Aberrant mesolimbic dopamine–opiate interaction in obesity

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ABSTRACT

Dopamine and opioid neurotransmitter systems share many functions such as regulation of reward and pleasure. μ-Opioid receptors (MOR) modulate the mesolimbic dopamine system in ventral tegmental area and striatum, key areas implicated in reward. We hypothesized that dopamine and opioid receptor availabilities correlate *in vivo* and that this correlation is altered in obesity, a disease with altered reward processing.

Twenty lean females (mean BMI 22) and 25 non-binge eating morbidly obese females (mean BMI 41) underwent two positron emission tomography scans with [^{11}C]carfentanil and [^{11}C]raclopride to measure the MOR and dopamine D2 receptor (DRD2) availability, respectively.

In lean subjects, the MOR and DRD2 availabilities were positively associated in the ventral striatum (r = 0.62, p = 0.003) and dorsal caudate nucleus (r = 0.62, p = 0.004). Moreover, DRD2 availability in the ventral striatum was associated with MOR availability in other regions of the reward circuitry, particularly in the ventral tegmental area. In morbidly obese subjects, this receptor interaction was significantly weaker in ventral striatum but unaltered in the caudate nucleus. Finally, the association between DRD2 availability in the ventral striatum and MOR availability in the ventral tegmental area was abolished in the morbidly obese.

The study demonstrates a link between DRD2 and MOR availabilities in living human brain. This interaction is selectively disrupted in mesolimbic dopamine system in morbid obesity. We propose that interaction between the dopamine and opioid systems is a prerequisite for normal reward processing and that disrupted cross-talk may underlie altered reward processing in obesity.

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Introduction

Endogenous dopamine and opioid neurotransmitters are involved in processing of reward and contribute to the pathophysiology of addiction. Alcohol and cocaine dependent subjects have decreased DRD2 densities (Hietala et al., 1994; Volkow et al., 2012) and increased μ-opioid receptor (MOR) densities (Gorelick et al., 2005; Heinz et al., 2005). Obesity shares many behavioral characteristics with addictions (Volkow et al., 2013) and also involves abnormalities in the brain reward circuitry (Balodis et al., 2013). In contrast to substance addictions, we have recently shown that obesity is associated with decreased MOR availability in the brain, but unaltered DRD2 availability (Karlsson et al., 2015).

Used alone, neither opioid receptor antagonist nor dopamine transporter inhibitor leads to weight reduction. However, when used together these drugs are effective at treating obesity (Greenway et al., 2010), suggesting that interaction of the dopamine and opioid systems might be crucially involved with pathophysiology of obesity. In healthy subjects, these neurotransmitter systems operate in a coordinated manner. The MOR modulates the release of dopamine by disinhibition through GABAergic interneurons in the ventral tegmental area (VTA) (Jalabert et al., 2011). Accordingly, the MOR agonist alfentanil triggers dopamine release in the VST in humans (Hagelberg et al., 2002). Conversely, amphetamine releases endogenous opioids in the VST (Colasanti et al., 2012; Mick et al., 2014), confirming the interdependence of the two systems. Moreover, dopamine and opioid neurotransmitter systems interact not only in the midbrain but also in their projection areas such as the striatum, where medium spiny neurons express both dopamine and opioid receptors (Ambrose et al., 2004; Pollard et al., 1977). Blocking the striatal opioid receptors leads to attenuated amphetamine-induced locomotion and impulsivity (Gonzalez-Nicolini et al., 2003; Wiskerke et al., 2011) whereas dopamine D2 receptor (DRD2) blockade inhibits the rewarding effects of morphine in opiate dependent rats (Laviolette et al., 2002).

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In this study, our aims were twofold. First, we set out to establish regional coupling of the DRD2 and the MOR in healthy human brain using the methods we have developed earlier when exploring regionally dependent interactions of opioid and serotonin systems in vivo in humans (Tuominen et al., 2014). Second, we wanted to test whether coupling between MOR and DRD2 systems is altered in morbid obesity. For these ends, we studied 45 female participants (20 lean, 25 morbidly obese) who underwent PET scans with [11C]raclopride and [11C]carfentanil to quantify the DRD2 and the MOR availabilities, respectively.

