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Neuroimaging and Neuropsychological Features of Hypothalamic Syndrome

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This thesis has been:
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All the results presented here were obtained by myself.

Part of the introduction has already been published in the article "Balestrino R, Losa M, Albano L, Barzaghi LR, Mortini P (2023) Intranasal oxytocin as a treatment for obesity: safety and efficacy. *Expert Rev Endocrinol Metab* 18: 295-306".

All sources of information are acknowledged by means of reference.

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Abstract

Hypothalamic obesity (HO) and neuropsychological deficits have been reported in 35-65% of craniopharyngioma (CP) patients and can significantly impact the quality of life and long-term prognosis. The mechanism underlying these complications is complex, and not fully explained by hypothalamic damage. Other factors, such as altered function of other networks, damage in other areas connected to the hypothalamus, oxytocin deficit might participate to the pathogenesis of these complications. The primary objective of this study was to elucidate potential shared pathogenetic pathways between obesity and psychological disturbances in patients diagnosed with HO following CP. 14 CP patients, 7 with HO (CP HO) and 7 age and sex matched subjects without HO (CP) underwent extensive neuropsychological evaluation, functional magnetic resonance imaging (fMRI) with eating behaviour task and an emotional recognition task, cortical thickness analysis, which was correlated with the expression of genes of the oxytoninergic pathway. In CP HO patients compared with CP, we observed a reduced cortical thickness bilaterally in the insula, which showed a significant correlation with eating behaviour questionnaire, and an increased activity of the insula as well as other regions implicated in the hedonic network of feeding behaviour, while CP patients showed greater activation of the anterior cingulate cortex. These findings are consistent with previous studies on obesity, which show an increased activation of brain areas involved in the pleasurable and emotional processing of food (as seen in CP HO), without the inhibitory control mediated by prefrontal regions and cingulate cortex (as seen in CP). Interestingly, previous fMRI in obesity have shown that oxytocin might revert this pattern. During the emotional recognition task, we observed increased activation of prefrontal regions in CP HO subjects compared to CP: consistently with previous studies, while CP subjects showed greater activation of the caudate nucleus: overall, the greater activation of the caudate in subjects without HO represents a physiological emotional processing activation, as opposed to a less physiological overactivation of frontal circuits and hypoactivation of basal ganglia activation in subjects with HO. Previous fMRI has shown that oxytocin increases the caudate nucleus activity during emotion recognition tasks.

We found -as expected- no correlation of cortical thickness with the expression of the *OXT* (oxytocin) gene and a significant correlation with the expression of *OXTR* (oxytocin receptor) gene, suggesting that the areas expressing more *OXT* receptors are conserved, and that oxytocin administration could potentially restore dysregulated activation pattern in subjects with HO after CP.

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ACRONYMS AND ABBREVIATIONS

- Anterior cingulate cortex (ACC)
- Body Mass Index (BMI)
- Craniopharyngiomas (CP)
- Functional Magnetic Resonance (fMRI)
- Glucagon-like Peptide 1 (GLP-1)
- Grey Matter (GM)
- Growth Hormone Releasing Hormone (GHRH)
- Hypothalamic Obesity (HO)
- Hypothalamic Syndrome (HS)
- Luteinizing Hormone Gonadotropin Releasing Hormone (LHGR)
- Oxytocin (*OXT*) gene
- Oxytocin Receptor (*OXTR*) gene
- Thyrotropin Releasing Hormone (TRH)
- Transcranial Surgery (TC)
- Transsphenoidal Access (TNS)

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INTRODUCTION

The hypothalamus is a structure comprised of 11 bilateral nuclei distributed across three zones surrounding the third ventricle and the mammillary bodies which serves distinct functions: it maintains homeostasis by controlling endocrine, autonomic, and somatic outputs, operating as a vital hub for sensory integration and behavioural output. To exert these functions, the hypothalamus forms connections with multiple brain regions through various pathways: positioned centrally within the brain, it is connected to the brainstem via the dorsal longitudinal fasciculus, to the cerebral cortex via the medial forebrain bundle, with the hippocampus through the fornix, to the amygdala through the stria terminalis, to the thalamus via the mammillothalamic tract, to the retina through the retino-hypothalamic tract, and interfaces the pituitary gland via the median eminence; its role is crucial in maintaining homeostasis involves receiving both internal and external stimuli.

The hypothalamus possesses receptors for circulating hormones, enabling the reception of signals concerning the body's energy status and blood osmolarity. Simultaneously, it processes external sensory information through the spinothalamic tract and integrates limbic sensory information by utilizing connections like the fornix, mammillothalamic tract in the Papez circuit, and the stria terminalis, which links to the amygdala. Furthermore, the retino-hypothalamic tract transmits light signals to the suprachiasmatic nucleus, contributing to the regulation of hormone release patterns in a diurnal cycle. These diverse inputs converge within the hypothalamus to coordinate appropriate physiological and behavioural responses vital for sustaining life. By integrating both internal and external signals, the hypothalamus exerts regulatory control over the endocrine system, autonomic system, and somatic behaviour. This control is primarily exerted via the pituitary gland, comprising distinct anterior and posterior lobes. The hypothalamic parvocellular neurons, situated between the preoptic area and the infundibulum, are in charge of modulating the adenohypophysis through the secretion of thyrotropin (TRH), luteinizing hormone (LHGR), growth hormone (GHRH), and inhibiting factors like somatostatin or SRIF for growth hormone and prolactin (PIH). The posterior pituitary lobe contains axonal neurosecretory endings of the magnocellular neurons located in the supraoptic and paraventricular nuclei of the hypothalamus, which produce the antidiuretic hormone (ADH) and oxytocin along with their carriers, the neurophysins.

The hypothalamic syndrome (HS) is an uncommon condition resulting from damage to the hypothalamus due to either disease-related factors or treatment-related causes. Its primary association is with infrequent benign parasellar masses like craniopharyngiomas (CP), other rare causes include germ cell tumours, gliomas, Rathke's pouch cysts, and histiocytosis. Also inflammatory or infectious origins, stroke, radiation therapy, traumatic brain injury and genetic anomalies impacting hypothalamic function, such as monogenetic obesity syndromes and intricate genetic conditions like Prader-Willi Syndrome (PWS) can cause this condition. HS manifests through persistent weight gain leading to severe obesity (see below), hormonal abnormalities, and an elevated susceptibility to cardiovascular and metabolic disorders, as well as cognitive affective and behavioural alterations (Balestrino *et al*, 2023).

Hypothalamic obesity (HO) represents a severe and challenging form of obesity originating from lesions within the hypothalamic region. The causes overlap with those of HS, with CP being the most frequent; notably in these cases the presence of hypothalamic involvement at the time of diagnosis serves as an independent risk factor for the development of HO (van Iersel *et al*, 2019).

CPs are rare intracranial tumours situated either intrasellar or suprasellar, originating from the craniopharyngeal duct epithelium (remnants of Rathke's pouch). CPs can manifest at any point along the pituitary-hypothalamic axis, from the sella turcica to the third ventricle of the brain, but approximately 50% of CPs emerge from the third ventricle floor, primarily within the infundibulum and/or tuber cinereum regions, eventually expanding into the third ventricle cavity (Müller *et al*, 2019) (Figure 1 a-b and 2 a-b).

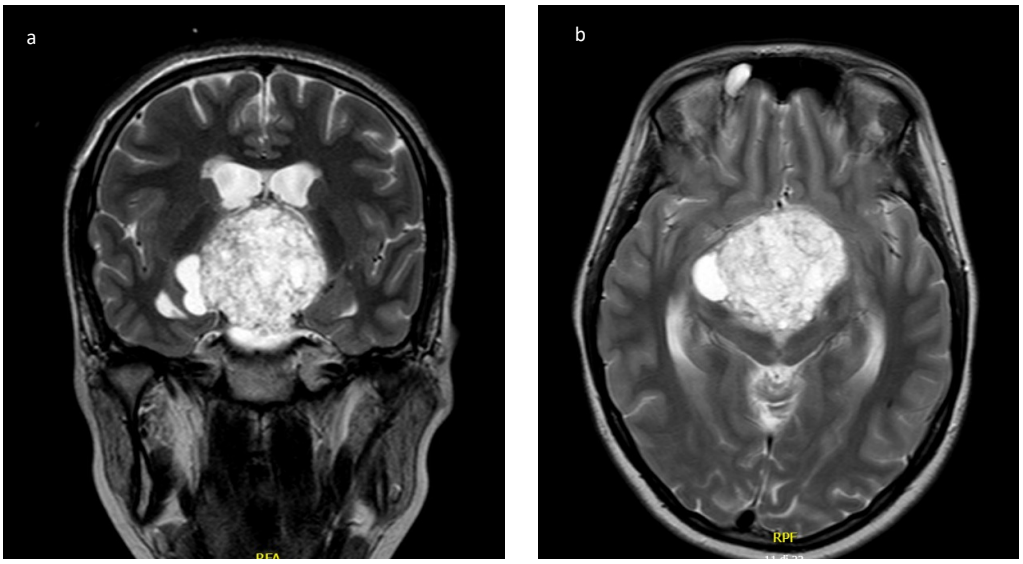

Figure 1: Clinical case, F, 21yo displaying >20 kg weight gain and behavioural disturbances. Brain MRI showed a round ∅ 5.5 cm suprasellar lesion with calcifications, cystic-necrotic aspect, contrast enhancement. She underwent transcranial resection of the lesion, the histological diagnosis being adamantinomatous CP.

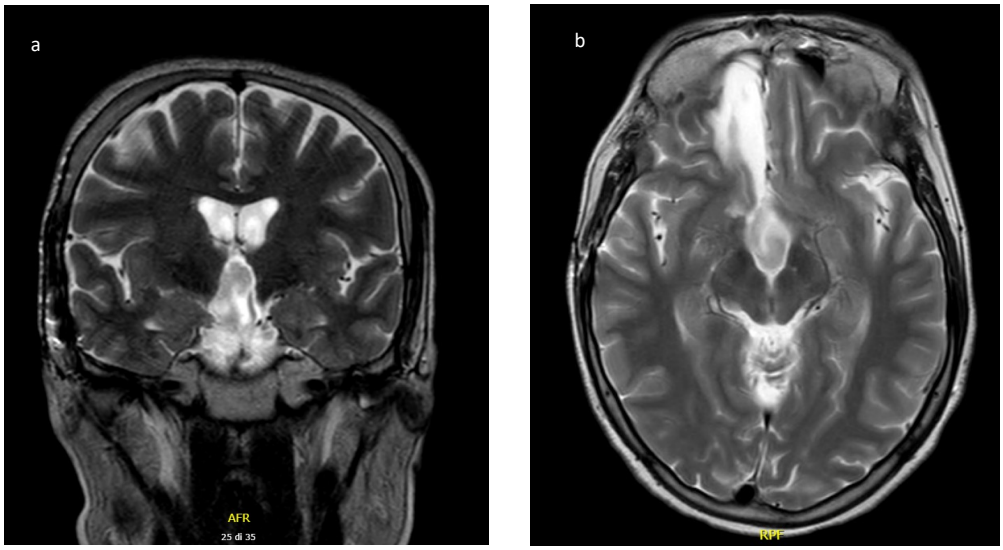

Figure 2: This Figure shows a long term post-operatory follow up, 9 years and 4 month after the CP resection.

HYPOYTHALAMIC OBESITY AFTER CRANIOPHARINGIOMA

Hypothalamic dysfunction, such as HO, neuropsychological deficits, and disruptions of circadian rhythms, occurs in 35% of CP patients at diagnosis and

increases to approximately 65% after surgical and/or radiation treatments, potentially due to hypothalamic damage(Müller, 2017). Weight gain typically manifests 6/12 months post-surgery. HO significantly impacts the quality of life and long-term prognosis in CP patients, leading to an elevated risk of cardiovascular disease, metabolic syndrome, increased mortality, social life limitations, and diminished emotional well-being(van Iersel *et al.*, 2019). The mechanism underlying HO is complex.

In a previously study conducted in our centre the main factors associated with postoperative obesity in CP were hypothalamic hyperintensity (in T2), mammillary body involvement, unidentifiable pituitary stalk, unrecognizable supra-optic recess or infundibular recess, and retrochiasmatic tumor extension, dislocated chiasm (Mortini *et al*, 2016).

In another study conducted in our centre, no significant difference was observed in terms of risk of developing HO between two surgical approaches, transcranial (TC) surgery vs transsphenoidal (TNS) access, and only tumour volume and basal BMI were identified as independent predictors for HO (Gallotti *et al*, 2022).

In a preliminary retrospective study, we aimed at evaluating the association between neuropsychological complications and HO in subjects who underwent CP resection. We collected data from of 54 patients (32 males). Histological diagnosis was adamantinomatous CP in 46 subjects, papillary CP in 8. The mean volume was 633.44 cc±540.39. Hypothalamic involvement was seen in 28 subjects. 15 patients showed psychological and/or behavioural disturbances within 1-year post-surgery. The mean BMI was 25.6±5.2 before surgery, 30.38 +-6.8 after ($p<0.001$). BMI was>30 in 11 subjects before surgery and in 25 subjects after surgery. 33 subjects gained more than 10% of their body weight after surgery. Subjects with psychological and/or behavioural disturbances showed (vs subjects without) a greater CP volume ($p<0.001$) and higher post-surgical BMI ($p<0.005$) (Fig.4 a-b). Subjects with psychological complication showed a greater change in BMI compared to those without neuropsychological complications (respectively: BMI pre=26.70±6.15, BMI post=35.67±8.99, $p=0.002$; BMI pre=25.25±4.755, BMI post=28.20±4.50, $p=0.001$); 14/15 patients with neuropsychological complications gained more than 10% of their body weight within 1 year; 11/15 had BMI>30 within 1 year after surgery (vs 4/15 before surgery). Neuropsychological complications correlated with obesity before-surgery (0.349, $p<0.001$), hyperphagia (0.336, $p<0.005$), hypothalamic involvement (0.343, $p<0.005$), obesity after-surgery (0.334,

p<0.005). In a logistic regression analysis with post-operative obesity as dependent variable including BMI before surgery, age at diagnosis, sex, hypothalamic involvement, pituitary hormones deficiency, tumour volume and psychological complications at diagnosis, tumour volume (P <0.005) and pre-operative BMI (P < 0.001) were significant. In a logistic regression analysis with neuropsychological complications as dependent variable including BMI before surgery, age at diagnosis, sex, hypothalamic involvement, pituitary hormones deficiency, and tumour volume, only tumour volume was significant (P <0.005). Our preliminary results obtained in the retrospective study showed an association between neuropsychological complications and obesity: in agreement with previous studies, the volume of the lesion is the main determinant of both metabolic and neuro-psychological complications (unpublished data).

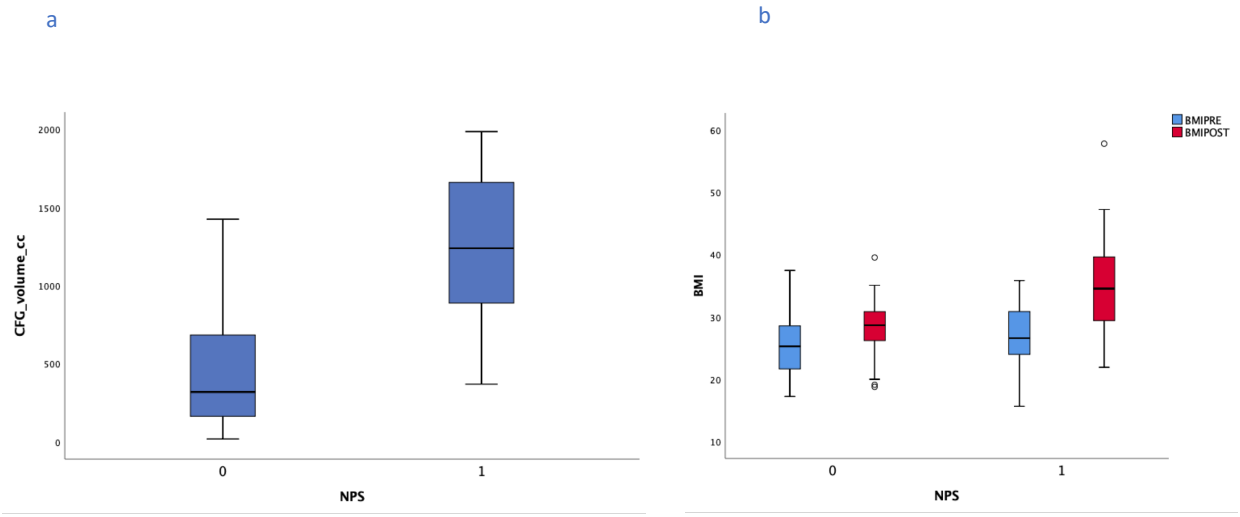

Figure 3 Comparison between subjects with neuropsychological complications and subjects without. subjects with psychological and/or behavioural disturbances showed a greater CP volume (p<0.001) (a) and higher post-surgical BMI (p<0.005) (b).

PATHOGENESIS OF HYPOYHALAMIC OBESITY

The clinical manifestations and pathogenetic mechanisms of HO fall into different domains: alteration of satiety mechanism and subsequent hyperphagia, psychological/affective/behavioural alterations, disruptions in sleep patterns, reduced energy expenditure, insufficient pituitary gland function (hypopituitarism), increased levels of insulin in the bloodstream (hyperinsulinemia) and unbalance of orthosympathetic/parasympathetic autonomous nervous system activity.

