2013a, 2015; Leidy et al., 2013; Lennerz et al., 2013; Li et al., 2012; Little et al., 2014; Liu et al., 2000; Page et al., 2013; Pannacciulli et al., 2007; Schilling et al., 2014; Spetter et al., 2014; Sun et al., 2014, 2015; Tataranni et al., 1999; Wright et al., 2016; Zhang et al., 2015), in three studies a nasogastric tube was used (Lassman et al., 2010; Spetter et al., 2014; Wölnerhanssen et al., 2015), while in

three studies no administration was performed (Grosshans et al., 2012; Savage et al., 2014; Wallner-Liebmann et al., 2010). The time between nutrient administration and brain imaging examination varied as well: in 14 studies, the neuroimaging examination started immediately after nutrient administration (Batterham et al., 2007; van Bloemendaal et al., 2014; Douglas et al., 2015; Gautier et al., 2000; Jastreboff et al., 2016; Jones et al., 2012; Lassman et al., 2010; Li et al., 2012; Malik et al., 2008; Page et al., 2013, 2011; Purnell et al., 2011; Spetter et al., 2014; Zhang et al., 2015), while in the other 20 brain signals were recorded 5–120 min after nutrient administration (De Silva et al., 2011; Eldeghaidy et al., 2016; Gautier et al., 2000; Goldstone et al., 2014; Heni et al., 2014, 2015; Karra et al., 2013; Kroemer et al., 2013a,b; Lennerz et al., 2013; Little et al., 2014; Page et al., 2009; Pannacciulli et al., 2007; Schilling et al., 2014; Sun et al., 2014, 2015; Tataranni et al., 1999; Wölnerhanssen et al., 2015; Wright et al., 2016). Three studies investigated long-term effects by focusing on an administration period between 6 days and 5 weeks (Hinkle et al., 2013; Leidy et al., 2013; Rosenbaum et al., 2008). As stated above, three studies did not administer any treatment (Grosshans et al., 2012; Savage et al., 2014; Wallner-Liebmann et al., 2010).

13 studies included obese participants beside healthy subjects (van Bloemendaal et al., 2014; Gautier et al., 2000; Grosshans et al., 2012; Heni et al., 2014, 2015; Hinkle et al., 2013; Jastreboff et al., 2016; Lennerz et al., 2013; Rosenbaum et al., 2008; Savage et al., 2014; Sun et al., 2015; Wallner-Liebmann et al., 2010; Zhang et al., 2015), while 27 studies focused only on healthy controls(Batterham et al., 2007; De Silva et al., 2011; Douglas et al., 2015; Eldeghaidy et al., 2016; Goldstone et al., 2014; Jones et al., 2012; Karra et al., 2013; Kroemer et al., 2013a,b, 2015; Lassman et al., 2010; Leidy et al., 2013; Li et al., 2012; Little et al., 2014; Liu et al., 2000; Malik et al., 2008; Page et al., 2009, 2011, 2013; Pannacciulli et al., 2007; Purnell et al., 2011; Schilling et al., 2014; Spetter et al., 2014; Sun et al., 2014; Tataranni et al., 1999; Wölnerhanssen et al., 2015; Wright et al., 2016). Details are shown in Table 1.

3.3. Effects of appetite-inducing hormones on the brain: ghrelin

Of the 10 fMRI studies investigating the effects of ghrelin on healthy subjects, four used a food cue paradigm (Table 2). The ‘food cue paradigm’, also called “food-picture paradigm”, refers to a block design in which high/low-energy-dense food pictures were shown in alternation to non-food pictures in a randomised fashion during the fMRI examination.

This approach was used for the first time by Malik et al. (2008) to investigate the effect of ghrelin on brain areas controlling appetite. After placebo (saline) administration, 0.5 mg/kg of ghrelin were injected with a peripheral venous cannula to 21 male healthy participants over a period of 20 min. In a food-cue paradigm, fMRI was performed during both the placebo and ghrelin conditions. Appetite scores were taken regularly during the blood-fMRI examination. Ghrelin increased the neural response to food pictures in different regions of the brain, including the amygdala, orbitofrontal cortex (OFC), anterior insula, and striatum, which are all implicated in encoding the incentive value of food cues. Moreover, the amygdala and OFC responses to ghrelin were positively correlated with subjects’ self-rated hunger ratings. The relationship between enhanced levels of plasma ghrelin and corticolimbic activity is confirmed by a similar study of Goldstone et al. (2014) on 21 healthy participants receiving ghrelin or saline injection, in which increased OFC and hippocampus activity were observed after acute ghrelin administration.

Furthermore, two overlapping fMRI studies of Kroemer et al. (2013a, 2015) using the same study population (26 healthy controls, 13 women) investigated how glucose and nicotine induced changes in ghrelin plasma levels and in brain responses during the presentation of food-related cues. In the first study (Kroemer et al., 2013a), fMRI in a food-cue paradigm was performed after overnight fasting and after a standardised caloric intake (75 g of glucose). Fasting levels of ghrelin correlated positively with food-cue reactivity in the OFC and in the limbic and paralimbic regions, in which ghrelin receptors are densely concentrated. Moreover, fasting ghrelin levels were associated with an increase in subjective appetite.

In the second study (Kroemer et al., 2015), nicotine (2 mg) was administered to fasting subjects and after meal consumption. During fasting, nicotine administration weakened the correlations between ghrelin levels and brain activity in the mesocorticolimbic system (hypothalamus and nucleus accumbens). In contrast, after meal administration, nicotine increased the correlation between ghrelin plasma levels and activity in the ventromedial prefrontal cortex (vmPFC) and in the amygdala. These results confirm that nicotine affects how ghrelin modulates the neural responses of appetite.

Furthermore, five studies used an ‘on-off treatment related block design’ during fMRI examination to investigate the effects of ghrelin on brain areas controlling appetite and satiation. Nutrients are administered during the fMRI examination and the timing of ghrelin plasma absorption is used to investigate the brain response. This approach was used for the first time by Batterham et al. (2007) to investigate the effects of ghrelin on brain activity after placebo and PYY administration (Batterham et al., 2007) on eight healthy males. Ghrelin levels were negatively correlated with activation in the hypothalamus, ventral tegmental areas and brainstem after PYY administration. Furthermore, a negative correlation was shown between activity in these areas and satiety levels. These findings are confirmed by the study of Jones et al. (2012) using the same paradigm, in which an intravenous infusion of ghrelin (1.25 pmol/kg/min) was injected before and after intragastric administration of lipids (dodecanoate, C_{12}) to 20 healthy subjects. During digestion, a decrease in appetite was negatively correlated with activity in the midbrain, thalamus, hypothalamus, insula, amygdala and hippocampus.

Two studies using the same sample size (Sun et al., 2014, 2015) investigated effects of ghrelin on 32 healthy individuals before and after meal ingestion using the same paradigm. During the fMRI examination, two different milkshake flavours (chocolate and strawberry) were administered. Larger post-prandial reductions in ghrelin plasma levels were associated with a reduced response to the chocolate milkshake in brain regions, including the midbrain, amygdala, pallidum, hippocampus, insula and medial OFC. Using the same paradigm, Li et al. (2012) investigated how ingested fat, glucose, protein, and water modulated brain activation in 14 healthy men. In line with previous findings (Sun et al., 2014), activation in the middle insula, amygdala and lateral OFC also correlated with changes in ghrelin levels after fat administration and glucose. Although this study did not demonstrate a direct correlation between cerebral activity and plasma ghrelin levels and appetite, it showed that ghrelin levels decreases after nutrient administration.

Leidy et al. (2013) used fMRI to confirm these results, by exploring brain activation in response to food cues in 20 late adolescent girls who consumed either a normal protein breakfast, a high protein breakfast, or who skipped breakfast continuously for six days. In agreement with previous evidence, ghrelin plasma levels decreased after the high protein breakfast, and reduced activation was observed in the amygdala, hippocampus and para-hippocampus.

Finally one PET study focused on ghrelin and brain-related neurochemical changes (Savage et al., 2014). This study included 8 subjects of normal weight and 19 obese subjects and investigated midbrain dopaminergic neurons (DA type 2/type 3 receptor (D2/D3R)). In healthy individuals, fasting ghrelin correlated negatively with dopaminergic binding potential in the midbrain and nucleus accumbens.

3.4. Effects of glucose and satiety inducing hormones on the brain: glucose, insulin, peptide YY, leptin, GLP-1, and CCK

19 fMRI studies used a food cue paradigm to explore the effect in

Table 1
Study characteristics.

Author and year	Nutrients received	Amount of nutrients received	Administration	Hormones investigated	Neuro-imagingmodality	Paradigm	Time after treatment administration
Batterham et al. (2007)	• PYY • Placebo	PYY was dissolved in 0.9% saline containing 5% by volume Haemaccel (Beacon)	Intravenous	• Ghrelin • Leptin • Insulin • PYY • Glucose	fMRI	On-off treatment related block design	Immediately
De Silva et al. (2011)	• Placebo • Standard breakfast • PYY • GLP-1 • PYY and GLP combined	• A 90 min saline infusion (fasted saline, control visit). • Standard breakfast, then a 90 min saline infusion (the fed saline visit). • A 90 min PYY3-36 infusion at 0.3 pmol/kg/min. • A 90 min GLP-1 7–36 amide infusion at 0.8 pmol/kg/min. • A 90 min combined PYY3-36 and GLP-1 7–36 amide infusion at 0.3 pmol/kg/min and 0.8 pmol/kg/min, respectively.	Intravenous	• PYY • GLP	fMRI	Food-cue	20 min after the start of the infusion
Douglas et al. (2015)	• Fiber–matched (MF) meal • Soy serving size–matched (SS) meal	400-kcal	Orally	• PYY • GLP	fMRI	Food-cue	Immediately
Eldeghaidy et al. (2016)	• High-fat meal • Water load • Fat	520 kcal	Orally	CCK	fMRI	On-off treatment related block design	45 min
Goldstone et al. (2014)	• Saline injection (Fed-Saline): before breakfast • Saline injection (Fasted-Saline): after breakfast • Acyl ghrelin (Fed-Ghrelin): after breakfast	3.6 nmol/kg	Intravenous	• Glucose, • PYY, • GLP-1 • Ghrelin • Insulin	fMRI	Food-cue	95 min.
Grosshans et al. (2012)	/	/	/	Leptin	fMRI	Food-cue	/
Heni et al. (2014)	• Glucose ingestion • Water ingestion	• 75 g glucose • 300 mL water	Orally	• Glucose • Insulin	fMRI	Food-cue	• 30 min • 120 min
Heni et al. (2015)	• Glucose ingestion • Water ingestion	• 75 g glucose • 300 mL water	Orally	• Glucose • Insulin	fMRI	Food-cue	• 30 min • 120 min
Hinkle et al. (2013)	• After a six weeks diet: 1. Leptin 2. Placebo (saline)	The leptin dose = leptin before the diet	Intravenous	Leptin	fMRI	Food-cue	5 weeks
Jastreboff et al. (2016)	• Glucose • Fructose	75 g	Beverage	• Glucose • Fructose • Leptin • Ghrelin	fMRI – ASL	Resting state	Immediately
Jones et al. (2012)	• Post-prandial state: 1. Ghrelin bolus 2. Saline • Fasting state:	• Ghrelin bolus (0.3 nmol/kg) • Ghrelin injection (1.25 pmol/kg/min)	Intravenous	Ghrelin	fMRI	On-off treatment related block design	Immediately

(continued on next page)

Table 1 *(continued)*

Author and year	Nutrients received	Amount of nutrients received	Administration	Hormones investigated	Neuro-imagingmodality	Paradigm	Time after treatment administration
	1. Ghrelin injection 2. C12 + Ghrelin						
Karra et al. (2013)	test meal	1840 kcal	Orally	Ghrelin	fMRI	Food-cue	45 min
Kroemer et al. (2013)	Glucose	75 g	Beverage	Ghrelin	fMRI	Food-cue	5 min.
Kroemer et al. (2013)	Glucose	75 g	Beverage	• Insulin • Glucose	fMRI	Food-cue	5 min.
Kroemer et al. (2015)	• Fasting 1. Placebo 2. Nicotine • Glucose: 1. Placebo 2. Nicotine	2 mg	Beverage	1. Leptin 2. Ghrelin	fMRI	Food-cue	6 min.
Lassman et al. (2010)	• Lipid (dodecanoic acid, 250 mL) • saline (control) • CCK-1 receptor antagonist dexloxiglumide (600 mg orally)	• Lipid (dodecanoic acid, 250 mL) • saline (control) • CCK-1 receptor antagonist dexloxiglumide (600 mg orally)	Intragastric	CCK	fMRI	On-off treatment related block design	Immediately
Leidy et al. (2013)	• 350-kcal NP (13 g protein) cereal-based breakfasts • 350-kcal HP egg- and beef-rich (35 g protein) breakfasts • breakfast skipping	• cereal-based breakfasts: 13 g protein • egg- and beef-rich breakfasts: 35 g protein	Orally	• Ghrelin • Peptide YY (PYY)	fMRI	Food-cue	6 days
Lennerz et al. (2013)	• High glycemic meal • Low glycemic meal	• High glycemic meal 84% of predictive glucose • Low glycemic meal 37% of predictive glucose	Orally	• Glucose • Insulin	fMRI – ASL	/	4 h
Li et al. (2012)	• glucose • soybean oil emulsion • whey protein • water	• glucose: 250 g • soybean oil emulsion: 111 g • whey protein: 257 g • water	Beverage	• Glucose • Insulin • Ghrelin • GLP-1 CCK	fMRI	On-off treatment related block design	Immediately
Little et al. (2014)	• 1 M glucose + predosing with dexloxiglumide (CCK1 receptor antagonist) • 1 M glucose + placebo • 0.9% saline (control) + placebo	• 250 gucose • 250 water • 600 mg of dexloxiglumide	Orally	CCK	fMRI	On-off treatment related block design	1h
Liu et al. (2000)	• D-dextrose • water	75 g	Beverage	Insulin	fMRI	On-off treatment related block design	Immediately
Malik et al. (2008)	• Ghrelin • Placebo	0.5 mg/kg for 20 min.	Intravenous	• Insulin • Glucose	fMRI	Food-cue	Immediately
Page et al. (2011)	• euglycemic-hypoglycemic (insulin) • euglycemic–euglycemic (glucose)	Insulin = 2 mU/kg/min + 20% glucose	Intravenous	• Leptin • Insulin • Ghrelin	fMRI	Food-cue	Immediately
Page et al. (2013)	• Glucose • Fructose	75 g	Beverage	• Glucose • Insulin	fMRI – ASL	Resting state	Immediately

(continued on next page)

Table 1 *(continued)*

Author and year	Nutrients received	Amount of nutrients received	Administration	Hormones investigated	Neuro-imagingmodality	Paradigm	Time after treatment administration
				• Leptin • Ghrelin • Peptide YY • GLP-1			
Page et al. (2009)	• Insuline • Glucose	• Euglycemia (plasma glucose ~95 mg/dl) • Hypoglicemia(plasma glucose ~50 mg/dl)	Intravenous	• Insuline • Glucose	fMRI – ASL	/	• 30 min after the start of the plasma glucose decline toward hypoglycemic levels • 90 min during the euglycemic session
Pannaciulli et al. (2007)	• Fasting state • Satiety state	Ensure-Plus 1.5 kcal/ml (1 Ca = 4.18 J)	Orally	• Glucose • Insulin • GLP-1	PET	/	25 min
Gautier et al. (2000)	• Fasting state • Satiety state	Ensure-Plus 1.5 kcal/ml (1 Ca = 4.18 J)	Orally	• Insulin • Leptin • GLP-1	PET	/	25 min
Purnell et al. (2011)	• Glucose • Fructose • Saline	0.3 mg/kg	Intravenous	• Insuline • Glucose	fMRI	On-off treatment related block design	Immediately
Rosenbaum et al. (2008)	• After a six weeks diet: 1. Leptin 2. Placebo (saline)	The leptin dose = leptin before the diet	Intravenous	Leptin	fMRI	Food-cue	5 weeks
Savage et al. (2014)	/	/	/	Ghrelin	PET	/	/
Schilling et al. (2014)	• Cortisol 1. Insulin 2. Placebo	• Cortisol: 30 mg • Insulin: 100 I.E./ml	• Cortisol: orally • Insulin Nasogastric tube	Insulin	fMRI – ASL	/	30 min
Spetter et al. (2014)	• Water • naso-gastric chocolate milk infusion • oral chocolate milk administration	per 100 mL: energy content of 354 kJ, 3.5 g proteins, 12 g mono and disaccharides, 2.5 fat g, 0.5 g fibres	• Nasogastric tube • Orally	• Insulin • Glucose • Ghrelin	fMRI	On-off treatment related block design	Immediately
Sun et al. (2014)	• Milkshake chocolate • Milkshake strawberry	• Milkshake chocolate • Milkshake strawberry	Orally	• Glucose • Insulin • Ghrelin	fMRI	On-off treatment related block design	30 min
Sun et al. (2015)	• Milkshake chocolate • Milkshake strawberry	• Milkshake chocolate (12 fl oz each of whole milk, Garelick Farms brand Chug Chocolate Milkshake, and Garelick Farms brand Chug Cookies and Cream Milkshake) • Milkshake strawberry (32 fl oz of whole milk to which 6 fl oz of Hershey's brand strawberry syrup was added)	Orally	• Glucose • Insulin • Ghrelin	fMRI	On-off odour (food-non food) block design	65 min
Tataranni et al. (1999)	• Fasting state • Satiety state	Ensure-Plus 1.5 kcal/ml (1 Ca = 4.18 J)	Orally	• Glucose • Insulin • GLP-1	PET	/	25 min
van Bloemendaal et al. (2014)	• GLP-1 receptor agonist exenatide	• Intravenous exendin 9–39 or placebo was	Intravenous	GLP-1	fMRI	Food-cue	Immediately

(continued on next page)

Table 1 *(continued)*

Author and year	Nutrients received	Amount of nutrients received	Administration	Hormones investigated	Neuro-imagingmodality	Paradigm	Time after treatment administration
	• exenatide together with the GLP-1 receptor antagonist exendin 9–39	started 30 min after the start of the clamp at an infusion rate of 600 pmol/kg/min. • Intravenous exenatide or placebo infusion was started 60 min after the start of the clamp at an infusion rate of 50 ng/min for 30 min					
Wallner-Liebmann et al. (2010)	/	/	/	• Insulin • Glucose	fMRI	Food-cue	/
Wölnerhanssen et al. (2015)	• Glucose • Fructose • Placebo	• Glucose 75 g • Fructose 25 g • Placebo 300 m pure tap water	Nasogastric tube	• Glucose • Insulin • GLP-1	fMRI	Resting state	5 min
Wright et al. (2016)	• Fasted night • Standard breakfast (cornflakes, semiskimmed milk, toast, margarine, strawberry jam and orange juice)	• Fasted night • Standardize breakfast (531 kcal for females, 670 kcal for males)	Orally	Glucose	fMRI	Resting state	20 min
Zhang et al. (2015)	• fasted • liquid formula meal	Liquid meal:55% carbohydrate, 30% fat, 15% protein; Ensure-Plus 1.5 kcal/ml	Orally	• Glucose • Insulin	fMRI	Resting state	Immediately

