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Decreased cerebral opioid receptors availability related to hormonal and psychometric profile in restrictive-type anorexia nervosa

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ABSTRACT

Purpose: The opioid system role in anorexia nervosa (AN) pathophysiology is still unclear since conflicting results were reported on peripheral and cerebrospinal fluid opioids levels. The study main aim was to evaluate cerebral AN opiate receptor availability by using [^{11}C] diprenorphine, a ligand with non-selective binding.
Methods: In vivo [^{11}C]diprenorphine cerebral non-displaceable binding potential (BP_{ND}) evaluated by PET imaging was compared between three groups : 17 undernourished restrictive-type AN patients (LeanAN), 15 AN patients having regained normal weight (RecAN) and 15 controls. A lower BP_{ND} may account for an increased opioid tone and vice versa. Serum hormones and endogenous opioids levels, eating-related and unspecific psychological traits were also evaluated.
Results: Compared to controls, LeanAN and RecAN patients had decreased [^{11}C]diprenorphine BP_{ND} in middle frontal gyrus, temporo-parietal cortices, anterior cingulate cortex and in left accumbens nucleus. Hypothalamo-pituitary (H-P), left amygdala and insula BP_{ND} was found decreased only in LeanAN and that of putamen only in RecAN. LeanAN presented higher dynorphin A and enkephalin serum levels than in controls or RecAN. Inverse correlations were found in total group between : 24 h mean serum cortisol levels and anterior cingulate gyrus or insula BP_{ND}; eating concern score and left amygdala BP_{ND}. Positive correlation were found between leptin and hypothamus BP_{ND}; LH and pituitary BP_{ND}.
Conclusions: Low opiate receptor availability may be interpreted as an increased opioid tone in areas associated with both reward/aversive system in both AN groups. The relationship between the opioid receptors activity and hypercorticism or specific psychometric scores in some of these regions suggests adaptive mechanisms facing anxiety but also may play a role in the disease perpetuation.

1. Introduction

Restricting-type anorexia nervosa (AN) is characterized by several behavioral and psychiatric symptoms, such as intense fear of being fat, restrictive food intake, body image distortion (American Psychiatric Association, 2013), hyperactivity, perfectionism and mood changes, associated with a low body mass index in most of the patients and amenorrhea in premenopausal female patients (American Psychiatric Association, 1994). Neuroscience model of anorexia nervosa integrate predisposing, precipitating and perpetuating phases and factors (Nunn et al., 2011). Thus strong evidence indicate gene environment interaction may contribute to the risk of developing anorexia nervosa (Karwautz et al., 2011), while subsequently triggered neurobiological mechanisms seem to be involved in the onset and the maintenance of the disease (Kaye et al., 2009). In PET brain imaging era such influences were suggested for some important neurotransmitter systems including serotoninergic (Galusca et al., 2008; Kaye et al., 2013), dopaminergic (Frank et al., 2005), or endocannabioid one (Gérard et al., 2011).

The role of the opioid system in the pathophysiology of anorexia nervosa is of particular interest because of its involvement or interaction with several altered functions or processes in this disease : emotions (Nummenmaa and Tuominen, 2018), reward (Le Merrer et al., 2009; Nummenmaa et al., 2018) or aversive processing (Petrovic et al., 2008), stress reactivity (Bilkei-Gorzo et al., 2008), food intake (Hagan

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et al., 2001) and gonadal function regulation (Genazzani et al., 1993). In AN patients, conflicting results on endogenous opioids levels in serum or cerebrospinal fluid have been reported (Brambilla et al., 1991; Kaye et al., 1982; Lesem et al., 1991; Marrazzi et al., 1997). Treatment with opioid antagonists in AN could influence abnormal eating behavior (Marrazzi et al., 1995) and restore menses (Wildt and Leyendecker, 1987). However, there are no data on opioid cerebral activity and its relationship with hormonal adaptive changes, psychometric features or nutritional status in AN, yet these are necessary to understand the place of this system within the physiopathology of AN.

The aim of the current study was to evaluate by PET imaging the cerebral opioid receptor binding in restrictive-type AN patients, either undernourished or after having regain normal weight, in comparison with healthy controls. [^{11}C]diprenorphine is a radioligand with reversible and non-selective binding to opiate receptors μ, κ and δ (Sadzot et al., 1991). Despite a higher affinity for opioid receptors (Sadée et al., 1982) no competition between this ligand and endogenous opioids will occur as injected [^{11}C] diprenorphine reach low cerebral levels. [^{11}C] diprenorphine will be fixed only on non-internalized receptors. Consequently, higher absolute or relative endogenous opioid tone, defined as the result of interaction between opioid receptors and endocgenous opioids (Borsook, 2017), would lead to a lower binding of [^{11}C] diprenorphine and vice versa (Frost et al., 1990; Sadée et al., 1982). Therefore, in a context of presumed higher opioid tone in AN patients, we hypothesize lower binding compared to controls. Correlations between cerebral opioid receptor binding and psychological scores, hormonal levels, and endogenous opioid levels were also evaluated.

2. Methods

2.1. Subjects

The study included three groups of young Caucasian women (18–30 years, see Table 1 for details): undernourished restrictive-type AN patients, patients clinically recovered from AN, and controls.

Seventeen patients with restrictive-type AN according to both DSM IV criteria (American Psychiatric Association, 1994) and DSM-5 criteria (American Psychiatric Association, 2013) a body mass index (BMI) between 13 and 16.5 kg/m^2 (LeanAN) were recruited before any therapeutic intervention.

A separate group of fifteen patients recovered from restrictive-type AN (RecAN) was recruited according to following criteria : weight gain to a BMI $>$ 18.5 kg/m^2 and recovered hypothalamic-pituitary-gonadal axis (HPG axis) activity, stable for at least one year, no eating behavior disorder over this period according to psychiatrist follow up (Deep et al., 1995).

