Cerebral μ-opioid and CB_1-receptor systems have distinct roles in human feeding behavior

Short title: Eating behavior and central opioid and cannabinoid receptors

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Abstract

Eating behavior varies greatly between healthy individuals, but the neurobiological basis of these trait-like differences in feeding remains unknown. Central μ-opioid receptors (MOR) and cannabinoid CB_1-receptors (CB_1R) regulate energy balance via multiple neural pathways, promoting food intake and reward. Because obesity and eating disorders have been associated with alterations in brain's opioid and endocannabinoid signaling, the variation in MOR and CB_1R systems could potentially underlie distinct eating behavior phenotypes, also in non-obese population. In this retrospective positron emission tomography (PET) study, we analyzed [^{11}C]carfentanil PET scans of MORs from 92 healthy subjects (70 males and 22 females), and [^{18}F]FMPEP-d_2 scans of CB_1Rs from 35 subjects (all males, all also included in the [^{11}C]carfentanil sample). Eating styles were measured with the Dutch Eating Behavior Questionnaire (DEBQ). We found that lower cerebral MOR availability was associated with increase in external eating – individuals with low MORs reported being more likely to eat in response to environment's palatable food cues. CB_1R availability was negatively associated with multiple eating behavior traits. We conclude that although MORs and CB_1Rs overlap anatomically and functionally in the brain, they have distinct roles in mediating individual feeding patterns.

Introduction

Obesity is one of the leading public health issues, resulting from individuals' long-term excessive energy intake in relation to energy expenditure (1). Yet, humans vary greatly in their choices and habits related to food intake quantity and quality i.e. eating behavior (2, 3). Trait-like eating behaviors have been associated with multiple clinical eating disorders in addition to obesity (4-7), but also non-obese individuals vary in how they control their feeding (8). Interacting with peripheral hormones, central nervous system (CNS) integrates hunger and satiety signals with environmental stimuli for regulating food intake (1). Large-scale genome-wide association studies have identified limbic system, hippocampus and hypothalamus to be key regions in the CNS contributing to individual's body mass index (BMI) and eating behavior (9, 10). Central regulation of feeding is however constantly challenged by the modern environment characterized by abundance of palatable and energy-dense food products, promoting feeding independently of metabolic needs (11, 12).

Palatability and hedonic properties of food are centrally mediated by μ-opioid receptor (MOR) system (13, 14). Both endogenous and exogenous opioids stimulate feeding, especially via hedonic hotspots of nucleus accumbens, insula and frontal cortex (15-18). Conversely, opioid antagonists reduce food intake and related hedonic responses in rodents (18) and humans (19, 20). Human positron emission tomography (PET) studies have revealed that obesity associates with decrease of MORs in appetite regulating brain areas (21, 22), and insular MORs are lowered in patients with bulimia nervosa proportionally to fasting behavior (23). Central MOR system function varies considerably also in healthy humans (24), and traits linked with feeding control such as impulsivity are associated with MOR availability (25). Nevertheless, the association between the MOR system and specific patterns of eating behavior remains elusive.

Feeding is also regulated by brain's endocannabinoid system, which overlaps anatomically and functionally with the central MORs (26). The most abundant central cannabinoid receptors are the CB_1-receptors (CB_1Rs), which regulate food intake through circuits of ventral striatum, limbic

system and hypothalamus (27, 28). Functional interplay between MOR and CB_1R systems is highlighted in animal studies, where CB_1R-antagonists and MOR-antagonists have synergistic effect on reducing food intake (29), and CB_1R-antagonist can be used to block MOR-agonist induced food intake and vice versa (30). MOR-agonists also directly increase endocannabinoid concentration and CB_1R-agonists increase opioid concentration in the brain, including nucleus accumbens (31, 32). In humans, CB_1R-antagonist rimonabant showed promise as an anti-obesity drug, but was withdrawn due to psychiatric side effects (33). More nuanced understanding of CB_1R system and feeding is clearly required to enable further pharmacological advancement.

The current study

Accumulating evidence suggests that variation in central MOR and CB_1R function could be linked to feeding and pathological eating behavior traits in humans, but it remains unresolved what facets of feeding they govern in humans. Individual differences in eating patterns can be conceptualized based on the psychological mechanisms that contribute to or attenuate development of overweight. In such conceptualization, *emotional eating* refers to reactive overeating to distress or negative emotions, while *external eating* refers to tendency to overeat in response to attractive food-cues. Finally, *restrained eating* refers to the tendency to eat less than desired (34-36). The emotional and external overeating are based on psychosomatic and externality theories of eating behavior, while restrained eating dimension centers around food intake self-inhibition (37). Such consistent patterns contribute to differences in weight gain and maintenance (34, 38), and they can be measured using The Dutch Eating Behavior Questionnaire (DEBQ) (37). Here we compiled 92 [^{11}C]carfentanil PET scans targeting the MOR system and 35 [^{18}F]FMPEP-d_2 PET scans of CB_1R system and corresponding Dutch Eating Behavior Questionnaire (DEBQ) data in a retrospective PET database study. We tested whether the MOR and CB_1R availabilities in food-intake-regulating brain areas associate with individual eating behavior traits measured with DEBQ.