Author and year	Sample size HC			Sample size Obese		
	N(m)	age	BMI (kg/m2)	N(m)	age	BMI
Batterham et al. (2007)	8(8)	29.6 ± 2.1	21.7 ± 0.7	/	/	/
De Silva et al. (2011)	15(10)	29.5	22.1	/	/	/
Douglas et al. (2015)	21(?)	23 ± 1	23.4 ± 0.6	/	/	/
Eldeghaidy et al. (2016)	17/11)	25 ± 2	22.4 ± 0.8	/	/	/
Goldstone et al. (2014)	22(17)	/	18.0–29.9	/	/	/
Grosshans et al. (2012)	23(8)	18–65	18.5–24.0	21(6)	18–65	36.9
Heni et al. (2014)	12(6)	21–29	19.4–22.5	12(6)	21–28	28.8–34.4
Heni et al. (2015)	12(6)	21–30	19.4–22.6	12(6)	21–29	28.8–34.5
Hinkle et al. (2013)	/	/	/	10(2)	38 ± 2	> 30
Jastreboff et al. (2016)	14(10)	15.8 ± 1.6	21.8 ± 2.3	24(11)	15.3 ± 1.8	34.4 ± 4.7
Jones et al. (2012)	20(?)	34.1	25.1			
Karra et al. (2013)	12AA(FTO)	23.0 ± 0.8	22.3 ± 0.5	12TT(FTO)	22.1 ± 1.0	21.6 ± 0.3
Kroemer et al. (2013)	26(13)	24.4 ± 3.4	18.5–24.9			
Kroemer et al. (2013)	26(13)	24.4 ± 3.5	18.5–24.10			
Kroemer et al. (2015)	26(13)	24.4 ± 3.6	18.5–24.11			
Lassman et al. (2010)	19					
Leidy et al. (2013)		19 ± 1	28.6 ± 0.7			
Lennerz et al. (2013)				12(12)	18–35	> 25
Li et al. (2012)	14(14)	21–25	21.2			
Little et al. (2014)						
Liu et al. (2000)	21(11)	34 ± 3				
Malik et al. (2008)	21(21)	24.1 ± 1.1	22.3 ± 0.7			

Table 1 (*continued*)

Author and year	Sample size HC			Sample size Obese		
	N(m)	age	BMI (kg/m2)	N(m)	age	BMI
Page et al. (2011)	21(12)	31.4 ± 7.9	25.2 ± 4			
Page et al. (2013)	20(10)	31 ± 7	22 ± 2.5			
Page et al. (2009)	9(8)	28 ± 5	23.6 ± 2			
Pannacciulli et al. (2007)	42(22)	31 ± 8	31 ± 9			
Gautier et al. (2000)	11(?)	35 ± 8	< 25	11(?)	27 ± 5	< 35
Purnell et al. (2011)	9(3)	29 ± 4.3	22.0 ± 2.2			
Rosenbaum et al. (2008)	/	/	/	6(2)	38 ± 2	> 30
Savage et al. (2014)	8(0)	38 ± 4.3	22	19(0)	/	38
Schilling et al. (2014)	48(48)	23.96 ± 3.4	20 < BMI < 25	/	/	/
Spetter et al. (2014)	16(16)	24.6 ± 3.8	22.3 ± 1.6			
Sun et al. (2014)	32(14)	25.5 ± 5.7	25.3 ± 4.4			
Sun et al. (2015)	20(9)	26 ± 5.9	21.7 ± 1.4	13(7)	28.2 ± 6.6	28.1 ± 2.5
Tataranni et al. (1999)	11(11)	35 ± 8	19 ± 6% body fat			
van Bloemendaal et al. (2014)	16(8)	57.8 ± 1.9	23.2 ± 0.4	16(?)	58.0 ± 2.1	32.6 ± 0.7
Wallner-Liebmann et al. (2010)	12(6)	18.3 ± 3.4	20.9 ± 1.6	12(6)	18.0 ± 3.7	34.1 ± 5.6
Wölnerhanssen et al. (2015)	12(12)	24.8	22.9			
Wright et al. (2016)	19(9)	24.8 ± 3.8	< 30			
Zhang et al. (2015)	20(20)		18.5–23.9	20(20)		> 28

healthy subjects of glucose and satiety hormones on brain activation (Table 3).

Six studies investigated the effects of glucose plasma levels on the brain. In 2011, Page et al. (2011) administered glucose and insulin to induce a hypoglycaemic or euglycemic status in 21 healthy subjects. A food cue paradigm was used to investigate brain responses during these two conditions. Hypoglycaemia preferentially activated limbic-striatal brain regions (such as the insula, putamen, hypothalamus, caudate) in response to food cues to produce greater desire for high calorie food, while euglycemia preferentially activated the medial prefrontal cortex and resulted in less interest in food stimuli.

In the milestone fMRI study of Liu et al. (2000), glucose was administered to 21 healthy volunteers in an 'on-off treatment related block design'. Temporal clustering analysis showed increased activation in the OFC, frontal lobe and decreased activation in the hypothalamus after glucose intake. Moreover, before glucose intake, plasma insulin levels correlated negatively with activity in the hypothalamus.

Woelnerhanssen et al. (2011) used an RS paradigm to explore the effects of acute glucose and fructose administration on the connectivity within the basal ganglia network of 12 healthy participants. They found that after glucose and fructose administration, a glucose-induced increase in rsFC was present in the left caudatus, left putamen, precuneus and lingual gyrus and − relative to placebo − the glucose-induced increase in functional connectivity within the basal ganglia/limbic network correlated positively with glucose-induced insulin release. Wright et al. (2016) confirmed these results by showing that the connectivity between the left hypothalamus and the superior frontal gyrus was negatively correlated with glucose plasma levels during fasting sessions.

In a 2009 study on nine healthy subjects (Page et al., 2009), Page used an fMRI-ASL sequence to show that increases in glucose blood levels lead to regional increases in cerebral blood flow (CBF) in the cerebellum and decreases in the hypothalamus, inferior frontal gyrus, and anterior cingulate cortex. In a study of 2013 (Page et al., 2013) on 20 HC, the author confirmed the previous results. After a drink containing glucose or fructose, regional CBF was reduced within the hypothalamus, thalamus, insula, anterior cingulate, and striatum after glucose or fructose compared to baseline. Moreover, changes in the levels of plasma insulin correlated negatively with changes in regional CBF in the caudate and putamen in response to glucose ingestion.

Seven studies investigated the effects of insulin plasma levels on brain activity.

In a study conducted in 2013 (Kroemer et al., 2013b), Kroemer used the "food cue paradigm" to investigate brain modifications after changes in insulin levels. fMRI was used to investigate reactivity to food cues after overnight fasting and following a standardised caloric intake (i.e., a 75 g oral glucose) in 26 participants. Increased plasma insulin levels correlated negatively with activity in the bilateral fusiform gyrus, superior temporal gyrus, medial frontal gyrus and the limbic system. In addition, activation in these regions was accompanied by lower subjective appetite ratings. In the same line, Wallner-Liebmann et al. (2010) showed that during high caloric food cues, insulin levels are positively associated with hippocampal activity and negatively with activity in the right superior frontal gyrus and left thalamus in 12 healthy adolescents.

Using an 'on-off treatment related block design' in a study on 14 healthy subjects, Li et al. (2012) showed that levels of plasma insulin after glucose administration correlated negatively with activity in the middle insula, thalamus, amygdala and lateral OFC, and – after protein administration – with activity in the caudate. In the same line, in the study of Purnell et al. (2011) on nine healthy individuals, increased activation in the OFC and increases in plasma glucose and insulin levels were observed during glucose infusion. Spetter et al. (2014) demonstrated that insulin responses following naso-gastric infusion of chocolate milk to 16 healthy individuals correlated positively with brain activation in the anterior cingulate cortex (ACC) and putamen and

Table 2
Effects of appetite-inducing hormones on the brain: Ghrelin. Decreased activation: "↓". Increased activation: "↑".

Authors and year of publication	Neuro-imaging modality	Brain region investigated	Type of analysis	Threshold	Results (HC, if not indicated otherwise)
Batterham et al. (2007)	fMRI	Whole brain + ROIs (Hypothalamus, substantia nigra, nucleus accumbens, solitary nucleus and tract, parabrachial nucleus)	GLM	Uncorrected	• Hypothalamus ↓ • VTA ↓ • Brainstem ↓
Goldstone et al. (2014)	fMRI	ROIs (Orbito-frontal cortex, hippocampus, nucleus accumbens, caudate, anterior insula, amygdala)	GLM	FDR at $P < 0.05$	• Ghrelin: orbitofrontal cortex↑, Hippocampus↑
Jastreboff et al. (2016)	fMRI – ASL	Whole brain analyses	GLM	$p < 0.05$, FWE whole-brain corrected	• Main effect: Ghrelin: putamen↑, thalamus↑, insula↑, hypothalamus ↑ • Obese vs. lean: Ghrelin: hypothalamus↑, thalamus↑ , hippocampus ↑
Jones et al. (2012)	fMRI	Whole brain analyses	GLM	$p < 0.05$, FWE whole-brain corrected	• Post-prandial state, ghrelin vs. saline: 1. decrease medulla, midbrain and pons regions of the brainstem↓, cerebellum↓, hypothalamus (upper)↓, insula↓, parahippocampal gyrus (amygdala/ hippocampus), postcentral gyrus, thalamus (ventral anterior nucleus)↓ 2. motor cortex and precentral gyrus↑ • Pre-prandial state, ghrelin vs. saline: 1. decrease medulla, midbrain and pons regions of the brainstem↑, cerebellum↑, hypothalamus (upper) ↑, insula↑, parahippocampal gyrus (amygdala/ hippocampus), postcentral gyrus, thalamus (ventral anterior nucleus) ↑ • Effects of ghrelin on C12-induced BOLD signal: 1. In the midbrain, pons and hypothalamus, ghrelin blocked the increase in BOLD signal in response to C12, and in the insula and amygdala/hippocampus the C12 response was reduced by ghrelin to baseline
Karra et al. (2013)	fMRI	Whole brain analyses	Regression	$p < 0.05$, FWE whole-brain corrected	• Fasted condition: 1. TT group: hypothalamus↑, nucleus accumbens↑ 2. AA group: hypothalamus↓, nucleus accumbens↓ • Fed condition (ghrelin suppression): 1. TT group: fusiform gyrus↑, the postcentral gyrus↑, the cuneus↑, caudate ↓ 2. AA group: fusiform gyrus↓, the postcentral gyrus↓, the cuneus↓, caudate ↑
Kroemer et al. (2013)	fMRI	Whole brain + ROIs (ventral striatum, hypothalamus, midbrain)	• GLM • Correlations	whole brain uncorrected $P < 0.001$/ROIs FWE correction	• Middle occipital/temporal gyrus ↑ • Fusiform gyrus ↑ • Superior/medial frontal gyrus ↑ • Middle occipital/temporal gyrus R ↑ • Inferior frontal gyrus L ↑ • Postcentral g., supramarginal gyrus, rolandic operculum L ↑ • Midbrain (i.e. substantia nigra, red nuclei, mammilary bodies, ventral tegmental area) L ↑ • Subthalamic nucleus R ↑ • Thalamus R ↑ • Hypothalamus R ↑ • Superior occipital gyrus L ↑ • Middle frontal gyrus R ↑ • Pallidum, amygdala L ↑ • Inferior frontal gyrus R ↑ • Inferior temporal g., fusiform gyrus L ↑ • Caudate body R ↑ • Thalamus (anterior nucleus) L ↑ • Middle/superior frontal gyrus L ↑ • Medial/superior frontal gyrus, anterior cingulate ↑
Kroemer et al. (2015)	fMRI	Whole brain + ROIs (ventral striatum, hypothalamus, midbrain)	• GLM • Correlations	whole brain/ROIs uncorrected $P < 0.001$	• Fasting state: 1. Hypothalamus ↑ 2. Nicotine administration impact on ghrelin: 3. nucleus accumbens ↓ 4. amygdala ↓ 5. right hypothalamus ↓ • Fed state: 1. Nucleus accumbens L ↑ 2. Amygdala R ↑ 3. Hypothalamus R ↑ 4. Ventro-medial pre-frontal cortex ↑
Leidy et al. (2013)	fMRI	ROIs	GLM	$p < 0.05$, multiple comparisons corrected	• Amygdala ↓ • Hippocampus ↓

(continued on next page)

Table 2 *(continued)*

Authors and year of publication	Neuro-imaging modality	Brain region investigated	Type of analysis	Threshold	Results (HC, if not indicated otherwise)
Li et al. (2012)	fMRI	ROIs (hypothalamus, insula, thalamus, parahippocampal/hippocampal cortex, caudate, putamen, amygdala, and OFC)	• GLM • Correlations	P < 0.05 corrected with Monte Carlo simulations	• Middle Frontal Gyrus ↓ 1 Soybean oil emulsion: • Middle insula↑ • Amygdala↑ • Latera orbito-frontal cortex↑ 1 Glucose: • Middle insula↑ • Latera orbito-frontal cortex↑ 1 Whey protein: • Amygdala↑
Malik et al. (2008)	fMRI	Whole brain	• GLM • Correlations	p < 001 uncorrected	• Amygdala↑ • Orbitofrontal cortex ↑ • substantia nigra↑ • ventral tegmental area↑ • caudate↑ • hippocampus ↑ • insula↑ • occipital gyrus↑ • left pulvinar ↑ • left fusiform ↑
Savage et al. (2014)	PET	ROIs (substantia nigra)	Correlations	P < 0.05	• HC: ○ Substantia nigra↑ • Obese: • no correlation
Sun et al. (2014)	fMRI	Whole brain + ROIs (insula, hippocampus, amygdala, caudate, putamen, midbrain, pallidum, nucleus accumbens, and hypothalamus)	• GLM • Correlations	p < 0.05 Family Wise Error	• Midbrain↑ • Amygdala↑ • Pallidum↑ • Insula↑ • Hippocampus↑ • Middle orbito-frontal cortex ↑
Sun et al. (2015)	fMRI	Whole brain + ROIs (insula, amygdala)	• GLM • Correlations	p < 0.05 Family Wise Error	• Odor > OL • Higher satiety than hunger • cerebellum ↓

negatively in the insula.

The opposite results were found by Schilling et al. (2014) using fMRI-ASL. Intranasal administration of insulin led to increased CSF in the insular cortex and putamen in 48 male volunteers.

Finally, one study used PET to investigate the effects of insulin plasma changes on brain activity. Tataranni et al. (1999) investigated brain neurochemical changes after satiation (liquid meal intake) or in the fasting state in 11 healthy subjects. Satiation was associated with increased CBF in the ventromedial prefrontal cortex, dorsolateral prefrontal cortex, and inferior parietal lobule. Furthermore, changes in plasma insulin concentrations in response to the meal were negatively correlated with changes in CBF in the insular and OFC.

A recent study of Kromer, as previously discussed (Kroemer et al., 2015), used fMRI to investigate effects of leptin on food-cue reactivity before and after a caloric load (oral glucose tolerance test, OGTT) in 26 healthy normal weight never-smokers. During fasting, nicotine administration increased correlations between leptin levels and activation in the mesocorticolimbic system. After the OGTT, nicotine increased the effects of leptin on food-induced neural activity, positively correlating with activity in the ventromedial prefrontal cortex (vmPFC) and the amygdala. Nicotine therefore enhances the effect of leptin, which might in turn reduce appetite.

Five studies investigated the effects of PYY and GLP-1 plasma levels on the brain.

De Silva et al. (2012), using the “food cue paradigm” during fMRI examination, and demonstrated that PYY and GLP-17-36 administration to 16 healthy subjects reduced appetite and in turn altered brain activity was present in areas as the amygdala, caudate, insula, nucleus accumbens, OFC and putamen. Similar findings were also found in the study of Leidy, as previously described (Leidy et al., 2013), which demonstrated that increased PYY plasma concentrations were negatively correlated with activity in the amygdala, hippocampal and para-hippocampal areas. Douglas et al. (2015) confirmed these results, using the same paradigm, and showed that high protein meal (beef lunch) increased GLP-1 and PYY3-36 plasma levels and in turn reduced activity in the anterior cingulate and insula in 21 healthy subjects. Moreover, GLP-1 levels correlated negatively with activation in the middle insula and lateral OFC after both glucose and protein administration. On the contrary, Batterham et al. (2007), using the ‘on-off treatment related block design’, showed that with high plasma PYY concentrations, mimicking the fed state, there was increased neural activity in the caudolateral OFC (as insula and anterior cingulate cortex).

In a fMRI-ASL on 42 healthy participants, Pannacciulli et al. (2007) showed that, in the postprandial state, there was an increased plasma concentration of GLP-1, which was positively correlated with increased rCBF in the left dorsolateral prefrontal cortex (including the left middle and inferior frontal gyri) and hypothalamus.

Finally, four studies investigated CCK effects at the brain level. A study previously reported by Li et al. (2012) using an ‘on-off treatment related block design’ on 14 healthy subjects, showed that levels of plasma CCK after glucose administration correlated negatively with activity in the caudate and in the thalamus. Moreover, in a work of Eldeghaidy on 17 healthy adults, an fMRI examination was performed assessing how prior consumption of an HFM or water load modulates reward, homeostatic, and taste brain responses to the subsequent delivery of oral fat. Their findings show that an individual’s plasma CCK concentration correlated negatively with brain activation in taste and oral somatosensory areas, insula, amygdala and thalamus. A similar study of Little et al. (2014) administering to 12 healthy subjects an intragastric infusion (250 mL) of 1 M glucose and predosing with dexloxiglumide (CCK receptor antagonist) or 1 M glucose + placebo, or 0.9% saline (control) + placebo, highlighted a CCK1-receptor

Table 3
Effects of satiety inducing hormones and nutrients on the brain: glucose, insulin, peptide YY, leptin and GLP-1. Decreased activation: "↓". Increased activation: "↑".