It is well known that hypothalamic lesions can induce pathological hunger: this, coupled with impulse control issues, leads to strong desires for food and incoercible

eating. While various nuclei within the hypothalamus integrate signals of fullness from both internal and external sources, the VMH (ventromedial hypothalamus) and the arcuate nucleus are believed to have the most significant functions in this process, as demonstrated by several animal models: lesions of the VMH cause hyperphagia, adiposity, and heightened parasympathetic tone (Parkinson & Weingarten, 1990). The regulation of body weight necessitates a highly integrated neurohumoral system encompassing circulating signals, gut-derived and brain peptides (Heisler & Lam, 2017). It's hypothesized that the lack of normal appetite inhibition in HO results from disruption of hypothalamic receptors governing the negative feedback loop of leptin and other peripheral hormones (Müller, 2020). Functional MRI studies suggest altered perception of food cues in individuals with HO after food intake (Roth *et al*, 2012) (see next sections). The emergence of eating disorders further contributes to HO development, often linked to psychological/psychiatric disturbances commonly presented by these patients(Müller, 2020).

Additionally, neuropsychological disturbances linked with HO highlight common mechanisms such as hypothalamic damage and the influence of psychological complications on weight gain (e.g., eating disorders), although the precise relationship between these conditions remains to be fully understood (Balestrino *et al.*, 2023).

Indeed, approximately 57% of patients (Zada *et al*, 2013) with CP exhibit behavioural disruptions, compromised social, emotional, and cognitive functioning, resulting in an overall reduction in Quality of Life (QoL). Moreover, compared to other benign intracranial tumours in children that undergo treatment, these patients tend to exhibit notably higher levels of both psychological, behavioural and social disturbances(Carpentieri *et al*, 2003), likely due to multiple factors, which are not yet fully comprehended: concurrent conditions, such as involvement of the hypothalamus, hormonal effects (like hypopituitarism and diabetes insipidus), obesity, and the presence of hydrocephalus seem to participate to the development of these complications. Various potential risk factors need consideration in understanding the origins of neurobehavioural disorders, including the age when diagnosed and treated, the duration before surgery, tumour size, need for CSF shunting, past surgeries, radiation exposure, and other relevant factors(Zada *et al.*, 2013). It is probable that neurobehavioural impairments stem not from a single cause but from a combination of these elements. Lesions to areas implicated in specific functions could participate to different aspects of psychological, cognitive, and behavioural disturbances. Hypothalamic lesions themselves, as also

demonstrated in animal models, are associated with increased aggressiveness, rage, and reduced impulse control, and might be directly responsible for these manifestations in CP patients (Falkner *et al*, 2020; Haller, 2013).

Damage to the prefrontal cortex or disruptions in connections between this area and the hypothalamus, due to tumour presence or surgical damage, are believed to underlie impairments in social abilities or emotions(Ondruch *et al*, 2011). Moreover, additional damage to frontal lobe areas could be implicated in the pathogenesis of deficits in attention, executive functions, and processing speed that can also occur in this population. As previously illustrated, the hypothalamus is part of the limbic system, which regulates affective and emotional processing as well as mood: therefore, lesions affecting this system and other adjacent structures can contribute to observed psychological, behavioural and cognitive alterations in CP patients (Anderson *et al*, 1997). The posterior part of the hypothalamus and the mammillary bodies are part of the hippocampus-centered limbic network: therefore, episodic memory deficits are also seen in CP, with some extremely severe cases which resemble Korsakoff-like deficits (anterograde amnesia, disorientation, confabulation)(Ricci *et al*, 2021; Savastano *et al*, 2018). Another theory is that, beside of direct damage due to the tumour or surgery, cognitive and emotional deficits might depend on remote effects on connected areas, trough different mechanisms such as diaschisis (alteration of function in distal regions due to alterations in functional connectivity) and trans neuronal degeneration (structural degeneration in distant brain regions)(Fornito *et al*, 2015). The hypothalamus has direct connections with major cortical and subcortical regions, including the orbitofrontal cortex, cingulum, insula, hippocampus, ventral striatum, amygdala, and thalamic nuclei. It also has some indirect connection, as it exerts its influence on the ventral striatum and orbital/medial prefrontal cortex also via thalamic nuclei (Kullmann *et al*, 2014; Lemaire *et al*, 2011; Toni *et al*, 2004). Studies on animal models demonstrated that lesions of the mammillary bodies cause degeneration of frontal cortex and hippocampus, (Beracochea *et al*, 1995) and that interruption of the connections between the medial mammillary and anterior thalamic nuclei determine degeneration in the hippocampus, the retrosplenial and prefrontal cortex (Vann, 2009). Only one study investigated distal structural abnormalities following CP resection, compared to healthy controls patients showed significant differences were found in one cluster composed of the ventral striatum, orbital frontal cortex, medial prefrontal cortex, sub- and perigenual anterior cingulate cortex (ACC), and another cluster composed of the anterior, mid and posterior cingulate cortex, with a

correlation between gray matter volumes in the left posterior cingulate cortex and long-term memory performance (Ozyurt *et al*, 2017).

Other factors, such as dynamics in families such as the persistent concerns about tumour recurrence and anxiety related to prior treatments seem to contribute to affective and mood disturbances in CP survivors (Shiminski-Maher & Rosenberg, 1990).

Finally, cranial irradiation post-tumour removal is another important risk factor for changes in neurocognitive function(Meyers *et al*, 2000).

Another factor that might be associated with both obesity and psychological alterations in CP is thought to be the lack of oxytocin. This topic is discussed in the next sections.

BRAIN NETWORKS IMPLICATED IN OBESITY

The control of feeding behaviour traditionally falls into two distinct systems which exhibit extensive anatomical and functional overlap, rather than a clear separation:

- homeostatic processes aimed at addressing caloric and metabolic deficits in energy intake.
- "non-homeostatic" processes driving energy intake independent of metabolic needs, often referred to as "hedonic feeding."

The homeostatic process is commonly associated with the hypothalamus, housing nuclei that can be classified as either orexigenic (stimulating appetite) or anorexigenic (suppressing appetite), which receive input from the gastrointestinal system and adipose tissue. This system is governed by signals concerning energy availability and needs, receiving feedback on nutritional status and energy reserves (Balestrino *et al.*, 2023). Key hypothalamic nuclei implicated in this process include: the Arcuate Nucleus with Pro-opiomelanocortin (POMC)/Cocaine- And Amphetamine-Regulated Transcript (CART) neurons (anorexigenic), and Agouti-Related Peptide (AgRP)/neuropeptide Y (NPY)/GABA neurons (orexigenic); the dorsal hypothalamus, predominantly orexigenic (with a smaller anorexigenic population); the ventral hypothalamus, primarily anorexigenic; the Paraventricular Nucleus (PvN), predominantly anorexigenic; the Lateral (LHA) and Peri-Fornical (PFA) regions of the Posterior Hypothalamus, mainly orexigenic. The nucleus of the solitary tract plays a role in feedback during eating and meal termination, receiving direct input from the

vagus nerve and relaying information from the gastrointestinal tract regarding food's nutritional, osmotic, and volumetric properties (Heisler & Lam, 2017).

The regulation of "non-homeostatic/hedonic eating" involves various neuroanatomical networks, such as the prefrontal cortex responsible for cognitive control, cortical regions processing sensory information (visual, olfactory, auditory, and oral taste systems), and polymodal association areas for integrating sensory input (orbitofrontal, prefrontal, insular cortex). The reward system, including the dorsal striatum, ventral striatum, substantia nigra/ventral tegmental area, and limbic structures like the insula, amygdala, and hippocampus, plays a key role in associating emotions and memory with feeding. Additionally, food intake modulation involves stress-related, emotional, and cognitive processes, adding complexity to this system (Heisler & Lam, 2017). This is a highly intricate system, integrating external factors, interoceptive cues, and cognitive aspects relevant to feeding.

Animals (and humans) integrate food ingestion with both internal cues (hunger, satiety, metabolic states) and external cues (spatial location, social cues), coupled with the reward system, forming learned associations that influence future food-related behaviours (Heisler & Lam, 2017). The two networks are closely interconnected both anatomically and functionally: in fact, stimuli affecting the hedonic system directly regulate hypothalamic structures, and stimuli influencing the homeostatic system impact regions involved in the hedonic system, illustrating the close connection between feeding and higher cognitive functions. Oxytocin plays a modulatory role in both these systems (see next section) (Balestrino *et al.*, 2023). A key characteristic of feeding behaviour is the willingness to work for food. Indeed, there is a strict connection with motivational circuits: how an organism's internal condition guides its behaviour is a complex interplay. For instance, an animal exhibits various behaviours to meet its survival needs. It may engage in high-energy activities like climbing trees or leaping between branches while foraging for food or opt for ground travel to seek water or a mate, despite potential exposure to predators. These actions incur significant energy expenditure or physical risks, and their likelihood is markedly influenced by the organism's internal state. Specifically, an animal facing an energy deficit prioritizes the search for food, while a dehydrated one seeks water. The intricate interaction between an organism's physiological condition and its motivational processes significantly shapes the intensity and purpose of its behaviour. Interestingly, orexigenic and anorexigenic nuclei have intrinsic motivational or inhibitory properties: for example, AGRP neurons are also entry point to motivational processes, engage brain sites controlling multiple levels of feeding behaviour, and their downstream circuit connections (e.g. the PVH) are clue

regulators of motivationally important brain regions(Atasoy *et al*, 2012; Krashes *et al*, 2011). These nuclei might also play a regulatory effect on the reward system: nutrients are rewarding, even in the absence of deprivation, however energy deficit, possibly acting through AGRP neurons, may increase the reward value of food (Sternson *et al*, 2013).

FUNCTIONAL NEUROIMAGING IN HO

Only four studies have delved into the examination of brain activity among patients diagnosed with CP using functional magnetic resonance (fMRI). In one investigation, 4 individuals diagnosed with CP (2 males, BMI 25.3–43.8 kg/m2) and 4 healthy controls (1 male, BMI 20.2–47.7 kg/m2) underwent fMRI after exposure to stimuli comprising images portraying both high- and low-calorie foods(Roth *et al.*, 2012). CP patients displayed heightened activation within Regions of Interest (ROIs), including the insula, Nucleus Accumbens, and Frontal Cortex. Furthermore, post a high-calorie meal, CP patients exhibited increased caloric intake during the subsequent ad-libitum meal. In another study, 29 individuals who had undergone CP surgery and 31 controls who had undergone surgeries for non-functional pituitary adenoma (constituting the control group) were compared in terms of overall brain reactions to food-related visuals, cognitive processes associated with food perception, psychological attributes intertwined with food perception, dietary habits, and changes in body weight. Comparisons drawn between the two groups unveiled that patients with CP exhibited diminished attention toward food, decreased sensations of fullness, and a proclivity toward controlled eating behaviours. Within the fMRI observations, when exposed to food images, CP individuals displayed higher occipital and inferior frontal gyrus but decreased activation in the left caudate nucleus compared to controls. As per the authors' interpretations, the reduced activation of the caudate nucleus might represent the lower attention to food and reduced sense of satiety, indicating an altered processing of rewarding stimuli. Furthermore, the diminished attention toward food might contribute to decreased feelings of fullness (resulting in increased hunger) and irregular eating patterns. Activation within the fusiform gyrus, orbitofrontal cortex, hippocampus, and amygdala among CP patients correlated with attitudes towards disordered eating and changes in weight (Lee *et al*, 2023). One study focused on emotional recognition: 10 CP patients with hypothalamic damage were compared to matched healthy controls, showing higher activation in the medial-frontal cortex among

patients compared to controls: the authors hypothesize that this activation reflects a mechanism which requires the recruitment of more cognitive skill to compensate for less efficient utilization in patients when encountering a task demanding emotional content. (Özyurt *et al*, 2014) In another study, a group of twenty-eight individuals diagnosed with CP underwent testing using the multi-source interference task (MSIT) alongside a matched control group. This task is commonly utilized to assess cognitive interference processing. The participants' behavioural performance, including response times and accuracy, was observed during fMRI between groups. (Svard *et al*, 2021)

ROLE OF OXYTOCIN IN HYPOTHALAMIC SYNDROME

Oxytocin, a nonapeptide hormone predominantly acknowledged for its reproductive functions, holds significance in regulating social and emotional behaviours, along with influencing body weight and metabolism (Balestrino *et al.*, 2023). Sir Henry Dale first discovered this hormone in 1906, primarily elucidating its effects during labor, hence its nomenclature originating from the Greek "ὠκυτόκος" (ōkutókos), combining "ὀξύς" (oxús) meaning "sharp," and "τόκος" (tókos) meaning "childbirth". Notably, oxytocin contributes to cervical ripening, uterine contraction, and clotting in the postpartum period. Synthesized principally in the hypothalamic paraventricular and supraoptic nuclei by magnocellular neurons, oxytocin's release into the periphery occurs in the posterior pituitary gland. Additionally, it is produced by parvocellular neurons projecting to regions such as the spinal cord and hindbrain, vital in regulating thermogenesis, energy expenditure, food intake, and cardiovascular function. Oxytocin is also released in various brain regions through dendritic diffusion, influencing emotional, social functions, and food intake, as well as metabolism (Bhargava *et al*, 2019). Studies by Kosfeld et al. (Kosfeld *et al*, 2005) highlighted that intranasal administration of oxytocin elevates trust among humans. Another study by Kirsch et al. (Kirsch, 2005) demonstrated oxytocin's role in modulating amygdala activation and its connection with autonomic and behavioural fear manifestations, suggesting with other evidences its involvement in enhancing sociality, bonding, trust, empathy, and altruism(Marsh *et al*, 2021). However, recent research in this area has revealed a more intricate perspective, acknowledging diverse effects of oxytocin based on factors like genetic background, sex, age, and context. Contrary to its supposed exclusive promotion of positive social behaviours, oxytocin may trigger protective or even defensive-aggressive responses(Marsh *et al.*,

2021). Known for its anxiolytic and antistress effects, oxytocin influences reward-related and fear-related neurocircuits (Marsh *et al.*, 2021). Its therapeutic potential in psychiatric conditions such as obsessive-compulsive disorder (OCD), schizophrenia, anxiety disorders, eating disorders, and autism spectrum disorders (ASD) is under investigation, although recent trials of intranasal oxytocin in ASD children and adolescents have shown limited efficacy in improving social or cognitive functioning (Sikich *et al*, 2021). Initially recognized for its anorexigenic properties(Arletti *et al*, 1989), oxytocin's role in modulating feeding behaviour and metabolism has gained substantial attention. Oxytocin receptors are widely distributed in regions that are pivotal for energy homeostasis, both in terms of caloric intake and energy consumption, both in the brain and in peripheral regions(McCormack *et al*, 2020). Studies indicate oxytocin's modulation of both "homeostatic" and "hedonic" feeding regulation networks, impacting caloric consumption, meal size, and frequency, with suggestions of increased meal frequency but compensated by reduced meal sizes (Arletti *et al.*, 1989; Blevins *et al*, 2015; Blevins *et al*, 2016; Deblon *et al*, 2011; Maejima *et al*, 2014; Ott *et al*, 2013; Ye *et al*, 2022). It's believed to promote meal termination, reduce food reward, and enhance cognitive control of food intake by affecting specific brain regions (Plessow *et al*, 2021; Striepens *et al*, 2016). Moreover, oxytocin's interacts with other hormones that regulate feeding behaviours, regulates energy expenditure thus further influencing metabolic regulation (Lawson, 2017; Rinaman & Rothe, 2002; Wu *et al*, 2008). Oxytocinergic neurons sensitive to nutrient status and hormones receive regulatory inputs from melanocortinergic signalling pathways affecting appetite (Kerem & Lawson, 2021; McCormack *et al.*, 2020). Peripherally, oxytocin exhibits a profile promoting fat metabolism and lean mass preservation, potentially reducing visceral and liver fat (Blevins *et al.*, 2015; Blevins *et al.*, 2016; Ding *et al*, 2019; Maejima *et al*, 2011). These actions also affect glucose uptake and utilization in various tissues, reducing diabetes-related mechanisms (Brede *et al*, 2019; Ding *et al.*, 2019; Klement *et al*, 2017). Studies in animal models indicate that disruptions in oxytocin signalling and receptor expression lead to obesity despite normal food intake, shedding light on its role in maintaining body composition (Ding *et al.*, 2019). Additionally, oxytocin influences muscle and bone homeostasis, exhibiting effects on muscle regeneration and bone maintenance (Camerino, 2021; Tamma *et al*, 2009). Beyond its role in metabolic homeostasis, oxytocin modulates the "non-homeostatic system" governing food behaviour by impacting emotional processing, reducing food reward values, attentional bias to food stimuli, and improving cognitive control over food intake (Burmester *et al*, 2022; Lawson *et al*, 2015; Onaka & Takayanagi, 2019;

Plessow *et al.*, 2021; Spetter *et al*, 2018). Recent studies examining intranasal oxytocin administration in humans have shown reduced food intake in non-psychiatric subjects, with minimal effects in psychiatric disorders highlighting its nuanced impact on food craving and hunger (Chen *et al*, 2021). Finally, it's suggested that oxytocin may enhance energy expenditure by influencing complex behaviours related to metabolism (McCormack *et al.*, 2020). This summary is illustrated in Figure 3.