Materials and Methods

Subjects

The study subjects were historical controls retrieved from the AIVO neuroinformatics database (http://aivo.utu.fi), a large-scale molecular image database hosted by Turku PET Centre. We identified all the [^{11}C]carfentanil and [^{18}F]FMPEP-d_2 baseline PET studies accompanied with completed DEBQ form (37). Exclusion criteria were neurologic and psychiatric disorders, current use of medications that could affect CNS or abuse of alcohol or illicit drugs. Subjects were not preselected on the basis of weight or eating habits. Final sample consisted of 92 subjects (70 males and 22 females) scanned with [^{11}C]carfentanil from five distinct projects with three different PET scanners. The [^{18}F]FMPEP-d_2 sample consisted of 35 males, all of which were also all included in the [^{11}C]carfentanil male sample. All subjects of the [^{18}F]FMPEP-d_2 subsample were nonsmoking males, while in the [^{11}C]carfentanil sample seven females smoked. All [^{18}F]FMPEP-d_2 scans were carried out with GE Discovery VCT PET/CT (GE Healthcare). The original data were acquired between 2011 and 2019. Characteristics of the study sample are summarized in **Table 1**, and the information of smoking status and PET scanners are detailed in **Supplementary Table 1**. The study was conducted in accordance with the Declaration of Helsinki and approved by the Turku University Hospital Clinical Research Services. The subjects had signed written informed consent forms and completed the DEBQ forms as a part of the original study protocols.

Table 1. Characteristics of the studied subjects.

	[^{11}C]carfentanil scans					
	Males (n = 70)			Females (n = 22)		
	mean	SD	range	mean	SD	range
Age (years)	27.4	7.5	19−58	47.7	10.0	20−59
BMI (kg/m^2)	24.5	2.8	19−31	23.7	3.1	18−31
Total DEBQ score	67.0	12.8	40−109	73.4	12.8	46−97
Emotional eating score	21.0	6.8	13−40	22.1	5.7	13−32
External eating score	24.7	6.5	10−43	25.0	5.4	13−34

Restrained eating score	21.3	5.5	11−39	26.2	5.5	10−33
Injected activity (MBq)	277.0	77.9	223−508	352.3	125.5	234−519

	[^{18}F]FMPEP-d_2 scans Males (n = 35)		
	mean	SD	range
Age (years)	25.9	4.3	21−35
BMI (kg/m^2)	24.5	3.1	19−31
Total DEBQ score	68.6	14.5	43−109
Emotional eating score	20.7	7.4	13−40
External eating score	27.1	6.0	14−43
Restrained eating score	20.8	5.6	12−32
Injected activity (MBq)	187.9	12.8	147−215

Eating behavior assessment with the DEBQ

The Dutch Eating Behavior Questionnaire (DEBQ) (37) was used to quantify eating behavior. The DEBQ is a 33-item questionnaire with Likert-type scoring in each item (response options ranging from 1−5, from "Never" to "Very often"). It is divided in three dimensions measuring different behavioral traits: Emotional eating, External eating and Restrained eating (34-36). The DEBQ subscales have good internal consistency, dimensional validity and test-retest reliability (4, 7, 37, 39).

Image processing and modeling

PET images were pre-processed similarly using automated processing pipeline, Magia (40). [^{11}C]carfentanil data preprocessing has been described previously (24). MOR availability was expressed as [^{11}C]carfentanil binding potential (BP_{ND}), which is the ratio of specifically bound radioligand to that of nondisplaceable radioligand in tissue (41). Occipital cortex served as the reference region (42). CB_1R availability was expressed as [^{18}F]FMPEP-d_2 volume of distribution (V_T), which was quantified using graphical analysis by Logan (43). The frames starting at 36 minutes and later since injection were used in the model fitting, since Logan plots became linear after 36

minutes (43). Plasma activities were corrected for plasma metabolites as described previously (44). The code for PET data processing pipeline (Magia) is available at https://github.com/tkkarjal/magia.

Statistical analysis

The primary study question was whether the DEBQ subscales (Emotional eating, External eating, Restrained eating) or Total DEBQ scores are associated with [^{11}C]carfentanil BP_{ND} or [^{18}F]FMPEP-d_2 V_T values. First, we used nonparametric approach with SnPM13 (http://nisox.org/Software/SnPM13/) to create full-volume linear regression models for BP_{ND} and V_T values. We used $p < 0.05$ as the cluster-defining threshold, and only report clusters large enough to be statistically significant at FWE $p < 0.05$. 5000 permutations were used to estimate the null distribution. We created distinct models for Total DEBQ score and all the subscale scores. Because age influences [^{11}C]carfentanil binding (24, 45) and different PET scanners may yield slightly different BP_{ND} estimates (46), both age and PET scanner were added as nuisance covariates to [^{11}C]carfentanil models. Only age was added as a covariate in [^{18}F]FMPEP-d_2 V_T models, since all [^{18}F]FMPEP-d_2 scans were acquired with the same PET scanner. To rule out the possible effects of sex, smoking and BMI, we replicated the [^{11}C]carfentanil full volume analysis with these additional covariates. The [^{18}F]FMPEP-d_2 models were also replicated with BMI as additional covariate (there were no smokers or females in the [^{18}F]FMPEP-d_2 data).