Materials and methods

Subjects

20 healthy females (mean BMI 22.3 ± 2.7) and 25 morbidly obese females (mean BMI 41.3 ± 4.1) participated in the study (Table 1). Clinical screening of the subjects included clinical interview, medical history, physical examination, anthropometric measurements, and laboratory tests. Exclusion criteria involved binge-eating disorders (BEDs); current neurological or severe mental disorders; pathological findings in the MRI scan; prescribed opiate drug use; illicit substance abuse and excessive alcohol consumption (>8 units per week).

Group differences in receptor availabilities and demographic characteristics have been previously reported for a subset of the subjects (14 healthy and 13 morbidly obese; (Karlsson et al., 2015)). For the current study, additional six healthy subjects and 12 morbidly obese subjects were included in order to increase statistical power for receptor interaction analyses. All subjects gave written informed consent prior to participation. The study was conducted according to the Declaration of Helsinki and the study protocol was approved by the Joint Ethical Committee of the University of Turku and the Turku University Central Hospital.

Imaging procedures

Imaging procedures have been described earlier (Karlsson et al., 2015). Briefly, each participant underwent two PET scans on separate visits. The scans were on average 3.2 ± 6.3 days apart. The two groups had similar interval between the scans (t-test p > 0.05). Each subject also underwent an MRI scan. A selective dopamine D2/D3 antagonist ligand [11C]raclopride (Farde et al., 1986) was used to quantify dopamine D2-like receptors (DRD2 and DRD3) or DRD2 for short. MOR agonist tracer [11C]carfentanil (Frost et al., 1985) was used to quantify the MOR in the brain. Radiochemical production protocols of both tracers have been previously described (Karlsson et al., 2015).

Both tracers were injected as bolus (258.3 ± 15.7 MBq [11C]raclopride and 251.2 ± 8.4 MBq [11C]carfentanil for healthy subjects, 247.9 ± 20.8 MBq [11C]raclopride and 253.2 ± 11.6 MBq [11C]carfentanil for morbidly obese subjects). Both tracers were given as tracer doses (injected mass of carfentanil 0.08–1.06 μg, injected mass of raclopride 0.12–1.05 μg in the whole sample). There were no group differences in the injected masses of either of the ligands (p < 0.05). Radioactivity of the tracers was measured for 51 min using GE Healthcare Discovery™ 690 PET/CT scanner (General Electric Medical Systems, Milwaukee, WI, USA), providing a 4.7 mm transaxial resolution (Bettinardi et al., 2011). Anatomical MRI scans were acquired with Philips Gyroscan Intera 1.5 T CV Nova Dual scanner to exclude any structural abnormalities and for an anatomical reference.

Image preprocessing

Head motion during the scans was corrected by realigning all the frames in each scan with SPM8 running on Matlab R2012a (The Mathworks Inc., Sherborn, Massachusetts). PMOD 3.4 software (PMOD Technologies Ltd., Zurich, Switzerland) was used to derive time activity curves from reference regions: cerebellar gray matter for [11C]raclopride and occipital cortex for [11C]carfentanil. We used non-displaceable binding potential BP_{ND} (Innis et al., 2007) as a measure of receptor availability. Parametric BP_{ND} maps were calculated with basis function implementation of simplified reference tissue model (Gunn et al., 1997) using in-house software running on Matlab R2013a (The Mathworks Inc., Sherborn, MA, USA). BP_{ND} maps were then transformed into MNI space combining linear transformation from PET image into T1 weighted MR image and nonlinear transformation from MRI to MNI space with SPM8. Transformation of the images into MNI space included reslicing of the images into $2 \times 2 \times 2$ mm^3 voxel dimensions. Normalized parametric maps were smoothed with 8 mm Gaussian kernel. Finally, parametric [11C]raclopride BP_{ND} images were thresholded at >0.5 and parametric [11C]carfentanil BP_{ND} images at >0.2 to reduce spurious correlations in low-signal regions. Mean thresholded BP_{ND} images are shown in the Fig. 1. Masking of parametric [11C]raclopride BP_{ND} images resulted in inclusion of voxels only in the striatum and thalamus for subsequent correlation analyses. Finally, the AAL atlas (Tzourio-Mazoyer et al., 2002) was used to derive average BP_{ND} values for bilateral VST, caudate nucleus, and putamen.

