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Effects of leptin and ghrelin on neural cue-reactivity in alcohol addiction: Two streams merge to one river?

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ABSTRACT

Leptin and ghrelin and a "cross-talk" between both hormones were implicated in the pathophysiology of alcohol dependence, both modulating alcohol craving and drug-seeking. To date, the neurobiological mechanisms underlying those effects are still little-known. We thus investigated the effect of leptin and ghrelin on alcohol cue-induced brain response, alcohol craving and relapse risk in alcohol-dependent subjects.

Seventy abstinent alcohol dependent individuals underwent a functional magnetic resonance imaging (fMRI) alcohol cue-reactivity task and patients` alcohol craving was assessed. Plasma levels of leptin, total and acylated, active ghrelin were measured prior to the fMRI session. Additionally, relapse data was collected during a three-month follow-up. Associations between hormone levels, mesolimbic cue-reactivity, alcohol craving and relapse risk were tested.

Leptin levels showed a significant negative association to alcohol cue-induced brain response in the striatum and alcohol craving. In addition, there was a significant effect of leptin on time to first heavy relapse in which higher leptin levels predicted longer times to first heavy relapse. Moreover, positive associations between acylated ghrelin and increased cue-reactivity in bilateral insulae as well as increased craving for alcohol during the fMRI task were revealed.

Leptin and acylated ghrelin show opposing effects on mesolimbic cue-reactivity and alcohol craving. We suspect that the reduced striatal cue-reactivity might be the neurobiological correlate of leptin's effect on relapse-risk. The reported results further support the relevance of appetite regulating hormones in the pathophysiology of addiction and their potential role as future treatment targets.

1. Introduction

Increasing evidence supports the role of appetite-regulating hormones in the pathophysiology of alcohol addiction. Amongstthose, leptin and acylated ghrelin seem to play key roles in mediating craving and relapse (Hirth et al., 2016).

Leptin is secreted by adipose tissue and transported across the blood-brain barrier by a unidirectional transporter (Banks et al., 1996). Studies demonstrated elevated leptin levels during acute withdrawal that were related to increased alcohol craving (Hillemacher et al., 2007; Kiefer et al., 2001), while no positive association was found during protracted abstinence (14 days) (Kiefer et al., 2001). Available data on the time course of leptin levels after withdrawal suggest that leptin levels are increased acutely after withdrawal and decrease over fourteen to thirty days to normal levels (Kiefer et al., 2001; Kim et al., 2013). Persistent elevations of leptin and increases over four to twelve weeks after withdrawal were associated with increased relapse risk (Kiefer et al., 2005). Results of a recent study document that the pathophysiological effects of leptin in alcohol addiction are complex, showing that intravenous administration of ghrelin was associated to increased alcohol craving (Leggio et al., 2014) and a reduction of leptin levels that in turn was negatively correlated with the change in the urge to drink alcohol (Haass-Koffler et al., 2015) (i.e. the percent change in leptin levels from baseline showed a negative association to percent change in the urge to drink alcohol), indicating that increases in ghrelin levels are associated with increased craving, while a reduction in leptin

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levels seem to be associated with reductions in alcohol craving.

Regarding leptin's effects on brain function, animal studies showed that leptin receptors are expressed in extra-hypothalamic sites, such as the ventral tegmental area (VTA), and that VTA neurons that project to the NAc are modulated by leptin (Fulton et al., 2006). Human imaging studies in patients with genetic leptin-deficiency found that a leptin-deficient state was associated with increased mesolimbic food cue-induced brain response, while supplementation of leptin attenuated this hyperactivation (Farooqi et al., 2007). These findings were corroborated by a study of Baicy and colleagues that found a reduction of food cue-induced brain response in the insula and parts of the temporal and parietal cortex by leptin supplementation (Baicy et al., 2007).

Acylated ghrelin is synthesized and secreted as precursor by entero-endocrine cells of the stomach and intestine, and is transformed into its active form by proteolytic cleavage. Increasing evidence supports the positive association of ghrelin and alcohol craving and its role during alcohol withdrawal (for a review, see (Koopmann et al., 2016)). A recent randomized clinical trial of our own workgroup demonstrated that a modulation in the ghrelin system following acute oral water intake reduces alcohol craving during early alcohol abstinence (Koopmann et al., 2017). The majority of preclinical studies suggest that ghrelin receptor antagonists decrease alcohol consumption (Koopmann et al., 2016; Lee et al., 2018), while the intravenous administration of ghrelin enhances alcohol craving in humans compared to a placebo administration (Kaur and Ryabinin, 2010; Leggio et al., 2014). Acylated ghrelin crosses the blood-brain barrier and activates growth-hormone secretagogue receptors (GHS-R1A) that are found in high density in the hypothalamus (Banks et al., 2002), but ghrelin signals have also been identified in mesolimbic dopaminergic pathways (Andrews, 2011), specifically in the VTA, where ghrelin promotes the firing rate of dopaminergic neurons that project to the nucleus accumbens (NAc), the ventral striatum (VS) and further into the prefrontal cortex (PFC) (Abizaid et al., 2008). A very recent functional magnetic resonance imaging (fMRI) study of our workgroup found that acylated ghrelin is positively correlated with alcohol craving and significantly predicts alcohol cue-induced brain response in the VS in recently detoxified alcohol dependent patients, with the effect of acylated ghrelin on craving being mediated via a modulation of cue-induced mesolimbic brain response (Koopmann et al., 2018).