Authors and year of publication	Neuroimaging modality	Tested hormone	Brain region investigated	Type of analysis	Threshold	Results (HC, if not indicated otherwise)
Batterham et al. (2007)	fMRI	PYY	Whole brain + ROIs (Hypothalamus, substantia nigra, nucleus accumbens, solitary nucleus and tract, parabrachial nucleus)	GLM	$p < 0.05$ cluster-level corrected	• Cerebellum ↑ • Anterior cerebellum ↑ • Cingulate ↑ • Anterior cingulate ↑ • Globus pallidus ↑ • Inferior parietal lobule ↑ • Middle frontal gyrus ↑ • Medial superior frontal gyrus ↑ • Caudolateral orbitofrontal cortex ↑ • Peri-aqueductal grey ↑ • Precentral gyrus ↑ • Substantia nigra ↑ • Ventral tegmental area ↑
De Silva et al. (2011)	fMRI	PYY	ROIs (amygdala, caudate, insula, nucleus accumbens, orbitofrontal cortex and putamen)	GLM	$p < 0.05$ cluster-level corrected	• Insula ↓ • ACC ↓
Douglas et al. (2015)	fMRI	PYY/GLP-1	Whole brain	GLM	$P = 0.01$ cluster-level, $a = 0.05$ corrected	Insula, ACC, but no direct link between the hormones and the studies
Eldeghaidy et al. (2016)	fMRI	CCK	ROIs (amygdala, caudate, insula, nucleus accumbens, orbitofrontal cortex and hypothalamus)	GLM	$p < 0.05$ cluster-level corrected	• Supramarginal gyrus ↑ • Insula ↑ • Amygdala ↑ • Operculum ↑ • Temporal gyrus ↑ • Cerebellum ↑ • Thalamus ↑
Grosshans et al. (2012)	fMRI	Leptin	Whole brain + ROIs (striatum)	Correlations	$P < 0.005$, uncorrected; cluster size ≥ 10 voxels	Ventral striatum ↑
Heni et al. (2014)	fMRI	• Glucose • Insulin	Whole brain	Regressions	$P < 0.05$, corrected for multiple comparisons	• Correlations: 1. Plasma glucose * brain response to high caloric food cues 30 min post load: Hypothalamus↓ 2. Plasma insulin * brain response to high caloric food cues 120 min post load: Inferior frontal gyrus↓, middle frontal gyrus↓, cingulate gyrus↓, Inferior parietal gyrus↓
Heni et al. (2015)	fMRI	GLP-1	Whole brain	Regressions	$P < 0.05$, corrected for multiple comparisons	• Correlations: 1. In lean subjects: Plasma glucose * brain response to glucose 30 min post load: orbitofrontal cortex↓ 2. In lean and obese subjects: Plasma glucose * brain response to high caloric food cues 120 min post load: orbitofrontal cortex↓
Hinkle et al. (2013)	fMRI	Leptin	• Whole brain, seeds: 1. Hypothalamus 2. Nucleus accumbens	PPI	$p < 0.05$ and cluster-level corrections	• Right mid- and posterior insula↑, right central and parietal operculae↑, precunous↑ • Frontal Pole↓, superior frontal gyrus↓, dorsal anterior cingulate cortex↓, superior division of the lateral occipital cortex↓, inferior parietal lobule↓, orbital frontal cortex↓, medial frontal gyrus↓
Jastreboff et al. (2016)	fMRI – ASL	• Insulin • Leptin	Whole brain	• GLM • Correlations	$p < 0.05$ and cluster-level corrections	• Main effect: Insulin: visual regions ↑ Leptin: no effects • Obese vs. lean: Insulin: hypothalamus↑, thalamus↑, hippocampus ↑ Leptin: PFC ↓
Kroemer et al. (2013)	fMRI	Insulin	Whole brain	• GLM • Correlations	whole brain uncorrected $P < 0.001$/ROIs FWE correction	• Cerebellum, parahippocampal gyrus L ↓ • Precentral gyrus ↓ • Middle frontal gyrus L ↓ • Superior temporal gyrus L ↓ • Thalamus↓ • Amygdala↓ Hippocampus↓

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Table 3 *(continued)*

Authors and year of publication	Neuroimaging modality	Tested hormone	Brain region investigated	Type of analysis	Threshold	Results (HC, if not indicated otherwise)
						• Superior temporal gyrus R ↓ • Cerebellum R ↓ • Precentral gyrus↓ • Middle frontal gyrus ↓ Insula L ↓ • Cingulate cortex, anterior cingulate cortex L ↓ • Fusiform gyrus ↓ • Caudate nucleus body ↓ • Middle temporal gyrus, posterior cingulate ↓ • Precuneus, cuneus ↓ • Inferior/middle frontal gyrus ↓ • Putamen ↓ • Precentral gyrus L ↓ • Superior frontal gyrus L↓ • Parahippocampal gyrus L ↓
Kroemer et al. (2015)	fMRI	Leptin	Whole brain + ROIs (ventral striatum, hipothalamus, midbrain)	• GLM • Correlations	whole brain/ROIs uncorrected P < 0.001	• Fasting state: 1. Nicotine administration impact on leptin: vmPFC↑ Nucleus accumbens↑ Dorsal striatum↑, amygdala↑ Ventral tegmental area↑ Hypothalamus↑ • Fed state: ○ Middle temporal gyrus↓ Thalamus↓ Inferior parietal lobule↓ ○ Midbrain↓ Orbitofrontal cortex↓ Amygdala↓
						• Fasting state: 1. Nicotine administration impact on leptin: vmPFC↑ Nucleus accumbens↑ Dorsal striatum↑, Amygdala↑ Ventral tegmental area↑ Hypothalamus↑ • Fed state: ○ Middle temporal gyrus↓ Thalamus↓ Inferior parietal lobule↓ ○ Midbrain↓ Orbitofrontal cortex↓ Amygdala↓
Lassman et al. (2010)	fMRI	CCK	Whole brain	Correlations	Uncorrected P < 0.005	• Brain stem ↑ • Hypothalamus ↑ • Motor cortex ↑ • Precuneus ↑ • Cingulate gyrus ↑ • Temporal gyrus, middle ↑ • Caudate ↑ • Thalamus ↑
Leidy et al. (2013)	fMRI	PYY	ROIs (hippocampus, insula, amygdala, cingulate, striatum, OFC and PFC)	• GLM • Correlations	ROIs corrected P < 0.01	Amygdala – Hippocampus-Middle – Frontal Gyrus↓
Lennerz et al. (2013)	fMRI	• Glucose • Insulin	Whole brain + ROIs (ventral striatum, hipothalamus, midbrain)	• GLM • Correlations	whole brain/Bonferroni corrected P < 0.002	Nucleus accumbens↑
Li et al. (2012)	fMRI	Insulin • Ghrelin • GLP-1	ROIs (hypothalamus, insula, thalamus, parahippocampal/hippocampal cortex, caudate, putamen, amygdala, and OFC)	• GLM • Correlations	P < 0.05 corrected with Monte Carlo simulations	• glucose: 1. Insuline: Middle insula↓ Thalamus↓ Amygdala↓ 2. Latera orbito-frontal cortex↓ 3. Glucose/CCK: Thalamus↓ 4. GLP-1: Middle insula↓ Latera orbito-frontal cortex↓ • whey protein: 1. Insulin/CCK: Caudate↓ 2. GLP-1: Lateral orbito-frontal cortex↓
Little et al. (2014)	fMRI	CCK	Whole brain	Correlations	False Discovery Rate level (pFDR < 0.05)	Motor cortex↓
Liu et al. (2000)	fMRI	Insulin	Hypothalamus	Temporal clustering analysis		• glucose: 1. Insuline:

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Table 3 *(continued)*

Authors and year of publication	Neuroimaging modality	Tested hormone	Brain region investigated	Type of analysis	Threshold	Results (HC, if not indicated otherwise)
						• the orbitofrontal cortex ↑ • pre-frontal cortex ↑ • hypothalamus ↓
Page et al. (2011)	fMRI	Glucose	Whole brain	• GLM • Correlations	FWE correction for multiple comparisons	ventromedial-preforntal corex/anterior cingulate cortex↑
Page et al. (2013)	fMRI – ASL	Insulin	Whole brain/hypothalamus	• GLM • Correlations	FWE correction for multiple comparisons	• Glucose: ○ Insula↓ ○ Putamen↓ ○ Anterior cingulate cortex↓ ○ Hypothalamus↓ ○ Caudate↓ • Insulin: • Putamen L↑
Page et al. (2009)	fMRI – ASL	• Glucose • Insulin	Whole brain/hypothalamus	Whole brain analyses	/	• Hypoglicemic vs. euglicemic: ○ Hypothalamus↑ ○ Inferior frontal gyrus L↑ ○ Right anterior cingulate cortex↑ ○ Caudate ↑ ○ Superior temporal gyrus L↑ ○ Pars triangolaris L↑ ○ Viasual association cortex L↑ ○ Cerebellum↓ ○ Medial Frontal Gyrus↓
Pannaciulli et al. (2007)	PET	GLP-1	Whole brain	Correlations	P ≤ 0.001 uncorrected for multiple comparisons	• Hypoglicemic vs. euglicemic: ○ Middle frontal gyrus L↑ ○ Inferior frontal gyrus L↑ ○ Hypothalamus ↑
Gautier et al. (2000)	PET	• Insulin • Glucose	ROIs (from main effect results)	Correlations	P ≤ 0.005 uncorrected for multiple comparisons	• Obese subjects: 1. Insulin: posterior orbitofrontal cortex L ↓ Hippocampus L↓ Precunoeus R↓ Putamen R↓ Thalamus L ↓ • Lean subjects: 1. Insulin: posterior orbitofrontal cortex L ↓ Hippocampus ↓ Precuneus ↑ Putamen ↓ Thalamus L↓ Dorsolateral prefrontal cortex L↑ Dorsolateral prefrontal cortex R↓ 2. Glucose: Anterior prefrontal cortex ↓
Purnell et al. (2011)	fMRI	Glucose	Hypothalamus	GLM – hormone absorption	p < 0.002 uncorrected	Cortical control areas(as the orbitofrontal cortex, pre-frontal cortex)↑
Rosenbaum et al. (2008)	fMRI	Leptin	Whole brain/hypothalamus	GLM	*p* values of 0.005 corrected for multiple comparisons	• Leptin > Placebo: ○ Cingulate gyrus ↑ Hypothalamus ↑ Inferior frontal gyrus ↑ ○ Lingual gyrus ↑ Middle frontal gyrus ↑ Middle temporal gyrus ↑ Postcentral gyrus ↑ Precuneus ↑ Putamen ↑ Thalamus ↑ • Placebo > Leptin ○ Brain stem ↑ Cingulate gyrus ↑ Inferior frontal gyrus ↑ ○ Insula ↑ Lingual gyrus ↑ Middle frontal gyrus ↑ Middle temporal gyrus ↑ Middle occipital gyrus ↑ Precuneus ↑ ○ Superior frontal gyrus ↑ Superior temporal gyrus ↑
Schilling et al. (2014)	fMRI – ASL	Insulin	Whole brain + ROIs (insula, hippocampus, putamen)	GLM	*p* values of 0.05 corrected for multiple comparisons	• Insulin: ○ Insula↑ ○ Putamen↑ ○ Caudate nucleus↑ ○ Inferior frontal gyrus↑
Spetter et al. (2015)	fMRI	Insulin	ROIs (amygdala, insula, inferior frontal gyrus, anterior cingulate cortex, hypothalamus and	Correlations	p < 0.05 FWE-corrected for multiple comparisons	• Insulin: ○ Anterior cingulate cortex↑ Putamen↑ Insula ↓

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Table 3 *(continued)*

Authors and year of publication	Neuroimaging modality	Tested hormone	Brain region investigated	Type of analysis	Threshold	Results (HC, if not indicated otherwise)
			striatum)			
Tataranni et a. 1999)	PET	Insulin	Whole brain	Correlations	$p \leq 0.001$ uncorrected for multiple comparisons	● Satiation insulin changes ○ Insula L ↓ Orbitofrontal cortex L↓
van Bloemendaal et al. (2014)	fMRI	● GLP-1 ● insulin	ROIs (insula, striatum, amygdala, and OFC)	● GLM ● Correlations	$p < 0.05$ FWE-corrected for multiple comparisons	● GLP-1 Obese: ○ Amygdala↑ ○ Insula↑
Wallner-Liebmann et al. (2010)	fMRI	● Insulin ● Glucose	ROIs (frontal lobe and the limbic system including: amygdala, thamalus hippocampus, nucleus caudatus,putamen, and gyrus cinguli)	● GLM ● Correlations	$p > 0.001$ with a minimum cluster size of 15 voxels	● High caloric food images, insulin: ○ Hippocampus R ↑ Insula L↑ Superior frontal gyrus R ↓ ○ Thalamus L ↓
Wölnerhanssen et al. (2015)	fMRI	Insulin	ROIs (Thalamus)	● Dual regression ● Correlations	$p < 0.05$ uncorrected	● Thalamus ↑
Wright et al. (2016)	fMRI	Glucose	Whole brain	Seed based functional connectivity (insula)	$p < 0.05$ FWE corrected	● Glucose (fed state vs. fasting state): ○ L Insula – Superior frontal gyrus ↓ ○ Middle insula – Posterior cingulate cortex↑
Zhang et al. (2015)	fMRI	Insulin	ROIs (dACC and precuneus)	● Low-frequency fluctuations ● Correlations	$p < 0.05$ Monte Carlo corrected	dACC ↓

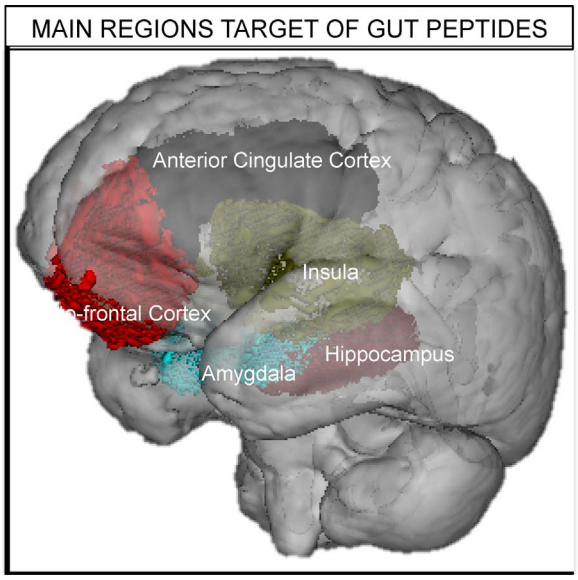

Fig. 2. Main target regions of gut peptides.

dependent increase in Blood oxygenation level dependent (BOLD) signal in the motor cortex. Lastly, a study of Lassman et al. (2010), investigating the brain activation responses to ingested lipid (dodecanoic acid) or saline (control) on 19 healthy subjects with and without prior administration of the CCK receptor antagonist dexloxiglumide, showed significant interaction of dexloxiglumide before treatment on brain stem, hypothalamus, precuneus, cingulate cortex, temporal gyrus and caudate.

The main areas involved in the neural circuit of appetite and target of the gut peptides are shown in Fig. 2.

3.5. *Effects of gut peptides on the brain in patients with obesity*

13 of the included studies focused on the neural effects of gut peptides in obese subjects (van Bloemendaal et al., 2014; Gautier et al., 2000; Grosshans et al., 2012; Heni et al., 2014, 2015; Hinkle et al., 2013; Jastreboff et al., 2016; Lennerz et al., 2013; Rosenbaum et al., 2008; Savage et al., 2014; Sun et al., 2015; Wallner-Liebmann et al., 2010; Zhang et al., 2015). Three studies were conducted to test the effects of ghrelin on brain areas of obese subjects in comparison to lean subjects (Jastreboff et al., 2016; Karra et al., 2013; Savage et al., 2014). Karra et al. (2013) investigated the relation between changes in plasma ghrelin concentrations and obesity, focusing on the obesity-associated gene (FTO). This fMRI study examined how brain responses to food cues differed between 12 carriers of the AA genotype and 12 carriers of the TT allele after consumption of a standard meal. During the fasted state, activation in the hypothalamus, nucleus accumbens, cingulate gyrus and OFC correlated positively with ghrelin levels in the AA group and with greater feelings of hunger. These results show that the FTO gene and ghrelin are key mediators of ingestive behaviour.

Jastreboff et al. (2016) tested how glucose and fructose administration modulated brain perfusion in 14 lean and 24 obese subjects. Obese patients showed high levels of perfusion in the hypothalamus and thalamus that was related to high plasma concentrations of ghrelin, while low levels of perfusion in the prefrontal cortex and anterior cingulate cortex were linked to low plasma levels of ghrelin (Jastreboff et al., 2016; Savage et al., 2014). Furthermore, Savage and colleagues found different brain responses between lean and obese subjects with respect to ghrelin plasma levels (Savage et al., 2014). Using positron emission tomography (PET) imaging, they investigated the expression of DA type 2/t 3 receptors (D2/D3R) in eight subjects with normal weight compared to 19 obese subjects. Ghrelin levels and D2/D3R binding potential (BPND) in the substantia nigra were positively

correlated in normal weight but not in obese participants.

On the other hand, 11 studies investigated differences in brain activation in obese subjects compared to lean subjects in relation to changes in glucose and satiety hormones (van Bloemendaal et al., 2014; Gautier et al., 2000; Grosshans et al., 2012; Heni et al., 2014, 2015; Hinkle et al., 2013; Jastreboff et al., 2016; Karra et al., 2013; Lennerz et al., 2013; Rosenbaum et al., 2008; Zhang et al., 2015). In one study, both ghrelin and insulin were measured (Jastreboff et al., 2016).

Lennerz et al. (2013) focussed on glucose plasma levels and explored resting state connectivity in 12 overweight men after a high glycaemic (high GI) or a low glycaemic meal (low GI). Compared with a low GI meal, a high GI meal decreased plasma glucose, increased hunger and enhanced activation in the nucleus accumbens, striatum and olfactory area.

Heni et al. (2014, 2015) explored the effects of glucose ingestion on brain activity in 12 lean and 12 obese subjects, using a fMRI food-induced paradigm. The hypothalamic response to high caloric food cues correlated negatively with changes in blood glucose levels 30 min after glucose ingestion, while activation in the ACC and OFC correlated negatively with increased plasma insulin levels 120 min after glucose ingestion. These effects can be observed in both the obese and lean groups. In a similar study, Jastreboff et al. (2016), confirmed these results, by showing that obese adolescents exhibited decreased CBF in the PFC, striatum and hypothalamus after drinking glucose. The hippocampus, an area implicated in the processing of high caloric food pictures, was also identified by Wallner-Liebmann et al. (2010)), which found a positive correlation between hippocampus activity and waist circumference.