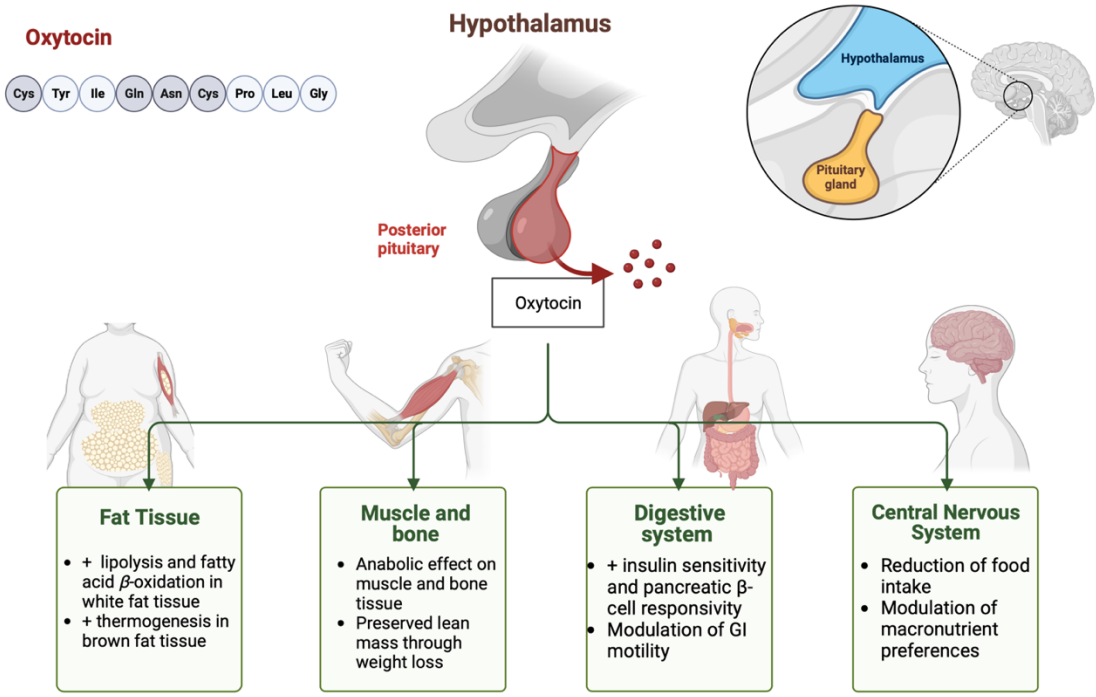

Figure 4 Oxytocin in the regulation of body weight and energy homeostasis. Made with biorender.com

OXYTOCIN IN THE PATHOGENESIS OF HO IN CP

It has been hypothesized that patients with CP may experience disturbances in oxytocin synthesis and release, similar to other pituitary gland hormones. Oxytocin is synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and then released by the posterior pituitary. Unfortunately, these structures are often affected by CP due to tumour involvement or the subsequent surgical intervention (Müller *et al.*, 2019). Evidence indicates a potential correlation between reduced oxytocin levels and HO, which in turn are associated with Body Mass Index (BMI) alterations and neuro/psychological disturbances (Bhargava *et al.*, 2019). Despite the challenges in accurately measuring oxytocin levels and the tenuous relationship

between central and peripheral oxytocin levels, several studies have sought to explore oxytocin deficits in individuals with HO resulting from CP (Balestrino *et al.*, 2023). Daubenbüchel et al. conducted a cross-sectional case-control study involving 34 CP patients and 73 healthy controls, investigating the association between hypothalamic involvement, eating behaviours, and salivary oxytocin concentrations. They found reduced postprandial oxytocin levels to be associated with higher BMI in CP patients and adverse eating behaviours in both CP patients and healthy controls (Daubenbüchel *et al*, 2016). Other studies delved into oxytocin's role concerning psycho-behavioural complications linked to CP beyond HO. In one pilot cross-sectional study, Hoffmann et al. administered intranasal oxytocin to 10 CP patients with anterior hypothalamic lesions, observing improvements in emotional identification after oxytocin administration(Hoffmann *et al*, 2017). Gebert et al. investigated variations of oxytocin levels in following physical exercise in 26 CP patients, revealing a reduced oxytocin release in all CP patients compared to controls, particularly in those with hypothalamic damage (Gebert *et al*, 2018). This study also indicated associations between oxytocin levels, anxiety traits, and emotional responses. Subsequent research observed higher autistic traits and reduced hedonic levels for social interactions in CP patients with blunted oxytocin-release, along with difficulties in rapid emotion recognition tasks. (Brandi *et al*, 2020) Özyurt et al. evaluated social-cognitive skills in 31 childhood-onset CP patients compared to healthy controls, showing impairment in understanding vocal expressions and mental states among CP patients, though without any direct correlation found between oxytocin levels and social-cognitive skills (Özyurt *et al*, 2020; Özyurt *et al*, 2015). In another study comparing oxytocin concentrations in patients with anterior hypopituitarism, reduced oxytocin levels were associated with impaired empathy tasks (Daughters *et al*, 2017). Overall, these studies collectively indicate a complex relationship between oxytocin alterations, HO and various aspects of social, emotional, and cognitive functioning in CP patients.

TREATMENT OF HO

To date, no specific treatment exists for HO, which typically shows poor response to conventional lifestyle modifications and treatment of other hormonal deficiencies in hypopituitarism (Haliloglu & Bereket, 2015; Shah *et al*, 2016). Central stimulant therapies have been reported to reduce body weight, but their long-term use may be limited due to adverse effects. Octreotide treatment has shown no efficacy and

increased adverse events in managing HO. Encouraging outcomes have been observed with glucagon-like peptide 1 (GLP-1) receptor agonists. Diazoxide and metformin have displayed some benefits in controlling weight gain, albeit with side effects, while fenofibrate and metformin were found ineffective. Beloranib, a selective methionine aminopeptidase 2 inhibitor, induced weight reduction but led to venous thromboembolic complications. Bariatric surgery has shown efficacy in weight reduction; however, its high invasiveness and associated severe adverse events limit its application, particularly in children with HO. Deep brain stimulation of the nucleus accumbens in HO due to CP resulted in weight loss (Balestrino *et al.*, 2023; Haliloglu & Bereket, 2015; Müller, 2020; Shah *et al.*, 2016; van Iersel *et al.*, 2019).

OXYTOCIN IN THE TREATMENT OF HO IN CP

Despite numerous patient anecdotes detailing positive outcomes with off-label oxytocin therapy, the existing literature on oxytocin in CP is relatively scarce (Balestrino *et al.*, 2023). In one case, an 8-year-old boy underwent a gross total resection of a CP without subsequent radiation therapy. Post-surgery, the patient experienced visual impairment, adipsic diabetes insipidus, panhypopituitarism, and developed hyperphagia leading to obesity. Lifestyle changes and dietary modifications were ineffective due to the severe behavioural aspects of hyperphagia. At the age of 13, intranasal oxytocin was initiated as an off-label therapy at an initial dose of 6 IU/day. After 10 weeks, improvements in eating behaviours and weight loss were observed, resulting in a reduction of the BMI z-score from 1.77 to 1.49. To further address the patient's craving for highly palatable foods, a regimen of 100 mg/day naltrexone was introduced, leading to a further reduction in BMI z-score from 1.49 to 0.82 over a span of 38 weeks. No significant side effects were reported during this treatment (Hsu *et al*, 2018). In another case report, a 6-year-old girl underwent CP resection, subsequently developing panhypopituitarism, HO, and significant psychological and behavioural disturbances, including social isolation, obsessive, and compulsive behaviours post-surgery. The patient commenced intranasal oxytocin at a dose of 5 IU twice daily for one week. Within the initial 24 hours, the parents noted a remarkable improvement in the child's inclination and ability to socialize. Due to severe thirst, the dose was then adjusted to 2 IU twice daily. At this dose, significant changes were observed in behaviours and social functions, but no effect was observed on HO. No side effects were reported at this adjusted dose level (Cook *et al*, 2016).

Recently, a pilot randomized, double-blind, placebo-controlled study with a crossover design was conducted on 10 CP overweight patients. Subjects were treated for 8 weeks with 16 to 24 IU 3 times daily at mealtimes and for 8 weeks with placebo. Analysis revealed a non-significant within-subject weight change of -0.6 kg attributed to oxytocin compared to placebo. Adverse events were similar between oxytocin and placebo groups. Exploratory analyses hinted at potential benefits of oxytocin in addressing anxiety and impulsivity. Although in this pilot study intranasal oxytocin did not demonstrate a significant impact of on body weight in individuals with HO, it was well-tolerated suggesting prospects for future larger-scale studies exploring longer treatment, different posology, combination with other therapies (McCormack *et al*, 2023).

AIMS OF WORK

The primary objective of this study is to elucidate potential shared pathogenetic pathways between obesity and psychological disturbances in patients diagnosed with hypothalamic obesity following CP. Our focus extends to investigating neuroimaging features linked to these conditions, aiming initially to identify potential neural networks susceptible to oxytocin modulation. Additionally, we seek to explore the plausibility of oxytocin's intervention within these networks as a prospective therapeutic approach. We conducted a cross sectional study, the aims of which were delineate a fMRI pattern in patients who develop HO or neuropsychological complications following CP, to examine structural changes in grey matter among patients with HO in comparison to patients without HO ; to investigate whether structural changes coincide with regions expressing the *OXT* (Oxytocin) or *OXTR* (oxytocin receptor) genes, in order to delve into the relationship between alterations in functional and structural MRI and the clinical features observed in patients.

RESULTS

POPULATION

7 subjects who underwent CP removal without development of HO (CP) and 7 subjects with CP who developed HO (CP HO) were included. Subjects were matched for age and sex. There were 3 females and 4 males in each group. There was no difference in terms of age (CP 40,4 (25-53) ±10,5; CP HO=39,0 (20-52) ± 11,09; p=0,608), time from surgery (CP= 15,0 (2-15) ± 8.9;CP HO=12,8 (0.5-24) ± 8,6;

p=0,537), type of surgery (CP= 5 TNS,CP HO= 2 TNS; p=0,286), education (CP= 13,9 (11-18) ± 3,0; CP HO=12,8 (0.5-24) ± 8,6; p=0,620) between groups.

NEUROPSYCHOLOGICAL TESTS

No significant differences were found between the CP group and the CP HO group. No significant differences based on the type of surgery. Table 1 displays the results of tests investigating cognition, Table 2 displays the results of tests investigating eating behaviours, Table 3 displays the results of tests investigating mood and emotional disorders, Table 4 displays the results of tests investigating quality of life.

	CP		**CP HO**		
Test	**N**	**Avg±SD (min-max)**	**N**	**Avg±SD (min-max)**	**p-value**
MMSE	7	29,7±0,8 (28,0-30,0)	7	25,9±8,4 (7,0-30,0)	0,417
FAB	7	17,9±0,4 (17,0-18,0)	6	17,0±0,6 (16,0-18,0)	0,11
Trail Making Test A	7	23,4±7,4 (15,2-35,7)	6	42,7±27,3 (19,3-94,0)	0,285
Trail Making Test B	7	87,7±34,2 (42,8-143,0)	6	88,0±31,8 (47,5-124,0)	0,326
Trail Making Test B-A	7	63,9±32,6 (27,6-117,9)	6	45,2±19,3 (2,7-7,7)	0,946
Digit Span	7	6,1±0,7 (5,0-7,0)	6	6,7±1,0 (6,0-8,0)	0,295
Span inverse	7	5,9±1,3 (4,0-8,0)	6	5,0±1,3 (3,0-7,0)	0,147
Modified Card Sorting Test (categories)	7	4,9±0,7 (4,0-6,0)	6	4,8±1,2 (3,0-6,0)	0,825
Modified Card Sorting Test (perseveration)	7	1,7±2,8 (0,0-7,0)	6	4,0±4,0 (0,0-10,0)	0,967

Stroop test-interference time	7	0,7±2,7 (-2,9-5,7)	6	0,2±8,8 (-6,4-1,7)	0,211
Stroop test-interference errors	7	0,4±0,8 (0,0-2,0)	6	0,0±0,0 (0,0-0,0)	0,687

Table 1: cognition tests. This table format includes the tests, the respective N, average with standard deviation (Avg±SD), the minimum-maximum range within parentheses, and the p-value of the comparison.

	CP		**CP HO**		
Test	**N**	**Avg±SD (min-max)**	**N**	**Avg±SD (min-max)**	**p-value**
Eating Disorder Inventory-3	6	5.75±5.315 (0-12)	7	11.75±9.743 (1-24)	0,995
Tendency to lose weight	6	4.33±6.772 (0-17)	7	8.29±5.992 (0-15)	0,952
Bulimia	6	0.17±0.408 (0-1)	7	1.29±2.360 (0-6)	0,368
Body dissatisfaction	6	8.17±8.796 (0-25)	7	14.14±10.590 (1-27)	0,712
Three-Factor Eating Questionnaire	6	20.83±5.037 (16-29)	7	20.00±5.020 (13-25)	0,982
Restriction	6	13.17±6.080 (2-20)	7	11.57±5.412 (3-18)	0,290
Disinhibition	6	4.33±2.944 (2-10)	7	5.29±2.289 (2-8)	0,631
Appetite	6	1.67±1.862 (0-5)	7	4.14±3.338 (1-11)	0,244

Table 2: eating disorders tests. This table format includes the tests, the respective N, average with standard deviation (Avg±SD), the minimum-maximum range within parentheses, and the p-value of the comparison.

	CP		**CP HO**		
Test	**N**	**Avg±SD (min-max)**	**N**	**Avg±SD (min-max)**	**p-value**

Anxiety Control Questionnaire - SC	6	52.5±9.6 (37.0-66.0)	7	49.4±10.8 (29.0-60.0)	0,449
Anxiety Control Questionnaire - DC	6	48.3±8.1 (38.0-58.0)	7	62.4±7.9 (49.0-71.0)	0,135
Difficulties in Emotion Regulation Scale	6	75.3±14.8 (59.0-101.0)	7	78.4±18.0 (59.0-107.0)	0,362
SET-Global Score	7	16.1±1.9 (13.0-18.0)	6	17.0±1.1 (15.0-18.0)	0,320
SET-Intention Attribution	7	5.6±0.8 (4.0-6.0)	5	5.8±0.4 (5.0-6.0)	0,872
SET-Causal Inference	7	5.3±1.0 (4.0-6.0)	5	5.6±0.5 (5.0-6.0)	0,673
SET-Emotion Attribution	7	5.3±1.1 (3.0-6.0)	5	5.6±0.9 (4.0-6.0)	0,105
Beck Depression Inventory	6	6.8±5.6 (1.0-16.0)	6	10.8±10.8 (2.0-29.0)	0,944
The State-trait Anxiety Inventory	6	35.1±8.0 (28.0-49.0)	6	31.2±21.0 (3.0-63.0)	0,922
Epworth Sleepiness Scale	6	5.0±4.2 (1.0-12.0)	6	9.7±5.9 (4.0-21.0)	0,051

Table 3: mood, emotions and sleep scales. This table format includes the tests, the respective N, average with standard deviation (Avg±SD), the minimum-maximum range within parentheses, and the p-value of the comparison.

	CP		**CP +HO**		
Test	**N**	**Avg±SD (min-max)**	**N**	**Avg±SD (min-max)**	**p-value**
Clinical impairment assessment questionnaire	6	4.5±6.6 (0.0-13.0)	6	9.8±1.1 (0.0-30.0)	0,679
SF-36 Health Survey					
Physical functioning	6	80%±20% (0.5%-100%)	6	80%±40% (0.1%-100%)	0,136

Role limitations due to physical health	6	70%±50% (0%-100%)	6	70%±40% (0.3%-100%)	0,578
Role limitations due to emotional problems	6	80%±40% (0%-100%)	6	80%±40% (0%-100%)	0,638
Energy/fatigue	6	60%±20% (0.3%-90%)	6	50%±30% (0.1%-90%)	0,148
Emotional well-being	6	70%±10% (0.6%-90%)	6	70%±30% (0.1%-100%)	0,550
Social functioning	6	80%±20% (0.4%-100%)	6	70%±30% (0.3%-100%)	0,378
Pain	6	80%±40% (0%-100%)	6	60%±40% (0.1%-100%)	0,883
General health	6	50%±30% (0%-90%)	6	50%±30% (0.3%-90%)	0,080

Table 4: quality of life scales. This table format includes the tests, the respective N, average with standard deviation (Avg±SD), the minimum-maximum range within parentheses, and the p-value of the comparison.

CORTICAL THICKNESS

Two subjects in the CP HO group were excluded due to artifacts. Patients with hypothalamic obesity (5) were compared with patients without hypothalamic obesity (7). Significant differences were observed: bilaterally in the superior temporal cortex and in the insula; in the left hemisphere in the superior frontal cortex and temporal pole; in the right hemisphere in the frontal pole (p-values are reported in Table5-6) (Figure 5 a-e).