We used Bayesian hierarchical modeling to analyze effects of eating styles on MOR and CB_1R availability in *a priori* regions of interest (ROIs). FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) was used to extract 10 bilateral ROIs based on known regions with high density of MORs (24) and CB_1Rs (47): amygdala, caudatus, cerebellum, dorsal anterior cingulate cortex, insula, middle temporal cortex, nucleus accumbens, orbitofrontal cortex, putamen, and thalamus. The Bayesian models were estimated using the R package brms (https://cran.r-project.org/web/packages/brms/index.html) that utilizes the Markov chain Monte Carlo sampling of RStan (https://mc-stan.org/users/interfaces/rstan).

For both tracers, we created models separately for the Total DEBQ score as well as its subscales, adjusting for age. [^{11}C]carfentanil BP_{ND} and [^{18}F]FMPEP-d_2 V_T were log-transformed to improve model fit (24). For both tracers, we estimated varying (random) intercepts for the subjects and ROIs, and varying (random) slopes across ROIs for the predictor of interest (e.g. Total DEBQ score) and age. Compared to a model where the regionally specific effects would be estimated using interaction term for ROI, the hierarchical model produces results that are partially pooled towards each other, thus accounting for the multiple comparisons performed (48). For both tracers, we also estimated regionally varying (random) residual variances. For [^{11}C]carfentanil data, we also estimated regionally varying (random) intercepts for the scanners. For [^{18}F]FMPEP-d_2 data, the scanner-adjustment was not needed because the data were acquired using a single scanner. We used the standard normal distribution as a prior distribution for the regression coefficients of the predictors to provide regularization. The standard normal distribution was also used as the prior distribution for the standard deviation of the group-level (random) effects. Otherwise we used the default priors of brms. We used 1000 warmup samples, 1000 post-warmup samples and 10 chains, thus totaling 10 000 post-warmup samples. The sampling parameters were slightly modified to facilitate convergence (*adapt_delta* = 0.999; *max_treedepth* = 20). The samplings produced no divergent iterations and the Rhats were all 1.0, suggesting that the chains converged successfully.

Results

Mean distribution of MORs and CB_1Rs is shown in **Figure 1**. Total DEBQ score correlated with all the subscales of DEBQ (r = 0.76−0.62). Correlations between the DEBQ subscales were positive but only modest, strongest being between Emotional and External eating (r = +0.33). BMI had a significant correlation only with Restrained eating (r = +0.27). Correlations with p-values are presented in **Supplementary Figure 1** and **Supplementary Table 2**.