Insulin-related brain changes were also investigated by Gautier et al. (2000) in a study on 11 lean and 11 obese subjects, that combined PET and fMRI-ASL sequences after fasting or satiation (liquid meal). A converse correlation was found between changes in plasma insulin concentrations and changes in rCBF in the precuneus, orbitofrontal cortex, putamen and thalamus in obese and lean subjects. This study raises the possibility that activation in OFC (involved in the inhibition of inappropriate response tendencies) and limbic/paralimbic areas (associated with the regulation of emotion) during eating may be different in obese and lean men.

As regards leptin, and using fMRI in a food cue paradigm, Grosshans et al. (2012) showed that plasma leptin levels were associated with brain activation in the ventral striatum and with BMI in 21 obese subjects. According to this study, leptin is therefore a satiety hormone linked to increased activation in subcortical regions and to weight gain.

In the same line, and with fMRI in a food-cue paradigm, Rosenbaum et al. (2008) examined how brain responses to food cues were modulated after subcutaneous injection of leptin in six obese subjects following a diet. During weight loss, leptin-related increases in neural activity in response to visual food cues were observed in the brain stem, parahippocampal gyrus, inferior and middle frontal gyri, middle temporal gyrus, and lingual gyrus. Leptin-related decreases were observed in the hypothalamus, cingulate gyrus, and middle frontal gyrus.

A recent study by Hinkle et al. (2013) confirms these results and investigated changes in the connectivity of the right hypothalamus in respect to leptin plasma levels in 10 obese subjects. Using fMRI with a food cue paradigm, the functional connectivity of the right hypothalamus with the mid-insula and the central and parietal operculae increased after leptin injections, while it decreased with the OFC, frontal pole and the dorsal ACC.

Apart from insulin, effects of changes in GLP-1 plasma levels on the brain have also been investigated in obese subjects. van Bloemendaal et al. (2014) explored how the administration of the GLP-1 receptor agonist exenatide modulated brain responses to food pictures during a somatostatin pancreatic-pituitary clamp in 16 obese and 16 normal-weight subjects. Relative to lean subjects, obese subjects showed increased brain responses to food pictures in the insula, amygdala, putamen, and OFC. In the same line, in a second study performed in 2015, Heni et al. (2015) administered 75 g of glucose to promote GLP-1 secretion. Food cue-induced brain activity was assessed with fMRI and GLP-1 concentrations measured before, 30 and 120 min after glucose intake. The significant increase in GLP-1 levels correlated negatively with a change in the food cue-induced brain activity in the OFC in lean and overweight participants. In contrast, postprandial changes in plasma insulin were associated with OFC activations in lean individuals only. Finally, using rsfMRI, Zhang et al. (2015) investigated the amplitude of low frequency fluctuations of spontaneous signals during both hunger and satiety states in 20 lean and 20 obese males. Before food intake, obese men had significantly higher baseline activity in the precuneus and lower activity in the dorsal anterior cingulate cortex (dACC) relative to lean subjects. After food intake, obese males had significantly lower activity in the dorsal anterior cingulate cortex (dACC) than lean males. Moreover a significant positive correlation was found between precuneus activation and hunger ratings before food intake, while dACC activity was negatively correlated with plasma insulin levels before and after food intake in both groups. These results indicated that both precuneus and dACC may play an important role in eating behaviour. While precuneus seemed to mediate subjective satiety, dACC activation rather reflected indirect measures of glucose utilisation.

4. Discussion

To our knowledge, this is the first study to systematically review the effects of different gut peptides on brain activation in healthy and obese subjects. Forty original studies were retrieved, which addressed how key gut hormones or nutrients, such as ghrelin, glucose, insulin, leptin, PYY, GLP-1 and CCK, modulate functional brain activation after food intake. Plasma levels of the appetite-promoting gut hormone ghrelin positively correlate with activity in the PFC, amygdala and insula and negatively correlate with activity in subcortical areas such as the hypothalamus. In contrast, satiety-regulating gut hormones or nutrients like glucose, insulin, letpin, PYY, GLP-1 and CCK affect the same brain regions in the opposite directions. Nevertheless, the lack of reproducible studies and the existence of multiple methodological approaches prevent definitive conclusions and explains some discrepancies in the results between the different studies. The present review is to be considered as the basis for a future meta-analysis of brain-gut interactions.

4.1. Nutrient administration

Individual nutrients were administered to stimulate the plasma release of the investigated hormones. In particular, 22 studies used direct administration of the target substance (i.e. glucose) (Batterham et al., 2007; van Bloemendaal et al., 2014; De Silva et al., 2011; Eldeghaidy et al., 2016; Goldstone et al., 2014; Heni et al., 2014, 2015; Hinkle et al., 2013; Jastreboff et al., 2016; Jones et al., 2012; Kroemer et al., 2013a,b, 2015; Lennerz et al., 2013; Malik et al., 2008 Page et al., 2009, 2011, 2013, p. 200; Purnell et al., 2011; Rosenbaum et al., 2008; Schilling et al., 2014; Wölnerhanssen et al., 2015) and 16 the administration of a nutrient (for instance chocolate milkshake) that subsequently stimulated the production of gut peptides (i.e. ghrelin and PYY) (Douglas et al., 2015; Gautier et al., 2000; Grosshans et al., 2012; Karra et al., 2013; Leidy et al., 2013; Li et al., 2012; Liu et al., 2000; Pannacciulli et al., 2007; Savage et al., 2014; Spetter et al., 2014; Sun et al., 2014, 2015; Tataranni et al., 1999; Wallner-Liebmann et al., 2010; Wright et al., 2016; Zhang et al., 2015). Studies also employed different administration schemes: while in 12 studies the administration was intragastric or intravenous (Batterham et al., 2007; van Bloemendaal et al., 2014; De Silva et al., 2012; Goldstone et al., 2014; Hinkle et al., 2013; Jones et al., 2012; Malik et al., 2008; Page et al., 2009, 2011; Purnell et al., 2011; Rosenbaum et al., 2008; Schilling et al., 2014; Wölnerhanssen et al., 2015), in 24 investigations the

nutrients were ingested orally (Douglas et al., 2015; Eldeghaidy et al., 2016; Gautier et al., 2000; Grosshans et al., 2012; Heni et al., 2014, 2015; Jastreboff et al., 2016; Karra et al., 2013; Kroemer et al., 2013a,b, 2015; Leidy et al., 2013; Lennerz et al., 2013; Li et al., 2012; Liu et al., 2000; Page et al., 2013; Pannacciulli et al., 2007; Savage et al., 2014; Spetter et al., 2014; Sun et al., 2014, 2015; Tataranni et al., 1999; Wallner-Liebmann et al., 2010; Wright et al., 2016; Zhang et al., 2015). Oral intake aimed to mimic the consumption of daily meals, whereas intragastric/intravenous administration aimed to directly assess the effects of the target hormones. The difference in the acquisition procedure leads to two main consequences in the comparison of the studies: a) intragastric/intravenous administration could lead to uncomfortable feelings and therefore influence data acquisition, b) differences in the timing of nutrient absorption leads to differences in the timing of the fMRI examination (immediately after nutrient administration or after 10, 30, 120 min).

4.2. Differences in the paradigm during the fMRI examination

Different paradigms were used during the neuroimaging examination to investigate the effects of gut peptides on brain activation. 16 studies (van Bloemendaal et al., 2014; De Silva et al., 2011; Douglas et al., 2015; Goldstone et al., 2014; Grosshans et al., 2012; Heni et al., 2014, 2015; Hinkle et al., 2013; Karra et al., 2013; Kroemer et al., 2013a,b, 2015; Leidy et al., 2013; Malik et al., 2008; Page et al., 2011; Rosenbaum et al., 2008; Wallner-Liebmann et al., 2010) used a “food-cue paradigm” to investigate effects of gut peptides on feelings of appetite and neural activity during high and low caloric food cues. The “food-cue paradigm” refers to a block design in which high/low energy dense food pictures were shown alternatively to non-food pictures in a randomised fashion during the fMRI examination.

On the other hand, 9 studies (Batterham et al., 2007; Eldeghaidy et al., 2016; Jones et al., 2012; Li et al., 2012; Liu et al., 2000; Purnell et al., 2011; Spetter et al., 2014; Sun et al., 2014, 2015) used an ‘on-off treatment related block design’ to assess the direct effect of the target compound on the brain. Nutrients are administered during the fMRI examination and the timing of hormonal plasma absorption is used to investigate the brain response. The best example is a milestone study by Liu et al. (2000), in which the statistical model to investigate the BOLD signal is based on the increasing insulin plasma levels after glucose administration. The last paradigm used during fMRI examination was that of the classical resting state. At the highest point of plasma hormone absorption, an fMRI sequence is performed; the subjects had to relax and not think about anything in particular. Differences within brain networks involved in appetite and satiety regulation were then investigated.

Although these paradigms are different, the absence of any cognitive tasks makes the results rather comparable. The focus is on brain activity changes associated with variations in hormonal plasma concentrations.

4.3. Neuroimaging results

In line with subjective feelings of appetite, neuroimaging results demonstrate that the two classes of gut hormones have opposite effects on the neural circuit of appetite. In particular, activation in frontocortical regions, such as OFC, ACC and insula correlates positively with ghrelin plasma levels, and with increased hunger feelings. Subcortical areas like the thalamus, hippocampus, striatum and hypothalamus correlated negatively with ghrelin levels. These results have consistently been reported in 8 studies (Batterham et al., 2007; Goldstone et al., 2014; Jones et al., 2012; Kroemer et al., 2013a, 2015; Li et al., 2012; Sun et al., 2014, 2015, p. 2), while 2 studies (Leidy et al., 2013; Savage et al., 2014) found associations in different directions. This discrepancy can perhaps be explained by the use of the food-cue paradigm that could discriminate between high caloric and low caloric food cues and therefore be more specific.

In contrast, plasma levels of satiety-stimulating hormones correlate negatively with the same cortical areas and positively with subcortical areas.

The present findings fit with a model proposed by Woods (Woods et al., 1998), which embeds gut-brain interactions during food-intake within the framework of homeostasis regulation. After food intake, the circulating adipose signals (ghrelin and insulin) penetrate the blood brain barrier and stimulate receptors on neurons in the hypothalamus (Woods et al., 1998). Satiety signals generated by ingested food enter subcortical areas, such as amygdala and striatum, where they influence reflexes related to the acceptance or rejection of food. In a second step, the hypothalamus sends signals to cortical areas, such as the OFC, ACC and insula, as part of the reward mechanism, where cognitive information is integrated with adiposity signals. A higher cognitive evaluation is performed and the prospective eating behaviour is determined. This model of integration between gut peptides, brain responses and subjective feelings explains the opposite direction of the correlations between cortical and subcortical brain activation, subjective satiety and appetite feelings and hormonal plasma levels.

Increased activity of adiposity signals enhances the ability of satiety signals to terminate a meal or of appetite signals to continue eating.

Although this pattern is clear in the majority of the included studies (De Silva et al., 2012; Douglas et al., 2015; Gautier et al., 2000; Grosshans et al., 2012; Heni et al., 2014, 2015; Hinkle et al., 2013; Jastreboff et al., 2016; Kroemer et al., 2013b, 2015; Lennerz et al., 2013; Li et al., 2012; Page et al., 2013; Rosenbaum et al., 2008; Spetter et al., 2014, p. 20; Tataranni et al., 1999; Wright et al., 2016; Zhang et al., 2015), discrepancies across studies may be due to the peculiarity of the different satiety stimulating hormones that have intrinsic properties and therefore affect different brain areas in different ways.

4.4. Differences between a clinical obese and a healthy lean population

Finally, our last result concerns the effects of gut peptides on brain activation in obese subjects. The included studies provide little, if any, evidence for alterations in obese compared to lean subjects. In particular, the results of gut hormones on brain regional activity in the obese population is not reproduced by any study using the same amount of ingested nutrients and the same paradigm. Moreover, it is very hard to compare brain changes in obese and lean subjects due to a lack of statistical comparisons between the two groups within each study.

Moreover, the discrepancies of results can be explained by methodological issues (the different nutrients administered, different peptides investigated and different paradigms used during the fMRI examination) and by the low number of studies performed and the lack of reproducibility of the results. Further investigations on the differential effects of gut peptides on the appetite circuit between obese and lean population are therefore needed.

4.5. Limitations

A first limitation that we want to highlight is that most studies didn’t control for possible pre-existing preferences for the participants for certain type of foods and this can impact the studies results. Moreover, the results might be influenced by psychopathological states, such as mood disturbances, which have not been systematically assessed in the included studies. Also the use of cannabinoids or psychoactive substances was self-reported and consequently not necessarily accurate (Becker et al., 2015). These factors may confound the neuroimaging results.

The amount of nutrients ingested varied in several studies and this hampers comparability. Moreover, the timing of the fMRI examination was very different across studies. It varied from an examination immediately after substance intake to 6 weeks post-administration. Although (as stated above) the timing was in accordance with the aim

of the investigation, it cannot be denied that this may result in a confounding factor and make the studies poorly comparable. Furthermore, in neuroimaging studies addressing brain-gut interactions in healthy subjects, the sample sizes were modest because the design of the study makes recruitment of subjects relatively difficult.

Finally, we suggest that studies including randomised samples that express preferences for specific food have to be conducted. Moreover, psychopathological states, such as mood disturbances in the participants, have to be previously screened in order to avoid confounding factors that can affect the results. Furthermore, studies on eating disorders, such as anorexia and bulimia, can greatly enhance the clinical relevance of studies of the effects of specific nutrients on brain regions regulating appetite. It would also be interesting to investigate cognitive changes (such as working memory performance) after nutrient administration, as shown by pioneering studies (Borgwardt et al., 2012; Schmidt et al., 2014).

Finally, studies using a standardised amount of ingested nutrient should be performed, since the amount of ingested nutrients can also lead to differences in the strength of brain activation

5. Conclusion

The present article systematically reviewed the existing literature investigating how gut peptides influence brain regions regulating appetite and satiety in healthy and obese subjects. The activation of brain areas controlling the brain-gut matrix occurs in opposite directions in respect to satiety or appetite regulation. The present review can enhance our understanding of the physiology of eating behaviour and the pathophysiology of obesity and eating disorders and is the basis for a future meta-analysis in the field.

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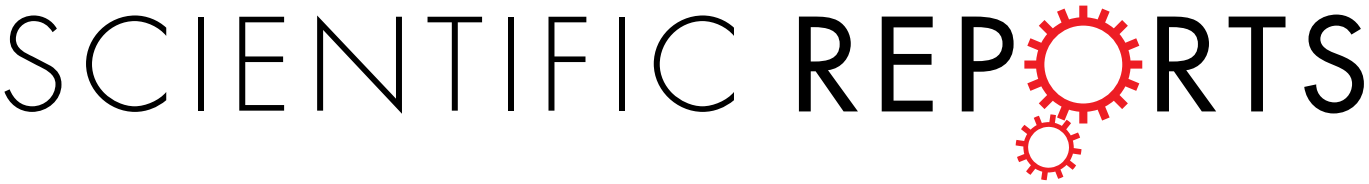

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Differential effects of L-tryptophan and L-leucine administration on brain resting state functional networks and plasma hormone levels

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Depending on their protein content, single meals can rapidly influence the uptake of amino acids into the brain and thereby modify brain functions. The current study investigates the effects of two different amino acids on the human gut-brain system, using a multimodal approach, integrating physiological and neuroimaging data. In a randomized, placebo-controlled trial, L-tryptophan, L-leucine, glucose and water were administered directly into the gut of 20 healthy subjects. Functional MRI (fMRI) in a resting state paradigm (RS), combined with the assessment of insulin and glucose blood concentration, was performed before and after treatment. Independent component analysis with dual regression technique was applied to RS-fMRI data. Results were corrected for multiple comparisons. In comparison to glucose and water, L-tryptophan consistently modifies the connectivity of the cingulate cortex in the default mode network, of the insula in the saliency network and of the sensory cortex in the somatosensory network. L-leucine has lesser effects on these functional networks. L-tryptophan and L-leucine also modified plasma insulin concentration. Finally, significant correlations were found between brain modifications after L-tryptophan administration and insulin plasma levels. This study shows that acute L-tryptophan and L-leucine intake directly influence the brain networks underpinning the food-reward system and appetite regulation.

Luminal enteral communication is a key factor in the regulation of appetite, food intake and metabolism. Protein digestion to dipeptides or tripeptides and free amino acids modulate digestive functions, glycemia and appetite[1–6]. Protein is currently believed to exert the greatest appetite-suppressing effect of the three macronutrients (carbohydrates, fats and proteins) in animals and humans[7]. High-protein diets have been extensively studied for their ability to reduce total energy intake and body weight[7]. Mechanisms that have been suggested include stimulation of insulin release[3], postprandial thermogenesis[1], intestinal gluconeogenesis[7], and direct effects of amino acids in regions of the brain[6]. In addition, it has been hypothesized that protein-induced satiation could be due to alterations in the release of gastrointestinal satiation peptides, such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY). Already in 1956, it was suggested that an elevated concentration of plasma amino acids serve as a satiation signal for food intake and thereby results in depressed food intake[8]. To date the effect of specific amino acids on satiation and appetite is only rarely studied.

Amino acids, including L-leucine[9,10], L-glutamine[11,12], and L-phenylalanine[13] modulate appetite and/or glycemia in lean, obese, or type 2 diabetic subjects. The aromatic amino acid, L-tryptophan, is of particular interest, as previous studies have reported effects on digestive functions[14] and food intake[15].

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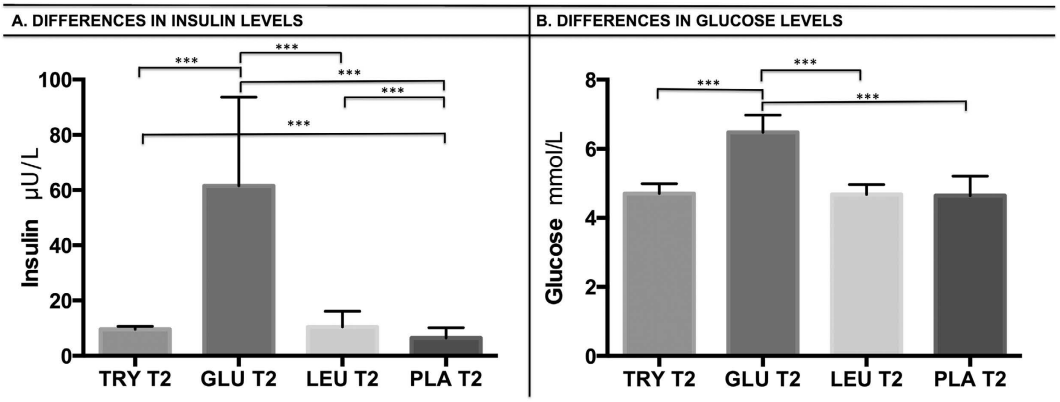

Figure 1. Physiological results. After treatment administration, plasma hormones levels were compared though a paired t test. (**A**) Higher insulin concentrations were found after glucose ingestion than with L-tryptophan ($p < 0.001$), L-leucine ($p < 0.001$) or placebo ($p < 0.001$). Moreover, after L-tryptophan and L-leucine administration, significantly higher insulin levels were found than with placebo ($p < 0.001$). No statistical differences were found between insulin levels after L-tryptophan and L-leucine intake. (**B**) Higher glucose levels were found after glucose than with L-tryptophan ($p < 0.001$), L-leucine ($p < 0.001$) or placebo ($p < 0.001$). No significant differences in glucose levels were found between other treatments.