Cortical Thickness (CP vs CP HO), left hemisphere	
Brain Region	**P-value**
Banksst	0,128
Caudal Anterior Cingulate	0,195
Caudal Middle Frontal	0,3
Cuneus	0,67

Entorhinal	0,429
Fusiform	0,406
Inferior Parietal	0,471
Inferior Temporal	0,579
Isthmus Cingulate	0,909
Lateral Occipital	0,259
Lateral Orbitofrontal	0,66
Lingual	0,188
Medial Orbitofrontal	0,663
Middle Temporal	0,801
Parahippocampal	0,812
Paracentral	0,285
Pars Opercularis	0,304
Pars Orbitalis	0,828
Pars Triangularis	0,385
Pericalcarine	0,266
Postcentral	0,17
Posterior Cingulate	0,225
Precentral	0,212
Precuneus	0,159
Rostral Anterior Cingulate	0,774
Rostral Middle Frontal	0,355
Superior Frontal	0,063

Superior Parietal	0,225
Superior Temporal	**0,026**
Supramarginal	0,265
Frontal Pole	**0,044**
Temporal Pole	0,099
Transverse Temporal	0,075
Insula	**0,01**

Table 5: This table presents the comparative analysis of cortical thickness measurements of the left hemisphere between patients with CP with and without HO, p-values for each region are reported. Significant values are bold.

Cortical Thickness (CP vs CP HO), right hemisphere	
Brain Region	**P-value**
Banksst	0,187
Caudal Anterior Cingulate	0,396
Caudal Middle Frontal	0,174
Cuneus	0,323
Entorhinal	0,579
Fusiform	0,131
Inferior Parietal	0,29
Inferior Temporal	0,366
Isthmus Cingulate	0,382
Lateral Occipital	0,259
Lateral Orbitofrontal	0,659
Lingual	0,369

Medial Orbitofrontal	0,074
Middle Temporal	0,17
Parahippocampal	0,736
Paracentral	0,4
Pars Opercularis	0,085
Pars Orbitalis	0,17
Pars Triangularis	0,581
Pericalcarine	0,27
Postcentral	0,134
Posterior Cingulate	0,75
Precentral	0,103
Precuneus	0,171
Rostral Anterior Cingulate	**0,05**
Rostral Middle Frontal	0,08
Superior Frontal	**0,023**
Superior Parietal	0,583
Superior Temporal	**0,02**
Supramarginal	0,349
Frontal Pole	0,215
Temporal Pole	**0,02**
Transverse Temporal	0,884
Insula	**0,025**

Table 6: This table presents the comparative analysis of cortical thickness measurements of the right hemisphere between patients with CP with and without HO, p-values for each region are reported. Significant values are bold.

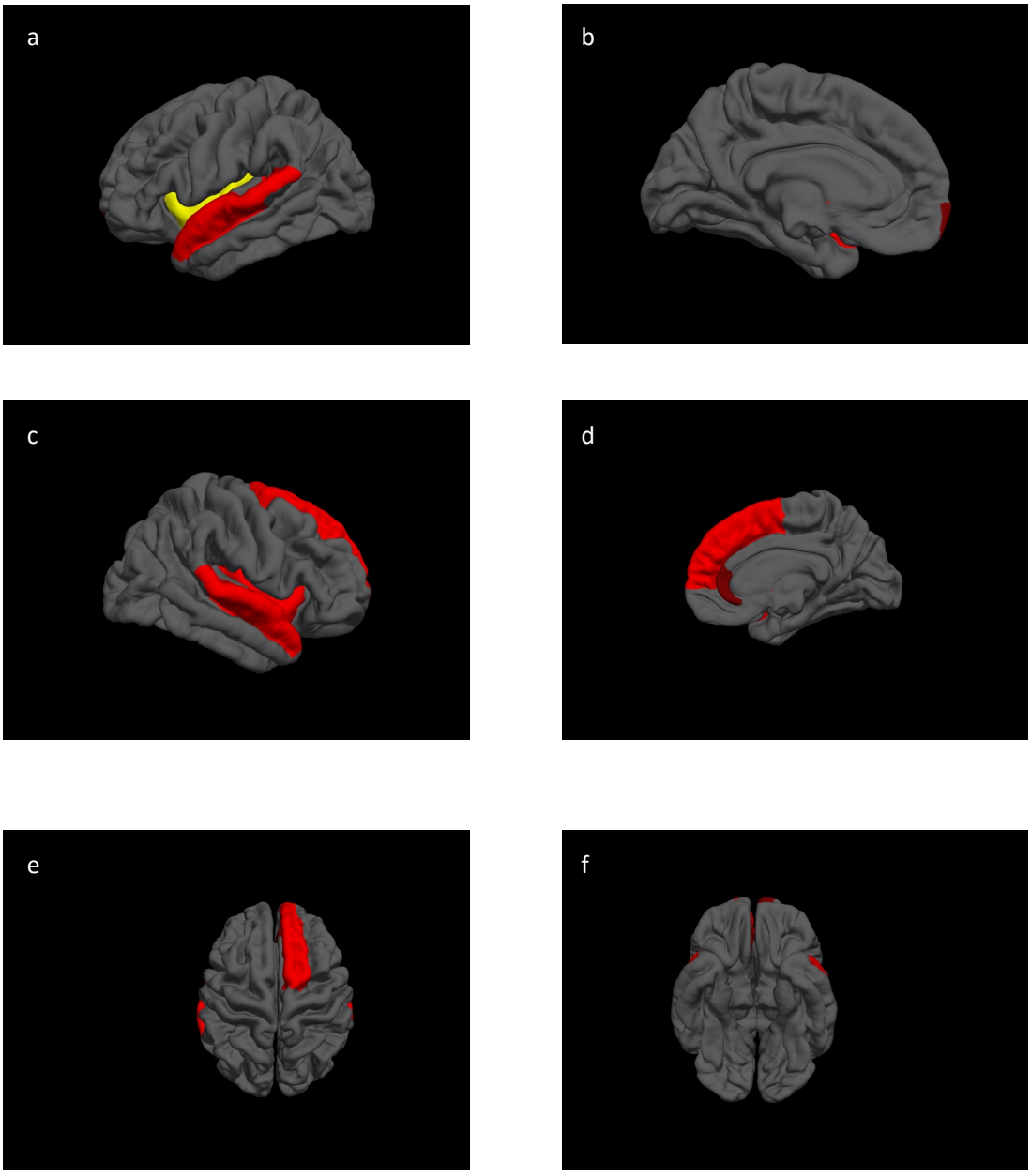

Figure 5 areas that showed a significant reduced thickness in CP HO vs CP subjects, as described in the tables 5-6. different colors indicate different levels of significance, yellow is for p=0,001; red 0,002<p<0,005; dark red p=0,005. a-b left hemisphere, c-d right hemisphere, e-f both hemispheres.

We compared surgical approaches: patients who underwent TNS removal of CP (6) were compared with patients who underwent TC (6). No significant difference was observed (Table 7 and 8).

Cortical Thickness (TNS vs TC), left hemisphere	
Brain Region	**P-value**
Bankssts	0,708
Caudal Anterior Cingulate	0,347
Caudal Middle Frontal	0,619
Cuneus	0,052
Entorhinal	0,630
Fusiform	0,843
Inferior Parietal	0,371
Inferior Temporal	0,723
Isthmus Cingulate	0,223
Lateral Occipital	0,417
Lateral Orbitofrontal	0,816
Lingual	0,579
Medial Orbitofrontal	0,284
Middle Temporal	0,177
Parahippocampal	0,957
Paracentral	0,072
Pars Opercularis	0,278
Pars Orbitalis	0,971
Pars Triangularis	0,116
Pericalcarine	0,058
Postcentral	0,232
Posterior Cingulate	0,935
Precentral	0,118
Precuneus	0,425
Rostral Anterior Cingulate	0,450
Rostral Middle Frontal	0,308
Superior Frontal	0,306
Superior Parietal	0,185
Superior Temporal	0,692

Supramarginal	0,153
Frontal Pole	0,098
Temporal Pole	0,894
Transverse Temporal	0,279
Insula	0,538

Table 7: This table presents the comparative analysis of cortical thickness measurements of the left hemisphere between patients with CP who underwent TNS vs TC surgery, p-values for each region are reported. Significant values are bold.

Cortical Thickness (TNS vs TC), right hemisphere	
Brain Region	**P-value**
Bankssts	0,226
Caudal Anterior Cingulate	0,882
Caudal Middle Frontal	0,637
Cuneus	0,159
Entorhinal	0,950
Fusiform	0,976
Inferior Parietal	0,087
Inferior Temporal	0,724
Isthmus Cingulate	0,733
Lateral Occipital	0,557
Lateral Orbitofrontal	0,594
Lingual	0,874
Medial Orbitofrontal	0,073
Middle Temporal	0,405
Parahippocampal	0,988
Paracentral	0,066
Pars Opercularis	0,522
Pars Orbitalis	0,303
Pars Triangularis	0,064
Pericalcarine	0,056
Postcentral	0,674
Posterior Cingulate	0,511
Precentral	0,277
Precuneus	0,255
Rostral Anterior Cingulate	0,075

Rostral Middle Frontal	0,229
Superior Frontal	0,287
Superior Parietal	0,206
Superior Temporal	0,759
Supramarginal	0,308
Frontal Pole	0,658
Temporal Pole	0,492
Transverse Temporal	0,527
Insula	0,157

Table 8: This table presents the comparative analysis of cortical thickness measurements of the right hemisphere between patients with CP who underwent TNS vs TC surgery, p-values for each region are reported. Significant values are bold.

CORRELATIONS BETWEEN CORTICAL THICKNESS AND NEUROPSYCHOLOGICAL TESTS

The correlation between areas that bilaterally showed a significant difference between CP and CP HO patients and tests evaluating the eating behaviours were analysed. A significant correlation was found between the thickness of the right insula and the score of Eating Disorder Inventory-3, rho -.829, p=0,042. No significant correlation was observed with other questionnaires investigating eating behaviours.

CORRELATION BETWEEN CORTICAL THICKNESS AND GENE EXPRESSION

There was no correlation between the expression of the *OXT* gene and cortical thickness (r=0.1839, p =0.1332) (Figure 12).

There was a significant direct correlation between the expression of the *OXTR* gene and cortical thickness (r=0.4741, p=0.000) (Figure 13, a-b).

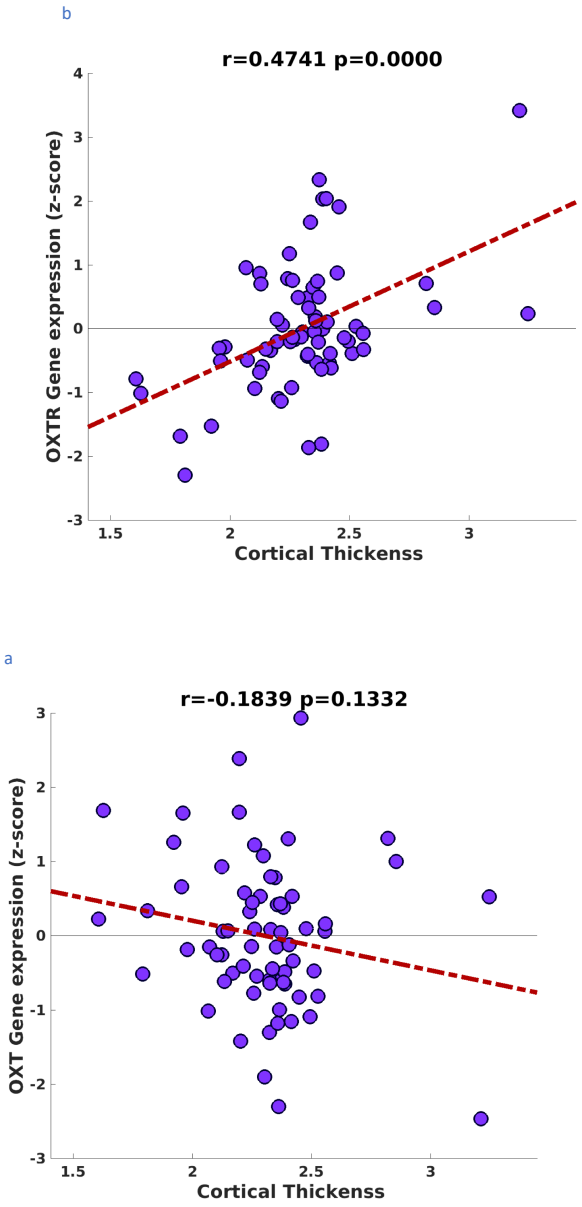

Figure 6 Correlations between thickness measures and expression of the oxt gene (a) and oxtr gene(b)

FUNCTIONAL MRI

EATING BEHAVIOUR

- High caloric foods

When exposed to picture showing high caloric food after removing the motor activation (see methods), CP subjects showed prevalent activation of the left ACC and superior frontal lobe (Fig 7), while CP HO patients showed activation the hippocampus and the cerebellum, as well as the visual cortex, bilaterally (Fig 8). The comparison between groups confirmed greater activation of the ACC in the CP group, and did not show significantly greater activation in any area in CP HO subjects (Fig 9)

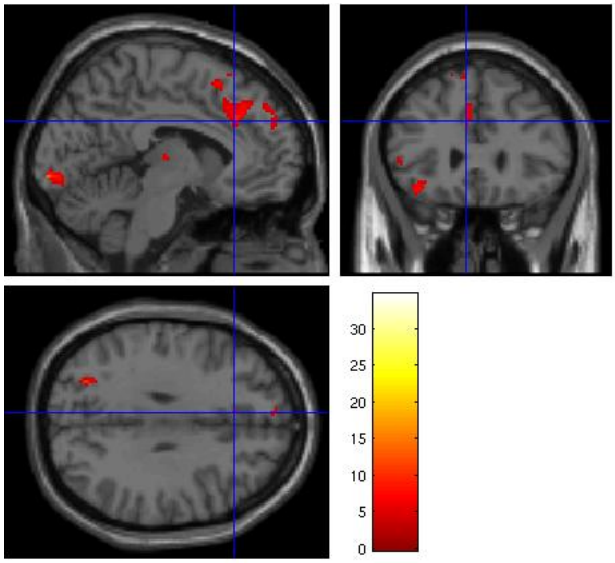

Figure 7 Activation of the left anterio cingulate and superior frontal cortex during the eating behaviours task, high caloric (no motor) condition, in the CP group. p<0,005, non FWE corrected.

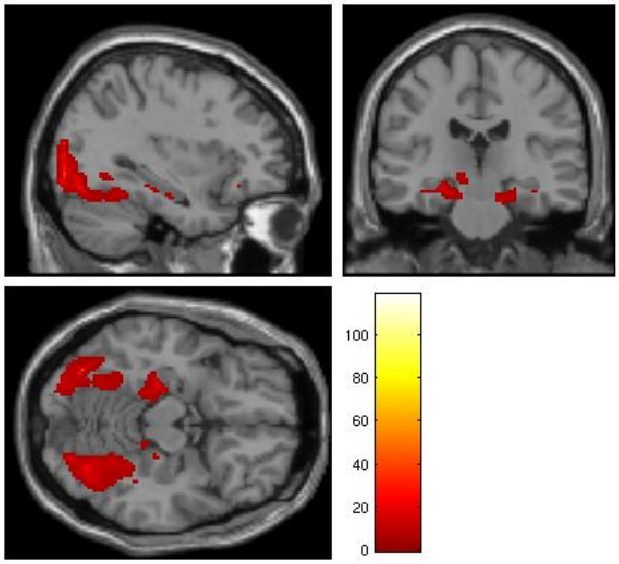

Figure 8 Activation of the hippocampus, visual cortex and cerebellum bilaterally during the eating behaviours task, high caloric (no motor) condition, in the CP HO group. p<0,005, non FWE corrected.

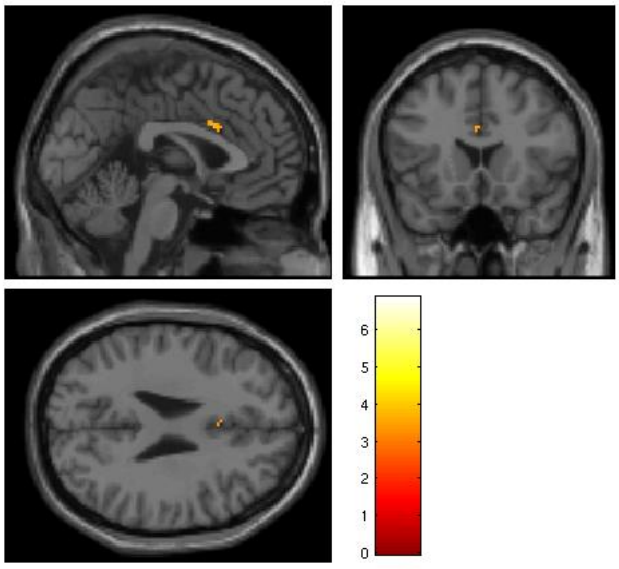

Figure 9 Activation of the left anterior cingulate cortex during the eating behaviours task, high caloric (no motor) condition, in the CP > CP HO group. p<0,005, non FWE corrected.