None of the patients had documented other chronic or psychiatric diseases including other eating disorders, and none of them were taking any medication, including oral contraception.

Fifteen controls (C) with normal weight (BMI 18.5 – 25 kg/m^2), spontaneous menses without oral contraception, and free of any psychiatric/neurological illness were included.

No subjects had contraindications to MRI and PET imaging. None of them were smokers or drug consumers. For eumenorrheic subjects, imaging was always carried out during the first phase of the menstrual cycle. A β-hCG test was carried out in every subject before imaging in order to exclude pregnant subjects.

The study was approved by the local ethics committee in accordance with the Helsinki declaration and French rules (registration number 2009-A00662-55). All subjects gave their written informed consent. Subjects were enrolled between January 2010 and September 2016.

2.2. Clinical, biological and psychological assessment

After an overnight fast, venous blood samples was obtained at 08h00 for measurement of serum IGF-I, 17β-Estradiol, free T3, LH and circulating opioid peptides including beta-endorphin, enkephalin-leucine and dynorphin A. Samples were collected every 4 h for a period of 24 h (08:00 h, 12:00 h, 16:00 h, 20:00 h, 24:00 h, 04:00 h) to assess cortisol and leptin.

Dual-energy x-ray absorptiometry allowed the quantification of body fat mass percentage (FM) (LUNAR, DPX-L, < 1%CV).

Hormonal parameters related to nutritional status including plasma cortisol, ACTH, IGF-1, 17-β estradiol, leptin, free T3 and LH were assessed using previously described techniques (Estour et al., 2010). Circulating opioid peptides were assessed using a RIA technique (Phoenix Pharmaceuticals, Burlingame, CA, USA; manufacturer's reference range: 10–1280 pg/mL).

Complementary psychological dimensions of eating behavior were assessed the same day in the morning using: EDE (Eating Disorders Examination) (Cooper and Fairburn, 1987), EDI (Eating Disorders Inventory) (Garner et al., 1983), DEBQ (Dutch Eating Behavior Questionnaire) (Van Strien et al., 1986) and SCL-90R (Symptom Checklist 90-Revised) (Derogatis, 1983).

2.3. Brain imaging assessment

The morning after the clinical and biological 24 h evaluation, patients served a standard breakfast and then were transported to CERMEP Lyon for brain imaging exploration. MRI and PET scan assessments were carried out after resting for one hour.

A three-dimensional T1-weighted structural MRI with a spatial

Table 1
Anthropometric, hormonal and circulating endogenous opioids characteristics of the groups.

	LeanAN (n = 17)	RecAN (n = 15)	C (n = 15)	p < 0.01
Age	24.1 ± 1.3	23.3 ± 1.2	23.5 ± 0.8	–
BMI	14.1 ± 0.4	18.9 ± 0.2	20.8 ± 0.4	a, b
% fat mass	14.9 ± 2.2	24.7 ± 2.3	27.9 ± 1.7	a, b
Leptin (μg/L)	1.4 ± 0.2	9.5 ± 2.2	14.0 ± 2.5	a, b
Albumin (g/L)	48.1 ± 10.1	47.5 ± 11.2	48.3 ± 10.8	–
IGF-I (μg/L)	110 ± 13	235 ± 18	232 ± 17	a, b
Free T_3 (pmol/L)	3.0 ± 0.1	4.0 ± 0.2	4.2 ± 0.1	a, b
ACTH (ng/L)	13.0 ± 1.6	11.5 ± 1.1	12.0 ± 1.2	–
24 h mean cortisol (ng/mL)	414 ± 27	283 ± 27	254 ± 19	a, b
17β-Estradiol (ng/L)	10.4 ± 2.0	40.2 ± 6.9	51.3 ± 17.0	a, b
LH (UI/L)	0.6 ± 0.2	7.8 ± 1.6	11.3 ± 3.6	a, b
β-endorphin (ng/L)	26.7 ± 3.7	25.9 ± 3.8	22.1 ± 2.9	–
Dynorphin A (ng/L)	195 ± 27	119 ± 8	111 ± 13	a, b
Enkephalin (ng/L)	397 ± 14	341 ± 16	334 ± 18	a, b

Inter-groups differences and were evalauted with Kruskal Wallis test followed by a post-hoc test (Steel–Dwass test) when significant – p < 0.01 : a – AN vs RecAN; b – AN vs C; c – RecAN vs C.

resolution of 1 mm × 1 mm × 1 mm consisting of 176 slices was acquired on a 1.5 T Siemens Magnetom scanner.

MRIs were processed using SPM8 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK, http://www.fil.ion.ucl.ac.uk/spm), running on MATLAB 2010 (MathWorks). MRIs were spatially normalized *via* linear and non-linear transformations to MNI (Montreal Neurological Institute) stereotaxic space using the Unified Segmentation algorithm (Ashburner and Friston, 2005), providing the deformation field from the individual MRI acquisition space to the MNI space, as well as the tissue probability maps for the grey matter (GM), white matter (WM), and Cerebro-spinal fluids (CSF).

2.3.1. ROI definition

83 regions of interest were automatically delineated with the maximum probability atlas created from 30 healthy subjects by Hammers et al. (Gousias et al., 2008; Hammers et al., 2003). Grey matter part of the ROIs was delineated by the intersection of the GM probability map thresholded at 0.3 and the segmented ROI. Because the pituitary region was not defined in the maximum probability atlas, a manual delineation of this region was performed on each subject' own MRI sagittal slices based on anatomic criteria of this gland situated in sella turcica, in-between the two cavernous sinuses and above sphenoidal sinus. For this delineation we used MNI Display software (McGill University, Canada).