It is unknown how amino acids affect specific brain regions. After eating, the brain senses a biochemical change and then signals satiation, but the precise sequence of events has not been determined. Even for established physiological systems such as glucose-insulin regulation, the timing of the interaction between hormonal processes and neural events has mostly been inferred from blood sampling studies. Recently, neuroimaging studies have provided *in vivo* information about the neuro-anatomical correlates of the regulation of energy intake. Temporal orchestration of such systems is, however, crucial to the integration of the neural and hormonal signals that control eating behaviour. In a landmark paper demonstrating eating-related neural activity in the brain, the response was shown to interact with an internal signal, plasma insulin[16]. As it was shown that most amino acids induce an increase in insulin (possibly due to an increase in GLP-1), amino acids could be one of the signals from the gut that interact with the brain through satiation peptides.

The present study was designed to further investigate the luminal influences of nutrients, which orchestrate gut-brain interactions. The objective was to compare the effects of intragastric L-tryptophan (L-Trp) and L-leucine (L-Leu) on brain networks and the release of insulin and glucose. We used plasma levels to represent physiological differences; this is a reliable measure of appetite and satiety[17,18]. In particular the association between brain activity in areas involved in appetite, appetite and satiety rating scores and levels of insulin and glucose was already demonstrated in previous studies[16,19].

On the basis of previous studies highlighting the differences between L-Trp and L-Leu on digestive functions[20], with L-leucine stimulating appetite and L-tryptophan stimulating satiety[21], we hypothesized that L-Trp would induce a different activation pattern in the brain, leading to modifications in brain networks related to appetite and metabolism regulation. The selection of the doses of L-tryptophan was based on the daily intake recommended by World Health Organization (WHO)[22]. The doses are comparable to the amount of L-tryptophan in soybeans in a normal portion of an Asian dish. For L-leucine an isocaloric approach to L-tryptophan was chosen. Glucose was chosen as a positive control and water as a negative control as we have previously shown effects on brain activity with both of them[23]. We have never intended to do an isocaloric comparison between glucose and amino acids.

Results

Physiological and psychological results. *Differences in insulin levels.* (Figure 1A) No significant differences were found between the treatments in insulin levels at baseline (Time1, before treatment). At Time2, the ANOVA was significant ($p < 0.001$) showing significant differences in insulin plasma levels after the treatments administration. In particular, significantly higher insulin concentrations were found after glucose administration than with L-tryptophan ($p < 0.001$), L-leucine ($p < 0.001$) or with placebo ($p < 0.001$). Significantly higher insulin levels were found after L-tryptophan and L-leucine administration than with placebo ($p < 0.001$). No statistical differences were found between insulin levels after L-tryptophan or L-leucine administration. Cohen's effect size (d): $d = 2.37$, $var(d) = 0.17$, $p < 0.001$.

Differences in glucose levels. (Figure 1B) At baseline (Time1, before treatment), no significant differences in glucose levels were found between the treatments At Time2, the ANOVA was significant ($p < 0.001$) showing significant differences in glucose plasma levels after the treatments administration. In particular, significantly higher glucose levels were found after glucose than after L-tryptophan ($p < 0.001$), after glucose than after L-leucine ($p < 0.001$) and after glucose than after placebo ($p < 0.001$). No significant differences in glucose levels were found between the other treatments. Cohen's effect size (d): $d = 3.66$, $var(d) = 0.27$, $p < 0.001$.

Functional connectivity results. From framewise displacement (FD) analyses, no significant effect of motion was found between the treatments.

At baseline (Time0), after permutation based non-parametric tests, no significant activation was found in the three networks for any group comparison, revealing that, before medication, there were no differences between the subjects in functional connectivity.

At Time2, after permutation based non-parametric tests, ANOVA showed significant differences between the treatments within each of the three pre-selected networks ($p < 0.001$). Cohen's effect size (d) for the default mode network was: $d = 1.97$, $var(d) = 0.15$, $p < 0.001$. Cohen's effect size (d) for the sensorimotor network was: $d = 2.72$, $var(d) = 0.23$, $p < 0.001$. Cohen's effect size (d) for the saliency network was: $d = 1.07$, $var(d) = 0.17$, $p < 0.001$.

In particular, the comparison "L-tryptophan vs. placebo" revealed increased connectivity in the cingulate cortex and in the precuneous within the default mode network (DMN), in the somatosensory cortex within the sensorimotor network (SMN) and in the bilateral anterior insula within the salience network (SN) (Fig. 2A).

For the comparison "L-tryptophan vs. glucose", group analyses showed reduced connectivity in areas overlapping those in the "L-tryptophan vs. placebo" contrast. In particular, the cingulate cortex and the precuneous show higher connectivity within the DMN, the somatosensory cortex within the SMN and the left anterior insula within the SN (Fig. 2B).

With respect to the sensorimotor network (SMN), the comparison "glucose vs. L-leucine" revealed significantly higher bilateral connectivity in the sensorimotor cortex (Fig. 2C), while the comparison "glucose vs. placebo" shows broader connectivity in areas including the precuneous (Fig. 2D).

The comparison "L-tryptophan vs. L-leucine" revealed significantly increased connectivity in the cingulate cortex within the DMN and in the somatosensory cortex within the SMN (Fig. 2E).

No significant activations where found for the remaining comparisons: "glucose vs. L-tryptophan", "placebo vs. L-tryptophan", "placebo vs. L-leucine", "placebo vs. glucose", "L-leucine vs. L-tryptophan", "L-leucine vs. glucose" and "L-leucine vs. placebo".

Finally, the interaction effect between time and treatments revealed no significant results.

These results reveal that amino acid administration has extensive effects on the brain, by modifying the connectivity of specific functional networks.

Correlations between imaging and physiological results. After L-tryptophan administration, a positive correlation ($p < 0.05$) was found between insulin plasma levels and bilateral activity in the insula within the saliency network (Fig. 3.1). Furthermore, after glucose administration, positive correlations were present between plasma insulin levels and activity in the sensorimotor area (SMA) within the sensorimotor network (SMN) (Fig. 3.2). These results established a link between the role of satiety hormones and connectivity changes in the brain after amino acid administration.

VBM Analysis of T1 Images. VBM analysis of the grey matter (GM) revealed no statistical differences between the visits at baseline.

Discussion

The current study provides novel insights into the effects of luminal amino acids on gut-brain interactions. The present findings suggest that intragastric L-tryptophan (L-Trp) and L-leucine (L-Leu) lead to differential modifications in insulin and glucose plasma concentrations and in brain networks connectivity, that are related to metabolic regulation and appetite sensations.

Our results from laboratory analyses indicate that different amino acids affect specific satiation hormones. In particular, L-tryptophan and L-leucine lead to a significant increase in insulin plasma levels when compared to water, but at the doses given they do not affect glucose levels. The differential effects of amino acids on insulin but not on glucose have been shown before in several studies[11]. On the other hand, and in line with previous studies[24], glucose is associated with both insulin and glucose plasma levels. As L-Trp and L-Leu have been proposed to be involved in food-intake and regulation of energy homeostasis[25–27], our findings suggest that L-tryptophan and L-leucine are key amino acids that affect satiety and appetite perception. To further explore these dynamic interactions, we investigated the effects of these amino acids on brain functional networks that are related to the food-reward mechanism.

Our additional findings are related to large-scale alterations in brain networks involved in metabolic regulation after amino acids ingestion. Our results reveal that - compared to glucose and placebo - L-tryptophan gives higher connectivity within the default mode network in the cingulate cortex and in the precuneous and within the salience network in the bilateral insula. In general, the finding that activity in brain areas regulating appetite can be influenced by different nutrients is consistent with previous reports[19,28] on modifications after glucose and fructose ingestion. Our study extends this research, by focusing for the first time on amino acids ingestion and suggesting that L-tryptophan may be a key amino acid that increases brain connectivity in areas controlling the metabolic state of the individual.

Moreover, the direct link between satiation hormones and brain areas involved in metabolism regulation is confirmed by the positive correlation between brain activity in the insular cortex and insulin plasma levels after L-tryptophan administration. These results resemble previous findings in studies with glucose intake and confirms that there is a direct link between these regions and food-reward mechanisms[16].

Furthermore, in comparison to placebo and glucose, L-tryptophan administration also increases activity within the sensorimotor network in somatosensory areas. This is the first study to report large-scale modifications in brain activity after L-tryptophan manipulation within the sensorimotor network. The reorganization in the sensorimotor network is also evident in the analysis of the effects of glucose administration compared to placebo that shows greater activation in somatosensory areas. As demonstrated in a previous study[16], the somatosensory

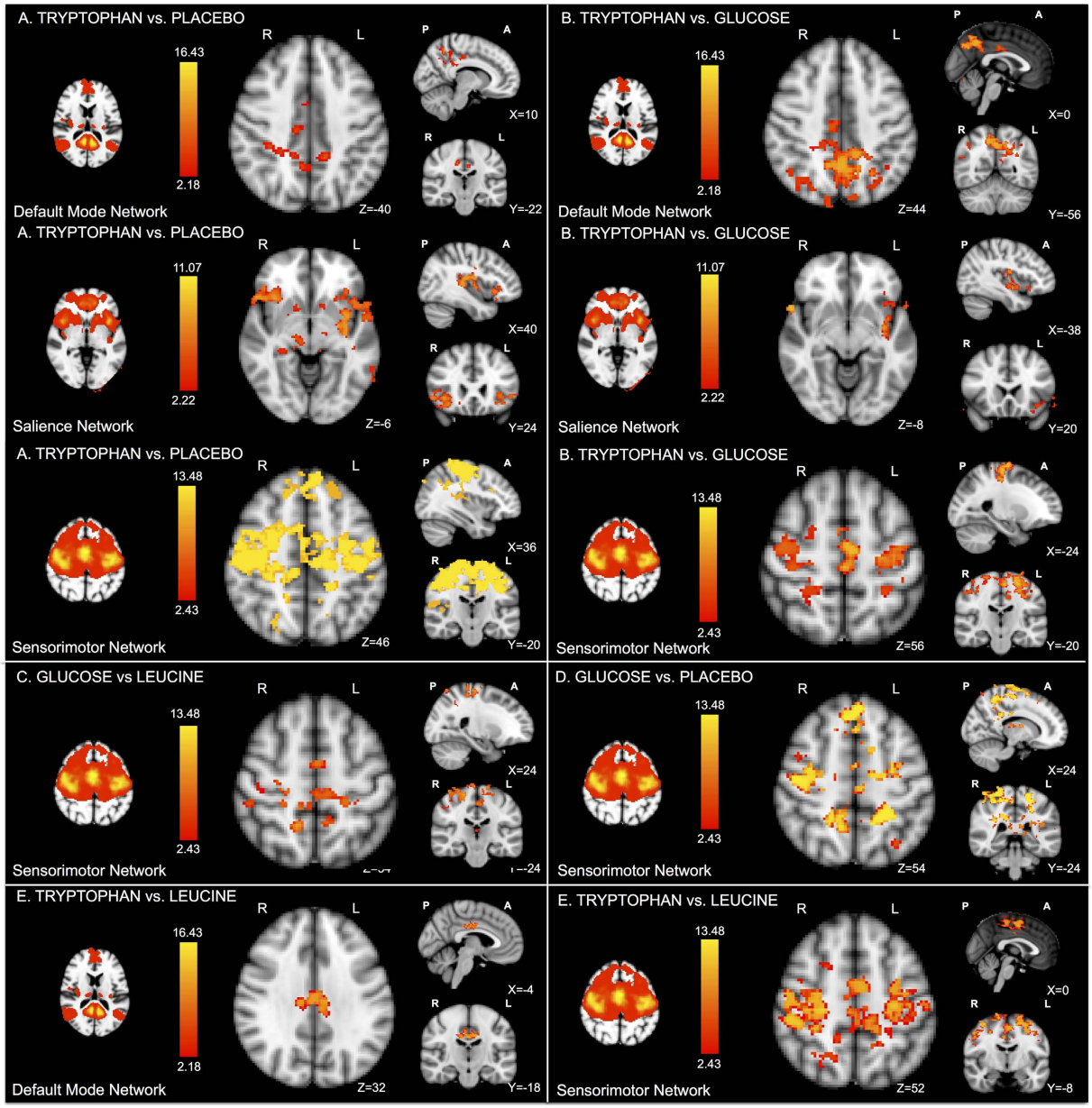

Figure 2. Functional connectivity results. After independent component analyses (ICA), the dual regression technique was performed to investigate differences between the different treatments in functional connectivity in the DMN, SN and SMN. (**A**) The comparison "L-tryptophan vs. placebo" revealed increased connectivity in the cingulate cortex and in the precuneous within the default mode network (DMN), in the somatosensory cortex within the sensorimotor network (SMN) and in the bilateral anterior insula within the salience network. (**B**) The comparison "L-tryptophan vs. glucose" showed altered connectivity in areas overlapping those of the previous contrast. In particular, the cingulate cortex and the precuneous show higher connectivity within the DMN, the left anterior insula within the SN and the somatosensory cortex within the SMN. (**C**) The comparison "glucose vs. L-leucine" revealed significantly higher connectivity in the bilateral sensorimotor cortex within the sensorimotor network. (**D**) Within the same network (SMN), the comparison "glucose vs. placebo" reveals an increase in connectivity in additional areas, including the precuneous. (**E**) The comparison "L-tryptophan vs. L-leucine" revealed significantly increased connectivity in the cingulate cortex within the DMN and in the somatosensory cortex within the SMN. No significant activations where found for the remaining comparisons. These results reveal that amino acids administration has an extensive influence on the brain, by modifying the connectivity of specific functional networks related to appetite perception and emotional regulation.

area (SMA) integrates sensory and visceral signals associated with protein intake and is therefore one of the main brain areas involved in appetite regulation and food-reward mechanism. In our study, its function is confirmed by the positive correlation found between changes in activity in SMA and in insulin.

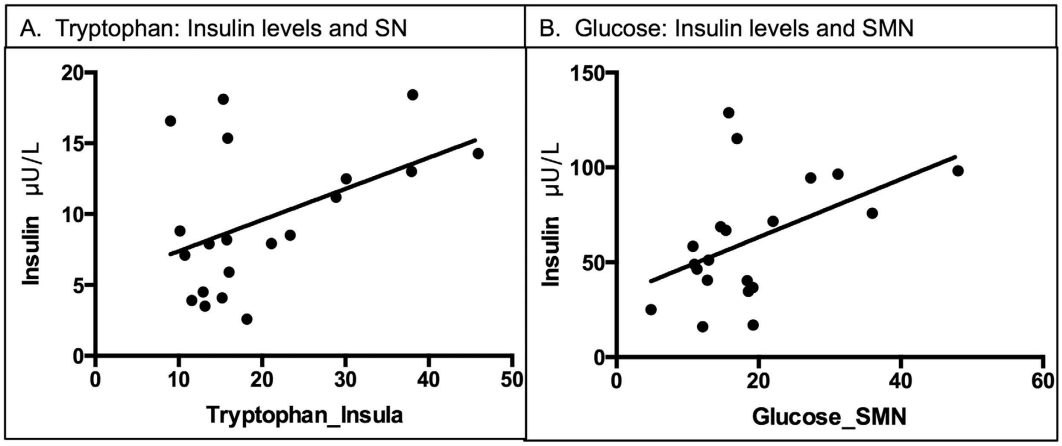

Figure 3. Correlations between imaging and physiological results. After L-tryptophan administration, a positive correlation ($p < 0.05$) was found between levels of insulin and connectivity in the saliency network (**A**). Furthermore, after glucose administration, positive correlations were present between insulin levels and connectivity in the sensorimotor network (SMN) (**B**). These results establish a link between the role of satiety hormones in amino acids synthesis and changes in functional connectivity in the brain.

Apart from brain modifications related to the food-reward system, we infer that L-tryptophan has an influence on cognitive functions and mood regulation. In fact, as demonstrated by different studies manipulating serotonin levels, changes in the activity in prefrontal regions can affect cognitive control and emotion processing[29–32]. In particular, as suggested by Kramer[33], L-tryptophan depletion is linked to reduced activity in the insula, that in turn regulates decision making in potential aggressive situations. On the other side, modulation of L-tryptophan leads to changes in the DMN that may reduce depressive mood[34]. Following this interpretation changes in the DMN and SN after L-tryptophan intake can be linked to changes in cognitive functions and emotion processing.

Our last result concerns the role of L-leucine on brain networks at rest. When L-tryptophan was compared to L-leucine, no differences were found in the activity in the insular cortex within the SN or within the DMN in the precuneous, while significant differences were present in the ACC within the DMN and within the SMN in the SMA. Moreover, comparison of glucose vs. L-leucine didn't find any differences in the DMN or SN.

Even if no previous studies have been conducted on the effects of L-leucine at brain level, these results suggest that this amino acid has an effect on brain network connectivity that is not equivalent to that of water, which suggests that L-leucine may influence areas responsible for cognitive and metabolic processing. Further investigations are needed to further clarify the effect of this amino acid on brain networks at rest.

It is important to note that this study has some limitations. As in previous neuroimaging studies of the brain-gut axis in healthy subjects, our sample size was modest because the design of the study makes recruitment of subjects relatively difficult. On the other hand Cohen's effect size analyses reveal that the treatments show already high magnitude. In addition, the present study focused on only two amino acids. Further investigations should be conducted on more amino acids. Moreover, plasma levels were measured for glucose and insulin, while other hormones might have better detected physiological differences between the different amino acids. Our results might potentially be influenced by mood variations not investigated by the oral examination of the health status of the participants. However, it is important to highlight that the aim of the present study is to investigate changes in brain networks involved in satiation and appetite regulation and changes in hormones levels also related to satiation and appetite (as glucose and insulin). Moreover, it is unlikely that there are systematic changes in mood associated with specific amino acids (the treatments were randomized), hence it is unlikely that potential mood variations systematically biased the current results. Furthermore, the fMRI examination in our study is not conditioned by a paradigm, so the results reflect pure resting state functional connectivity and they may not be comparable to other studies that use a tasks-related approach. Concerning the fMRI analyses, it is important to notice that smoothing may introduce spurious local functional connectivity and affect the subsequent conduction of ICA. Therefore, we performed the analyses without applying smoothing and we compared the three functional networks with and without smoothing. As expected smoothing improves the quality of the results, making the components less noisy. Therefore we decided to work on smoothed data. Lastly, T1 images were acquired at Time0 but not Time2, but the preprocessing of the Time2 images used the T1 image at Time0, so there were different space templates in segment. Since the subjects stayed in the MRI during the treatments administration, the position of the subjects remained the same during all the sequences, therefore the registration can be considered quite effective. Moreover, we repeated the analyses registering the EPI images directly to standard space. Slightly worse registration was seen in the brain networks for the registration directly to MNI, as expected (in fact this is the reason why the high-resolution 3DT1 was used). Despite this small reduction in the quality of the spatial normalization, we observed no relevant differences in the brain networks identified by ICA between the two different registrations. This shows that the registration first to T1 and then to MNI was the most effective one, and that the effect of the spatial registration on the results was marginal.