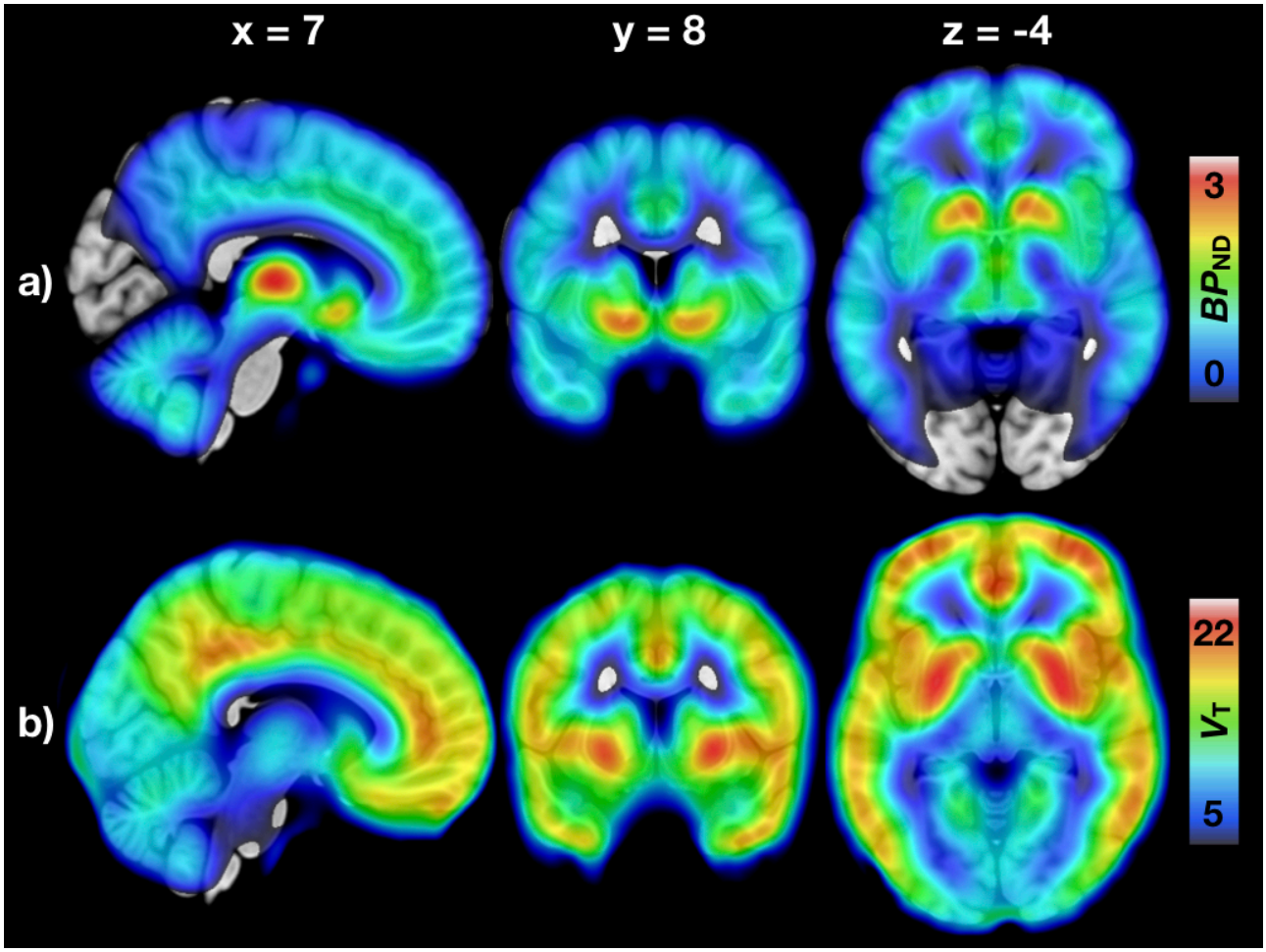

Figure 1. Mean distribution of central μ-opioid and CB_1-receptors. a) Mean binding potential (BP_{ND}) of the 92 subjects (70 males and 22 females) studied with [^{11}C]carfentanil. b) Mean volume of distribution (V_T) of the 35 males studied with [^{18}F]FMPEP-d_2.

Associations between μ-opioid receptor availability and eating behavior

Higher External eating score was associated with lower [^{11}C]carfentanil BP_{ND} bilaterally in multiple brain areas (**Figure 2**). Cortical associations were found in cingulate and frontotemporal areas, while subcortical effects were prominent in nucleus accumbens, caudate, putamen, insula, hippocampus and amygdala. Associations with Total DEBQ or other subscale scores were not statistically significant. Results were essentially similar when controlling for sex, smoking and BMI. We also checked the association of External eating and BP_{ND} in subsamples of 70 males and 22 females. In males, the association was similar to that of the full sample (**Supplementary Figure 2**), while in the female subsample there were no significant associations, likely due to limited statistical power.