### Normality tests and the effect of antidepressants and smoking

Normality of the BP_{ND} distribution across subjects was first confirmed with Shapiro–Wilk test. [11C]raclopride and [11C]carfentanil BP_{ND} values were normally distributed in the VST and caudate nucleus (p > .05), whereas [11C]carfentanil BP_{ND} values in putamen were not (p = .048) in healthy subjects. In morbidly obese subjects, all BP_{ND} values were normally distributed in the three brain regions (p > 0.05).

Table 1
Characteristics of the participants.

	Morbidly obese subjects (n = 25)		Non-obese subjects (n = 20)		*p*
	M	*SD*	*M*	*SD*	
Age (years)	41.24	9.17	42.00	13.20	0.83
BMI (kg/m^2)	41.30	4.14	22.40	2.62	**<0.001**
Percentage of fat (%)	50.34	3.69	30.57	6.43	**<0.001**
Amount of alcohol use [a]	1.66	1.83	2.89	2.25	0.07
Tobacco smokers (N)	8		0		**<0.001**

M = mean, SD = standard deviation. Last column indicates between-groups differences, significant differences in two-sample t-test are shown in boldface.
[a] In units per week.
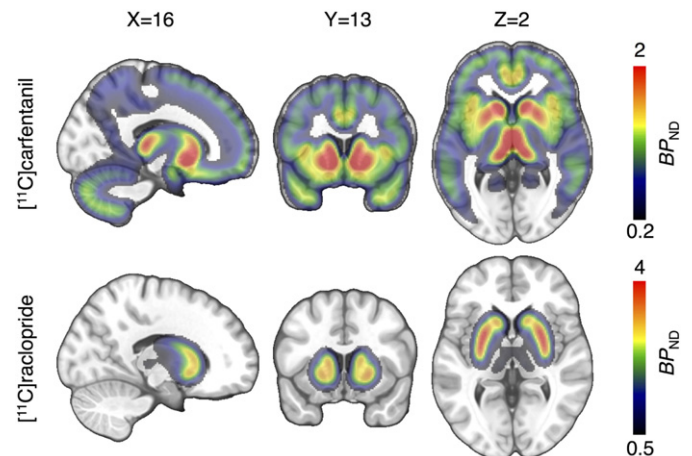
Fig. 1. shows average BP_{ND} values of [11C]carfentanil (top row) and [11C]raclopride (bottom row) in the healthy subjects (N = 20). Numbers on the top indicate MNI coordinates in mm. Colorbar shows the BP_{ND} values thresholded at 0.2 for [11C]carfentanil and at 0.5 for [11C]raclopride.
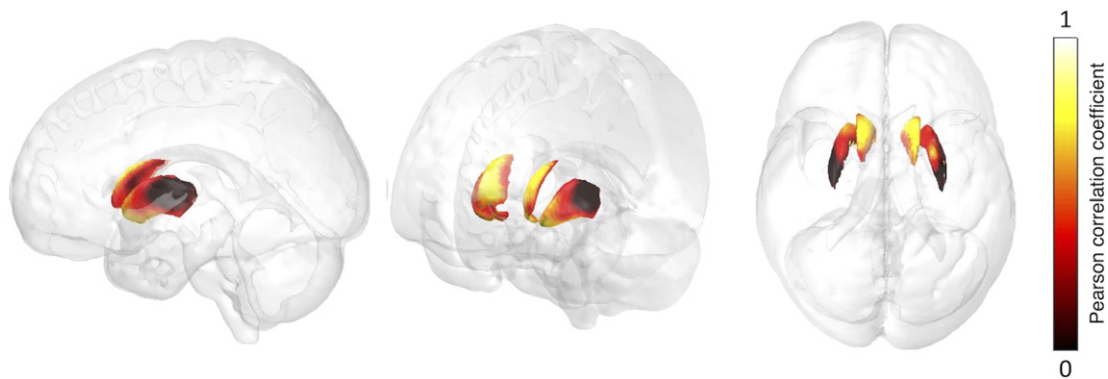
Fig. 2. Voxel-wise correlations between [^{11}C]raclopride BP_{ND} and [^{11}C]carfentanil BP_{ND} in healthy subjects (N = 20). Correlations are high in the ventral striatum and caudate nucleus. Colorbar indicates the Pearson correlation coefficient.