Finally, it is important to underline possible confounding results from structural changes in grey matter that can influence the response to the treatment administration. To avoid this, VBM analyses were conducted before treatment administration and no differences were observed between the visits, thus excluding this potential confounder.

Conclusion

The current work provides new insights into acute neural modifications after ingestion of amino acids. By linking satiety hormones and fMRI measurements, this study shows that acute L-tryptophan and L-leucine intake directly affect specific brain networks that underpin the food-reward system and appetite regulation.

Materials and Methods

Participants. The protocol was approved by the Ethics Committee of Basel, Switzerland (EKBB: 08/11) and conducted in accordance with the principles of the Declaration of Helsinki. All experimental procedures were carried out in accordance with the approved guidelines. All participants gave written informed consent prior to inclusion. Twenty-three (23) subjects were recruited through local and Internet advertising. Each participant underwent a medical interview, laboratory screening and gave written informed consent. Exclusion criteria were: lactose intolerance, smoking, substance abuse, regular intake of medications, medical or psychiatric illness, and any contraindication to MRI (e.g. claustrophobia, non-removable metal devices) or abnormalities detected upon laboratory screening. Of the 23 subjects originally recruited, three had to be excluded, as they did not meet the eligibility criteria. The final sample included 20 healthy volunteers (28.1 ± 6.2 years, 11 females). Estimation of statistical power in functional MRI requires knowledge of the expected percent signal change between two conditions, as well as estimates of the variability in percent signal change. We calculated the sample size for a strict statistical adjusted threshold of $p < 0.05$, 20 subjects were required to achieve 50% power at the single voxel level for brain activations in the a priori defined networks of interest.

Experimental Protocol. This was a randomized, placebo-controlled, double blind, crossover study and was carried out at the Phase I Research Unit of the University Hospital of Basel. L-tryptophan, L-leucine, glucose and placebo were administered to each subject on four different days, following the procedure described below. The treatment order was randomized and at least 7 days passed between the visits. Therefore no interaction was present between the four administrations.

The subjects started each physiological and imaging examination between 9 and 10am in the morning, after an overnight fast of at least 10 hours. The subjects consumed no breakfast before the visits.

The study was carried out in three phases: Baseline (Time0), treatment administration (Time1) treatment assessment (Time2) (Fig. 4).

Before each visit health status assessment was performed orally by a physician. At the beginning of the experiment an 8F polyvinyl nasogastric tube was inserted into the stomach through an anesthetized nostril and its intragastric position was confirmed by rapid injection of 10 ml of air and auscultation of the upper abdomen. Then two blood samples were taken through a peripheral venous cannula and stored for laboratory analyses. Baseline examination (Time0) was then conducted, in order to control for possible differences in hormone levels and brain functions and structures before treatment administration. To assess for possible functional and structural differences at the brain level, subjects underwent an fMRI examination, including a functional resting state (RS) sequence and a T1 sequence. Before scanning a pillow in dotation of the MRI PRISMA was set in the head-coil behind the head of the subjects to prevent subjects head movements.

After the fMRI examination, the treatment was administered (Time1). The solutions were freshly prepared and were at room temperature when administered. L-tryptophan and L-leucine were purchased from Sigma Aldrich Chemical Company, Germany (>97% pure) and glucose monohydrate was purchased from Haenseler AG (Herisau, Switzerland). Different persons prepared and administered the treatment, in order to maintain the double blindness of the study. Subjects received 300 ml tap water with 1.56 g (7.5 mmol) L-tryptophan, 1.56 g (11.89 mmol) L-leucine, 75 g glucose, and 300 ml pure tap water (placebo) via a nasogastric tube, over 2 minutes while sitting in the MR room.

15 minutes after administration, the tube was removed. To evaluate treatment effects, the subjects underwent a second physiological and brain imaging examination (Time2): blood sampling assessed through a peripheral venous cannula and fMRI examination (RS sequence) was repeated. No T1 sequence was used in this phase, since structural changes were not expected, due to the short period of time after treatment administration.

Laboratory analyses. *Plasma glucose* concentration was measured by a glucose oxidase method (Rothen Medizinische Laboratorien AG, Basel, Switzerland). The intra- and inter-assay coefficients of variation are below 2.9% and below 3.9%, respectively.

Plasma Insulin was measured with a commercially available electrochemiluminescence immunoassay (Cobas/Roche Diagnostics GmbH, Mannheim, Germany). The intra- and inter-assay coefficients of variation for this assay are below 2.0% and below 2.8%, respectively.

fMRI acquisition. Images were obtained using a 3T scanner (Prisma; Siemens, Erlangen, Germany) with a standard 32-channel head-coil. fMRI imaging of the whole brain was acquired by echo planar imaging, using the following parameters: whole brain coverage, TR = 1.8 s, TE = 28 ms, 35 slices, slice thickness 3.5 mm, 168 repetitions. The 3D T_1-weighted structural scan had the following parameters: 256×256 matrix size, 176 sections, $1 \times 1 \times 1\,mm^3$, TE = 3.37 ms, TR = 2000 ms).

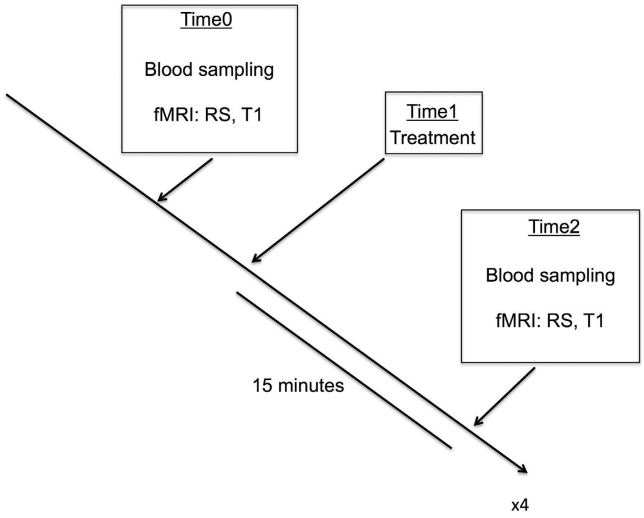

Figure 4. Study design. After an overnight stay of at least 10 hours, the study was carried out in three phases: Baseline (Time0), treatment administration (Time1) treatment assessment (Time2). The baseline examination (Time0) was conducted to control for possible differences in hormone levels and brain functions and structures before treatment administration. Two blood samples were taken through a peripheral venous cannula and stored for laboratory analyses (for blood collection method refer to 38. To assess for possible functional and structural differences at the brain level, the subjects underwent an fMRI examination, including a functional resting state (RS) sequence and a T1 sequence. After the fMRI examination, the treatment was administered (Time1). An 8F polyvinyl nasogastric tube was inserted into the stomach through an anaesthetized nostril. Subjects received 300 ml tap water with 1.56 g (7.5 mmol) L-tryptophan, 75 g glucose, 1.56 g (11.89 mmol) L-leucine and 300 ml pure tap water (placebo) via the nasogastric tube over 2 minutes while sitting in the MR room. 15 minutes after administration, the tube was removed. To evaluate treatment effects, the subjects underwent a second physiological and brain imaging examination (Time2): blood sampling assessed through a peripheral venous cannula and fMRI examination (RS sequences) were repeated. No T1 sequence was used in this phase, since structural changes were not expected in the short period of time after treatment administration.

Statistical Analysis. The statistical analyses were conducted using GraphPad Prism (Version 6, GraphPad Software, San Diego, USA), FSL (Version 5.0.9, FMRIB, Oxford, UK) and R (Version 0.99.896, The R-Project for Statistical Computing).

Analysis of physiological data. To compare hormones levels between the different treatments at Time1 and Time2 separately, a repeated measure analysis of variance (ANOVA) was performed with Tukey correction for post-hoc pair-wise comparisons. Cohen's effect size was also calculated using compute.es package in R (https://cran.r-project.org/) to assess the strength of the difference in hormones plasma levels between the treatments.

Functional connectivity analysis. *Pre-processing of functional data.* Processing and analysis of imaging data of Time1 and Time2 were performed using FSL. Preprocessing included brain extraction using FSL's BET (Brain Extraction Tool), motion correction using FSL's MCFLIRT (intra-modal motion correction tool)[35], spatial smoothing of 5 mm using FSL's SUSAN (noise reduction uses nonlinear filtering)[36]. High-pass temporal filtering of 100 seconds was used according to the standard MELODIC ICA procedure in FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC). Functional images were first co-registered to structural images (acquired during Time1) using linear transformation and later normalized to MNI space using linear transformation. In addition, for each subject, we computed a maximum of the framewise displacement[37,38] from the realignment parameters and subjected this to group (treatments) comparison (ANOVA).

RSNs and Subcortical structures identification. To define brain networks at rest, independent component analysis (ICA) was carried out on the resting state data of Time1 using FSL's multi-session multivariate exploratory linear optimized decomposition into independent components (MELODIC Multi-session temporal concatenation)[39]. First automatic estimation of components was used to explore resting state networks, but due to high parcellation of the signal, the number of components was set to 25 as suggested by previous studies and as common practice in ICA for fMRI data[40]. Out of these 25 components, we decided to select and focus our analyses on 3 resting-state networks (RSNs) identified as consistent by previous studies[41,42] and involved in appetite regulation and control of metabolism[43]: Default Mode Network (DMN), Sensorimotor Network (SMN) and Saliency Network (SN).

RSNs group comparison and correlations with clinical scores. A dual regression approach with non-parametric permutation (5000) tests (randomize, FSL) was carried out on the resting state data at Time1 and

separately at Time2 to detect statistically significant differences between treatments (placebo vs. L-tryptophan, L-tryptophan vs. glucose, glucose vs. L-leucine, etc.) within the boundaries of the three RSNs identified at Time1. A repeated measure ANOVA was performed.

Results were corrected for multiple comparisons using threshold free cluster enhancement (TFCE) and p values < 0.05 were considered as significant. TFCE is similar to cluster-based thresholding, but generally more robust and avoids the need for the arbitrary initial cluster-forming threshold. It is recommended when randomize is performed[44]. Moreover, we performed an ANOVA comparing the visits in respect to time to test whether any interaction effect between times and treatments is present. Finally, Cohen's effect size was also calculated for each comparison within each network using compute.es package in R (https://cran.r-project.org/).

Correlations between imaging and physiological results. Additionally, we tested for possible correlations between regions that were significantly active in the previous contrasts and hormones levels.

On the basis of the results of the dual regression, we defined regional masks and extracted region-averaged time courses of each subject for the three resting state networks. These values were then correlated with the insulin and glucose levels of each subject, using Pearson correlations.

VBM Analysis of T1 Images. To assess differences in grey matter density between groups, a voxel-based morphometric (VBM) analysis was performed in FSL (FSL Version 5.0.9; http://fsl.fmrib.ox.ac.uk), using standard processing steps[45,46]. Firstly, BET extraction and tissue-type segmentation were performed using the corresponding FSL tools (Brain Extraction Tool and FAST4). Secondly, non-linear transformation into Montreal Neurological Institute (MNI) reference space was applied and a study-specific grey matter (GM) template was created. The native GM images were then non-linearly registered to this template. Finally, the images were smoothed with an isotropic Gaussian kernel of 2 mm sigma. A voxel-wise GLM was implemented using permutation-based nonparametric testing (Randomise, part of FSL). Results were corrected for multiple comparisons using TFCE[44] and p values < 0.05 were considered as significant.

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Author Contributions

Conceived and designed the experiments: B.K.W., A.C.M.G., C.B. and S.B. Performed the experiments: B.K.W. A.C.M.G. and K.J. Data analyses: D.Z., C.S., S.H., C.L.R. and J.D. Wrote the paper: D.Z., C.B. and S.B.

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Acute Effects of Glucose and Fructose Administration on the Neural Correlates of Cognitive Functioning in Healthy Subjects: A Pilot Study

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The present randomized double-blinded cross-over study aims to extensively study the neural correlates underpinning cognitive functions in healthy subjects after acute glucose and fructose administration, using an integrative multimodal neuroimaging approach. Five minutes after glucose, fructose, or placebo administration through a nasogastric tube, 12 participants underwent 3 complementary neuroimaging techniques: 2 task-based functional magnetic resonance imaging (fMRI) sequences to assess working memory (N-back) and response inhibition (Go/No-Go) and one resting state fMRI sequence to address the cognition-related fronto-parietal network (FPN) and salience network (SN). During working memory processing, glucose intake decreased activation in the anterior cingulate cortex (ACC) relative to placebo, while fructose decreased activation in the ACC and sensory cortex relative to placebo and glucose. During response inhibition, glucose and fructose decreased activation in the ACC, insula and visual cortex relative to placebo. Resting state fMRI indicated increased global connectivity strength of the FPN and the SN during glucose and fructose intake. The results demonstrate that glucose and fructose lead to partially different partially overlapping changes in regional brain activities that underpin cognitive performance in different tasks.

Keywords: functional magnetic resonance imaging, glucose, fructose, brain–gut, working memory, cognition

INTRODUCTION

The mammalian brain depends upon sugars as the main source of energy, and the regulation of sugar metabolism is critical for brain physiology (1). Glucose and fructose, two of the most important monosaccharides, have a roughly equal number of calories but are metabolized differently (2). Glucose, a highly potent secretagogue, leads to the release of insulin and satiation hormones such as GLP-1 by enteroendocrine cells as well as inhibition of the appetite inducer ghrelin (3, 4). In contrast, fructose intake does not affect the release of insulin to the same extent (5, 6) and chronic fructose consumption may adversely affect human health by leading to increased *de novo* lipogenesis in the liver, hyperuricemia, and obesity (7, 8).

The differences in the metabolism of glucose and fructose may also explain their differential effects on neuronal pathways. Page's milestone study (9) has documented reduced relative cerebral blood flow and increased functional connectivity after the ingestion of sugars (both glucose and fructose) in the insula, anterior cingulate, striatum, and posterior cingulate cortex (appetite and food-reward regions). The effects from fructose were greater, and this resulted in increased brain activation in the visual cortex during a food-cue task (2). Similar results were found by a recent study conducted by our group investigating resting state functional connectivity in the basal ganglia network (4).

Whereas changes linked to appetite stimulation in the human brain are generally accepted (2, 9, 10), recent animal studies suggest that sugars may have different effects on brain regional activity underlying cognitive functioning (11–14). While extensive evidence indicates that increased glucose concentrations enhance learning and memory processes in rodents through the enhancement of hippocampal activity (15), recent studies indicate that the hippocampus may be particularly vulnerable to the effects of fructose, with impaired synaptic plasticity and consequent decreased working memory performance after high-fructose diets (16, 17). To our knowledge, no previous studies have investigated the effects of glucose and fructose on whole-brain activity during different cognitive functions in humans.

Therefore, while dietary energy intake, in particular the consumption of simple sugars such as fructose, has been increasing steadily in Western societies, the effects of such a diet on the human brain are still poorly understood (17). In particular, food intake (as sugars) can have a significant role beside age and gender in multimodal neuroimaging studies (18). The present study can be considered as a starting point for future investigations also outside the brain-gut field, suggesting to assess nutrients intake in the functional magnetic resonance imaging (fMRI) analyses.

In the present study, we employ a functional multimodal approach to study the effects of glucose and fructose on different cognitive functions. We administered glucose and fructose to the participants through a nasogastric tube inserted into the stomach. After 5 min, the participants underwent an extensive fMRI examination, performing one N-back task (to assess working memory), one Go/No-go task (to assess response inhibition), and one resting state sequence focusing on two cognition-related resting state networks, in particular the fronto-parietal network (FPN) and salience network (SN).

As glucose and fructose are subject to differential metabolic processes at the cellular level (2), we hypothesized that these monosaccharides would also induce dissociable effects on brain regional activity during cognitive functioning.

MATERIALS AND METHODS

Participants

The protocol was approved by the Ethics Committee of Basel, Switzerland (EKBB: 08/11) and conducted in accordance with the principles of the Declaration of Helsinki. All experimental procedures were carried out in accordance with the approved guidelines. The participants and the experiment protocol for the present study were already presented in a previous work of the same team (4). Fourteen (14) subjects were recruited through local and internet advertising. Each participant underwent a medical interview and laboratory screening and gave written informed consent prior to inclusion. Exclusion criteria were: lactose intolerance, smoking, substance abuse, regular intake of medications, medical or psychiatric illness, and any contraindication to MRI (e.g., claustrophobia, non-removable metal devices) or abnormalities detected upon laboratory screening. Of the 14 (14) subjects originally recruited, 2 had to be excluded as they did not meet the eligibility criteria. There was also one drop-out, who was replaced. The final sample included 12 healthy volunteers (mean age: 24.8 years, range: 21–31 years, and mean BMI: 22.9 kg/m^2, range: 21.0–24.0 kg/m^2).

Experimental Protocol

This was a randomized, double-blind, cross-over study and was carried out at the Phase I Research Unit of the University Hospital of Basel. Glucose, fructose, and a placebo were administered to each subject on three different days, following the procedure described below. The treatment order was randomized and at least 7 days passed between the visits.

After an overnight fast of at least 10 h, an 8 F polyvinyl nasogastric tube was inserted into the subjects' stomach through an anesthetized nostril and its intragastric position was verified by rapid injection of 10 ml air and auscultation of the upper abdomen.

The solutions were freshly prepared and were at room temperature when administered. Glucose monohydrate and fructose were purchased from Hänseler AG (Herisau, Switzerland). Different persons prepared and administered the solutions. Over 2 min, subjects received 300 ml of tap water with 75 g of glucose or with 25 g of fructose, or 300 ml pure tap water (placebo) *via* the nasogastric tube while sitting in the MR room. The administered doses were chosen on the basis of previous studies demonstrating lipogenesis increased in proportion after sugar intake (19).