Taken together, preclinical and clinical data thus far indicate that leptin and ghrelin interact with each other and that both modulate the signaling rate of dopaminergic neurons in reward networks. However, their role in alcohol dependence is far from being completely understood.

To address these issues, the current study aims to investigate the association between leptin and acylated ghrelin and mesolimbic brain response to alcohol cues, craving and relapse in alcohol-dependent patients in the post-acute withdrawal phase. We hypothesized that i) higher leptin levels during protracted abstinence are associated with dampened cue-induced brain response, while ii) ghrelin levels are positively associated with brain response. Further we explored effects of both peptides on alcohol craving and relapse risk.

2. Methods

2.1. Study sample

Participants for the study were recruited from the inpatient unit of the Department of Addictive Behaviour and Addiction Medicine at the Central Institute of Mental Health (Mannheim, Germany) as part of a larger German multi-center study. The sub-group of the current sample was recruited during the second recruiting phase at Mannheim site, where processing of blood samples for peptide testing was established. All participants were required to be aged 18 to 65 years and meet the diagnostic criteria for alcohol dependence, according to the Diagnostic Statistical Manual of Mental Disorders (DSM-IV). Subjects that i) met the criteria for any other Axis-I disorder, except from nicotine dependence in the last 12 months (as assessed using a standardized psychiatric interview (SCID-I)), or ii) took any psychoactive substances, anti-craving or anticonvulsive medication within the last months, or iii) had any comorbid severe internal or neurological condition (including liver cirrhosis, viral hepatitis and clinically relevant liver function deficits, i.e. altered clotting), or iv) had a positive drug-screening, or v) had any contraindications for receiving a MRI-scan (e.g. metal implants) were excluded from the study. A mere elevation of liver enzymes (e.g. GOT, GPT) above the respective norm was not considered as an exclusion criterion per se, as increased values are frequently observed in alcohol dependent patients as surrogate of a reversible alcoholic steatohepatitis. Still, participants with elevated enzymes received additional diagnostic check-up (e.g. ultrasound, hepatitis screening) or follow-up blood sampling to rule out any clinically relevant liver pathology. A total of 70 alcohol-dependent patients were included in the study. Breath alcohol was controlled on the day of scanning (and unheralded during inpatient treatment at the CIMH). In addition, unheralded drug urine screenings were conducted and no patient had a positive screening for any substance. The ethics committee of the University of Heidelberg approved all experimental procedures (number of the ethical committee decision: 2011-303N-MA).

2.2. Assessment

All participants completed several questionnaires, including the Beck Depression Inventory (BDI; Hautzinger et al., 2009), the Alcohol Dependence Scale (ADS; Kivlahan et al., 1989), the Obsessive Compulsive Drinking Scale for Alcohol Dependence (OCDS Anton et al., 1995), the Fagerstroem Test for Nicotine Dependence (FTND; Heatherton et al., 1991) and the State Trait Anxiety Inventory (STAI; Spielberger, 1983). To assess craving changes during the cue-exposure task, participants were asked to rate their intention and desire to drink alcohol and their expectancies towards positive effects of alcohol on negative mood using a short questionnaire (the scale for ranged from 0 – "strongly disagree" to 100 – "strongly agree") before and after the cue-reactivity task. Substance use during the 90 days before the experiment was assessed using a semi-structured interview (Form 90; Sobell et al., 1996). Peptide levels were determined from peripheral blood samples that were obtained by venepuncture after 8 h of fasting and anti-coagulated with sodium EDTA, and then immediately cooled on ice (for detailed description see supplementary material).

2.3. Relapse data

During the 3 months following the imaging sessions, patients were contacted monthly by telephone and given a semi-structured interview, which incorporated the Alcohol Timeline Followback (TLFB, Sobell et al., 1996), to obtain information about alcohol consumption. Relapses were considered as heavy-relapse if patients' alcohol consumption was $\geq$ 48 g per day for women and $\geq$ 60 g per day for men. In accordance to previous work, time to relapse to heavy-drinking served as outcome variable in our survival analyses (Bach et al., 2015). Follow-up data was available for 67 participants.