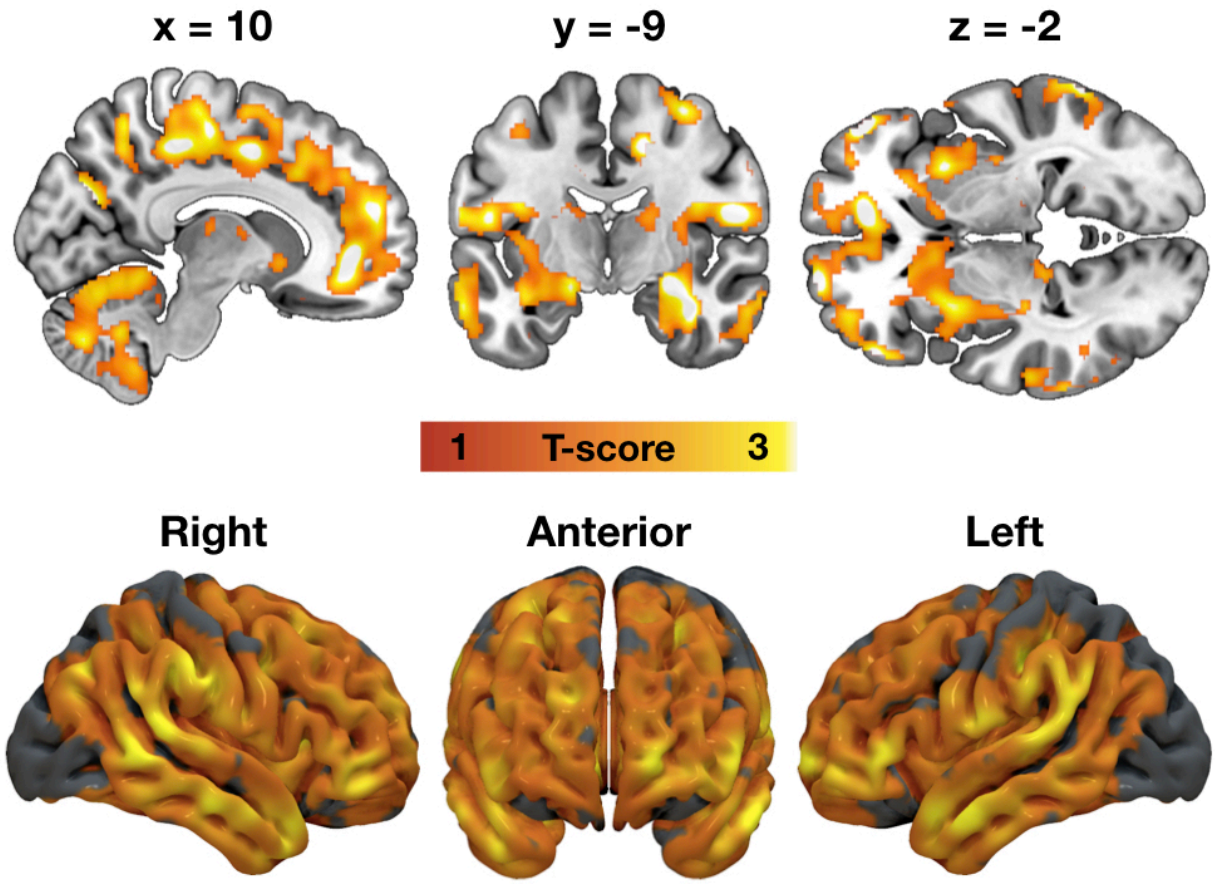

Figure 2. Association between External eating and decreased μ-opioid receptor availability. Brain regions where lower [^{11}C]carfentanil binding potential (BP_{ND}) associated with higher External eating score, age and PET scanner as nuisance covariates. Shown are clusters where $p < 0.05$, FWE corrected at cluster level.

Associations between CB_1-receptor availability and eating behavior

Total DEBQ score was associated with lower [^{18}F]FMPEP-d_2 V_T in multiple brain regions including bilateral anterior cingulate cortex, nucleus accumbens, caudate, putamen, insula, hippocampus, amygdala and thalamus (**Figure 3**). Full-volume associations with distinct DEBQ subscales and V_T were not statistically significant. Controlling for BMI did not alter the results.

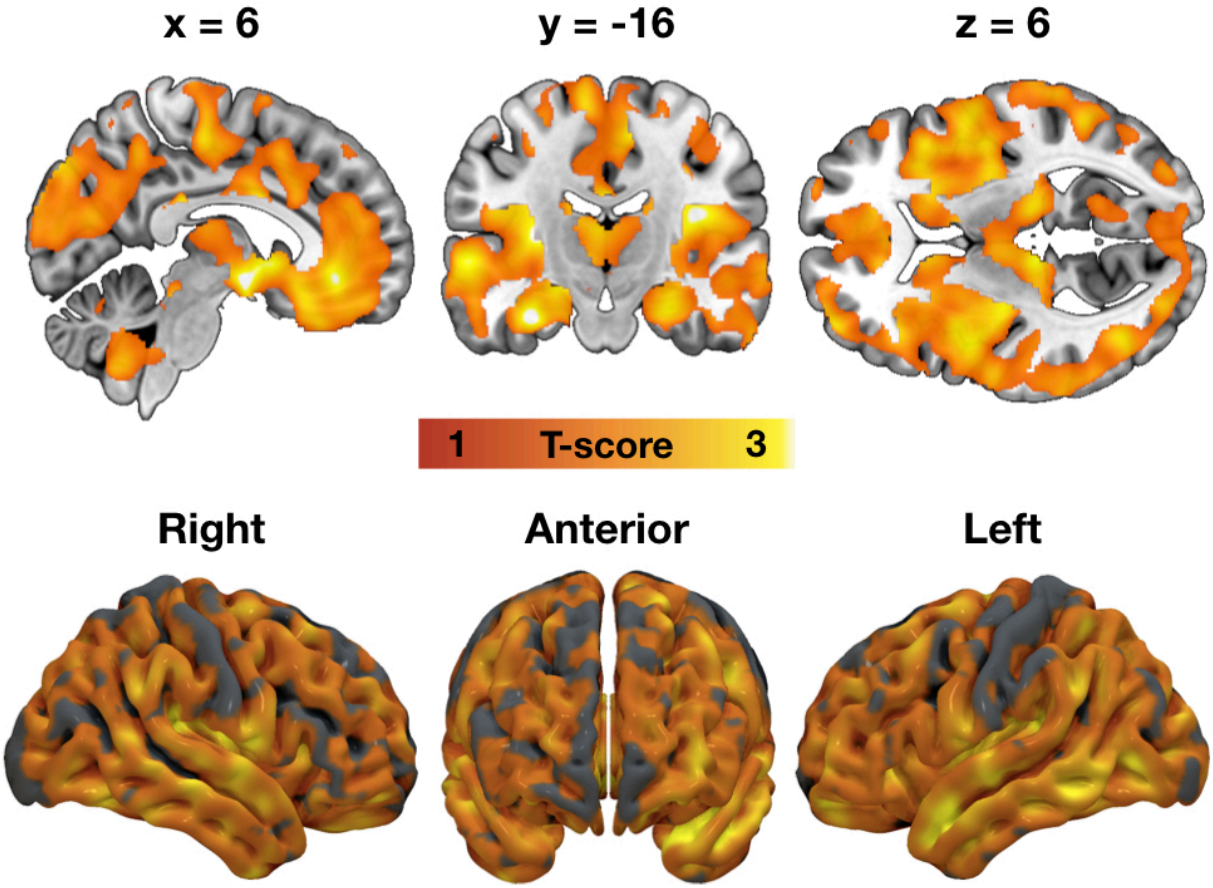

Figure 3. Total Dutch Eating Behavior Questionnaire (DEBQ) score associated with decreased CB_1-receptor availability. Brain regions where lower [^{18}F]FMPEP-d_2 volume of distribution (V_T) associated with higher Total DEBQ score, age as a nuisance covariate. Shown are clusters where $p < 0.05$, FWE corrected at cluster level.