Directly after administration, the tube was removed. To evaluate the treatment effect, the subjects underwent a brain imaging examination, including: three echo planar imaging (EPI) sequences (N-back task, Go/No-go task, and resting state sequence) and one T_1 sequence.

fMRI Acquisition

Scanning was performed on a 3T scanner (Siemens Magnetom Verio). The N-back task sequence was performed using an EPI sequence (TR = 2,500 ms, TE = 28 ms, flip angle = 83°, field of view = 228 mm × 228 mm, 32 slices, slice thickness: 3 mm; voxel size = 3.6 mm × 3.6 mm × 3.3 mm). In total, 126 EPI volumes were acquired. The Go/No-Go task sequence was performed using an EPI sequence (TR = 2,500 ms, TE = 28 ms, flip angle = 83°, field of view = 228 mm × 228 mm, 32 slices, slice thickness: 3 mm; voxel size = 3.6 mm × 3.6 mm × 3.3 mm). In total, 160 EPI volumes were acquired. The resting state EPI sequence had the following parameters: TR = 2,000 ms, TE = 28 ms, flip angle = 82°, field of view = 228 mm × 228 mm, 32 slices, slice thickness: 3.3 mm; voxel size = 3.6 mm × 3.6 mm × 3.3 mm. In total, 152 EPI volumes were acquired. Finally, the 3D T_1-weighted structural scan

had the following parameters: 256 × 256 matrix size, 176 sections, 1 mm × 1 mm × 1 mm TE = 3.37 ms, TR = 2,000 ms.

N-Back Task

During the N-back task (20–22), all participants saw series of letters with an interstimulus interval (ISI) of 2 s. Each stimulus was shown for 1 s. During a baseline (0-back) condition, participants were required to press the button with the right hand when the letter "X" appeared. During 1-back and 2-back conditions, participants were instructed to press the button if the currently presented letter was the same as that presented in one (1-back condition) or two trials previously (2-back condition). The three conditions were presented in 10 alternating 30-s blocks (2 × 1-back, 3 × 2-back, and 5 × 0-back), matched for the number of target letters per block (i.e., 2 or 3), in a pseudorandom order. Task performance was expressed by the accuracy (number of correct responses to the 2-back task). A repeated measure of analysis of variance (ANOVA) was performed across the three visits.

Go/No-Go Task

After the N-back task, all patients immediately underwent an event-related Go/No-Go fMRI paradigm that was conducted with jittered ISIs and containing infrequently presented oddball stimuli to optimize statistical efficiency. This is a well-validated paradigm (23, 24), requiring either the execution or the inhibition of a motor response, depending on the visual presentation of the stimuli. The basic Go task is a choice reaction time paradigm, in which arrows point either to the left or to the right for 500 ms, with a mean ISI of 1,800 ms (jitter range: 1,600–2,000 ms). During Go trials, subjects were instructed to press the left or the right response button according to the direction of the arrow. In 11% of the trials, arrows pointing upward appeared. During these so-called "No–Go" trials, participants were required to inhibit their motor response. During another 11% of the trials, arrows pointing left or right at a 23° angle were shown, and the subjects were told to respond in the same way as to Go stimuli (even though they pointed obliquely). These "oddball" stimuli were used as a control of the novelty effects associated with the low frequency and different orientation of the No–Go relative to the Go trials (stimulus-driven attention allocation). In total, there were 24 No–Go, 160 Go, and 24 oddball trials, with task durations of approximately 6 min.

Statistical Analysis Software

The statistical analyses were conducted using GraphPad Prism (Version 6, GraphPad Software, San Diego, CA, USA) and FSL (Version 5.0.9, FMRIB, Oxford, UK).

Analysis of Cognitive Performance

N-Back Task

To compare the performance during the N-back task, the reaction time and the number of correct answers (accuracy) were investigated for all conditions. A repeated measure ANOVA was performed with Tukey correction for *post hoc* pair-wise comparisons.

Go/No-Go Task

To compare the performance during the Go/No-go task, the reaction time and the "probability of inhibition" (ratio between No-Go correct and incorrect response) were investigated for all conditions. A repeated measure ANOVA was performed with Tukey correction for *post hoc* pair-wise comparisons.

Task-Based Functional Imaging Analyses

Pre-Processing

Processing and analysis of imaging data were performed using FSL FEAT (fMRI Expert Analysis Tool version 6.00, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT). Pre-processing included brain extraction using FSL's brain extraction tool, motion correction using FSL's MCFLIRT (intra-modal motion correction tool) (25) and smoothing using FSL's SUSAN (noise reduction uses non-linear filtering) (26). Images were finally normalized to MNI space.

N-Back Task

After pre-processing, the linear-model analysis of the N-back sequence included two levels. At the first level, the contrast "2-back vs. 0-back" was calculated separately for each participant. At the second level, group differences between glucose, fructose, and placebo were investigated. This resulted in a mixed-effects group model implementing FLAME 1 (FMRIB's Local Analysis of Mixed Effects). Finally, a repeated measures permutation-based non-parametric test (randomize, FSL tool) was applied, correcting for multiple comparisons by threshold-free cluster enhancement (27). p-Values <0.05 were considered as significant.

Go/No-Go Task

After pre-processing, general linear models (GLM) analysis were performed to investigate brain activation differences during the Go/No-go sequence. At the first level, the contrast "No-go vs. oddball" was calculated separately for each participant. At the second level, group differences between glucose, fructose, and placebo were investigated. As above, a repeated measures permutation-based non-parametric approach (randomized, FSL tool) was applied, correcting for multiple comparisons by threshold-free cluster enhancement (27). p-Values <0.05 were considered as significant.

Functional Resting State Connectivity Analysis

Resting State Network Identification

After pre-processing, to define brain networks at rest, an independent component analysis (ICA) was carried out on the resting state data using FSL's multi-session multivariate exploratory linear optimized partition into independent components (MELODIC multi-session temporal concatenation) (28), setting the number of components to 20, which is common practice in ICA for fMRI data. Out of these 20 components, we decided to select and focus our analyses on 2 resting state networks (RSN): the fronto-parietal (also called executive functions) (FPN) network and the SN—identified as consistent with our previous studies (29, 30)—due to their involvement in cognitive functions

and cognitive control (31–33). Cross-correlation of the two time-series, timepoint by timepoint, using as reference RS maps of Laird (30) were performed to compare the EF and SN networks to a major RSN template using a higher number of subjects.

RSNs Group Comparison

A dual regression approach (34) was carried out on the resting state data within the boundaries of the identified RSN. Region-averaged time courses of each subject for the three resting state networks were extracted and submitted to a repeated measure ANOVA to test for differences between the treatments, using Tukey correction for *post hoc* pair-wise comparisons.

Cross-Modalities Correlations

After the task-based and resting state studies, we performed cross-modalities correlations analyses.

The regional averaged time-course was extracted across the subjects for the N-back and the Go/No-go for the significant contrasts. Moreover, connectivity values from the identified component for the resting state analyses were used. Individual correlation analyses across modalities were performed. FDR multiple comparisons corrections were used (**Table 1**).

RESULTS

Behavioral Results

N-Back Task

The ANOVA showed no significant differences in accuracy across treatments.

Go/No-Go Task

No significant treatment differences were found for the probability of inhibition.

N-Back Activations

Absolute values for motion are (mean ± SD): glucose 0.14 ± 0.04, fructose 0.17 ± 0.05, placebo 0.12 ± 0.04. Relative values for motion are (mean ± SD): glucose 0.04 ± 0.01, fructose 0.046 ± 0.01, placebo 0.042 ± 0.01.

In the task-related GLM, we considered the contrast of "2-back versus 0-back." Relative to placebo, glucose significantly reduced activation in the anterior cingulate cortex (ACC)/dorsal pre-frontal cortex (**Figure 1A**; Table S1A in Supplementary Material). Relative to placebo, fructose significantly reduced activation in the ACC/dorsal pre-frontal cortex, sensory cortex, and cerebellum (**Figure 1B**; Table S1B in Supplementary Material). Glucose compared with fructose also significantly increased activation in the bilateral dorsal pre-frontal cortex and cerebellum (**Figure 1C**; Table S1C in Supplementary Material).

TABLE 1 | Cross-modality correlations.

	N-back	Go/No-go	FPN	SN
N-back		0.048	−0.316*	−0.341*
Go/No-go	0.048		−0.465*	−0.333*
FPN	−0.316*	−0.465*		−0.383*
SN	−0.341*	−0.333*	−0.383*	

After performing task-based and resting state analyses, we carried out cross-modalities correlations analyses. Significant correlations were found between the N-back results and resting state connectivity strength, both for the FPN ($p < 0.05$) and salience network (SN) ($p < 0.05$). For the SN, significant correlations were found between the Go/No-go results and the resting state connectivity values ($p < 0.05$). The results are corrected for FDR multiple comparison corrections. Pearson R values are displayed. Significant levels are reported using the conventional.*

Go/No-Go Activations

Absolute values for motion are (mean ± SD): glucose 0.16 ± 0.06, fructose 0.21 ± 0.07, placebo 0.17 ± 0.07. Relative values for motion are (mean ± SD): glucose 0.04 ± 0.01, fructose 0.04 ± 0.01, placebo 0.04 ± 0.01.

Relative to placebo, glucose significantly reduced activation in the ACC, dorsal pre-frontal cortex, right insula, and visual cortex (**Figure 2A**; Table S2A in Supplementary Material). Relative to placebo, fructose significantly reduced activation in the ACC, dorsal pre-frontal cortex, sensory cortex, and visual cortex (**Figure 2B**; Table S2B in Supplementary Material). No significant differences were found between glucose and fructose.

Functional Resting State Connectivity Analysis Results

Absolute values for motion are (mean ± SD): glucose 0.14 ± 0.03, fructose 0.17 ± 0.05, placebo 0.15 ± 0.04. Relative values for motion are (mean ± SD): glucose 0.06 ± 0.02, fructose 0.07 ± 0.02, placebo 0.06 ± 0.02.

Group analyses of frame wise displacement found no significant effect of motion between the visits. Cross value correlations are for the EF network: $r = 0.3$, $p < 0.01$ and for the SN $r = 0.4$, $p < 0.01$. Repeated measure ANOVA revealed a significant main effect in functional connectivity in the fronto-parietal network (FPN) network [$F(2, 11) = 13.69$, $p < 0.001$] (**Figure 3A**). Subsequent *post hoc* testing showed significantly higher connectivity strength after ingesting glucose than with placebo ($p < 0.05$) or fructose ($p < 0.05$), while an increase in connectivity in the FPN network was found after fructose ingestion compared with placebo ($p < 0.01$). Moreover, repeated measure ANOVA also revealed a significant main effect of treatment in functional connectivity in the SN network [$F(2, 11) = 6.117$, $p < 0.05$] (**Figure 3B**). In particular, significantly higher connectivity strength than with placebo was found after ingesting glucose ($p < 0.05$) or fructose ($p < 0.05$). No differences in connectivity were found between glucose and fructose ingestion.

Cross-Modalities Correlations Results

Significant correlations were found between the N-back results ($p < 0.05$) and resting state connectivity values, both for FPN ($p < 0.05$) and SN ($p < 0.05$). For the SN, significant correlations were found ($p < 0.05$) between the Go/-No-Go results and the resting state connectivity values. The results are corrected for false discovery rate multiple comparison corrections. Results are displayed in **Table 1**.

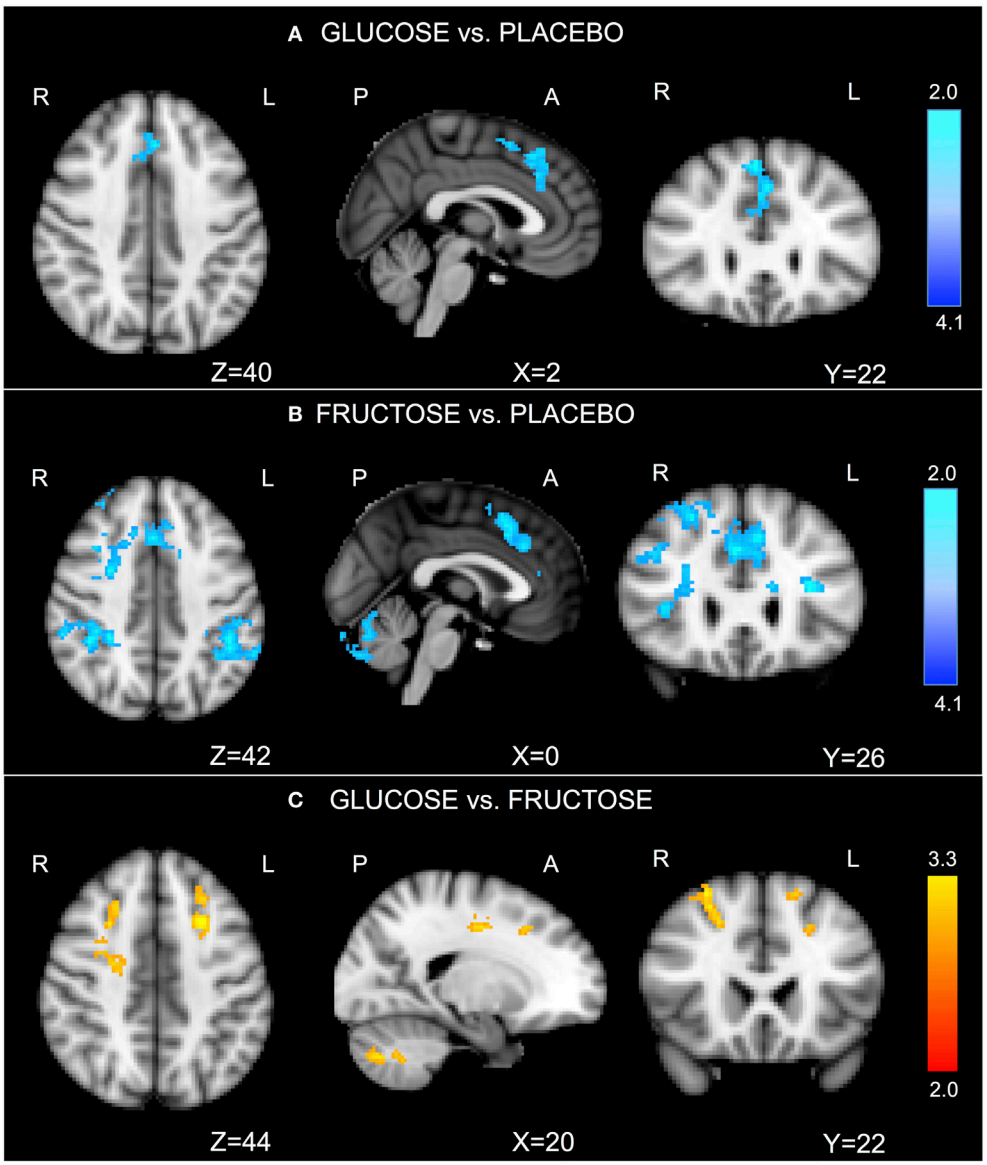

FIGURE 1 | N-back functional imaging results. In the task-related general linear models, we considered the contrast of "2-back versus 0-back." The comparison "glucose vs. placebo" revealed significantly reduced activation after ingesting glucose in the anterior cingulate cortex (ACC)/dorsal pre-frontal cortex [**(A)**, Table S1A in Supplementary Material]. The comparison "fructose vs. placebo" revealed significantly lower activations after ingesting fructose, particularly in the ACC/dorsal pre-frontal cortex, sensory cortex, and cerebellum [**(B)**, Table S1B in Supplementary Material]. The comparison "fructose vs. glucose" revealed significantly greater activations after ingesting fructose in the bilateral dorsal pre-frontal cortex and cerebellum [**(C)**, Table S1C in Supplementary Material]. *Z*-stat values are shown in the color bar. The results are given by repeated measures permutation-based non-parametric test (randomize, FSL tool) approach, correcting for multiple comparisons by threshold-free cluster enhancement (27). *p*-Values <0.05 were considered as significant.

DISCUSSION

The present study performs an extensive assessment of cognition-related brain functional changes after glucose and fructose administration. Although we found no significant differences in behavioral performance during working memory processing and response inhibition, both glucose and fructose decreased activation in frontal areas such as the ACC and dorso-lateral pre-frontal cortex (DLPFC) during working memory processing and response inhibition—especially after fructose intake. The connectivity of these regions as parts of the FPN and SN is in turn increased during glucose and fructose ingestion.

Our first group of results relate to the absence of differences in task performance during working memory processing and response inhibition after glucose and fructose intake compared with placebo. The absence of changes in performance after fructose intake is confirmed by animal studies that found no differences in cognitive/motor performance as measured by object recognition

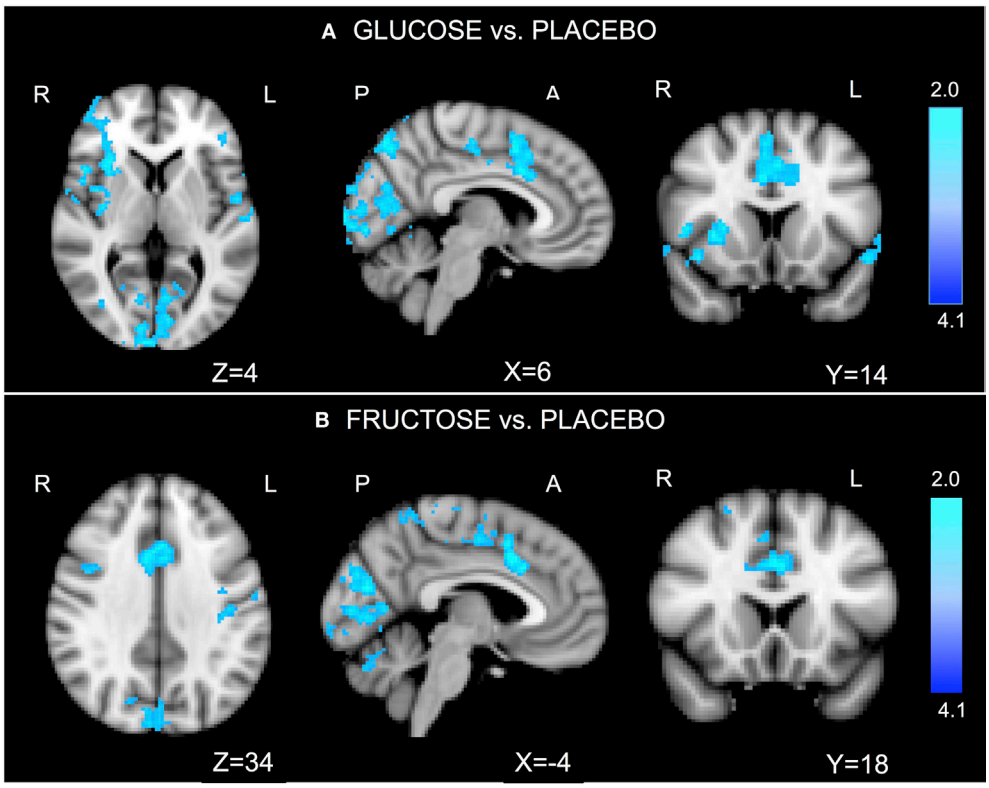

FIGURE 2 | Go/No-go functional imaging results. In the task-related general linear models, we considered the contrast of "No-go versus Oddball." The comparison "glucose vs. placebo" revealed significantly reduced activations after ingesting glucose, particularly in the anterior cingulate cortex (ACC), the dorsal pre-frontal cortex, right insula, and visual cortex **(A)**. The comparison "fructose vs. placebo" revealed significantly lower activations after ingesting fructose in the ACC, the dorsal pre-frontal cortex, sensor cortex, and visual cortex **(B)**. No significant differences were found for the comparison glucose vs. fructose. *Z*-stat values are shown in the color bar. The results are given by repeated measures permutation-based non-parametric test (randomize, FSL tool) approach, correcting for multiple comparisons by threshold-free cluster enhancement (27). *p*-Values <0.05 were considered as significant.

and fear conditioning in rodents (35, 36) and by a recent review (11) that concluded that fructose does not induce cognitive deficits. Published reports on behavioral differences after glucose administration are inconsistent with respect. Although a previous study (37, 38) reported improvements in object recognition and word-recall performance after glucose intake, other authors have found no differences in cognitive performance (39–41). In the present study, we confirm the absence of changes at the behavioral level after sugar administration. From our perspective, this is still an open field of research and our results with this small sample size are not definitive. Although fMRI data on small subject numbers are relatively robust (42), behavioral indexes are typically underpowered and could be confounded by many personal attributes that cannot be clearly assigned to the cognition required for adequate task performance (43).