2.4. fMRI alcohol cue-reactivity task

Individual alcohol cue-reactivity was assessed using a visual alcohol cue-reactivity task based on a task that was validated in previous studies (e.g. (Vollstadt-Klein et al., 2012)). Series of alcohol-related and neutral pictures were presented to participants in a pseudo-randomized order using MRI compatible goggles (MRI Audio/Video Systems, Resonance Technology Inc., Los Angeles, CA, USA). The pictures were presented in a block-design (12 alcohol-related blocks and 9 neutral blocks) that consisted of a series of five pictures (alcohol or neutral). Each picture was presented for four seconds. The entire task consisted

of 21 picture blocks and took approximately 12 min in total. Image presentation was controlled using the Presentation® software (Version 16.0, Neurobehavioral Systems Inc., Albany, CA, USA).

2.5. fMRI acquisition, pre-processing and statistical analyses

The fMRI measurement was performed using a Siemens MAGNETOM 3 T whole-body-tomograph (MAGNETOM Trio, TIM technology, Siemens, Erlangen, Germany). A total of 305 T2*-weighted echo-planar images (EPI) were acquired during the alcohol cue-reactivity task with standardized parameters (TR = 2.41 s, TE = 25 ms, flip angle = 80°, 42 slices, slice thickness = 2 mm, 1-mm gap, voxel dimensions 3 × 3 x 3 mm^3, FOV = 192 × 192 mm^2, 64 × 64 in-plane resolution).

In order to reduce artefacts due to magnetic saturation effects, the first five scans were dropped from all subsequent analyses. The remaining MRI data was pre-processed using the statistical parametric mapping software for Matlab (SPM, Wellcome Department of Cognitive Neurology, London, UK) version 5. All images were spatially realigned, corrected for micro-movements in the scanner and normalized to a standard a MNI [Montreal Neurological Institute, Quebec, Canada] EPI template. Subsequently, all data was smoothed using an isotropic Gaussian kernel for group analysis [8 mm Full Width at Half Maximum]). First level statistics were computed for each participant, modelling the different experimental conditions (alcohol-related and neutral picture stimuli) in a generalized linear model. Resulting contrast images ("alcohol – neutral") were imputed in second-level analyses using SPM version 8. One-sample t-tests were applied to investigate alcohol cue-induced brain responses across all participants. In addition, associations between peptide levels (ghrelin, leptin) and cue-induced neural activation were assessed using multiple regression analyses. Brain regions were labelled using the automated anatomical labelling atlas (aal) for SPM (Tzourio-Mazoyer et al., 2002). Previous research indicated associations between BMI and leptin levels (Haass-Koffler et al., 2015), as well as between the BMI and the posttranslational acetylation of ghrelin to its biologically active form (Goebel-Stengel et al., 2013). For this reason, individual BMI was included as a covariate in the multiple regression analyses. In addition, smoking and gender significantly affected ghrelin and leptin levels in past studies (Tomoda et al., 2012). Therefore, both were considered as a covariate in the statistical model. Moreover, past work has suggested that functional cue-induced brain response is influenced by the time that has elapsed since the last alcohol consumption (Fryer et al., 2013). Therefore, abstinence prior to MRI scanning (i.e. time till last alcohol consumption) was also included as a covariate in the analyses. In order to control for multiple comparisons, a combined voxelwise and cluster extent threshold, corresponding to a family wise error (FWE) rate of pFWE < 0.05, was determined using the AlphaSim module of the NeuroElf toolbox (www.neuroelf.net) for Matlab. For a pre-set voxelwise threshold of $p < 0.001$, the AlphaSim procedure determined a cluster extent threshold of 49 voxels (10.000 Monte Carlo simulations, estimated smoothness based on the residual images was x = 12.42 mm, y = 12.27 mm, z = 11.17 mm). All thresholds for the fMRI analyses were set accordingly. In addition to whole brain analyses, mean functional brain activation (contrast: alcohol – neutral) was extracted from brain areas that showed significant positive associations with acylated ghrelin levels, i.e. bilateral insulae and from brain areas that showed significant negative associations with leptin levels, i.e. bilateral caudate. Standard anatomical masks from the Wake Forest University PickAtlas (www.fmri.wfubmc.edu/downloads) were used to define the insula and caudate areas. Mean functional activation was extracted using a custom SPM toolbox that is described in detail by Reinhard and colleagues (Reinhard et al., 2015). Mean functional activation to alcohol cues (contrast: alcohol – neutral") was averaged across all mask voxels and imported into SPSS for further analyses.