Regional analysis of central receptor availability and eating behavior

Results of the ROI-analysis were consistent with the full volume results for both tracers (**Figure 4**). For [^{18}F]FMPEP-d_2, the association directions of all DEBQ subscales were negative, but the 95% confidence intervals overlapped with zero. Visualization of the regional relationships in representative ROIs is presented in **Figure 5**.

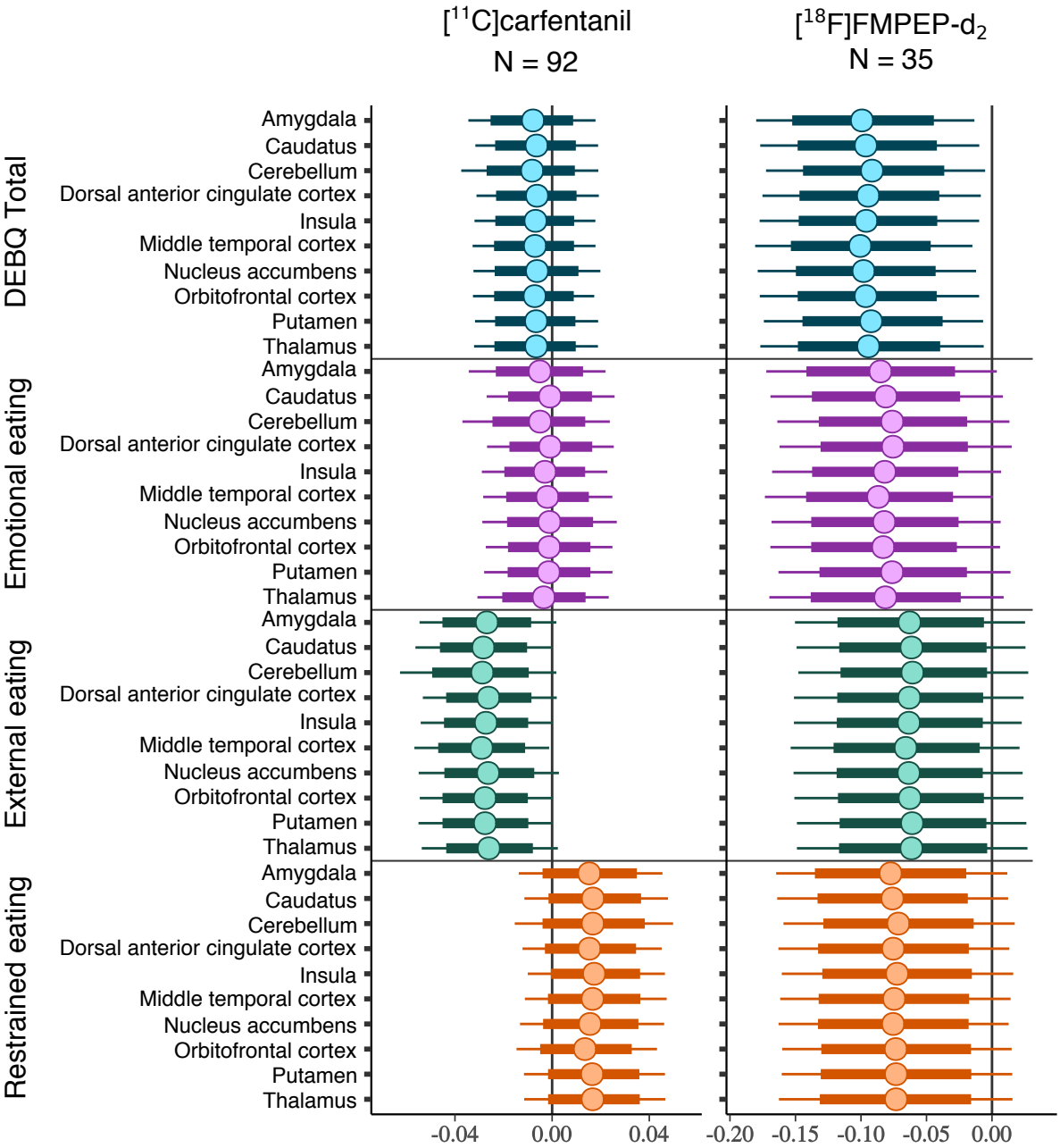

Figure 4. Regional associations between Total DEBQ and subscale scores with μ-opioid and CB_1-receptor availabilities. The figure shows posterior distributions of the regression coefficients for Total DEBQ and subscale scores on log-transformed [^{11}C]carfentanil binding potential (BP_{ND}) and

[^{18}F]FMPEP-d_2 volume of distribution (V_T) in 10 regions of interest. Age (and PET scanner for [^{11}C]carfentanil data) are controlled as covariates. The colored circles represent posterior means, the thick horizontal bars 80% posterior intervals, and the thin horizontal bars 95% posterior intervals.