A multi-frame PET acquisition was realized on a Siemens HR +camera at Lyon *in vivo* imaging research center (CERMEP). Thirty-seven frames were acquired over 70 min after injection of ~185 M Bq of [^{11}C]diprenorphine, a non-selective opioid radioligand (Frost et al., 1990; Lever et al., 1987), synthesized at the CERMEP. A preliminary 10 min transmission acquisition with rotating ^{68}Ge sources was performed before PET emission acqusition for tissue attenuation correction. PET images were reconstructed with all corrections (scatter, random, and attenuation) with a 3D filtered back projection and a Hanning filter (Cut-off at the Nyquist frequency). The dynamic PET image was converted from ECAT format to MINC format (McConnell Brain Imaging Centre, Montreal Neurological Institute). In case of subject' movement during the PET acquisition, a correction was applied (Costes et al., 2009). For registration purpose, a weighted summed image of the 70 min dynamic PET was computed. The individual PET sum and the MRI were automatically co-registered with rigid body transformation (Collins et al., 1994). The maximum probability atlas was resampled in the individual acquisition PET space by applying the inverse transformation from the MNI space to the individual space, then from the MRI space to the PET space. The time activity curves (TAC) of all ROIs where extracted from the dynamic PET, then submitted to kinetic modeling.

2.4. Modeling

The binding potential (BP) of the [^{11}C]diprenorphine on the opioid receptors were computed using the Simplified Reference Tissue Model (SRTM) (Gunn et al., 1997) with the occipital cortex used as the reference tissue. The SRTM estimates the non-displaceable binding potential (BP_{ND}) (Innis et al., 2007), Regional parameter values (ROI BP_{ND}), as well as voxel-wise parameter values (BP_{ND} parametric maps) were computed. To avoid arterial input function, SRTM modelization requires a reference region, which should present a density of receptors as low as possible, without variability among studied subjects. Previously, the occipital cortex was determined with the lowest accumulation of [^{1} ^{1}C]diprenorphine (Sadzot et al., 1991) and as a valuable reference region for [^{11}C]diprenorphine quantification with simplified reference method (Spilker et al., 2004). According to our data, medial occipital region, the cuneus, was precisely chosen because of its complete accordance regarding SRTM criteria.

2.5. Statistical analysis

Regional values of BP_{ND}, biological, anthropometric and psychometric parameters

All these parameters are presented as mean ± SEM. Giving the groups size non-parametric tests were further used for statistical analysis. Intergroup comparison was performed by using a Kruskal Wallis test followed by a post-hoc test (Steel–Dwass test) when significant. Correlations were calculated between [^{11}C]diprenorphine ROI BP_{ND} and psychiatric questionnaires, hormonal parameters and endogenous circulating opioids using a Spearman rank correlation test. Significance level set at 0.01 after correction for multiple tests.

Parametric maps of BP_{ND} were normalized to the MNI space, using the transformations computed in the MRI to PET coregistration and the MRI to MNI spatial normalization, then smoothed with a 12 mm full width at half maximum Gaussian filter. Whole –brain voxel-by-voxel analysis was performed with SPM8 in the frame of the general linear model. First, a ANOVA model ("Flexible factorial") was computed, with the group condition: RecAN, LeanAN and Controls, without global mean, without threshold but using an explicit mask of intra-cerebral regions. Simple post-hoc t-contrasts testing group main effects (RecAN and LeanAN versus Controls, and RecAN versus LeanAN) were computed. Secondly, an ANCOVA model ("Multiple regression") was computed to test for any covariation between voxel BP_{ND} and the circulating level of endogenous opioids as covariates. Statistical parametric maps resulting from the post-hoc contrasts were thresholded at $p < 0.001$ for illustrative views and comments.

3. Results

3.1. General and hormonal characteristics

General and hormonal characteristics are shown in the Table 1. Most of the anthropomorphic and hormonal measures were found abnormal in the LeanAN compared to the C group: lower BMI, % fat mass, leptin, IGF-I, free T_3, 17β-estradiol, LH, and higher cortisol, Enkephalin and Dynorphin A levels. All of them were found in the normal range in the RecAN group.

3.2. Psychological profile

Results of the DEBQ, EDE, EDI and SCL 90-R questionnaires are shown in Table 2. Most items of these questionnaires showed significant elevated scores in the LeanAN group compared to controls. Significant lower values were found in RecAN when compared with LeanAN for all the items, except EDI perfectionism and EDI interpersonal distrust.

3.3. Inter-groups [11C]Diprenorphine binding potential differences

Mean [^{11}C]Diprenorphine BP_{ND} cerebral distribution in each studied group is presented in Fig. 1.

3.3.1. LeanAN vs C

ROIs analysis indicated lower mean BP_{ND} in several cerebral areas (Table 3). Voxel-based analysis confirmed these results by revealing several clusters with lower BP_{ND} in LeanAN group patients compared to controls (Fig. 2, left column). SPM clusters covered symmetrically the both hemispheres: large part of anterior cingulate cortex and superior frontal gyrus ; part of insula, inferior frontal gyrus and precentral gyrus ; middle frontal gyrus ; parietal cortexes including postcentral gyri, superior parietal, supramarginal and angular gyri; posterior part of temporal lobes ; hypothalamus and pituitary gland, left accumbens nucleus. Lower BP_{ND} in medial area of cerebellum was revealed only by voxel based analysis.

No ROIs or clusters were found with an increased BP_{ND} in LeanAN group compared to the control group.

Table 2
Psychological evaluation scores.