2.6. Analyses of demographic data, peptide levels and relapse data

Data analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 24.0. Descriptive results are reported as means and standard deviations (SD). Statistical significance level was set to an α of 5% and corrected for multiple comparisons by the use of false discovery rate (FDR) estimation according to the Benjamini & Hochberg procedure as implemented in MATLAB version R2016b (Benjamini and Hochberg, 1995). Bivariate correlation analyses were implemented to test for associations between clinical variables and plasma peptide levels, and for associations between mean functional alcohol cue-induced activation that was extracted from the region of interests (insula and caudate) and clinical data. Previous research noted a body mass index (BMI) dependent activity of the ghrelin-O-acyltransferase (GOAT), which is responsible for the acylation of ghrelin to its biologically active form (Goebel-Stengel et al., 2013), and a strong association between leptin and BMI (Haass-Koffler et al., 2015). Hence, BMI was considered as covariate in all correlational analyses by: i) performing partial correlations using BMI as covariate, and ii) computing the leptin/BMI ratio (Haass-Koffler et al., 2015) to control for inter-individual BMI differences when analyzing associations between leptin and clinical parameters. Relapse data were analyzed using cox regression models, testing the effects of ghrelin and leptin on time to severe relapse. Cox regression analyses were performed using a stepwise forward selection approach. Based on our a-priori hypotheses, leptin, acylated ghrelin and BMI were included in the Cox-regression model and a separate model was tested implementing leptin/BMI ratio and acylated ghrelin.

3. Results

3.1. Sample characteristics

Demographic data and substance use patterns and the results of psychometric scales assessing levels of depression (BDI), symptoms of state and trait anxiety (STAI), severity of alcohol addiction (ADS), severity of nicotine addiction (FTND) and alcohol craving (OCDS) for all participants are shown in Table 1.

3.2. Hormonal analyses

The mean plasma concentration for total ghrelin was 1050.5 pg/ml (SD = 418.8 pg/ml), 111.5 pg/ml (SD = 67.8) for acylated ghrelin and 10.2 ng/ml (SD = 7.6) for leptin. There was no significant relationship between leptin and total ghrelin (r = -0.006, p = 0.328) or acylated ghrelin (r = 0.049, p = 0.352), but there was a significant association between leptin and BMI (r = 0.543, p < 0.001), while there was no association between acylated ghrelin and BMI (r = −0.171, p = 0.078).

3.3. Associations between hormone levels and clinical data

Leptin plasma levels were negatively correlated with OCDS sum scores (partial correlation, r = −0.305, p = 0.020, p_{FDR} = 0.040, covariate: BMI) and values of the OCDS compulsion subscale (partial correlation, r = −0.296, p = 0.009, p_{FDR} = 0.015, covariate: BMI), while the association with the obsession subscale did not yield significance (partial correlation, r = −0.178, p = 0.079, n.s., covariate: BMI). Corroborating this finding, there was a negative correlation between leptin/BMI ratio and OCDS sum scores (r = −0.211, p = 0.049, p_{FDR} = 0.05) and the OCDS compulsion subscale (r = −0.218, p = 0.038, p_{FDR} = 0.05), while the association with the obsession subscale did not yield significance (r = −0.175, p = 0.080, n.s). For ghrelin, we found a positive association between acylated ghrelin levels and changes in the intention to drink alcohol and the expectancies towards positive effects of alcohol on negative mood, such that higher

Table 1
Demographic and clinical data.

	Total Sample (n = 70) Mean/Median (SD)
Baseline	
Peptides	
Leptin (ng/ml)[#]	10.2/8.5 (7.6)
Acylated Ghrelin (pg/ml)[T]	111.5/104.4 (67.8)
Total Ghrelin (pg/ml)[T]	1050.5/973.0 (418.8)
Demographical variables	
Age (years)	47.3/49.0 (9.6)
BMI (kg/m2)	25.8/25.5 (4.4)
Substance use patterns	
Ethanol (g/day; mean of last 90 days)	196.1/163.6 (178.8)
Heavy-drinking days (%)	74.9/85.5 (28.0)
Abstinent days (%)	20.2/17.4 (25.0)
Drinks per drinking day (number)	20.8/15.1 (17.0)
gGT (U/l, normal range < 100 U/l)	336.8/139.0 (687.4)
GOT (U/l, normal range < 50 U/l)	68.2/46.0 (65.8)
GPT (U/l. normal range < 50 U/l)	63.7/45.0 (60.6)
Abstinence prior to MRI scanning (days)	11.6/9.5 (8.6)
Smoker (yes/no)	46:23
Cigarettes per day (smokers only)	1.7/2.0 (0.9)
Clinical scales	
OCDS (sumscore)	16.4/15.0 (7.1)
OCDS obsession subscale (sumscore)	6.0/6.0 (4.3)
OCDS compulsion subscale (sumscore)	10.5/10.0 (3.6)
STAI (trait sumscore)	45.5/46.0 (12.7)
FTND (sumscore)	5.8/6.0 (2.2)
ADS (sumscore)	16.1/16.3 (6.5)
BDI (sumscore)	15.9/14.0 (10.6)
Follow-up	
Ethanol (g/day; mean of last 90 days)	20.8/1.0 (48.7)
Heavy-drinking days (%)	7.8/0.2 (15.1)
Abstinent days (%)	88.8/98.4 (23.5)
Drinks per drinking day (%)	9.9/6.7 (9.8)