To justify the inclusion of the 8 morbidly obese subjects who smoked tobacco into the study, we compared their [^{11}C]raclopride and [^{11}C] carfentanil BP_{ND} values to those of the non-smoking morbidly obese using two sample t-test. Four morbidly obese subjects who used antidepressant were similarly compared to the morbidly obese subjects who did not use antidepressants. We found no statistically significant effect of smoking or antidepressant use on BP_{ND} values (cluster-level corrected p-value > 0.05). However, smoking as well as the use of antidepressants did have small to medium effects in the ventral striatum, dorsal caudate nucleus and putamen on [^{11}C]raclopride (effect size ranging between 0.27 and 0.66) and [^{11}C]carfentanil (effect sizes between 0.28 and 0.65) BP_{ND} values. That is, in this particular sample, morbidly obese subjects who either smoked or used antidepressants had lower [^{11}C]raclopride and [^{11}C]carfentanil BP_{ND} values in all striatal regions. However, excluding those morbidly obese subjects who smoked or used antidepressants did not change the associations between the tracers seen at the ROI-level (data not shown) and thus all analyses were carried out using all morbidly obese subjects.

Regional interactions between dopamine and opioid systems

In the full-volume analysis within the striatum and thalamus, voxel-wise Pearson correlations were calculated between the [^{11}C]raclopride and [^{11}C]carfentanil BP_{ND} using in-house tools as described earlier (Tuominen et al., 2014). In a complimentary ROI analysis, Pearson correlation was computed between the tracer-wise BP_{ND} values.

Group differences in the interaction between dopamine and opioid systems

Fisher's z-test was used for quantifying whether ROI-level Pearson correlations between the [^{11}C]raclopride and [^{11}C]carfentanil BP_{ND} values were statistically different between morbidly obese and lean subjects.

Interactions between the striatal DRD2 and the whole brain MOR availability

Finally, to test whether regional alterations in striatal DRD2 availability are associated with MOR availability in regions outside the striatum, we used [^{11}C]raclopride BP_{ND} in the VST, dorsal caudate nucleus and putamen to predict, in separate analyses, [^{11}C]carfentanil BP_{ND} in all other brain voxels using linear regression in SPM8.

Group differences in DRD2 and MOR availabilities

To replicate the previous finding (Karlsson et al., 2015) in our extended study sample, voxel-wise group differences in DRD2 and MOR BP_{ND} were compared with independent samples t-tests in SPM8. Statistical significance was set at p 0.05, false discovery rate (FDR) corrected at cluster-level.

Results

Interaction between dopamine and opioid systems

We found that DRD2 and MOR availability were positively associated ($p < 0.05$) in the VST and dorsal caudate nucleus, but not in the putamen, in the voxel-level analysis (Fig. 2). This association was confirmed in ROI-level analysis in the VST (r = .62, p = .0034, df = 18) and in dorsal caudate nucleus (r = .62, p = .0036, df = 18) (Fig. 3). Neither Pearson nor Spearman correlations were significant in the putamen (Pearson's r = .30, p = .19, df = 18).