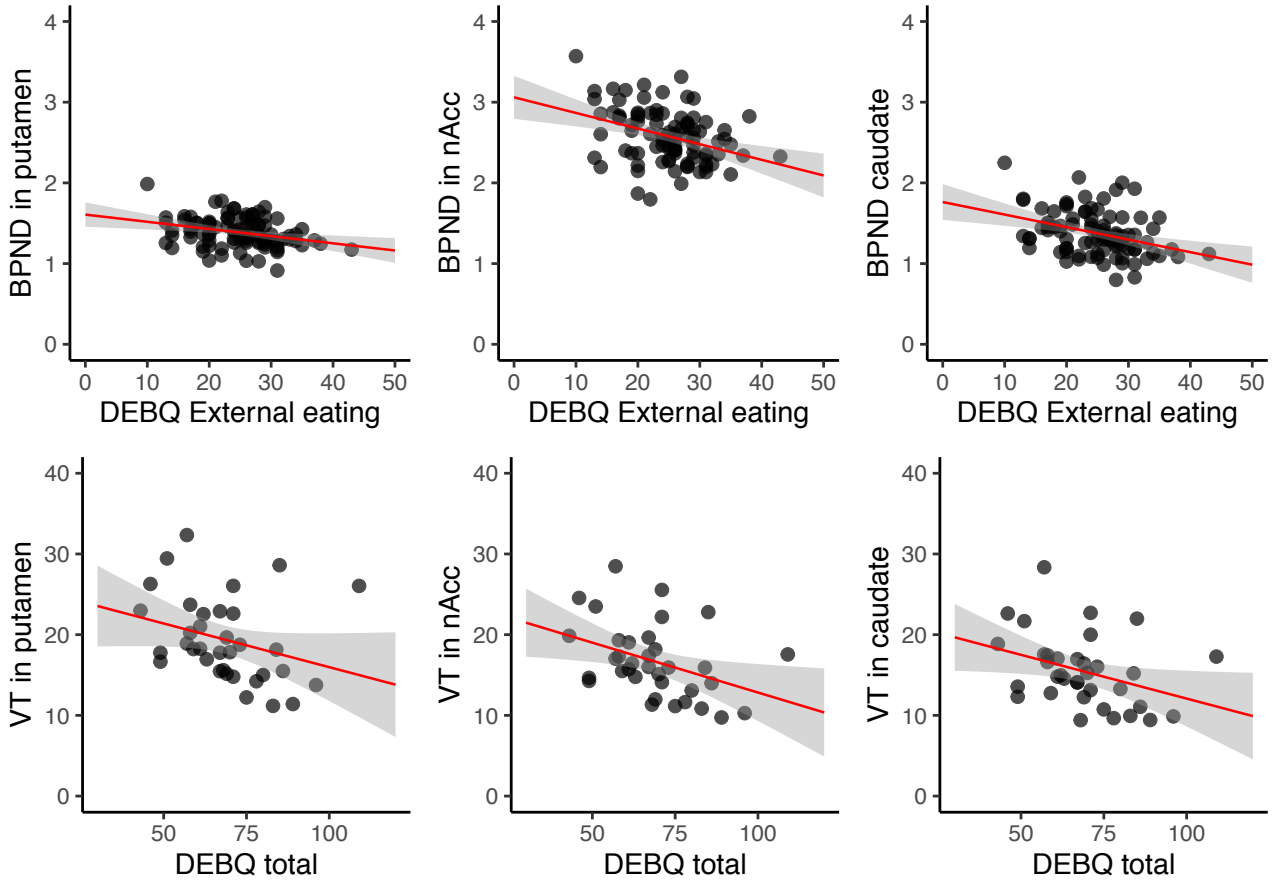

Figure 5. Visualization of regional associations in three representative regions of interest. Upper row: Scatterplots show the associations of External eating score and [^{11}C]carfentanil binding potential (BP_{ND}) in putamen, nucleus accumbens (nAcc) and caudate (92 subjects, LS-regression line with 95% CI). Lower row: Scatterplots show the associations of Total DEBQ score and [^{18}F]FMPEP-d_2 volume of distribution (V_T) in putamen, nucleus accumbens (nAcc) and caudate (35 subjects, LS-regression line with 95% CI).

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Discussion

Our main finding was that higher DEBQ scores were associated with lower central availability of μ-opioid and CB_1-receptors in healthy, non-obese humans. MOR and CB_1R systems however showed distinct patterns of associations with specific dimensions of self-reported eating: While CB_1Rs were associated in general negatively with different DEBQ subscale scores (and most saliently with the Total DEBQ score), MORs were specifically and negatively associated with externally driven eating only. Our results add support to the view that endogenous opioid and endocannabinoid systems underlie interindividual variation in feeding, with distinct effects on eating behavior measured with DEBQ.

Central μ-opioid receptors and external eating behavior

External eating – the tendency to feed when encountering palatable food cues such as advertisements – was associated with lowered MOR availability in cortico-limbic and striatal regions, which are major brain areas processing environmental food cues and mediating reward (49). A bulk of studies have shown that these regions are activated by mere perception of food cues or anticipation of feeding (50-52), and our recent work shows that lowered MOR availability is associated with amplified hemodynamic responses to food images in the same regions (14). Higher score on external eating is associated with increased food craving (53) and cue-induced palatable food intake (35, 54), and may also contribute to short-term weight gain (38). Altogether these results suggest that central MOR system has an important role in modulating particularly this kind of impulsive feeding that may lead to overweight.