ADS = Alcohol Dependence Scale; BDI = Beck Depression Inventory; FTND = Fagerstroem Test for Nicotine Dependence; OCDS = Obsessive-Compulsive Drinking Scale; STAI = State-Trait-Anxiety Inventory; SD = standard deviation; # = three participants had missing leptin values, [T] = two participants had missing ghrelin values, * = significant deviation from normal distribution $p < 0.05$, Kolmogorov-Smirnov test.

acylated ghrelin levels were associated with an increase in the intention to drink alcohol (r = 0.331, p = 0.006, p_{FDR} = 0.024). The association to the expectancy towards alcohol's effects on negative mood approached significance (r = 0.234, p = 0.041, p_{FDR} = 0.0547). No association with OCDS scores (r = −0.111, $p_{FDR} > 0.05$) was revealed. Furthermore, we found no association between total ghrelin plasma levels and the OCDS scores or craving dynamics during the scanning session (r = −0.061 to 0.034, $p_{FDR} > 0.05$).

3.4. fMRI alcohol cue-induced brain activation

3.4.1. Leptin

Multiple regression analyses controlling for BMI, abstinence prior to scanning, gender and smoking status revealed a significant negative association between leptin plasma levels and alcohol cue-induced activation as the dependent variable in left (77.2% of cluster) and right caudate (18.3% of cluster) (contrast: "alcohol – neutral") (see Table 2a and Fig. 1), with a relevant proportion being located in the dorsal striatum (20.6%), while only a small proportion was located in the ventral striatum (5.1%). In addition, mean alcohol cue-induced activation extracted from bilateral caudate, defined using a standardized anatomical mask from the WFU PickAtlas, negatively correlated with plasma leptin levels (partial correlation = −0.316, p = 0.016, p_{FDR} = 0.040, covariate: BMI) and the leptin/BMI ratio (r = −0.347, p = 0.002), thus corroborating the results of the whole brain analyses.

3.4.2. Acylated ghrelin

Acylated ghrelin showed a significant positive association to alcohol cue-induced activation in several clusters of brain areas, including the bilateral insulae and parts of the superior and middle frontal gyri, as well as the middle cingulum (see Table 2d and Fig. 2), even after controlling for BMI, abstinence prior to scanning, gender and smoking status. The mean functional activation (contrast: "alcohol – neutral") in the left and right insula, defined by anatomical masks, significantly correlated with acylated ghrelin levels (r = 0.279, p = 0.013, p_{FDR} = 0.026)

In addition, data show a significant association between insula activation and craving dynamics during the scanning session, such that higher insula activation was associated with an increase in desire to consume alcohol (r = 0.277, p = 0.016, p_{FDR} = 0.048).

3.4.3. Total ghrelin

There was no significant association between total ghrelin and alcohol cue-induced blood oxygenation level dependent (BOLD) response. Furthermore, total ghrelin plasma levels were not significantly correlated with the cue-induced brain response ($p_{FDR} > 0.05$).

3.5. Relapse to heavy-drinking

Cox regression analyses showed a significant association between leptin and time to heavy-relapse, such that high leptin levels during the post-acute phase of withdrawal (mean abstinence = 11.6 days, range = 5–25) were associated with a longer time to first heavy-relapse (Chi^2 overall model = 4.308, HR = 0.922, 95%CI 0.853 – 0.996, p = 0.039), while acylated ghrelin and BMI did not contribute to the prediction of time to heavy-relapse (p > 0.684). A separate model, testing leptin/BMI ratio and acylated ghrelin, found a significant association only between leptin/BMI ratio and relapse (Chi^2 overall model = 4.560, HR = 0.120, 95%CI 0.015 – 0.996, p = 0.033), supporting the association between leptin and relapse risk. For illustration purposes, survival curves for patients with low (< median) and high (> median) leptin levels are shown in Fig. 3.