In morbidly obese subjects, statistically significant positive correlations between the [^{11}C]raclopride BP_{ND} and the [^{11}C]carfentanil BP_{ND} was found only in the caudate nucleus at ROI-level (r = .43, p = .03, df = 23). Correlations in the VST (r = .12, p = .57, df = 23) and
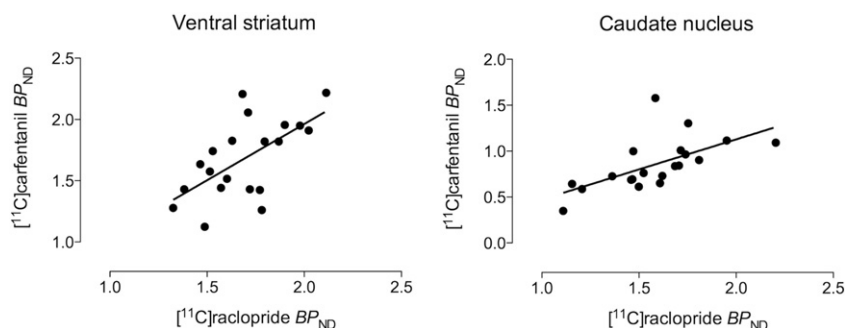
Fig. 3. [^{11}C]raclopride BP_{ND} correlates with [^{11}C]carfentanil BP_{ND} in the ventral striatum and in the dorsal caudatus in healthy subjects.
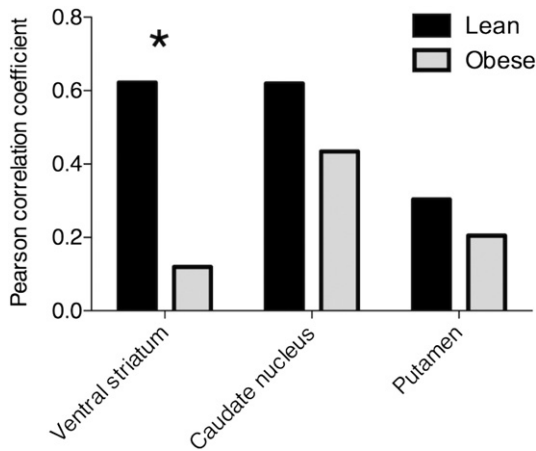
Fig. 4. Obesity lowers the association between [^{11}C]raclopride BP_{ND} and [^{11}C]carfentanil BP_{ND} in the ventral striatum but not in the dorsal caudatus or putamen. Correlations between [^{11}C]raclopride BP_{ND} and [^{11}C]carfentanil BP_{ND} are statistically significant ($p < 0.05$) in the ventral striatum and caudate nucleus in healthy subjects but only in caudate nucleus in the morbidly obese. Asterisk denotes significant between-groups differences (one-tailed Fisher's z-test $p = .031$).

in the putamen ($r = .21$, $p = .33$, $df = 23$) were not statistically significant.

Group differences in dopamine-opioid interaction

Correlation between the [^{11}C]raclopride BP_{ND} and the [^{11}C]carfentanil BP_{ND} in the VST was significantly weaker in the morbidly obese subjects than in the lean subjects (Fisher's $z = 1.872$, one-tailed $p = .031$) (Fig. 4). Correlations in other ROIs were not statistically different between the two groups (Fisher's z-test $p > .05$).