		LeanAN (n = 17)	RecAN (n = 15)	C (n = 15)	$p < 0.01$
EDE	Body shape concern	15.2 ± 2.3	3.9 ± 1.6	3.0 ± 1.2	a, b
	Weight concern	22.5 ± 2.8	10.9 ± 3.2	5.1 ± 1.0	a, b
	Eating concern	15.9 ± 2.7	6.7 ± 2.1	3.7 ± 1.0	a, b
	Restrictive around eating	19.8 ± 2.5	4.5 ± 1.9	2.2 ± 1.4	a, b
EDI	Drive for thinness	10.7 ± 2.2	4 ± 1.3	0.5 ± 0.2	a, b
	Bulimia	14.5 ± 1.9	6.8 ± 2.6	5.2 ± 1.5	a, b
	Body dissatisfaction	12.8 ± 1.9	4.9 ± 1.9	0.7 ± 0.4	a, b
	Ineffectiveness	7.4 ± 1.6	5.3 ± 1.6	1.1 ± 0.4	b
	Perfectionism	6.3 ± 1.0	4.0 ± 1.5	2.5 ± 0.7	b
	Interpersonal distrust	12.3 ± 1.7	3.8 ± 1.7	0.2 ± 0.2	a, b
	Interoceptive awareness	6.4 ± 1.4	3.2 ± 1.0	1.1 ± 0.4	a, b
	Maturity fears	15.2 ± 2.3	3.9 ± 1.6	3.0 ± 1.2	b
DEBQ	Restrained eating	38.1 ± 3.7	23.8 ± 3.5	22.2 ± 3.5	a, b
	Emotional eating	39.7 ± 5.2	25.3 ± 2.6	23.1 ± 2.9	a, b
	External eating	30.2 ± 2.2	28.8 ± 1.9	29.7 ± 1.5	–
SCL-90-R	Somatization	1.27 ± 0.29	0.37 ± 0.15	0.08 ± 0.03	a, b
	Obsessive-compulsive	1.69 ± 0.22	0.63 ± 0.18	0.11 ± 0.03	a, b
	Anxiety	1.49 ± 0.19	0.46 ± 0.18	0.08 ± 0.04	a, b
	Depression	2.14 ± 0.25	0.61 ± 0.20	0.16 ± 0.06	a, b
	Interpersonal sensitivity	2.13 ± 0.34	0.71 ± 0.24	0.22 ± 0.04	a, b
	Hostility	1.46 ± 0.23	0.36 ± 0.15	0.14 ± 0.08	a, b
	Phobic anxiety	0.97 ± 0.17	0.41 ± 0.16	0.03 ± 0.02	b
	Paranoid ideation	0.80 ± 0.43	0.38 ± 0.25	0.25 ± 0.10	–
	Psychotism	0.75 ± 0.27	0.36 ± 0.15	0.22 ± 0.12	–
	Global severity index	1.60 ± 0.21	0.51 ± 0.22	0.1 ± 0.02	a, b

Mean score values ± SEM for every subscale of Eating Disorders Examination (EDE), Eating Disorders Inventory (EDI) and Dutch Eating Behavior Questionnaire (DEBQ) and Symptom Checklist 90-item Revised (SCL-90-R); Kruskal Wallis test followed by a post-hoc test (Steel–Dwass test) when significant – $p < 0.01$: a – AN vs RecAN; b – AN vs C; c – RecAN vs C.

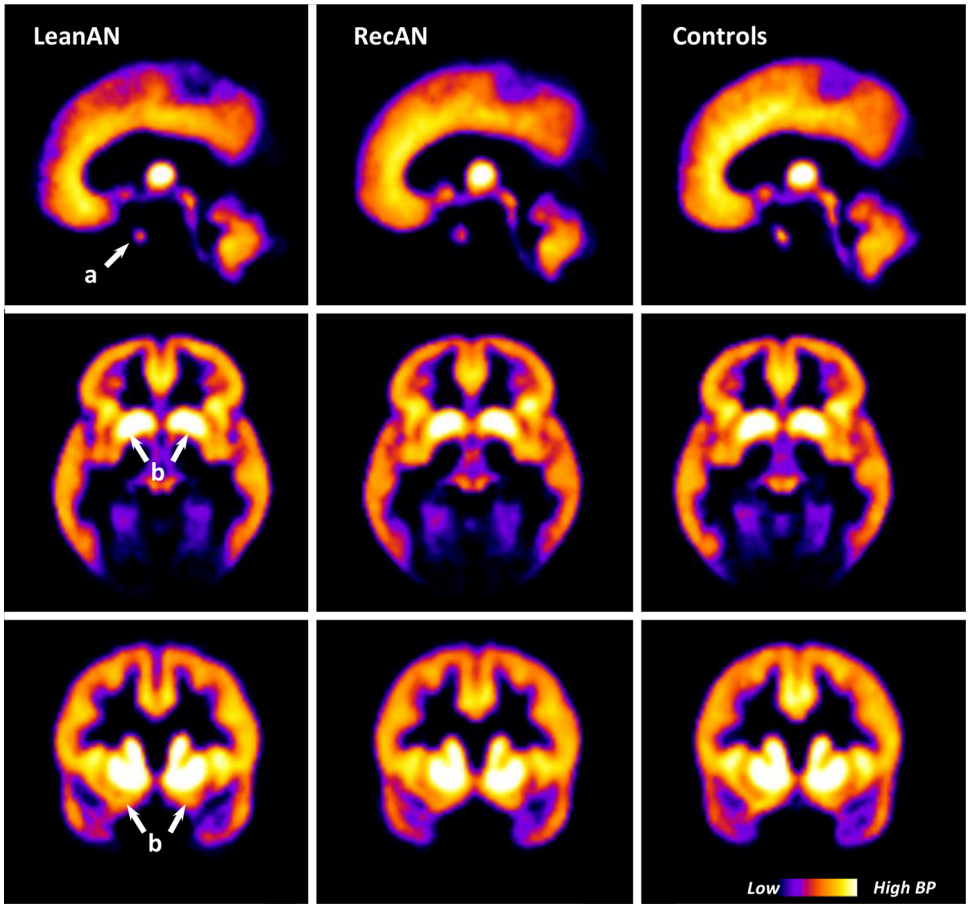

Fig. 1. Mean [^{11}C] Diprenorphine BP_{ND} cerebral distribution by group. Sagittal, axial and coronal PET images in LeanAN, Rec AN and controls; (a) – Pituitary gland; (b) – striatum region including putamen, accumbens and caudate nucleus; BP_{ND} intensity scale in right bottom corner.