4. Discussion

Current findings highlight for the first time that high leptin levels during the post-acute withdrawal phase are associated with decreased cue-induced brain response in the striatum and longer time to first heavy-relapse in alcohol dependent patients. Further, leptin levels were negatively correlated to OCDS scores. The role of the dorsal striatum in drug-seeking and habit-driven behavior is confirmed by previous laboratory and imaging studies (Di Ciano and Everitt, 2001; Vollstädt-Klein et al., 2010). Imaging studies also show that patients relapsing during follow-up had increased cue-induced activation in the dorsal striatum compared to abstinent patients (Grüsser et al., 2004), indicating the relavence of striatal cue-reactivity as phenotype in relapsing alcohol dependent paptients. Our finding of a negative association between leptin and cue-reactivity in the bilateral caudate and striatum is in line with previous evidence that supplementation of leptin attenuates mesolimbic hyper-activation in the NAc, caudate and putamen of leptin-deficient patients (Baicy et al., 2007; Farooqi et al., 2007). The present results also mirror findings of animal studies showing that leptin modulates firing of dopaminergic neurons in the VTA that project to the striatum (Fulton et al., 2006). In addition, leptin causes a reduction of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor and *N*-methyl-D-aspartate receptor-mediated excitatory postsynaptic currents in VTA dopamine neurons (Thompson and Borgland, 2013). Taken together, the negative association between leptin and cue-induced brain response might reflect a protective effect of leptin, such that it attenuates cue-reactivity, potentially reflecting a neural correlate of the reduced relapse risk.

Multiple lines of evidence support the hypothesis that leptin's effects

Table 2
Correlations between leptin, acylated ghrelin and alcohol cue-induced brain activation (contrast: "alcohol - neutral" and "neutral - alcohol", $n = 63$, combined voxel-wise- [$p < .001$] and cluster-extent-threshold [k > 49 voxel], corresponding to $p_{FWE} < .05$).

Side	Lobe	Brain Regions	Cluster Size	MNI Coordinates (x, y, z)			t_{max}
Leptin							
a) negative correlation with alcohol cue-induced brain activation							
L& R		Caudate (left Caudate 77.2% of cluster, right Caudate 18.3% of cluster)	136	−4	6	8	4.36
b) positive correlation with alcohol cue-induced brain activation							
–	–	–	–	–	–	–	–
Acylated Ghrelin							
c) negative correlation with alcohol cue-induced brain activation							
–	–	–	–	–	–	–	–
d) positive correlation with alcohol cue-induced brain activation							
L	Temporal	Insula (67.7% of cluster), Superior Temporal Gyrus, Transverse temporal Gyrus, Rolandic Operculum	226	−40	−20	20	4.62
R	Temporal	Insula (79.3% of cluster), Transverse temporal Gyrus	179	48	−18	14	4.19
R	Frontal	Middle Frontal Gyrus (81.6 % of cluster), Precentral Gyrus	98	−52	12	42	4.12
R	Frontal	Superior and Middle (56% of cluster) Frontal Gyrus	50	26	10	52	3.80
L & R		Middle Cingulum, Supplementary Motor Area	164	0	−6	56	3.67

on drug-craving and drug-seeking behavior seems to depend on the time since withdrawal from drug-consumption. Kiefer et al (2001) reported that intraperitoneal leptin infusion does not alter free-choice ethanol consumption during baseline, but increases alcohol intake in mice after alcohol deprivation for two days. Studies in alcohol dependent patients reported that high leptin levels during acute withdrawal are associated with increased craving (Hillemacher et al., 2007; Kiefer et al., 2001; Kraus et al., 2004), while no positive association was found during protracted abstinence. Data on the time course of leptin levels during protracted abstinence are still scarce, but multiple studies reported increased leptin levels acutely after withdrawal that decreased during abstinence (Kiefer et al., 2001; Kim et al., 2013). Multiple mechanisms have been implicated as basis of those changes, such as a regeneration of ghrelin-producing cells in the stomach, which attenuates leptin levels via ghrelin-induced suppression of leptin (Badaoui et al., 2008), an interaction with the HPA-axis (Kiefer et al., 2001) and changes in alcohol-induced sensitization of body fat tissue (Nicolas et al., 2001). A normal leptin signaling seems to be important to modulate reward-driven behavior and drug-taking. Fulton et al. (2000) reported that the effectiveness of a rewarding electrical stimulation in mice was attenuated by intracerebro-ventricular infusion of leptin (Fulton et al., 2000). In addition, experimental leptin receptor depletion in mice increased VTA dopamine response and cocaine-conditioned place preference in mice (Shen et al., 2016). This finding was complemented by studies showing that intra-VTA administration of leptin attenuates the rewarding effects of cocaine (You et al., 2016). A recent study in alcohol dependent patients demonstrated that an intravenous administration of ghrelin increased alcohol craving compared to placebo (Leggio et al., 2014) and was associated to a reduction in leptin levels (Haass-Koffler et al., 2015) that in turn showed a negative correlation to changes in craving (i.e. the change of leptin from baseline negatively correlated with changes in the urge to drink alcohol).