- Low caloric foods

When exposed to picture showing low caloric food after removing the motor activation (see methods), CP subjects showed prevalent activation of the left dorsal ACC and supplemental motor area (Fig 10), while CP HO patients showed activation the amygdala, dorsal enthorinal cortex and hippocampus bilaterally (Fig 11). The comparison between groups showed greater activation of the left dorsal posterior

cingulate cortex in the CP group, and greater activation in the left insula and superior temporal gyrus in CP HO subjects (Fig 12-13)

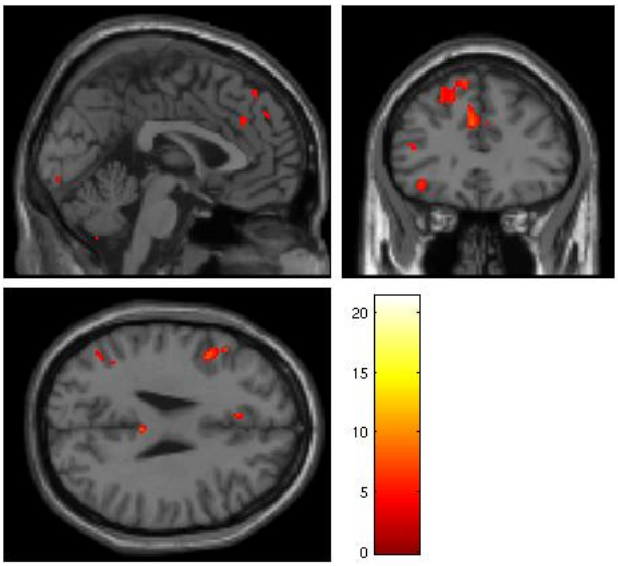

Figure 10 Activation of the left dorsal anterio cingulate and supplemental motor cortex during the eating behaviours task, low caloric (no motor) condition, in the CP group. p<0,005, non FWE corrected.

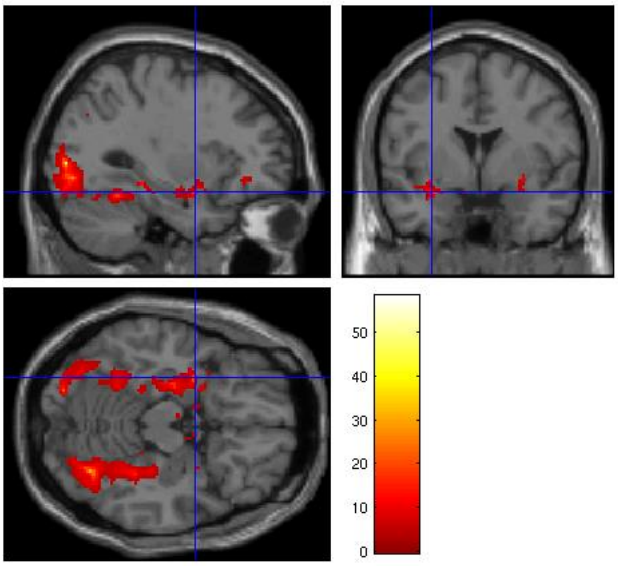

Figure 11 Activation of the the amygdala, dorsal enthorinal cortex and hippocampus bilaterally during the eating behaviours task, low caloric (no motor) condition, in the CP HO group. p<0,005, non FWE corrected.

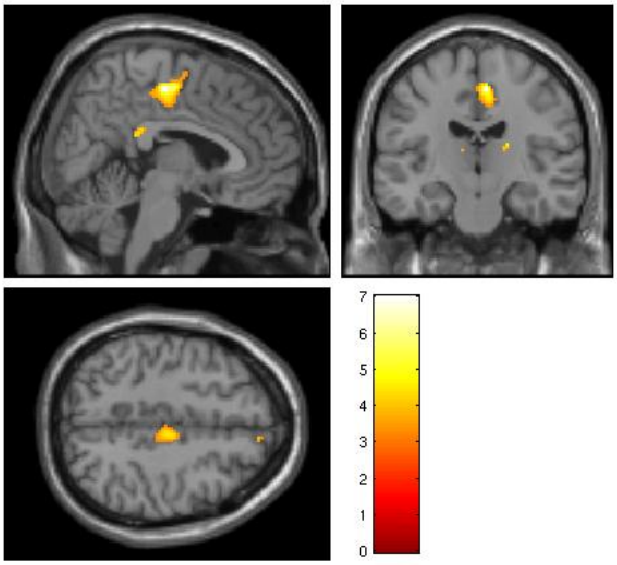

Figure 12 Activation of the left dorsal cingulate cortex during the eating behaviours task, low caloric (no motor) condition, in the CP > CP HO group. p<0,005, non FWE corrected.

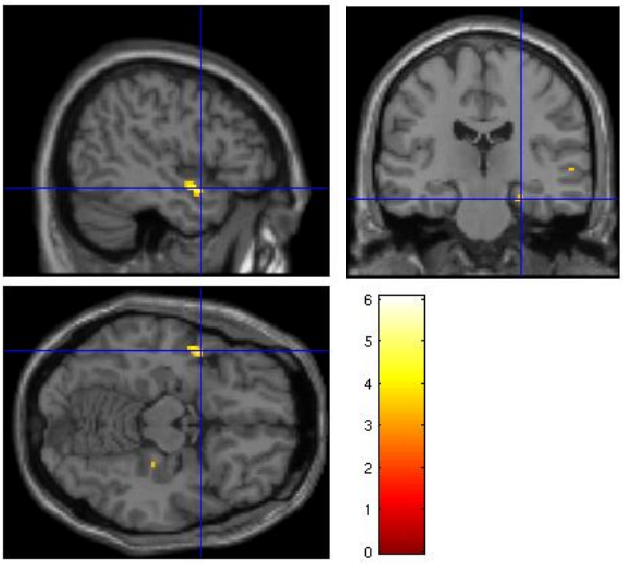

Figure 13 Activation of the left insula and superior temporal gyrus and right hippocampal cortex during the eating behaviours task, low caloric (no motor) condition, in the CP HO > CP group. p<0,005, non FWE corrected.

- **Food vs non-food images**

When exposed to picture showing food vs pictures showing objects (see methods), CP subjects showed bilaterally prevalent activation of the ACC (Fig 14), while CP HO patients showed activation of a cluster encompassing the limbic system, encompassing the insula, and hippocampus and amygdala bilaterally (Fig 15). The comparison between groups confirmed greater activation of the ACC in the CP group, and showed greater activation of the left insula in the CP HO subjects (Fig 16-17)

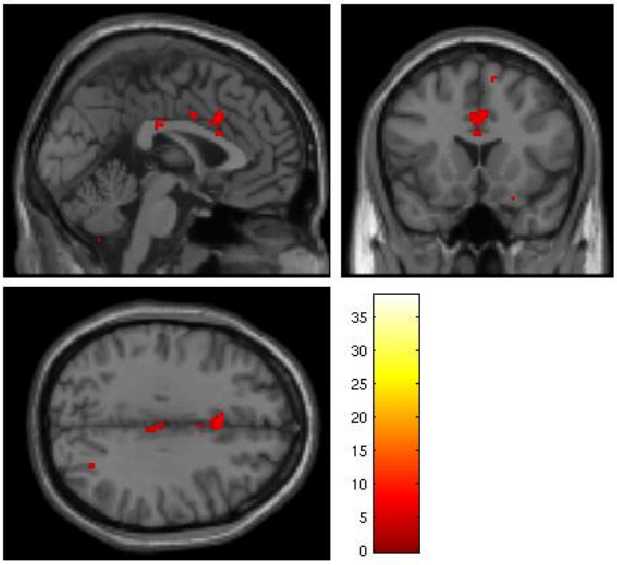

Figure 14 Activation of the bilateral ACC during the eating behaviours task, food vs non-food contrast, in the CP group. p<0,005, non FWE corrected.

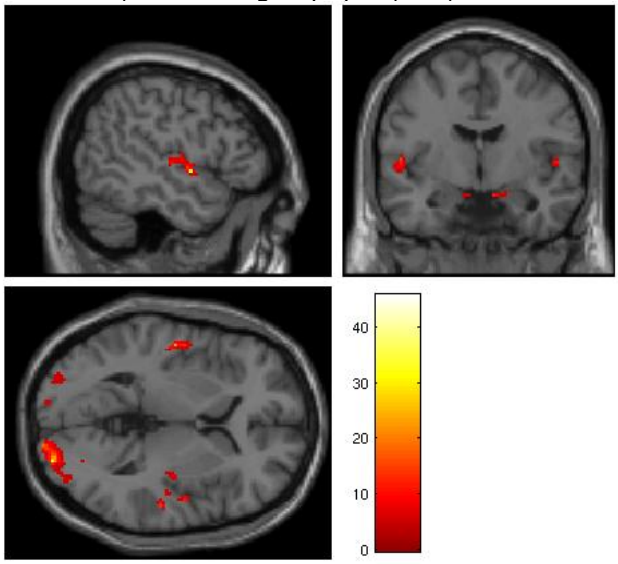

Figure 15 Activation of the insula, and hippocampus and amygdala bilaterally during the eating behaviours task, food vs non-food contrast, in the CP HO group. p<0,005, non FWE corrected.

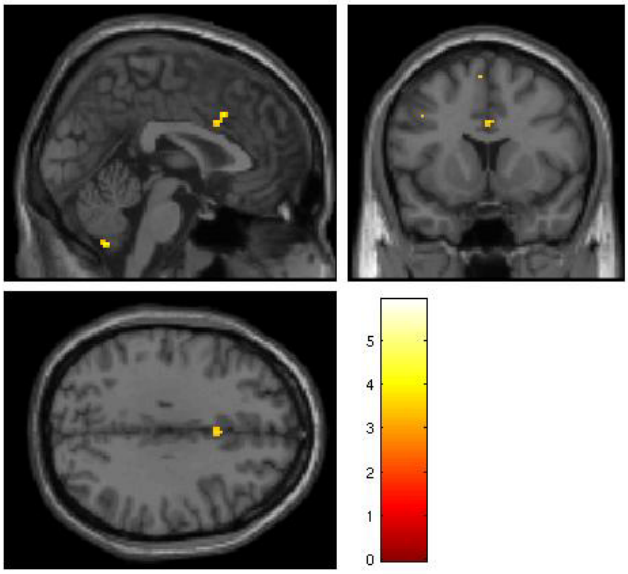

Figure 16: Activation of the ACC during the eating behaviours task, food vs non-food contrast, in the CP group> CP HO group. p<0,005, non FWE corrected.

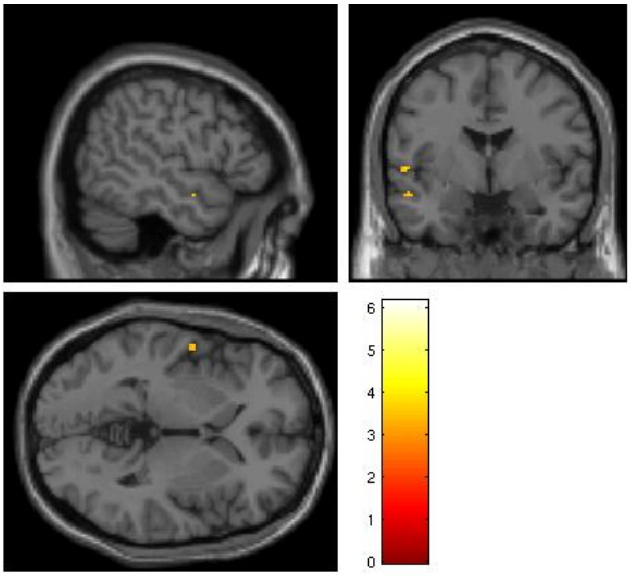

Figure 17: Activation of the left insula during the eating behaviours task, food vs non-food contrast, in the CP HO group> CP group. p<0,005, non FWE corrected.

- High caloric >low caloric foods

When exposed to picture showing high caloric food compared to low caloric foods (see methods), CP subjects showed prevalent activation of middle cingulate cortex (Fig 18), while CP HO patients showed activation the right thalamus (Fig 19). The comparison between groups showed greater activation of the middle cingulate cortex and the right caudate in the CP group, and did not show significantly greater activation in any area in CP HO subjects (Fig 20 a-b)

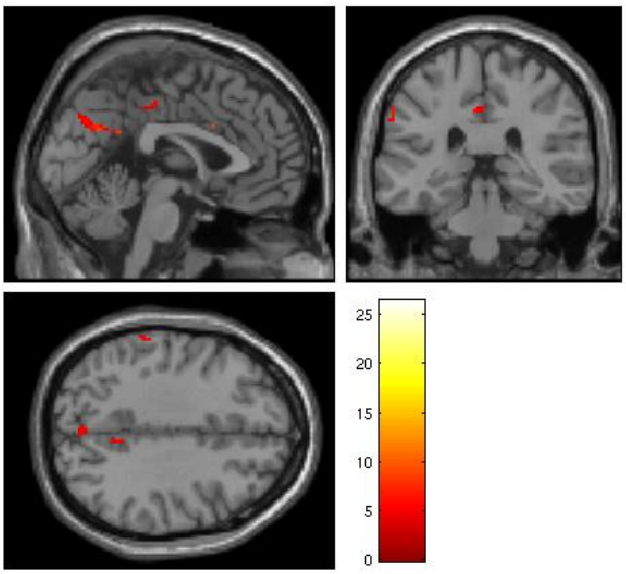

Figure 18 Activation of the middle cingulated cortex during the eating behaviours task, high caloric food vs low caloric foods food contrast, in the CP group. p<0,005, non FWE corrected.

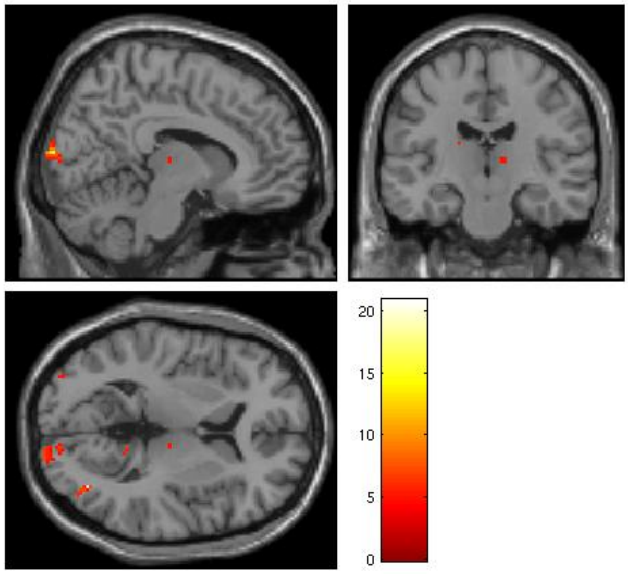

Figure 19 Activation of the right thalamus during the eating behaviours task, high caloric food vs low caloric foods food contrast, in the CP group. p<0,005, non FWE corrected.

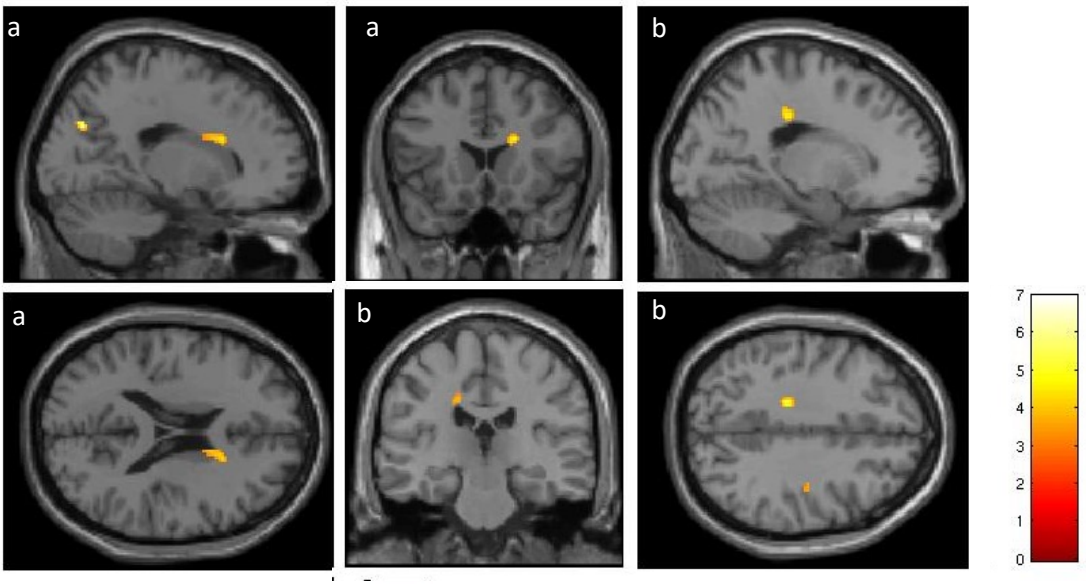

Figure 20 Activation of the right caudate (a) and left posterior dorsal cingulus (b) during the eating behaviour task, high caloric food vs low caloric foods food contrast, in the CP group > CP HO group. p<0,005, non FWE corrected.