Our second group of results relate to changes at the level of brain function. During working memory processing, decreased activation in the ACC and DPFC was shown after glucose administration. Less activation in the ACC/DPFC and in the sensory cortex was found after fructose administration than after glucose administration.

As previous studies on cognitive functions and induced-training suggested, decreased brain activation during a demanding cognitive load is associated with more efforts to perform a task (44–47). According to this interpretation, our results suggest the subjects show less demanding brain activation during the stimulus-response association task after glucose and fructose intake than with placebo (48, 49). Moreover, our findings are in line with a recent study that concluded that after glucose and fructose intake the participants showed significantly decreased cerebro-spinal fluid relative to placebo, particularly in the ACC, insula, and thalamus compared with Ref. (9).

In comparison with placebo, we found reduced functional activation in the ACC, DPFC, insula, DLPFC, and visual cortex after glucose and fructose administration. No differences between glucose and fructose were found, which was comparable with the results during working memory processing.

Although working memory involves temporary storage and manipulation of the information (50) and response inhibition involves the suppression of actions that are no longer required or inappropriate (51), our results indicate that acute glucose and fructose administration similarly modulates brain activation during these two cognitive processes.

Our third group of findings relates to differences in resting state functional connectivity after fructose and glucose intake. The connectivity within the FPN and the SN is increased during

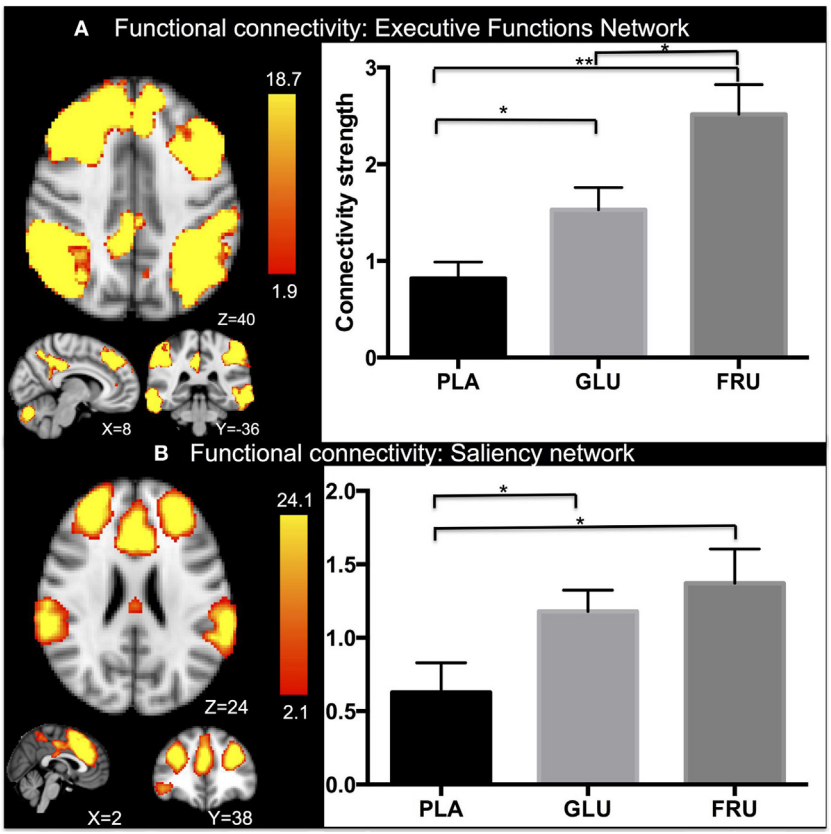

FIGURE 3 | Independent component analyses results. After dual regression on the executive functions network (EF) and extracting the connectivity strength values, repeated measure analysis of variance (ANOVA) revealed significant activation in the EC network for the three groups **(A)**. In particular, significantly higher connectivity strength was found after ingesting glucose than with placebo ($p < 0.01$) and fructose ($p < 0.01$), while an increase in connectivity was found in the EC network after fructose ingestion ($p < 0.05$) compared with placebo. Moreover, repeated measure ANOVA revealed significant differences in functional connectivity in the salience network too for the three groups **(B)**. In particular, significantly higher connectivity strength was found after ingesting glucose than with placebo ($p < 0.05$) and fructose compared with placebo ($p < 0.05$). No differences in connectivity were found between glucose and fructose ingestion. Mean and standard errors are reported. Significant levels are reported using the conventional*. *Z*-stat values are shown in the color bar. The results are given by repeated measures permutation-based non-parametric test (randomize, FSL tool) approach, correcting for multiple comparisons by threshold-free cluster enhancement (27). *p*-Values <0.05 were considered as significant, $^{**}p < 0.01$.

both fructose and glucose intake compared with placebo; this is comparable with the task-induced fMRI findings, but in the opposite direction.

The increase in connectivity after glucose intake has already been reported several times (4, 9, 52, 53), but ours is the first study to demonstrate increased functional connectivity in networks related to cognitive functions after fructose intake.

Our correlation analyses confirm that glucose and fructose intake lead to increased functional connectivity in the FPN and SN and to decreased efforts during working memory and response inhibition tasks.

We finally want to mention, as already suggested in Section "Introduction," that food intake may play a significant role beside age and gender in multimodal neuroimaging studies (18). The present work can be considered as a starting point for future investigations also outside the brain-gut field, suggesting to assess nutrient intake beside age, and gender changes on brain functional activity, using them for instance as covariates in the fMRI analyses.

Limitations

Some limitations of our study merit comment. As in previous neuroimaging studies of the brain-gut axis in healthy subjects, our sample size was modest since it is intended to be a pilot study. In addition, the present study focused only on glucose and fructose, while sucrose and other substances could also be investigated. Our results might potentially be influenced by external factors such as daily mood variations not investigated by examination of the health status of the participants. However, it is important to point out/emphasize that the aim of the present study was to investigate changes in brain networks involved in cognitive functions and this was why emotional changes were not studied in detail. In the fMRI analyses, it is important to notice that smoothing may introduce spurious local functional connectivity and affect the subsequent conduction of ICA, but we decided to keep the smoothing in order to reduce noise. We also want to stress that while we randomized for the treatment assignment order, and for the sequence of stimuli during the tasks, we did not randomize for the fMRI sequences ordering. We therefore

suggest future investigations to randomize also for the fMRI tasks ordering, to control for ordering effects.

To conclude, the results of the present work suggest the presence of two partially overlapping neural pathways related to cognitive functions after glucose and fructose ingestion. The working memory and the response inhibition pathways showed that glucose and fructose decrease activation and increase connectivity strengths in regions in the FPN and the SN. These results are to be considered as part of a preliminary and exploratory investigation of sugar effects on cognitive functions. Our findings suggest that future studies on diet-induced manipulations are plausible and efficient for pathologies affecting the cognitive dimension.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

BW, AM-G, CB, and SB conceived and designed the experiments. BW, AM-G, and KJ performed the experiments. DZ, AD, CS, AS, SH, CLR, and JD data analyses. DZ, CB, and SB wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/articles/10.3389/fpsyt.2018.00071/full#supplementary-material.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DISCUSSION

The present PhD thesis aims at investigating the brain gut matrix. First, through a systematic review of the literature, previous studies assessing the effects of nutrients on brain functions were examined. A global picture on the relationship between the brain and the gut is therefore available and a common research methodology can be identified.

Second, we performed a set of studies investigating the effects of sugars and amino acids on satiety hormones and RS functional networks involved in appetite regulation.

Third, we investigated more specifically the effects of sugars beyond appetite and satiety, focusing on cognitive functions and discriminating their effects on working memory and response inhibition.

These three studies therefore provide us with a general overview of the different processes occurring in the brain-gut interaction.

The brain-gut matrix: a systematic review.

To our knowledge, this is the first systematic review on the effects of gut peptides on brain functions in healthy and obese subjects. 349 studies were investigated and 40 were retrieved.

Due to the exploratory nature of the present review, it is important to highlight differences in the methodology between the studies.

A first distinction is the gut-peptides stimulation. Half of the studies used a direct administration of the target substance, as insulin, and the other half an indirect administration of a nutrient, as chocolate milkshake, that indirectly stimulated gut peptides. This choice impacts as well the aim of the investigations, the first is specifically useful for the direct assessment of gut peptides effects on brain functions and the second for assessment of the nutrients effects on gut peptides (Malik *et al*, 2008).

Moreover, differences in substance intake can also influence the procedure of data acquisition increasing the easiness for the participants (Zanchi *et al*, 2016), they can also lead to changes in the timing of nutrient absorption that can influence the fMRI paradigm (De Silva *et al*, 2012; Liu *et al*, 2000a; Malik *et al*, 2008). While the majority of the studies used a “food-cue paradigm”, presenting high and low caloric food cues, few used what we called an ‘on-off treatment related block design’ by the timing of hormonal plasma absorption to investigate the brain response. Finally, a classical resting state paradigm was used for the remained works (Zanchi *et al*, 2017).

Regarding neuroimaging results, ghrelin plasma concentration is positively associated with activity in areas part of the food-reward system as the Pre-frontal Cortex (PFC), amygdala and insula and negatively with activity in subcortical

areas as the hypothalamus. In contrast, satiety-regulating gut hormones or nutrients like glucose, insulin, letpin and GLP-1 affect the same brain regions in the opposite directions (Zanchi *et al*, 2017).

The results we found reflect the model proposed by Woods, showing activation in frontal regions as the Orbito-Frontal Cortex (OFC), Anterior Cingulate Cortex (ACC) and insula associated positively to ghrelin plasma levels and with increased hunger feelings. Subcortical areas like the thalamus, hippocampus, striatum and hypothalamus correlated negatively with ghrelin levels (Woods *et al*, 1998).

As Woods stated, adipose signals (ghrelin and insulin) penetrate the blood brain barrier and stimulate receptors on neurons in the hypothalamus, amygdala and striatum (Woods *et al*, 1998) after food intake where they influence reflexes related to the acceptance or rejection of food. Afterwards, the hypothalamus signals cortical areas part of the reward mechanism as the OFC, ACC and insula, where higher cognitive process begins. At this point the eating behaviour is determined (Higgs *et al*, 2017).

Effects of sugars and amino acids intake on brain resting state functional connectivity.

Our first study aims at investigating the effects of sugars and amino acids on brain resting state networks related to satiety regulation. In particular, we chose three resting state functional networks involved in food-reward mechanisms: the

Default Mode Network (DMN), the Sensorimotor Network (SMN) and the Salience Network(SN) (De Silva *et al*, 2012).

Our results reveal that sugars and amino acids intake show differential effects on RS functional connectivity. Specifically, tryptophan shows the highest increase in connectivity within the sensory-motor network in the somatosensory area, within the default mode network in the cingulate cortex and in the precuneus and within the salience network in the bilateral insula, when compared to placebo and glucose (Zanchi *et al*, 2016). The reorganization in the sensorimotor network is also present after glucose administration compared to placebo. The somatosensory area (SMA) is one of the main brain areas involved in appetite regulation, integrating sensory and visceral signals associated with protein intake (Liu *et al*, 2000a; Park *et al*, 2016, 2016). This is confirmed in our study by the positive correlation found between changes in activity in SMA and in insulin.

Moreover, due to changes in the DMN and SN connectivity, we speculate that tryptophan and glucose also impact brain areas involved in a broad class of functions, beyond satiety regulation. In particular, several works confirm the involvement of the DMN in mood regulation (Sheline *et al*, 2009; Shi *et al*, 2015; Uchida *et al*, 2015) and of the SN in cognitive functions (Cao *et al*, 2016; Menon and Uddin, 2010; Putcha *et al*, 2016). Manipulation of serotonin levels associated to tryptophan intake leads to changes in the activity in prefrontal regions that in turn can affect cognitive control and emotion processing (Dantzer *et al*, 2011; Passamonti *et al*, 2012; Seymour *et al*, 2012; Williams *et al*, 2007). Tryptophan

depletion is also associated with decision making processes and depressive mood (Krämer *et al*, 2011; Kunisato *et al*, 2011).

We finally want to highlight the role of L-leucine on brain network activity at rest. The comparison L-leucine vs. Placebo shows no significant differences. However, the visual differences between tryptophan and L-leucine when compared to water suggest that the effect of L-leucine on functional activity is not equivalent to the one of water. We believe that the absence of significant results is due to the modest sample size that decreases the statistical power. However, we can only speculate and we are not allowed to go further with our interpretation.

Further investigations are needed to clarify the effect of this amino acid on brain networks at rest.

Effects of sugars intake on brain activity underpinning cognitive functions.

Our second study explores brain activity changes related to sugars administration beyond the food-reward mechanisms, investigating the impact of glucose and fructose on cognitive functions. In particular working memory, assessed by the N-back task, is a cognitive function involved in temporary storage and manipulation of the information necessary for complex cognitive tasks (Baddeley, 1992; Jacola *et al*, 2014; Owen *et al*, 2005).Response inhibition, assessed using Go/no-go task, is involved in the suppression of no-

longer required or inappropriate actions, which supports flexible and goal-directed behaviors (Simmonds *et al*, 2008; Verbruggen and Logan, 2008).

Our first results show no differences in task performance during N-back and Go/no-go task tasks after glucose and fructose intake when compared to placebo. Although our results are confirmed by several previous studies (Beilharz *et al*, 2015), we believe that the small sample size does not allow an extensive investigation of the behavioral performance (Wilkinson and Halligan, 2004). Further studies with a bigger sample size are suggested to depict differences in performance also in light of our functional results. In fact, as several studies showed, the BOLD signal is more sensitive to tasks than the behavior (Balsters *et al*, 2013; Bonakdarpour *et al*, 2015).

Our second results related to decreased activation in frontal areas, as the ACC and dorso-lateral pre-frontal cortex (DLPFC), during working memory processing and response inhibition after both glucose and fructose ingestion compared to placebo. The connectivity of these regions as parts of the Fronto-Parietal Network (FPN) and Saliency Network (SN) is in turn increased during glucose and fructose ingestion.

As stated above, studies on cognitive functions and induced-training suggested decreased brain activation during a cognitive task is associated with more efforts to perform a task, while increased activation is associated with higher easiness to perform the task (Engström *et al*, 2013; Erickson *et al*, 2007; Heinzel *et al*, 2016; Lee *et al*, 2012). Following this interpretation, glucose and fructose administration

when compared to placebo facilitate the stimulus-response association and in turn lead to decreased activation in brain areas related to cognitive functions (Corbetta and Shulman, 2002; Erickson *et al*, 2005).

Our correlation analyses confirm that glucose and fructose intake lead to increased functional connectivity in the FPN and SN and to decreased efforts during working memory and response inhibition tasks.

Limitations and further directions.

Due to the exploratory nature of the present work, it is important to highlight the limitations of the investigated studies and suggest possible improvements for future works on the brain-gut interaction.

First, we suggest to decrease the variability of the administered substances. Although it can appear as counterintuitive, this will decrease the complexity of the studies (less nutrients ingested will lead to a precise discrimination of their direct and indirect effects on gut peptides) promoting an univocal methodology in terms of nutrients intake and timing of administration (Sizonenko *et al*, 2013).

This will also impact the easiness of the recruitment procedure increasing the sample size and therefore the statistical power, allowing to disentangle more consistent results in particular at the behavioral level. Decreasing the complexity of the studies and increasing the sample size is a straightforward improvement for the next generation of studies on the brain-gut interaction (Charan and Biswas, 2013).

A third important improvement is the different paradigms and imaging analyses involved. Due to the exploratory nature, different paradigms were used. A validation of more specific instruments will disentangle more specifically the effects of nutrients administration on brain functions (Francis and Eldeghaidy, 2015; Isaacs, 2013; Sizonenko *et al*, 2013).

This point is also connected to the statistical analyses used to analyze brain data. A work on the methodology of fMRI analyses where changes to the classical General Linear Model (GLM) model are explored is essential. In

particular, using as regressors the “area under the curve” of plasma concentration of gut hormones will model the statistical analyses accordingly to pharmacodynamics and pharmacokinetics effects. The milestone study of Liu demonstrated already this possibility (Liu *et al*, 2000a).

A further improvement would be the investigation of the impacts of the nutrients on brain activity beyond satiety regulation and cognitive functions, such as mood and emotional feelings, as previously suggested (Benton and Donohoe, 1999; Casper, 2004). As we demonstrated after sugars intake, nutrients can also impact large scale of neural functions.

This is may also be related to the effects of release of gut hormones that are associated with pathological conditions, as suggested by previous studies this may be of particular interest in psychiatry, due to the alteration of neural functions as mood and reward mechanisms, involved in depression and anxiety (Jacka, 2017; Lakhan and Kirchgessner, 2013). Moving towards this speculation, the idea that a diet can lead to improvements to normalize psychiatric condition acting on specific brain areas is a further direction to be explored.

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