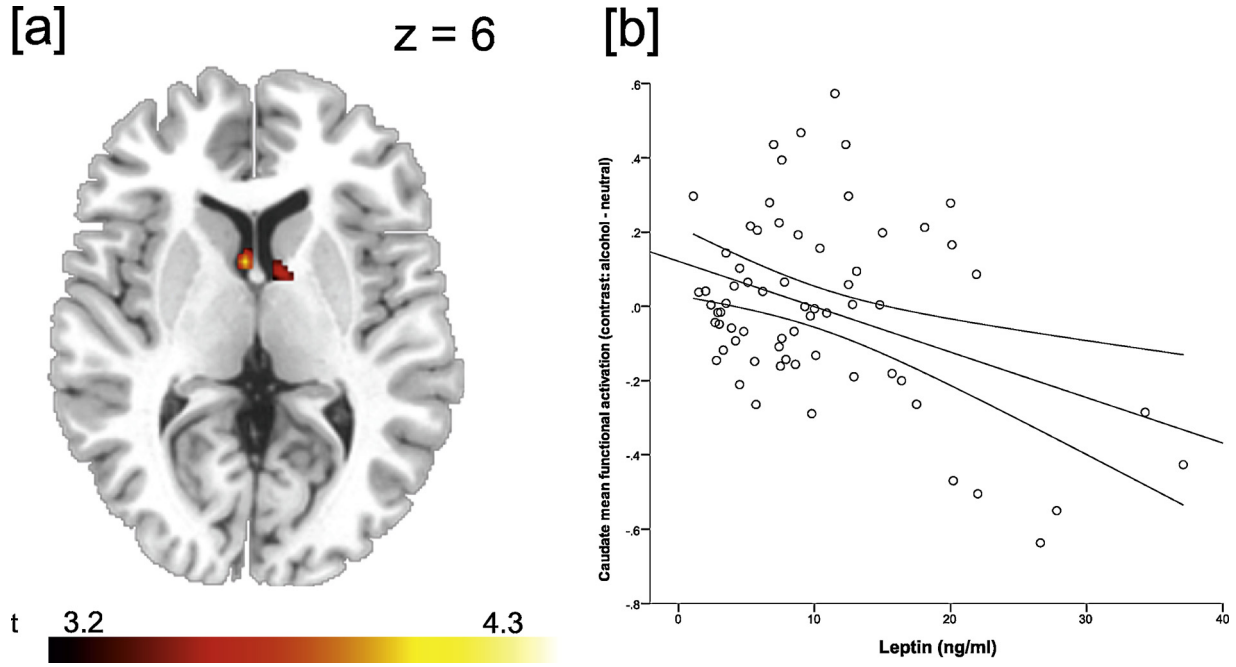

Fig. 1. Depiction of **[a]** brain areas that show a significant negative association between alcohol cue-induced brain response and leptin plasma levels (contrast: "alcohol - neutral", $n = 63$, height-threshold: $p < .001$, extent-threshold: cluster size ≥ 49 voxel, corresponding to $p_{FWE} < .05$) and **[b]** scatterplot depicting the negative association (partial correlation, r = -0.316, p = 0.016, covariate: BMI) between leptin levels and mean functional activation in the caudate (defined by anatomical mask from WFU-PickAtlas).