EMOTIONAL RECOGNITION TASK

- Emotional recognition

When asked to recognize the showed emotion, after removing the motor activation (see methods), CP subjects showed prevalent activation of the dorsolateral prefrontal cortex and the insula bilaterally (Fig 21), CP HO patients showed a similar activation pattern (Fig 22). The comparison between groups showed a greater activation of the posterior cingulate cortex bilaterally, and right superior temporal gyrus and associative visual cortex in the CP group and did not show significantly greater activation in any area in CP HO subjects (Fig 23).

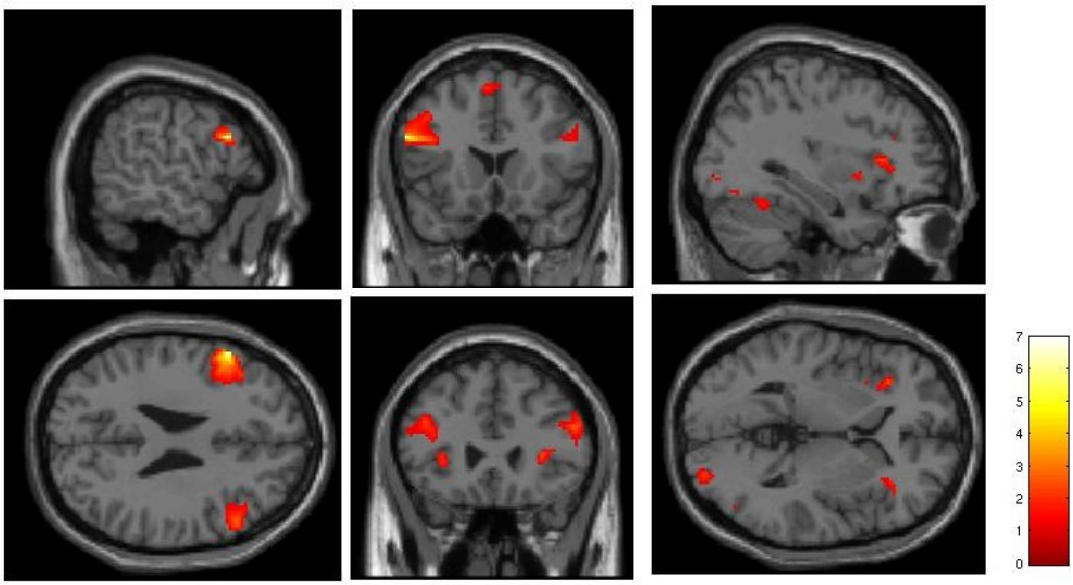

Figure 21 activation of the dorsolateral prefrontal cortex and the insula bilaterally during the emotion recognition task, no motor condition, in the CP group. p<0,005, non FWE corrected.

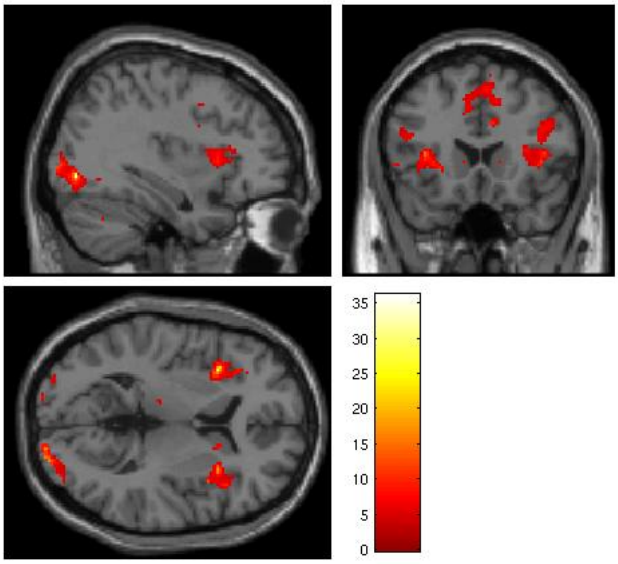

Figure 22 Activation of the dorsolateral prefrontal cortex and the insula bilaterally during the emotion recognition task, no motor condition, in the CP HO group. p<0,005, non FWE corrected.

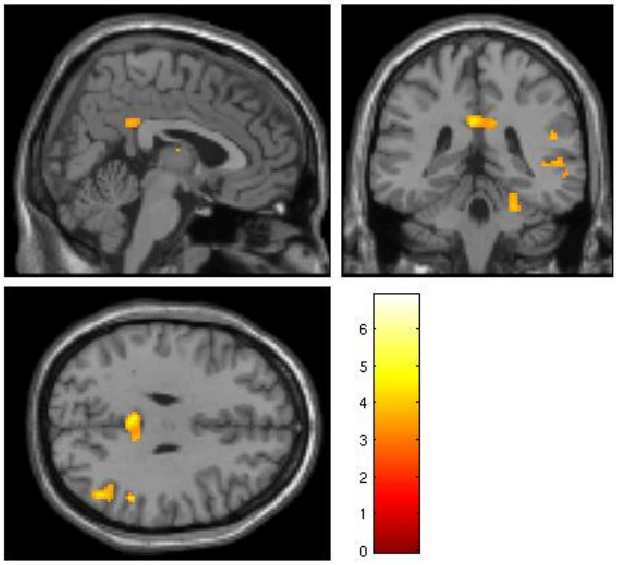

Figure 23 Activation of the posterior cingulate cortex bilaterally and right superior temporal gyrus and associative visual cortex during the emotion recognition task, no motor condition, in the CP > CP HO group. p<0,005, non FWE corrected.

- Identity recognition

When asked to recognize characteristics of the displayed face, after removing the motor activation (see methods), CP subjects showed prevalent activation of the bilateral dorsolateral prefrontal cortex and the right insula (Fig 24), CP HO patients showed a similar activation pattern, although with apparently milder activation (Fig 25). The comparison between groups showed a greater activation of the posterior cingulate cortex bilaterally, and right superior and middle temporal gyrus and insula in the CP group, and showed a slight increased activation in the left putamen and right caudate in CP HO subjects (Fig 26-27).

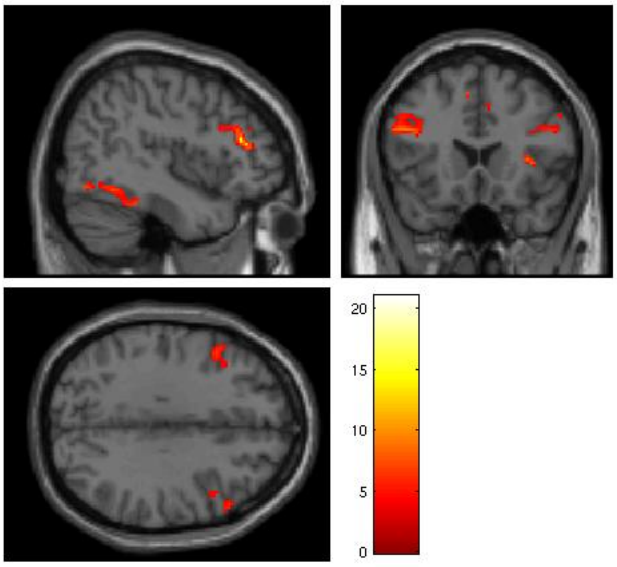

Figure 24 Activation of the dorsolateral prefrontal cortex and the right insula during the face recognition task, no motor condition, in the CP group. p<0,005, non FWE corrected.

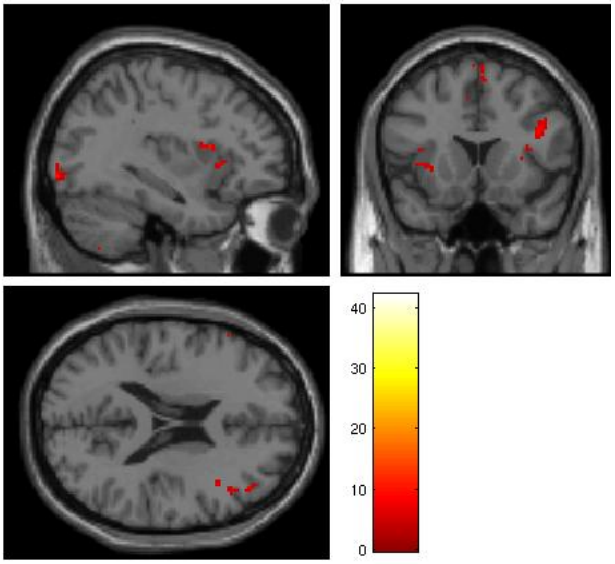

Figure 25 Activation of the right dorsolateral prefrontal cortex and the right insula during the face recognition task, no motor condition, in the CP HO group. p<0,005, non FWE corrected.

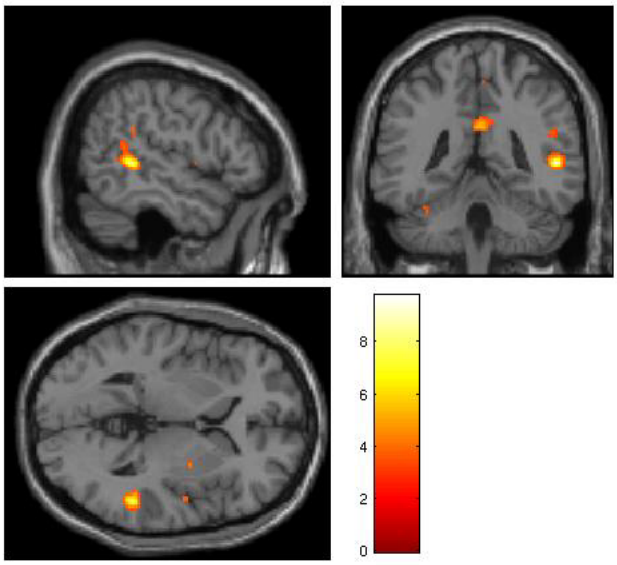

Figure 26 Activation of the posterior cingulate cortex bilaterally, and right superior and middle temporal gyrus and insula during the face recognition task, no motor condition, in the CP > co HO group. p<0,005, non FWE corrected.

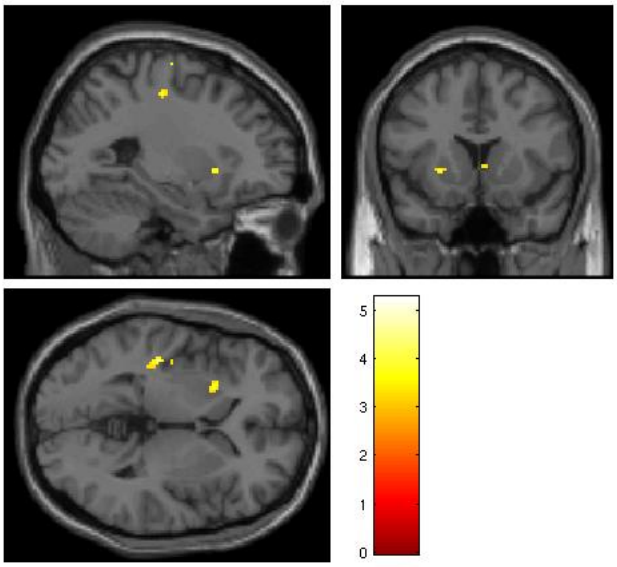

Figure 27 Activation in the left putamen and right caudate during the face recognition task, no motor condition, in the CP HO > co group. p<0,005, non FWE corrected.

- Emotion vs identity recognition

When asked to recognize the showed emotion vs the characteristics of the showed face (see methods), CP subjects showed activation of the left basal ganglia (Fig 28), while CP HO patients showed activation of the left insula (Fig 29). The comparison between groups showed greater activation of the left caudate in the CP group, and showed greater activation of ACC and medial frontal cortex bilaterally in the CP HO subjects (Fig 30-31)

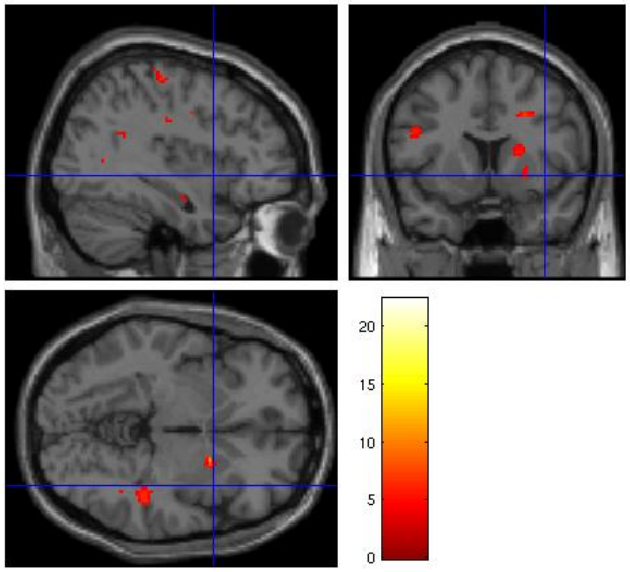

Figure 28 Activation of the left putamen and caudate in the emotion recognition task > the face recognition task, in the CP group. p<0,005, non FWE corrected.

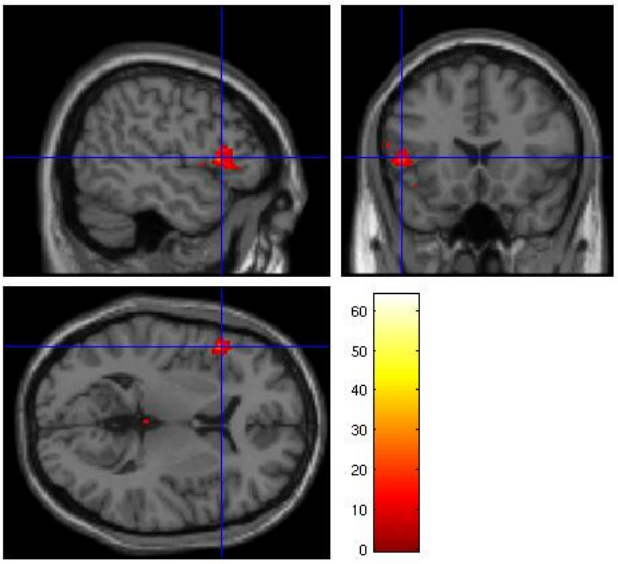

Figure 29 Activation of the left insula in the emotion recognition task > the face recognition task, in the CP HO group. p<0,005, non FWE corrected.

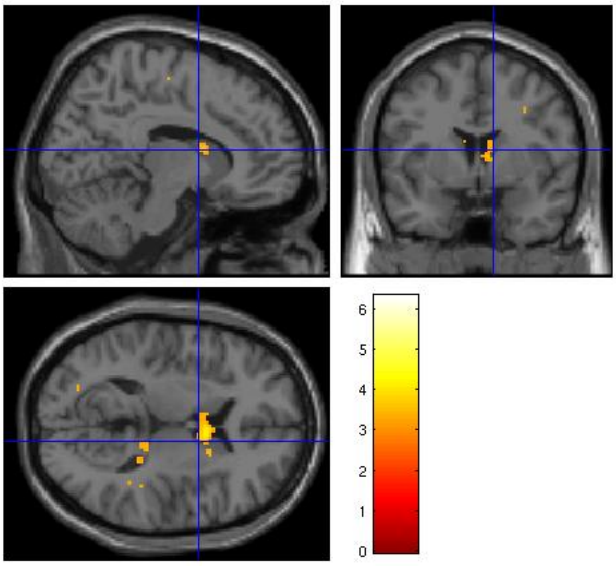

Figure 30 Activation of the left caudate in the emotion recognition task > the face recognition task, in the CP > CP HO group. p<0,005, non FWE corrected.

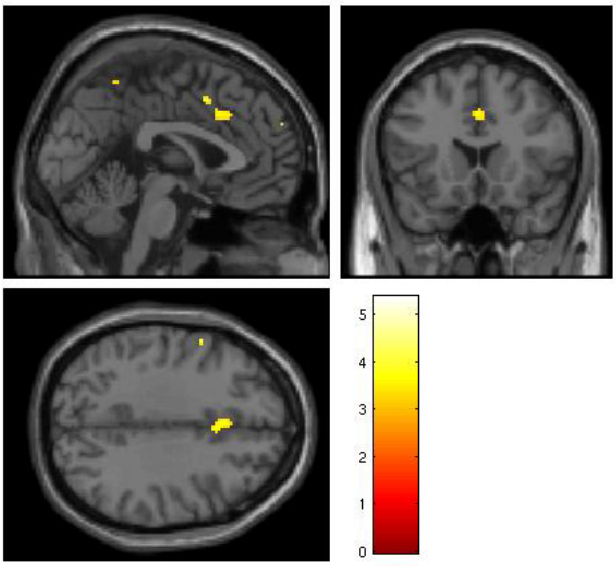

Figure 31 Activation of the anterior cingulate cortex and mediofrontal cortex bilaterally in the emotion recognition task > the face recognition task, in the CP HO > CP group. p<0,005, non FWE corrected.