Previous PET studies have established that feeding triggers endogenous opioid release in humans (21, 55). Binge eating disorder (BED) is accompanied with downregulated central MORs and high External and Emotional eating scores (56). Morbid obesity is also associated with lowered central MOR availability (21, 22), possibly reflecting receptor downregulation due to repeated

overstimulation following feeding. In minipigs, already 12 days of high sucrose intake and following central endogenous neurotransmitter release downregulates MORs in cingulate and prefrontal cortices, nucleus accumbens and elsewhere in striatum (57). The present findings extend the role of MORs in obesity and eating disorders to different feeding patterns in healthy subjects.

Healthy humans vary considerably in central MOR availability (24), and it is also possible that lowered MOR availability constitutes a genetically determined (58) risk factor for externally driven eating behavior. In healthy humans, trait impulsivity is associated with central MOR availability (25). Increased cue-reactivity is prevalent feature of behavioral addictions (59), and patients with BED and pathological gambling have reduced availability of central MORs as measured with *in vivo* PET (60). It is thus possible that subjects with lower MOR availability are susceptible for increased external eating in modern environment where they are consistently bombarded with feeding cues in advertisements and food shelves in supermarkets (11). However, the present data are purely cross-sectional and longitudinal human studies are needed to further disentangle the causes and the effects between the decrease of MORs in relation to external eating.

Central CB_1-receptors and eating behavior

Higher Total DEBQ score associated with lower availability of central CB_1Rs, and ROI-level modeling suggested a consistent negative association with all DEBQ subscales. Compared with the [^{11}C]carfentanil model, wider posterior distributions reflect the uncertainty arising from smaller [^{18}F]FMPEP-d_2 sample size. Brain's endocannabinoid system is a major homeostatic signaling system, with CB_1Rs abundant in all known food intake regulating central regions (61). In previous brain PET studies, lowered CB_1R availability has been associated with increased BMI (62), while globally upregulated CB_1Rs have been found in anorexia nervosa (63). These opposite phenotypes on body adiposity spectrum could potentially result from corresponding alterations from CB_1R-regulated food intake behaviors. Indeed, stimulation of CB_1Rs by pharmacological agonists or

endocannabinoids promotes food intake and amplifies the rewarding properties of feeding (64). In contrast, antagonism of the CB_1Rs by rimonabant (withdrawn anti-obesity drug, Acomplia) effectively reduces food intake and increases energy expenditure, but in many patients with the cost of psychiatric symptoms (e.g. depressive mood, suicidality, anxiety) (61). Accordingly, the endocannabinoid system function has been connected to several other essential behavioral processes in addition to feeding (e.g. stress-coping, emotion regulation, pain perception) (65, 66). Being this diverse and complex regulatory system, it may not be possible to pinpoint single distinct aspect of food intake behavior mediated by CB_1Rs. Rather, our results add support to central CB_1Rs role in regulation of multiple eating behavior traits, with implications on both homeostatic and hedonic feeding (67).

Limitations and methodological considerations

The [^{11}C]carfentanil data were pooled from three PET scanners, which may produce minor variance in outcome measures (46). However, this was accounted for in the analyses by adding the PET scanner as a nuisance covariate to all full-volume and regional analyses. The sample studied with [^{11}C]carfentanil consisted predominantly of males, and our statistical power was compromised for detecting potential sex differences. Also all subjects of the [^{18}F]FMPEP-d_2 subsample were males, and thus conclusions regarding CB_1Rs might not be generalizable to females. Eating behavior was assessed by self-reports, rather than by direct observations. DEBQ has however been found to successfully identify clinically relevant eating styles (4, 5). Our study had a cross-sectional design, and although we found evidence of eating behavior's association with MOR and CB_1R systems, whether these receptor systems' alterations directly promote future weight gain is to be examined in longitudinal studies. Finally, in a single PET scan it is not possible to determine the exact proportions for causal factors to the altered receptor availability, which could potentially be affected by changes in receptor density, affinity or endogenous ligand binding.

Conclusions

Low cerebral MOR availability is associated with increased externally triggered eating behavior. Modern obesogenic environment may promote food consumption via engaging the opioidergic link of the reward circuit whose tone is linked with this kind of impulsive eating. Central CB_1Rs are in turn associated with multiple eating behavioral traits measured with DEBQ, consistent with endocannabinoid system's role as a major homeostatic regulatory system at the intersection of feeding, emotion and behavior.

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Disclosures

The authors declare no competing financial interests.

Author contributions

TaK: Corresponding and first author, study design, study coordination, data acquisition, data modeling, statistical analysis, interpretation of the results, tables and figures, main writer of the manuscript. ToK: Study design, data modeling, statistical analysis, interpretation of the results, figures, writing of the manuscript. LP: Data acquisition, interpretation of the results, writing of the manuscript. JI: Data acquisition, interpretation of the results, writing of the manuscript. KK: Interpretation of the results, writing of the manuscript. VK: Interpretation of the results, writing of the manuscript. JH: Data modeling, interpretation of the results, writing of the manuscript. PN: Study design, study coordination, interpretation of the results, writing of the manuscript. LN: Study design, study coordination, statistical analysis, interpretation of the results, figures, writing of the manuscript, supervision of the study.

Supplementary Material

Supplementary Material accompanies this paper.

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