Table 3
ROIs (regions of interest) with inter-groups differences of [^{11}C]Diprenorphine BP_{ND}.

ROIs	Hemisphere	AN	RecAN	C	$p < 0.01$	Corresponding opioid receptor subtypes*
Anterior cingulate cortex	L	1.72 ± 0.07	1.87 ± 0.07	2.05 ± 0.11	b	δ
	R	1.79 ± 0.08	1.94 ± 0.09	2.12 ± 0.16	b	δ
Superior internal frontal gyrus	L	1.26 ± 0.05	1.19 ± 0.07	1.48 ± 0.10	b, c	δ, κ
	R	1.30 ± 0.05	1.27 ± 0.06	1.53 ± 0.10	b, c	δ, κ
Inferior frontal gyrus	L	1.27 ± 0.06	1.30 ± 0.06	1.51 ± 0.09	b, c	δ, κ
Precentral gyrus	L	0.93 ± 0.03	0.92 ± 0.04	1.11 ± 0.06	b, c	δ
Insula	L	1.69 ± 0.06	1.79 ± 0.06	1.96 ± 0.12	b	δ
Middle frontal gyrus	L	1.26 ± 0.05	1.19 ± 0.06	1.45 ± 0.09	b, c	δ, κ
	R	1.30 ± 0.04	1.20 ± 0.07	1.45 ± 0.09	c	δ, κ
Postcentral gyrus	L	0.98 ± 0.04	0.91 ± 0.04	1.14 ± 0.07	b, c	δ
	R	0.98 ± 0.02	0.96 ± 0.04	1.11 ± 0.06	b, c	δ
Superior parietal gyrus	L	0.88 ± 0.03	0.79 ± 0.04	0.97 ± 0.04	c	δ
	R	0.88 ± 0.03	0.77 ± 0.04	0.95 ± 0.05	a, c	δ
Angular & supramarginal gyri	L	1.20 ± 0.04	1.08 ± 0.04	1.33 ± 0.07	a, c	δ
	R	1.22 ± 0.03	1.07 ± 0.05	1.29 ± 0.07	a, c	δ
Posterior temporal lobe	L	1.05 ± 0.03	1.01 ± 0.04	1.15 ± 0.05	c	δ
	R	1.06 ± 0.03	0.99 ± 0.03	1.12 ± 0.06	c	δ
Amygdala	L	1.85 ± 0.08	2.16 ± 0.09	2.29 ± 0.21	a, b	μ, κ
	R	2.07 ± 0.11	1.85 ± 0.06	2.20 ± 0.13	c	μ, κ
Hippocampus	R	0.96 ± 0.09	0.90 ± 0.08	1.19 ± 0.12	c	μ, δ
Hypothalamus		0.24 ± 0.03	0.44 ± 0.04	0.40 ± 0.06	a, b	μ, κ
Pituitary		0.66 ± 0.12	0.83 ± 0.13	1.19 ± 0.10	b, c	μ, κ
Putamen	L	1.94 ± 0.08	1.84 ± 0.12	2.23 ± 0.16	c	μ, κ
	R	1.85 ± 0.07	1.80 ± 0.08	2.12 ± 0.16	c	μ, κ
Accumbens	L	2.27 ± 0.07	2.26 ± 0.08	2.61 ± 0.17	b, c	μ, κ
Thalamus	L	2.46 ± 0.09	2.48 ± 0.10	2.87 ± 0.14	b, c	μ, δ
	R	2.44 ± 0.08	2.40 ± 0.10	2.95 ± 0.14	b, c	μ, δ

Significance is calculated with an ANOVA followed by *post-hoc LSD Fisher*'s test: $p < 0.05$: a – AN vs RecAN; b – AN vs C; c – RecAN vs C.

* Corresponding opiate receptor subtype predominance in the region was displayed according to literature data (Hiller and Fan, 1996; Peciña et al., 2019; Valentino and Volkow, 2018).

3.3.2. RecAN vs C

Overall, ROIs and clusters with lower BP_{ND} were also found in RecAN group when compared to controls, most of them being similar to those described in LeanAN group with some exceptions : a cluster with lower BP_{ND} was found in both putamen, and only in the subgenual part of anterior cingulate cortex; no abnormalities were found in insula or hypothalamic-pituitary areas (Table 3, Fig. 2- right column). "C-RecAN" voxel based analysis contrast confirmed these similitudes.

No ROIs or clusters were found with an increased BP_{ND} in RecAN group compared to the controls.

3.3.3. Lean AN vs RecAN

Clusters with lower BP_{ND} were found in LeanAN group compared to RecAN group in hypothalamus area, and restrained area of anterior cingulated gyrus and temporal cortex (Fig. 3).

3.4. Regional BP_{ND} co-variations with plasmatic levels of endogenous opioid peptides

No significant co-variations were found between BP_{ND} and the circulating level of beta-Endorphin, Dynorphin A or Enkephalin.