DISCUSSION

In previous studies conducted in our centre, we identified several factors associated with HO, mainly related to the characteristics of the tumour, and pre-surgery BMI, while we did not observed differences based on different surgical approaches. (Mortini *et al.*, 2016) (Gallotti *et al.*, 2022) We have also conducted a preliminary study, which showed an association between neuropsychological complications and obesity: in agreement with previous studies, the volume of the lesion was the main determinant of both metabolic and neuro-psychological complications. Besides of the structural damage to these regions, endocrinological mechanisms leading to HO and neuropsychological complications have been postulated: for instance, considering the influence of oxytocin on eating behaviours, social and emotional regulation and metabolic functions, its depletion has been hypothesized as a common mechanism (Zada *et al.*, 2013). Whether the cause of this association is a common pathophysiological process (structural damage, oxytocin depletion) and the role of a reciprocal relationship (e.g. neuropsychological complications contributing to weight gain) remain open questions. Hence, we aimed to investigate shared pathogenetic mechanisms between obesity and psychological disturbances in patients diagnosed with craniopharyngioma. In contrast to prior literature focusing solely on hypothalamic damage, our particular interest lay in examining the "remote" effects of the lesion, encompassing both structural and functional alterations. Specifically, our focus was on delineating networks and activation patterns potentially correlated with oxytocin-related action mechanisms, while also conducting preliminary evaluations of its possible efficacy in treating these conditions

A key aspect of this study was the differentiation between subjects with CP with and without HO. The groups were matched for age, sex, time from surgery. There were no significant differences in neuropsychological and tests and questionnaires between the CP and the CP HO group, despite apparently different scores in Eating Disorder Inventory-3: this could be possibly reconducted to the small sample size and the high heterogenicity within each group in terms of age, time from surgery and age at the time of surgery, which represent the main limitations of the current study (see below).

We were interested in investigating remote effects of the damage caused by the tumor and/or the surgical removal that could unravel possible pathogenetic mechanism in the development of this complication.

Following previous reports that highlighted significant differences in patients with CP vs controls in grey matter atrophy in areas that are functionally connected to the hypothalamus yet distant from the area of the resection, namely the frontal and limbic regions, we compared the cortical thickness between the groups. We observed a significantly reduced thickness bilaterally in the insula and in the superior temporal lobe in CP patients who developed HO vs those who did not, and we found a significant correlation between the insular thickness (right) the scores of the Eating Disorders Inventory-3. Interestingly, during the eating behaviours fMRI task, patients in the CP HO group, showed a greater activation of the left insula when exposed to images of food, compared with subjects in the CP group.

An increased activation of the insula has already been reported in CP patients after exposure to stimuli comprising images portraying both high- and low-calories foods(Roth *et al.*, 2012). An increased activation of the insula in response to food images in obese subjects has already been observed compared with healthy subjects (Carnell *et al*, 2012): in one study, obese women showed greater activation in the anterior insula, hippocampus, putamen and parietal cortex in response to high-calorie foods vs. neutral images (Rothemund *et al*, 2007). Another study found greater activation in the insula, amygdala, hippocampus, nucleus accumbens /ventral striatum and caudate/putamen and orbitofrontal cortex in obese women vs controls in response to pictures of high-calorie foods vs. neutral images (Stoeckel *et al*, 2008). A model for interpreting these results in obesity hypothesizes that satiety signalling and negative feedback on food consumption from homeostatic areas is impaired, while hunger (and also hedonic) signals from sensory areas and areas implicated in emotion and memory, as reflected by greater activation in amygdala, hippocampus, insula and precentral gyrus in fMRI studies(van Bloemendaal *et al*, 2015). Moreover, a meta-analysis of grey matter (GM) structural abnormalities in obese patients showed that the most consistent GM reductions in patients with obesity in the left, middle, right inferior frontal gyrus and insula, the left middle temporal cortex, the left precentral gyrus, and the cerebellum (Herrmann *et al*, 2019).

The insula is a critical structure in this process: indeed, together with the hypothalamus, to which is strictly connected, it participates to the regulation of feeding, acting as a relay for interoceptive sensation and emotional processing. The insular cortex has been identified as a brain region linked to interoception and is involved in subjective feelings in a wide sense, ranging from attentional processes, cognitive decision-making, intentions, time perception, and notably, the conscious perception of sensations, movements, visual and auditory stimuli, to more complex aspects such as self-representation, reliability of sensory input, and subjective

anticipations (Rolls, 2016). The insular cortex holds a key role in taste processing during feeding. Studies suggest that within the primate anterior insula and adjacent frontal operculum, the primary taste cortex houses neurons responsive to diverse tastes like sweetness, saltness, bitterness, sourness, and umami. Additionally, this region contains neurons encoding oral somatosensory sensations, covering aspects like consistency, fat texture, temperature perception, and even sensitivity to capsaicin. Moreover, the insula serves as a crucial center for integrating reward signals linked directly to nutrient-related properties: unconscious reward cues from gut nutrient metabolism are conveyed to brain regions including the striatum, hindbrain and midbrain. Signals associated with taste, oral somatosensation, and retronasal olfaction are conveyed via cranial nerves V, VII, IX, and X to reach the sensory cortex, where they converge, forming perceptions of flavor within the insula (Rolls, 2016). Food choices are not only determined by taste, nor nutrients, but rather from a complex interaction of these aspects with other emotional, cultural, cognitive stimuli: in the insula, sensory information subliminal reward signals from nutrients converge, projecting to cortical areas that add more complex perception to further influence food choice (de Araujo *et al*, 2020).

Consistently with this hypothesis, in our study patients with HO also showed greater activation of the insula when exposed to food images, even solely high or low caloric foods, together with the activation of other areas such as the hippocampus, the amygdala and entorhinal cortex, which have been related, in obese individuals, with emotional and memory-related responses to food, which could potentially promote excessive eating. We hypothesize that in CP HO patients, following the reduction of the connections between the hypothalamus and the insula and the reduction of cortical thickness in this area, there might be a dysfunctional system, in which the hyperactivation of the insula in response to food stimuli and the lack of feedback from hypothalamic nuclei participates to the pathogenesis of HO.

Conversely, patients without HO, showed greater activation of the ACC compared to subjects with HO, both when exposed to picture of high caloric foods and when exposed to pictures of food vs non-food. It is hypothesized that the connections between the insula, the hypothalamus and subregions of the frontal cortex form part of a circuit motivating feeding, preventing overeating and terminating feeding upon satiation. The ACC has been implicated in conflict monitoring/error detection, cognitive inhibition, reward-based learning, it has been hypothesized that, together with the prefrontal cortex, it could be responsible for cognitive control and attention in eating behaviours (Carnell *et al.*, 2012).Reduced activation of the ACC has been

linked to disinhibited eating: in one fMRI study, reduced pre-meal ACC activation to visual food vs. non-food cues was observed in obese subjects, and there was a negative correlation between ACC activation and self-report measures of disinhibition (Martin *et al*, 2010). Indeed, the dorsomedial prefrontal cortex/dorsal ACC have been proposed as a target for transcranial magnetic stimulation (rTMS) and transcranial direct-current stimulation (tDCS) in obesity: the hypothesis behind this is that augmenting the activity within these brain regions could potentially shift the equilibrium between reward and cognition, leading to the promotion of cognitive control and potentially the dampening of reward-driven mechanisms responsible for food cravings and excessive eating (Val-Laillet *et al*, 2015). We hypothesize that in our sample patient who did not develop HO showed a greater activation of the ACC as an expression of greater control on feeding behaviours, despite the possible damage caused by CP.

Consistently with this hypothesis, we observed a reduction of cortical thickness in the rostral ACC in patients with HO compared with those without. This could participate to the less efficient control exerted from this region in this group. Moreover, interestingly, subjects in the CP HO group also showed reduced thickness in the superior temporal cortex, bilaterally. The recruitment of this area has been previously demonstrated during the process of resisting to a craving in addiction studies: as this region is an auditory processing area, it has been hypothesized that its activation may reflect one of the pattern of resisting craving, which is "self-talk" (Hartwell *et al*, 2011). However, as we did not observe any changes in the activation of this area we cannot speculate regarding its activation patterns in this sample.

Interestingly, a number of fMRI studies of intranasal oxytocin in feeding behavior demonstrate an increased activation of the ACC, together with other frontal region implicated in cognitive control of eating, while reducing the activity of areas implicated in reward or hedonic control of feeding: according to this hypothesis, oxytocin might exert its anorexigenic functions (see previous sections) improving the cognitive regulation of food craving via the increased activation of neurocircuitry implicated in top-down control and self-referential processing.

In one study, 15 normal-weight men were exposed to food stimuli and were given an ad-libitum breakfast, while in a fasting state. Intranasal oxytocin reduced calorie intake by 12% while it increased the activity in the anterior cingulate, ventromedial and ventrolateral prefrontal cortices in response to high- vs. low-calorie food stimuli (Spetter *et al.*, 2018).

In another fMRI investigation, 31 healthy women were administered either 24 IU of intranasal oxytocin or a placebo before being exposed to images of high caloric food. Oxytocin notably elevated activity levels in the middle and superior frontal gyrus, precuneus, and cingulate cortex. Furthermore, during the study, participants were directed to either visualize immediate consumption of the food or exercise cognitive control over their urge to eat it. Oxytocin administration specifically decreased food cravings during the cognitive control scenario. Additionally, a correlation was noticed between the behavioral impact of oxytocin and heightened activation in these brain regions (Striepens *et al.*, 2016). Another study demonstrated that, following oxytocin administration, compared to placebo, 10 obese subjects showed redcued activation in areas implicated in the "hedonic" network such as the orbitofrontal cortex, insula, basal ganglia, hippocampus and amygdala as well as in the hypothalamus and hyperactivation in areas implicated in cognitive control, namely the anterior cingulate and frontopolar cortex. In another study,following oxytocin administration, compared with placebo, overweight participants exhibited significantly attenuated functional connectivity between the VTA and the insula, oral somatosensory cortex, amygdala, hippocampus, operculum, and middle temporal gyrus in response to viewing high-calorie foods (Kerem & Lawson, 2021). Hence, an interesting hypothesis could be that the administration of oxytocin in patients with HO could restore the regulation of food cravings. This could occur by augmenting inhibitory control mediated by prefrontal regions and adjusting a self-focused processing bias driven by heightened activation in the precuneus and cingulate cortex, akin to what's observed in individuals without HO. Simultaneously, such oxytocin administration might reduce the activation of brain areas involved in the pleasurable and emotional processing of food, which tend to exhibit increased activation in individuals affected by HO.

Patients without HO also showed greater caudate activation in response to high caloric foods vs low caloric foods, compared with CP HO subjects. This is in line with a previous study on CP patients in which individuals with hypothalamic damage who showed greater weight gain displayed decreased activation in the left caudate nucleus when exposed to food images. As per the authors' interpretations, the reduced activation of the caudate nucleus might represent the lower attention to food and reduced sense of satiety, indicating an altered processing of rewarding stimuli and might contribute to decreased feelings of fullness (resulting in increased hunger) and irregular eating patterns (Lee *et al.*, 2023).

Aligned with these findings, additional research on obesity has documented an inverse relationship between BMI and the neural response within the caudate nucleus when individuals consume appealing, high-calorie foods. For example, in a specific study, a negative correlation between BMI and the brain's reaction to drinking a milkshake compared to a tasteless substance was noted in the caudate nucleus. When examining the correlation between brain response and measures of impulsivity and food reward, a significant negative correlation was found between the caudate nucleus response to milkshakes and self-reported impulsivity in the overweight group, although this association was not evident in the control group (Babbs *et al*, 2013).

In the emotional recognition task, we observed a greater activation in the ACC and medial frontal cortex in subjects with HO compared with CP. This is consistent with a previous study on CP patients with hypothalamic involvement, in which patients underwent fMRI when asked to recognize emotional and emotional faces: an over-recruitment of prefrontal regions was observed in CP patients compared with controls. This increased brain activity was seen as a compensatory mechanism, indicating that these patients might have needed to use more neural resources and might have has to recruit additional cognitive skill to complete the emotional task (Özyurt *et al.*, 2014).

Human social interaction stands as an intricate and multifaceted process, far more intricate than observed in many other animal species. It involves intricate mechanisms encompassing awareness of internal bodily sensations, understanding one's own identity, perceptions of others, and the motivations driving interpersonal relationships. This intricate set of processes, collectively termed as social cognition, has recently been linked to the functioning of various brain regions forming a network. Notably, this neural network includes the medial frontal cortex, the ACC, the temporoparietal junction, the superior temporal sulcus, and the temporal poles. Among these regions, the medial frontal cortex and the ACC play distinctive and pivotal roles specifically tied to social cognition. In contrast, the remaining brain areas in this network serve more generalized functions, particularly related to the regulation and supervision of actions within social contexts (Amodio & Frith, 2006). The ACC is a part of the brain’s limbic system. It has been classically related to affect, on the basis of lesion studies in humans and in animals, although more modern evidence highlighted its role in error detection and correction. Various models have been proposed to elucidate the collaborative functioning of brain regions in these tasks, notably highlighting the interaction between the ACC and specific sections of

the lateral prefrontal cortex during tasks demanding significant cognitive exertion. One such proposal suggests that the ACC could be involved in a neural pathway utilized when demanding cognitive control is needed. For instance, this may occur when formulating a response for the first time, in case of fear of making mistakes, or when feeling insecure. Additionally, it is theorized that activation in the cingulate cortex is associated with a diverse range of control processes, particularly when confronting new situations, addressing errors, and managing conflicting information (Bush *et al*, 2000).

On the other hand, subjects without HO showed greater caudate activation in the emotional recognition conditions the identity recognition condition compared with subjects with HO. This is in line with previous evidence emphasizing the role of basal ganglia circuits in emotional processing. The caudate nucleus contributes to the social decision-making process by integrating memories, social stimuli, feelings of trust and anticipated reward through its connections with the amygdala and cortical regions. Interestingly, it has been shown that oxytocin administration increased caudate activity while participants performed tasks measuring face processing, trust and affection (Baumgartner *et al*, 2008; Grace *et al*, 2018; Wang *et al*, 2017; Wittfoth-Schardt *et al*, 2012). Overall, the greater activation of the caudate in subjects without HO could represent the physiological emotional processing activation, as opposed to a less physiological overactivation of frontal circuits and hypoactivation of basal ganglia activation in subjects with HO: it could be speculated that the administration of oxytocin might restore this activation.

Finally, we were interested in investigating whether there where association between the alterations in cortical thickness and the expression of genes related to the oxytoninergic system, namely the *OXT* and the *OXTR* gene.

While we found, as expected, no correlation between the expression of the *OXT* gene and the cortical thickness, as we know that *OXT* is less expressed in cortical regions. Conversely, we found a significant positive association between the expression of *OXTR* gene and cortical thickness, hence suggesting that the areas expressing more *OXT* receptors are the conserved. *OXT* is supposed to mediate its effects, both when endogenously produced both when administered intranasally, via is receptors in the frontal regions (mainly prefrontal cortex and ACC), basal ganglia and reward circuit, and in the limbic system, besides of its expression in the hypothalamic nuclei and peripheral expression(Boccia *et al*, 2013; Busnelli & Chini, 2018). We conclude that the administration of oxytocin could still be beneficial for

patients and restore activation patterns that are pathologically altered in subjects with HO.

STRENGTH AND LIMITATIONS

The study has some limitations to consider. These include a small sample size leading to statistical underpowering, the heterogeneous nature of the sample comprising individuals with diverse surgical interventions, varying timeframes post-treatment, different ages, surgeries performed across various years and locations, which might introduce variability in surgical techniques and outcomes. The reliance on self-administered questionnaires could limit data sensitivity, especially when patients provide insufficient details. Moreover, the absence of interviews with caregivers, particularly relevant for patients operated on during childhood, might restrict the understanding of subjective differences in the postoperative experience. The influence of surgical interventions on structural MRI measurements presents an additional concern, as these procedures may introduce artifacts or alterations in brain structure, potentially impacting the interpretation of structural changes observed in the imaging data. However, to avoid this bias, we also compared patients who underwent different type of surgery, without finding significant differences. Additionally, some patients were excluded from either fMRI or structural MRI analysis due to artifacts, reducing the sample size for these specific imaging modalities and potentially introducing selection bias. Finally, the incomplete questionnaire from a patient with severe cognitive impairment further limits the comprehensiveness of the data and may affect the overall analysis and conclusions of the study.

On the other hand, the study's strengths lie in its multifaceted approach and unique comparative design, setting it apart from prior research endeavors. It must be noted that, although the sample size is relatively small, it is significant in consideration of the rarity of the condition under examination, HO, which is a relatively rare complication of a rare disease. Moreover, the study's inclusion of an extensive neuropsychological battery alongside functional MRI (fMRI) evaluation sets a new standard. Many studies tend to focus solely on either neuropsychological assessments or neuroimaging, whereas this research combines both approaches. This comprehensive evaluation allows for a more holistic understanding of the cognitive and neural mechanisms underlying hypothalamic obesity in craniopharyngioma patients. Secondly, the comparison between patients with CP and HO and those without HO within the same condition is a significant departure from

conventional studies. Rather than comparing patients to healthy controls, this approach elucidates the specific impact of HO within the context of the disease itself, thus minimizing confounding variables related to overall health status or unrelated medical conditions. Furthermore, the study's focus on examining remote effects of the condition distinguishes it from prior research, which often concentrates solely on immediate structural alterations within the hypothalamus. By exploring alterations beyond the immediate region of interest, this study aims to discern structural changes specifically linked to the development of obesity in these patients, distinguishing them from alterations induced by the tumor or surgical interventions. Finally, the integration of multiple data streams—combining neuropsychological assessments, functional and structural neuroimaging, and gene expression analysis—creates a cohesive model. This comprehensive approach not only helps delineate the neural underpinnings of hypothalamic obesity but also establishes a groundwork for potential therapeutic interventions, particularly in relation to oxytocin deficiency observed in these patients. This interdisciplinary approach strengthens the study's findings, providing a more comprehensive understanding and paving the way for targeted therapeutic strategies.