Interactions between striatal DRD2 and whole-brain MOR availability

The DRD2 availability in the VST was differently associated with MOR availability in the brain in healthy and morbidly obese subjects. In healthy subjects, DRD2 availability in the VST was positively associated with the MOR availability in the VST but also in some but not all other brain regions, namely the ventral tegmental area, amygdala, thalamus, insula and in the dentate nucleus of the cerebellum (FDR corrected at cluster-level $p < 0.05$) (Fig. 5). In morbidly obese subjects, DRD2 availability in the VST was not associated with MOR availability in any

brain region. The DRD2 availabilities in the caudate nucleus or putamen were not associated with MOR availability in the brain in neither healthy nor morbidly obese subjects (FDR corrected at cluster-level $p < 0.05$).

*Group differences in DRD2 and MOR availabilities*

Morbidly obese subjects had lower MOR availability across the brain, but unaltered DRD2 availability. MOR availability was lower in the VST, caudate nucleus, putamen, thalamus, cingulate cortex, and in prefrontal and temporal cortices (FDR corrected at cluster-level $p < 0.05$).

Discussion

We show for the first time that DRD2 and MOR availabilities are tightly coupled in healthy subjects in vivo as demonstrated by PET imaging. Subjects who have higher DRD2 availability in the VST and caudate nucleus also have higher MOR availability in these regions. We also found regional differences in dopamine-opioid interaction: the association was strongest in the VST and in the caudate nucleus, and markedly lower and not statistically significant in putamen. This significantly extends prior animal studies that have not described the regional differences in the MOR and DRD2 expression profiles. We propose that the regional coupling of the DRD2 and the MOR is higher in regions where these neurotransmitter systems contribute to similar functions, such as those related to reward processing.

The association between DRD2 and MOR has not previously been demonstrated in vivo in humans, yet these receptors are expressed by the same neurons in the rat striatum (Ambrose et al., 2004; Pollard et al., 1977). The outcome measure BP_{ND} is a product of receptor density and affinity, and we therefore cannot deduce whether the association is driven by dynamic changes in receptor densities or affinities. Especially, changes in MOR conformation between high and low affinity states could affect BP_{ND} because [^{11}C]carfentanil is an agonist tracer that prefers the high-affinity state (Henriksen and Willoch, 2008). However, animal studies have shown that the coexpression of the receptors is interdependent: dopamine agonists upregulate the expression of MOR mRNA (Azaryan et al., 1996), whereas opioid agonists down-regulate DRD2 binding (Brent and Bunn, 1994). We therefore propose that the detected associations in the striatum reflect interdependent coexpression of the DRD2 and the MOR in humans.

The link between DRD2 and MOR availabilities was not limited to the striatum, but DRD2 availability in the VST (but not in caudate nucleus or putamen) also predicted MOR availability in several other brain regions.
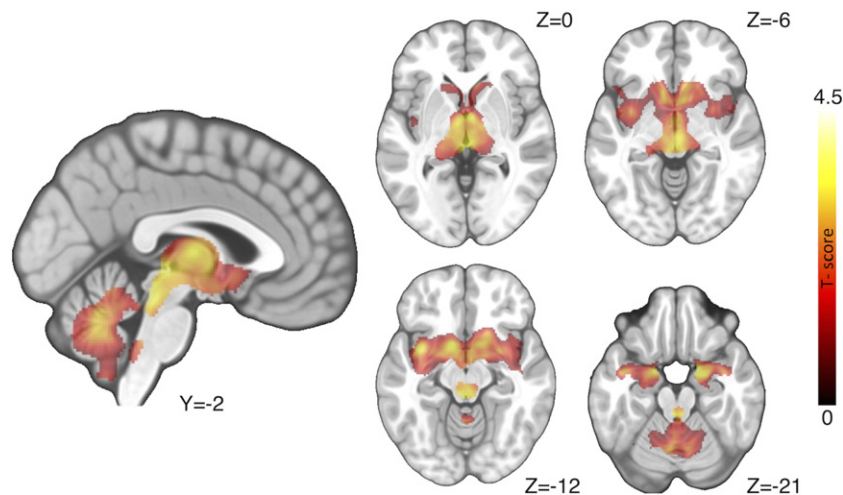
Fig. 5. In healthy subjects, [^{11}C]raclopride BP_{ND} in the ventral striatum predicts [^{11}C]carfentanil BP_{ND} in the ventral striatum, thalamus, midbrain, amygdala, insula and dentate nucleus of the cerebellum, ($p < 0.05$, FDR corrected at the cluster-level). Color bar indicates the T-statistic range. Y and Z denote the MNI coordinates in mm.