3.5. Hormonal parameters, BMI or psychiatric scores correlations with ROI BP_{ND} or plasma endogenous opioids

In total group (including LeanAN, C and RecAN) significant inverse correlations were noticed between 24 h mean cortisol level and BP_{ND} of hypothalamus (rho = −0.51, $p = 0.001$), left insula (rho = −0.44, $p = 0.008$) or left anterior cingulate gyrus (rho = −0.39, $p = 0.01$) (Fig. 4). A direct strong correlation was found between 24 h cortisol and dynorphin A levels (rho = 0.50, $p = 0.004$). Mean leptin levels correlated directly with hypothalamus BP_{ND} (rho = 0.58, $p = 0.0004$). Free T_3 correlated positively with left anterior cingulate gyrus BP_{ND} (rho = 0.51, $p = 0.002$). BMI correlated with pituitary BP_{ND} (rho = 0.43, $p = 0.004$). Inverse correlation were found between left amygdala BP_{ND} and EDE eating concern score (rho = −0.53, $p = 0.005$), pituitary BP_{ND} and EDI drive for thinness (rho = −0.51, $p = 0.008$)

When considered separately LeanAN presented with a positive correlation between SCL-90-R "phobic anxiety" score and left amygdala BP_{ND} (rho = 0,78, $p = 0.004$) and left insula BP_{ND} (rho = 0,70, $p = 0.01$), SCL-90-R "anxiety" and β-endorphin circulating level (rho = −0,76, $p = 0.008$).

4. Discussion

This is the first report of structured impairments of cerebral opioid activity in anorexia nervosa. A decreased [11C] diprenorphine binding was noticed in several areas of cortex, midbrain and limbic system. As mentioned above, the decreased BP_{ND} found in LeanAN or in RecAN patients may account for an increased opioid tone, defined as a results of the interaction between endogenous ligands and opiate receptors (Zubieta et al., 2003). This could be partially due to an increased endogenous opioids release triggered by food deprivation (Vaswani and Tejwani, 1986). In line with this hypothesis we found increased dynorphin A and enkephalin levels in undernourished restrictive type AN patients. Possible internalization or inactivation of opioid receptors, subsequent and persistent after food restriction (Jones et al., 2004), could also be involved in a relative increase of the opioid tone. One study showed a link between a decrease in brain opiate receptor density and hypo protein diet in rats (Kademian et al., 2002). Meanwhile anorexia nervosa is moreover a lipid undernourished state displaying in most of the cases normal albumin levels (Miller et al., 2005), feature also found in the current study.

Although the opioid system involves the whole brain structures, abnormal cerebral opioid activity in AN occurred only in certain regions. Interestingly, some of these cerebral abnormalities were presented in both LeanAN and RecAN patients, independently of nutritional status. These findings may suggest a link between the opioid tone and the organic background of this disease. Conversely, specific insula