In conclusion, despite the outlined limitations, the findings of this study hold promise in shedding light on the intricate neural mechanisms underlying craniopharyngioma-related hypothalamic obesity. Certainly, to further enhance the study and consolidate its findings, several aspects could be considered. Increasing the sample size with a more homogeneous cohort, considering factors such as age, time since surgery, and treatment specifics, would strengthen the study's statistical power and generalizability. Homogeneity within the sample would reduce confounding variables and aid in drawing more definitive conclusions regarding the observed effects. Encouraging collaboration between multidisciplinary teams, such as neuroscientists, endocrinologists, and psychologists, could facilitate a more comprehensive understanding of the complex interplay between neural, hormonal, and behavioral aspects in these patients. Moreover, implementing a longitudinal study design would offer insights into the progression of neural changes over time post-surgery. This would enable to track the development of HO alterations and discern any dynamic patterns or associations that might emerge. Incorporating interviews with caregivers, particularly in cases where patients were operated on during childhood, would provide valuable subjective information regarding postoperative experiences and potential behavioral or cognitive changes. This holistic perspective could offer comprehensive insights into patient outcomes.

By addressing these aspects and refining the study design accordingly, future research could build upon the current findings, ultimately providing a more nuanced understanding of the underlying mechanisms and potential therapeutic interventions for hypothalamic obesity in craniopharyngioma patients. Ultimately, the effectiveness of oxytocin in addressing these conditions can only be truly elucidated through the conduction of a well-designed clinical trial.

MATERIAL AND METHODS

SELECTION OF PATIENTS

Potential participants were identified in the routine neurosurgery/neurology clinic appointments at the Department of Neurosurgery and at the Department of Neurology, San Raffaele Hospital. Subjects that underwent surgery for hypothalamic lesions and are in active follow-up were recruited.

Adult subjects who have developed HO following CP (Weight gain of >15% body weight 12 months before or after the diagnosis of CP) and patients with CP that did not develop hypothalamic obesity were recruited. All subjects had to be in active radiological follow-up according to their physician; able and willing to give informed consent or assent if informed consent obtained by parents-guardian, older than 18 years of age, stable for at least 2 months on any pituitary replacement (e.g., glucocorticoid, thyroid hormone, oestrogen/progestin or testosterone, growth hormone, except for adjustments of less than or equal to 20%) or any medications that might influence the outcomes (es. antidepressants, anti-diabetics etc).

Subjects unable to lie still within the environment of the MRI scanner for the required period to perform the study and those where MRI scanning is contraindicated (metal implants, pacemaker, etc.), pregnant or breastfeeding women, subjects with uncontrolled psychiatric disease or other diseases that might hinder the results, any person unable to understand and follow the instructions of the investigators were excluded.

PROCEDURES

Subjects underwent brain MRI as scheduled for their follow-up, during the MRI patients underwent additional sequences for functional brain MRI. In this occasion, patients also underwent neuropsychological evaluation, which addressed cognitive functions, eating behaviour and social skills (see below).

Study subjects and controls underwent one visit, scheduled based on their MRI follow-up. Participants were asked to read through the most recent version of the Consent form. They were given the opportunity to ask any questions they might have had regarding the study. Once consent had been obtained and the participant was

happy to proceed, the clinical history, physiological anamnesis, concomitant and past medications, and neurological examination were obtained. Inclusion and exclusion criteria for MRI scanning were revised again, and subjects were asked to fill in the MRI safety questionnaire. The MRI scan included, during a single session, conventional structural MRI sequences to rule out the presence of recurrence. During the MRI, subjects underwent additional sequences for functional MRI.

FUNCTIONAL MRI

Using a 3.0 T scanner (Ingenia CX, Philips), the following brain MRI sequences were obtained from all participants: 3D T1-weighted (TFE) (TR=7 ms; TE=3.2 ms; flip angle=9 [degrees]; 204 contiguous sagittal slices with voxel size=1 x 1 x 1 mm, matrix size=256 x 240, FOV=256x240 mm2); 3D FLAIR (TR=4800 ms; TE=267 ms; TI=1650 ms; ETL=167; NEX=2; 192 contiguous sagittal slices with voxel size=0.89 x 0.89 x 1 mm, matrix size=256 x 256, FOV=256x256 mm2); 3D T2 (TR=2500 ms; TE=330 ms; ETL=117; NEX=1; 192 contiguous sagittal slices with voxel size=0.89 x 0.89 x 1 mm, matrix size=256 x 258, FOV=256x256 mm2). Patients performed two fMRI tasks: “feeding behaviour" and "emotional recognition". Subjects received instructions on how to perform the task before the MRI. Tasks were performed equally well by all the subjects. All patients were fasting >8h before performing the examination. For the "Eating behaviour" task, participants viewed 10 high-calorie food stimuli, 10 low-calorie food stimuli, 10 non-food objects and 10 motor control stimuli in a block design repeated for 3 runs, for a total of 120 dynamic periods. Each stimulus was presented once for 2.5’’ using Presentation software (Neurobehavioural Systems, Albany, CA, USA). Each image was paired with a question "is that good for you?" (è buono secondo te?) for the food images; "is it useful for you?" (è utile secondo te?) for the non-food objects; "where is the circle?" (dove è il cerchio?) for the motor-control condition. Participants were instructed to press a button (left for answering "yes/left”, right to answer "no/right") with their index finger (Figure 32, a-d).

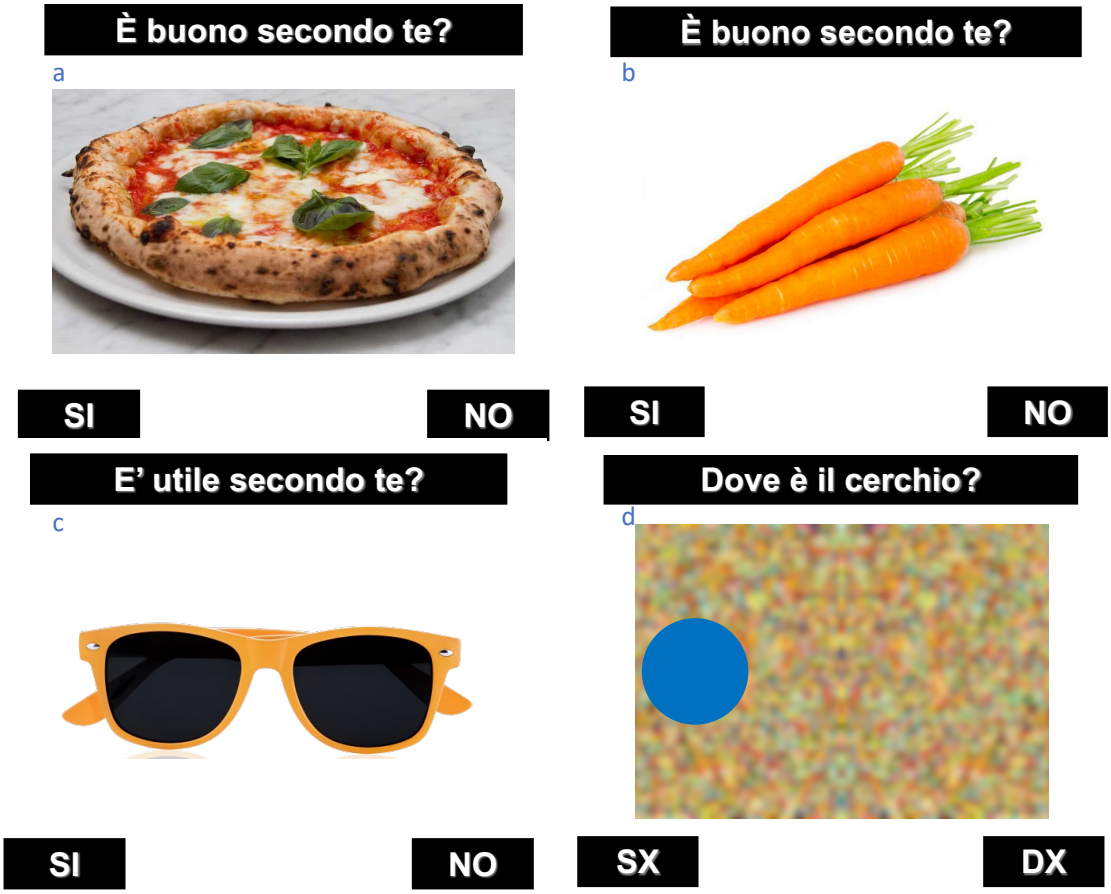

Figure 32 Examples of stimuli shown during the eating behaviour task, a: high-calorie food stimuli, b: low-calorie food stimuli, c: non-food objects, d: motor control stimuli

For the "emotional recognition" task, participants viewed: 25 emotional stimuli (disgust) (Figure 4), 25 identity stimuli (faces), 25 emotion stimuli (fear), 25 motor control; 25 emotional stimuli (sadness), 25 identity stimuli (faces), 25 emotional stimuli (anger), 25 motor control, in a block design repeated for 1 run, for a total of 200 dynamic periods. Each stimulus was presented once for 2.5'' using Presentation software (Neurobehavioural Systems, Albany, CA, USA). Each image was paired with a question: in the emotional condition, patients were asked to recognize the displayed emotion, es. "disgust?" (disgust?) and were instructed to press a button (left for answering "yes", right to answer "no ") with their index finger. For the identity condition, patients were asked about the "age/sex?" (età/sesso?) for the shown face and were instructed to press a button (left for answering "<30/female", right to answer ">30/male", respectively, for each question) with their index finger. For the motor-control condition, patients were asked "where is the circle?" (dove è il

cerchio?). Participants were instructed to press a button (left for answering "yes/left", right to answer "no/right") with their index finger (Figure 33 a-b).

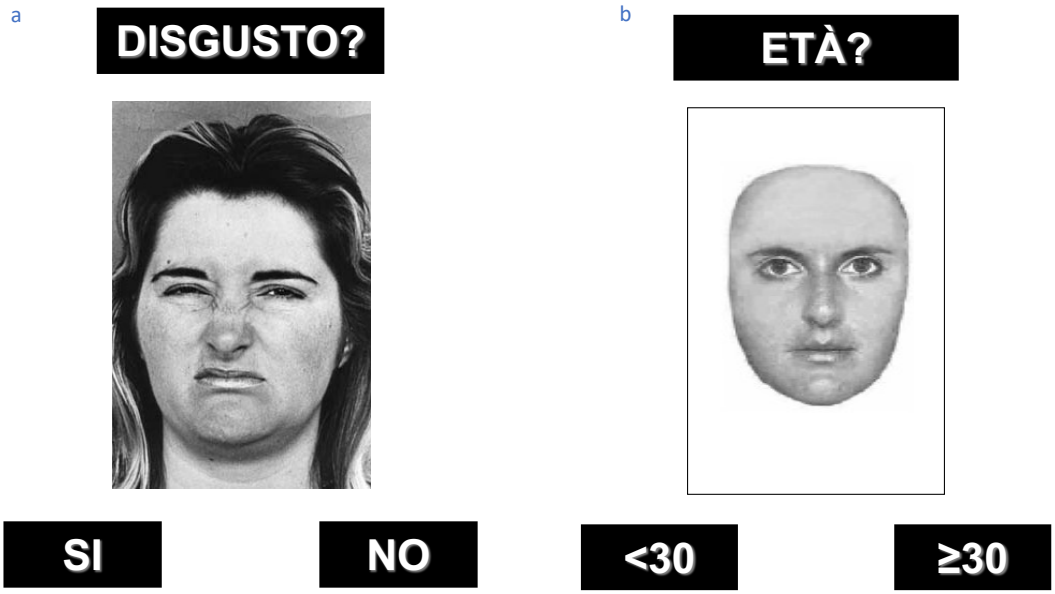

Figure 33 Examples of stimuli shown during the emotion recognition task, a.: emotional stimuli (disgust), b: identity stimuli (age)

fMRI

The SPM12 application (available at the Wellcome Trust Centre for Neuroimaging website, University College London) was employed to process the functional magnetic resonance imaging (fMRI) data. Each image underwent an alignment process to the initial scan for movement correction, ensuring no participant exceeded a 3 mm movement threshold in any axis. The images were then conformed to the standardized MNI (Montreal Neurological Institute) brain space and subsequently smoothed using a Gaussian kernel of 10 mm. To assess the blood oxygen level-dependent (BOLD) signal fluctuations corresponding to task performance (accounting for movement-related variables), we applied voxel-wise analysis using the General Linear Model (GLM) and Gaussian random field theory. Linear contrasts were utilized to probe specific effects.

Measurement of structural changes

Cortical thickness: The FreeSurfer image analysis software (version 5.3, available at the Massachusetts General Hospital-Harvard Medical School website) was utilized

to reconstruct the cortical surface and compute cortical thickness measurements from the three-dimensional T1-weighted turbo field echo (TFE) magnetic resonance images. Enhanced differentiation of gray matter (GM) and white matter (WM) was achieved by suppressing pixel values falling below the mean intensity level of the cerebrospinal fluid (CSF) and rescaling the remaining intensities to the baseline. After alignment with Talairach coordinates and uniformity in signal intensity, the methodology included an automated stripping of the skull using a combined approach of watershed algorithms and morphing surface models. The efficacy of the automatic process was meticulously verified, and eventual adjustments were performed manually on each image. Following this, segmentation into GM, WM, and CSF was conducted, with cerebral hemispheres being delineated and subcortical structures segregated from the cortical regions. The demarcation between WM and GM was discretized, creating a mesh that was refined to align with the intensity gradients, ensuring precise demarcation of WM-GM and GM-CSF boundaries and generating the corresponding WM and pial surfaces. Succeeding operations encompassed the inflation of these surfaces and their alignment with a spherical reference template, facilitating cortical parcellation into 34 anatomically defined regions per hemisphere based on the observed cortical convolutions.

Cortical thickness was then determined by calculating the mean of the shortest distances from the WM boundary to the pial surface across the cortex. For comparative purposes, individual cortical reconstructions were normalized to a standardized template, and the resulting surface maps were smoothed using a Gaussian filter with a full-width half-maximum of 10 mm to facilitate the analysis of cortical thickness across the study cohort.

GENE EXPRESSION

Local gene expression of the genes oxytocin (*OXT*) and oxytocin receptor (*OXTR*) was analysed through the Allen Human Brain Atlas (AHBA) dataset (available at http://human.brain-map.org).

NEUROPSYCHOLOGICAL EVALUATION

During the follow-up visit, patients also underwent general and neurological examination, medical history collection, and questionnaires assessing cognitive and

affective features, social skills, and eating behaviour. All tests were performed by experienced psychologists and were available in validated Italian translation.

Cognitive screening:

- MMSE (Mini-Mental State Examination)
- FAB (Frontal Assessment Battery)

Attention and Executive Functions:

- Working Memory
  - Digit Span
  - Reverse Span
- Decision Making
  - Modified Card Sorting Test, categories
  - Modified Card Sorting Test, perseverations
- Inhibition and Control
  - Stroop Test - Interference Time
  - Stroop Test - Interference Errors

Eating Disorders

- Eating Disorder Inventory-3
  - Tendency to lose weight
  - Bulimia
  - Body dissatisfaction

- Three-Factor Eating Questionnaire
  - Restriction
  - Disinhibition
  - Appetite

Mood and affective disorders

- Anxiety Control Questionnaire (ACQ) – SC/DC
- Difficulties in Emotion Regulation Scale (DERS)
- Beck Depression Inventory
- The State-Trait Anxiety Inventory

Social Cognition

- SET-Global Score
- SET-Intention Attribution
- SET-Causal Inference
- SET-Emotion Attribution

Quality of Life

- SF-36 Health Survey
  - Physical functioning
  - Role limitations due to physical health
  - Role limitations due to emotional problems
  - Energy/fatigue
  - Emotional well-being
  - Social functioning
  - Pain
  - General health

Clinical Severity

- The Clinical Impairment Assessment (CIA) Questionnaire

Sleep Quality

- Epworth Sleepiness Scale

STATISTICAL METHODS

Demographic, clinical, and neuropsychological variables were subjected to statistical analysis using the Pearson's chi-squared test for categorical variables and t-tests or ANCOVA models for continuous variables. Prior to analysis, normal distribution assumptions were assessed through the Kolmogorov-Smirnov and Shapiro-Wilk tests. Cortical thickness: the mean regional cortical thickness of 34 regions of interest (ROIs) per hemisphere were compared between different CP subgroups using ANOVA models, Bonferroni-corrected for multiple comparisons at level of 0.05 adjusting for age. fMRI: To ascertain significant activity responses to the contrasts, we adopted the methodology provided by Statistical Parametric Mapping (SPM12). The SPM's one-sample t-test identified significant average cerebral activations for each cohort during their respective tasks. For contrasting patients with and without HO and comparing different task conditions, we used a two-sample t-test. The results are presented with a significance threshold of $P < 0.005$ (uncorrected).

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