Remarkably, one of these regions was the VTA that includes the cell bodies of the dopaminergic neurons projecting to the VST. Our finding implies a close link between disinhibition exerted by MOR on dopaminergic neurons in the VTA and dopaminergic drive to the VST (Jalabert et al., 2011). Other regions found in this analysis (thalamus, amygdala, insula, dentate nucleus of cerebellum) have strong anatomical connectivity with the VST (Morgane et al., 2005) and activate in response to rewards alongside VTA (García-García et al., 2014; Moulton et al., 2014; Tomasi et al., 2014). As an important negative control, DRD2 availability in the VST did not associate with all brain regions that have high MOR availability such as putamen. Furthermore DRD2 availability in the putamen did not predict MOR availability in any region of the brain. Altogether, the findings suggest that dopamine and opioid systems are highly intertwined in the healthy brain and that MOR availability in the reward circuitry associates with the DRD2 availability in the VST.

Interaction between DRD2 and MOR in the morbidly obese subjects

Our second aim was to examine whether the interaction between MOR and DRD2 systems is altered in morbid obesity. We found that obesity is associated with regionally selective disruption of the dopamine-opioid interaction in the VST, whereas in the caudate nucleus the association remains intact. We also found that in morbidly obese, DRD2 levels in the VST are not associated with MOR levels in the VTA or in any other region in the brain. We have previously shown that obesity is associated with decreased MOR BP_{ND} both in the VST and in the dorsal caudate nucleus compared to healthy subjects, but DRD2 BP_{ND} levels in both regions remain unaltered (Karlsson et al., 2015); we replicated this finding here in an extended sample. The overall lower MOR BP_{ND} in the morbidly obese subjects cannot therefore explain the findings of this study. In other words, in the dorsal caudate nucleus, the morbidly obese subjects seem to have decreased MOR levels depending on DRD2s levels, where as in the VST such dependency between the receptors does not exist.

Virtually all drugs of abuse act via stimulating mesolimbic dopamine signaling from the VTA to the VST. Altered engagement of the VST has also been associated with obesity (García-García et al., 2014). For instance, in non-binge-eating obese subjects, hemodynamic responses of the VST are elevated in response to monetary rewards (Balodis et al., 2013). This may be attributed to the higher dopamine signaling in these subjects in response to rewards (Schott et al., 2008). In keeping with this, eating releases dopamine in the striatum (Small et al., 2003). Although, we were unable to detect any changes in DRD2 levels in the striatum in morbidly obese subjects, we found that the link between MOR and DRD2 is disrupted.

MOR modulates the mesolimbic dopamine system at several levels. Blocking MORs in the VST attenuates the behavioral effects of dopamine (Gonzalez-Nicolini et al., 2003; Wiskerke et al., 2011). Also MORs in the VTA modulate of the mesolimbic dopamine system: MOR agonist infused into the VTA causes dopamine release in the VST (Devine et al., 1993) whereas MOR antagonist applied to the VTA diminishes dopamine release in the VST (Spanagel et al., 1992). In addition to VTA, we found that obesity disrupts the link between DRD2 levels in the VST and the MOR levels in the extended reward circuit encompassing thalamus, amygdala, insula and the dentate nucleus of cerebellum. These regions have been implicated in pathological eating behaviors. Obese subjects have increased neural activity in amygdala and thalamus and decreased activity in insula in response to food stimuli (Brooks et al., 2013; Kennedy and Dimitropoulos, 2014) and patients suffering from bulimia have lower the MOR levels in insula (Bencherif et al., 2005). Taken together, we suggest that MOR-dependent modulation of the mesolimbic reward circuit is disrupted at multiple levels in morbid obesity. This may lead to increased release and altered behavioral effects of dopamine in response to eating in obese subjects.