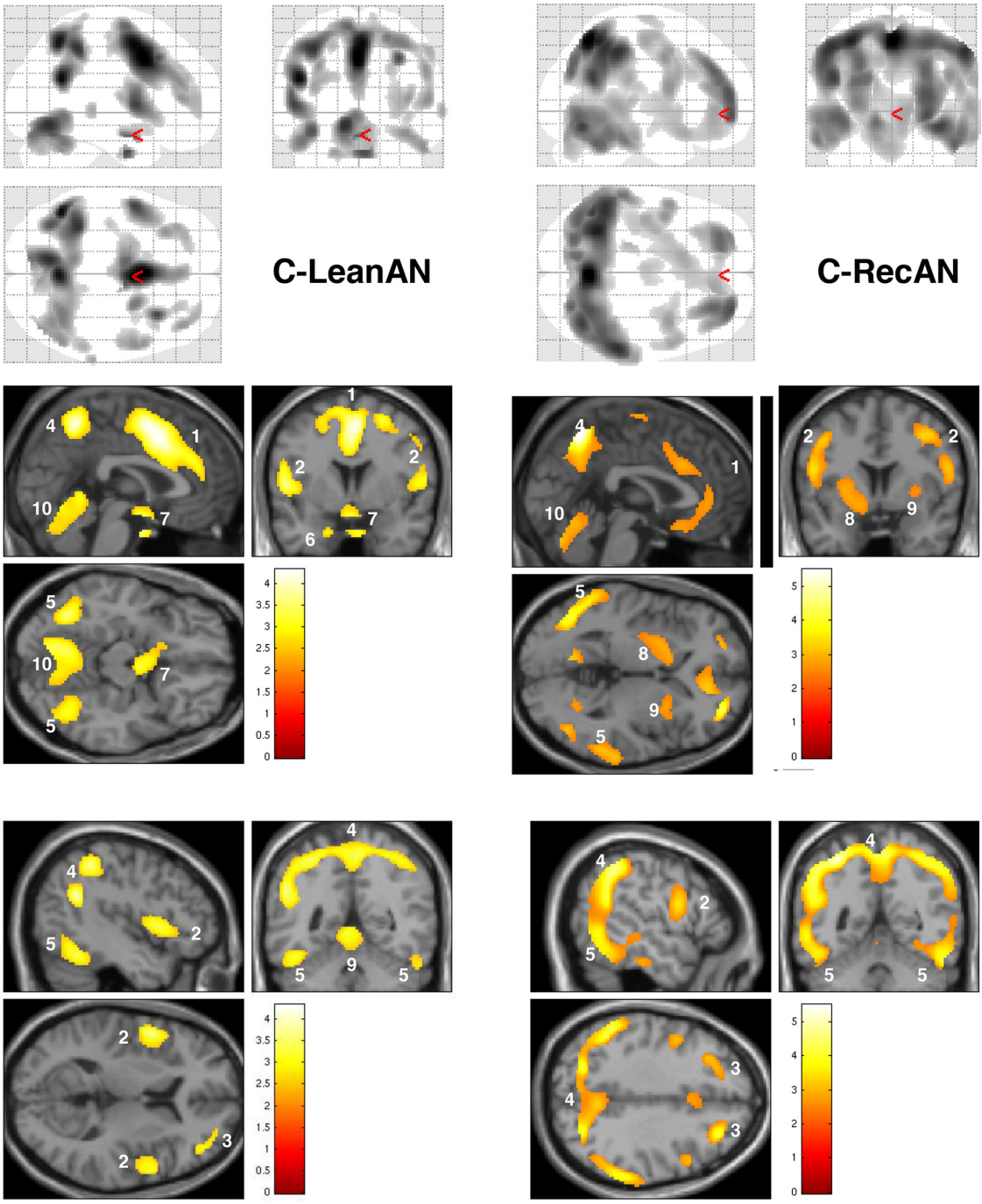

Fig. 2. Clusters of inter-groups [^{11}C] Diprenorphine BP_{ND} significant differences revealed by voxel-based ANOVA analysis. Left column – "C-LeanAN" contrasts; Right column – "C-RecAN" contrasts. Cluster 1 – areas of anterior cingulate cortex and superior frontal gyrus; Cluster 2 – areas of insula, inferior frontal gyrus and precentral gyrus; Cluster 3 – areas of middle frontal gyrus; Cluster 4 – areas of parietal cortex; Cluster 5 – posterior part of the temporal lobes; Cluster 6 – amygdala; Cluster 7 – hypothalamic-pituitary region; Cluster 8 – right putamen; Cluster 9 – left putamen and accumbens; Cluster 10 – areas of cerebellum.

or hypothalamic/pituitary region abnormalities were noticed only in undernourished AN patients while putamen abnormal activity was described only in RecAN patients. These abnormalities could be interpreted as a functional pattern, more related to nutritional status of these patients.

The hypothesis of a constitutional neurocircuity controlling several traits of anorexia nervosa was lately proposed (Galusca et al., 2008; Kaye et al., 2009). Specific dysfunction of fronto-pariental connectivity were revealed using resting state functional imaging in both undernourished and recovered AN patients (Boehm et al., 2014; Cowdrey et al., 2014). The current study detected symmetric clusters of decreased [^{11}C] diprenorphine binding in middle frontal cortex and superior parietal gyrus of LeanAN patients. Similar but larger clusters were detected in RecAN patients. The maintenance/intensification of abnormal opioid activity in these specific areas may be part of the organic background mechanisms subsidiary to long-lasting traits of AN after bodyweight recovery, including defected body image or interoceptive perception or feeding inhibition. Mentioned temporal and parietal cortices are characterized by higher densities of δ-receptors (Hiller and Fan, 1996; Peciña et al., 2019; Valentino and Volkow, 2018). The relationship of regional opioid activity abnormalities we found in these areas and δ-receptors genetic polymorphism previously

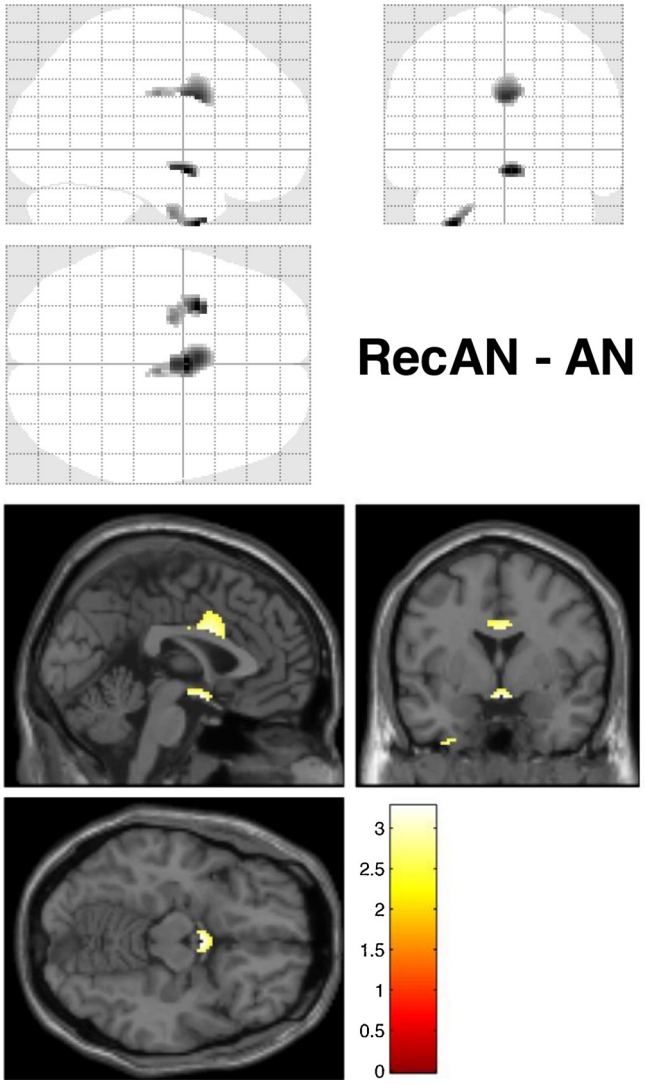

Fig. 3. Clusters of inter-groups [^{11}C] Diprenorphine BP_{ND} revealed by voxel-based ANOVA analysis in "RecAN-LeanAN" contrast.

described in AN (Bergen et al., 2003) remains to be explored.

Low regional opiate receptor availability found in specific brain areas including prefrontal cortex (inferior, middle and superior frontal gyrus), bilateral insula, anterior cingulate cortex, amygdala and accumbens nucleus in undernourished and/or recovered AN patients raise some questions about the role of opioid system in the pathophysiology of anorexia nervosa. In a recent review it was highlighted that, depending on the type of opioid receptors, the opioid system activity in these regions can have opposite effects on hedonia and can act on two systems with opposite functions: reward system and aversion or pain system (Darcq and Kieffer, 2018).