Finally, combining the opioid antagonist naloxone with the dopamine and noradrenaline uptake inhibitor bupropion has shown to be effective in treating obesity (Greenway et al., 2010). The authors suggest that the effects of this combination might be conveyed through modulation of mesolimbic reward pathways. Our findings, i.e. disrupted cross-talk between MOR and the DRD2 in the mesolimbic dopamine pathway in obesity accords with that hypothesis. Whether combined naloxone and bupropion treatment is effective especially for subjects who have aberrant interaction between the two neurotransmitter systems remains to be shown. In the future, measuring altered receptor interactions could be used to develop new drugs and augmentation strategies for treating other brain disorders as well.

Strengths and limitations

The number of subjects in the present study was reasonably high and allowed testing differences in correlation coefficients between the two groups. In comparison with substance abuse, obesity is better suited for studying altered interactions between dopamine and opioid systems, because these alterations cannot be due to direct effects by substances of abuse. We used well-established tracers and modeling to quantify receptor availabilities (Endres et al., 2003; Lammertsma and Hume, 1996). As both tracers have high selectivity for their targets (Andersen, 1988; Cometta-Morini et al., 1992), receptor cross-binding is unlikely to confound the correlation. The affinity of the [^{11}C]raclopride for DRD2 is too low to reliably quantify DRD2 binding potential in extrastriatal regions, and we could not reliably measure the association between MOR and DRD2 outside the striatum and thalamus. Both ligands have excellent test-retest reproducibility (Alakurtti et al., 2011; Hirvonen et al., 2009) and therefore the time interval between the scans should not affect the findings. Moreover, the time interval between the scans did not significantly differ between lean and morbidly obese groups. Eight morbidly obese subjects were light smokers and four morbidly obese subjects used antidepressants. Smoking and antidepressant use can affect dopamine and opioid neurotransmitter systems (Penttilä et al., 2004; Ray et al., 2011; Salokangas et al., 2000). In this study, receptor availabilities of the morbidly obese patients who either smoked or used antidepressants did not differ statistically significantly from those who did not smoke or use antidepressants. However, as the effect sizes ranged from small to medium, this may be only due to the small subsample sizes. Exclusion of the morbidly obese who smoked or took antidepressants did not change the associations seen at the ROI-level and it is therefore unlikely that inclusion of these subjects affected any of the results in this study. Menstrual cycle phase was not controlled for, but it was evenly distributed among the subjects and therefore unlikely affects the results. Finally, studying only females may restrict generalizability of the results to males. For instance, gender-specific differences in the connectivity of the VST have been shown in obesity (Atalayer et al., 2014). Moreover, males are more prone to develop addictions than females (Brady and Randall, 1999). Future studies should therefore examine receptor interaction in males and in other addictive disorders.

In summary, in this study we asked whether the known interactions of the dopamine and opioid systems translate into correlation between the DRD2 and the MOR in vivo. And if so, does morbid obesity disrupt this association. We tested these hypotheses with PET imaging, which is a powerful tool to explore regional interactions between different neurotransmitters. The study showed a robust association between the DRD2 and the MOR availabilities, suggesting coordinated expression that may be crucial for their shared functions. We also demonstrate a disrupted interaction of the mesolimbic dopamine system and the MOR in morbid obesity. We propose that disruption of this interaction may contribute to altered reward processing in obesity, and that mesolimbic dopamine system may be malfunctioning in obesity through changes in opioid system despite unaltered DRD2 levels per se. These findings underline that analyzing interactions between

neurotransmitter systems instead of mere regional receptor availabilities could emerge as powerful strategy for revealing systems-level neurotransmitter alterations in neurological and psychiatric diseases.

Conflict of interest

The authors declare no financial conflict of interest.

Acknowledgments

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