Accumbens nucleus, essential structure of the reward system (Haber and Knutson, 2010), presented with low receptor availability regardless the nutritional status of AN patients (Fig. 4). Regions like prefrontal cortex, amygdala or anterior cingulate cortex, even not the core of dopaminergic reward circuits, are considered key structures in reward system network (Haber and Knutson, 2010). The reduced opiate receptors availability we found in these areas may be potentially involved in reward circuit modulation in anorexia nervosa. It was previously showed the opioid receptor availability reduction after long distance running in both ACC and insula cortex were inversely correlated to euphoria ratings (Boecker et al., 2008). In line with these data, our observation suggests the concept of "behavioral addiction" in which self-restricition may procure some pleasure by releasing opioids in this reward system and may act as a perpetuating factor (Baldo, 2016). Opioid tone abnormalities were found in both ventral putamen only in recovered AN patients. Putamen revealed abnormal opioid receptor activity in early abstinence from opioid dependence (Williams et al., 2007). The disturbances found in recovered AN could also be associated with an craving dimension able to bring the patient back to undernutrition state. All these hypothesis needs confirmation via further studies combining specific imaging with pharmacological modulation of opioid system in these patients.

On the other hand the opioid system activity of insula, anterior cingulate cortex, amygdala or ventral putamen is involved in the modulation of other symptoms like pain, anxiety or depression, also specific for these regions (Friederich et al., 2007; Valentino and Volkow, 2018; Wey et al., 2014). High anxiety and depression scores were confirmed in the lean AN group of the current study. Interestingly, the direct correlation we found between left amygdala or left insula BP_{ND} and phobic anxiety score in these AN patients suggests an adaptive opioid tone reaction with potential efficacy on their anxious state. This modulation could also explain the correlations we found between the opioid activity inside the insula or anterior cingulate cortex and cortisol levels (anxiety marker) (Tabbert et al., 2010).

Hypothalamus and pituitary also presented with decreased [^{11}C] diprenorphine binding only in LeanAN patients. To our knowledge, this is the first imagistic report of neurotransmitter abnormalities in these

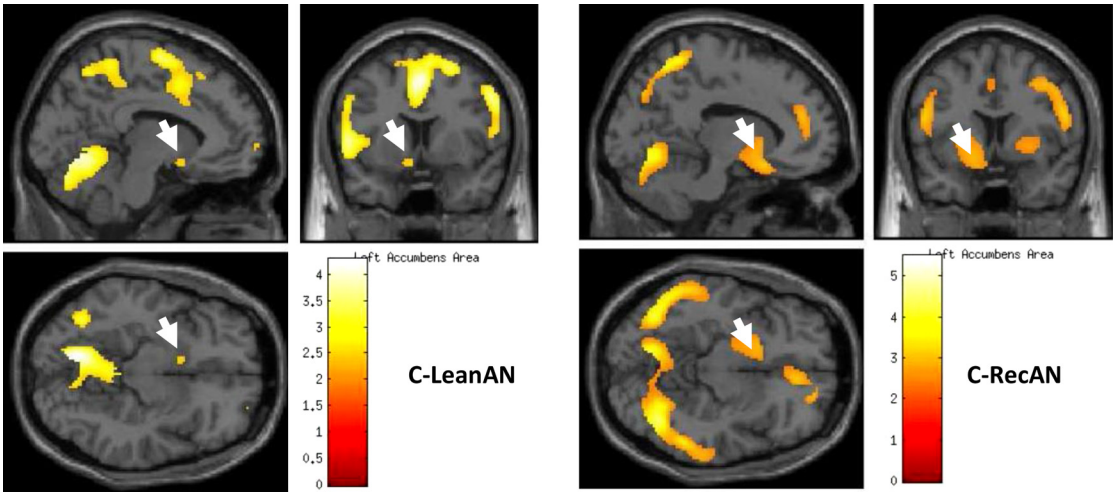

Fig. 4. Clusters of decreased [^{11}C] Diprenorphine BP_{ND} in left accumbens nucleus in both LeanAN and RecAN.

regions in anorexia nervosa. It is known the control of hypothalamic GnRH pulsatile function involves both central influences as the opioid tone (Genazzani et al., 1993) or peripheral signals like leptin (Nagatani et al., 1998). In line with these data the strong correlation we found between the low hypothalamus opioid receptor availability and plasma leptin levels underline the complex mechanisms of the central hypogonadism characterizing the undernourished state of the disease. Our imaging results corroborate indirectly with previous reports of transient menses restoration in anorexia nervosa after administration of naltrexone, a specific opiate antagonist (Wildt and Leyendecker, 1987). All mentioned structures located in the deep brain including pituitary and hypothalamic structures but also accumbens nuclei or amygdala preferentially display κ- and μ- receptors (Herkenham et al., 1986; Hiller and Fan, 1996). Interestingly, the opioid activity of all these regions in AN seem to be more related to the nutritional status of AN patients.

In conclusion, anorexia nervosa is characterized by a decreased [^{11}C] diprenorphine binding/opiate receptor availability in specific cortical and deep brain areas suggesting an increased cerebral opioid tone. The occurrence of similar cortical opioid activity disturbances in both lean and bodyweight recovered AN add evidence for an organic background hypothesis of this disease. Opioid abnormalities noticed in cerebral structures involved in both reward and aversion/pain systems indicate the dual role the opioid system might play in the neurophysiopathology of anorexia nervosa (Berner et al., 2019; Steinglass and Foerde, 2018), feature to take into account for further pharmacological interventions.

Data availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Bogdan Galusca: Conceptualization, Data curation, Funding acquisition, Investigation, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Bastien Traverse:** Formal analysis, Funding acquisition, Methodology, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Nicolas Costes:** Data curation, Formal analysis, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Catherine Massoubre:** Investigation, Validation, Visualization, Writing - original draft, Writing - review & editing. **Didier Le Bars:** Formal analysis, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Bruno Estour:** Conceptualization, Data curation, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Natacha Germain:** Conceptualization, Investigation, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Jerome Redouté:** Formal analysis, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

None.

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