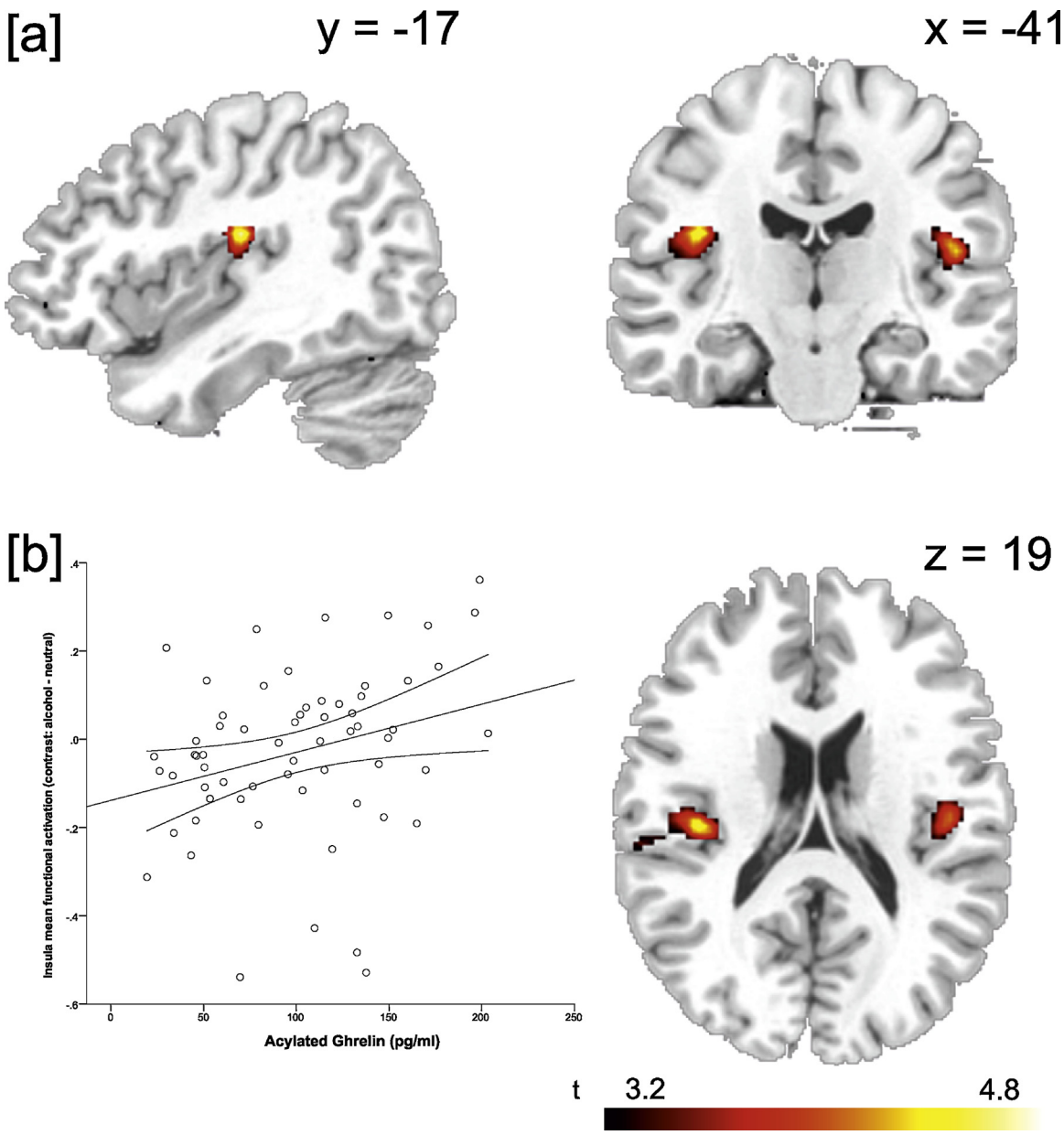

Fig. 2. Depiction of **[a]** brain areas that show a significant positive association between alcohol cue-induced brain response and acylated plasma ghrelin levels (contrast: "alcohol - neutral", $n = 63$, height-threshold: $p < .001$, extent-threshold: cluster size ≥ 49 voxel, corresponding to $p_{FWE} < .05$) and **[b]** scatterplot depicting the positive association (r = 0.279, p = 0.013) between acylated ghrelin levels and mean functional activation in the left and right insula (defined by anatomical mask from WFU-PickAtlas).

Further, studies in leptin-deficient patients showed that leptin supplementation reduces the liking of anticipated food-reward and mesolimbic cue-reactivity (Farooqi et al., 2007). Animal studies also demonstrated that leptin administration reduces stress-induced relapse to heroin-seeking (Shalev et al., 2001). Taken together, existing evidence shows that increased leptin levels during acute alcohol withdrawal are associated with increased craving and that this association is attenuated during protracted abstinence. Further a recent study of Haass-Koffler et al. (2015), found that an intravenous administration of ghrelin lowers leptin, whose change from baseline correlates with ghrelin-induced increase in craving, confirming that leptin is involved in the pathophysiological pathways underlying craving. Current findings of a negative association between leptin levels and OCDS scores during protracted abstinence (mean = 11.6 days) support the idea that normal leptin functioning modulates craving, potentially via attenuation of mesolimbic dopaminergic transmission. We speculate that especially the (OCDS) compulsion component of craving is attenuated by leptin, as indicated by a negative association. In addition, the negative association between leptin and the cue-induced caudate response, a region that has been implicated as key area for goal-directed and habitual responses and that has been related to compulsive behavior in addictive disorders and in obsessive compulsive disorders (Fineberg et al., 2018) might indicate that the effect of leptin on craving and especially on the compulsive component of craving (i.e. OCDS scores) is mediated by an attenuation of caudate cue-reactivity. While this assumption could not be verified in the current study, preclinical evidence that shows an attenuation of hyper-activation in the caudate by leptin supplementation support our speculation (Baicy et al., 2007), but further studies are needed to directly test this hypothesis.

A failing re-instatement of normal leptin levels has been associated with increased relapse risk. Kiefer et al. (2005) showed that alcohol dependent patients who showed an increase in leptin levels during

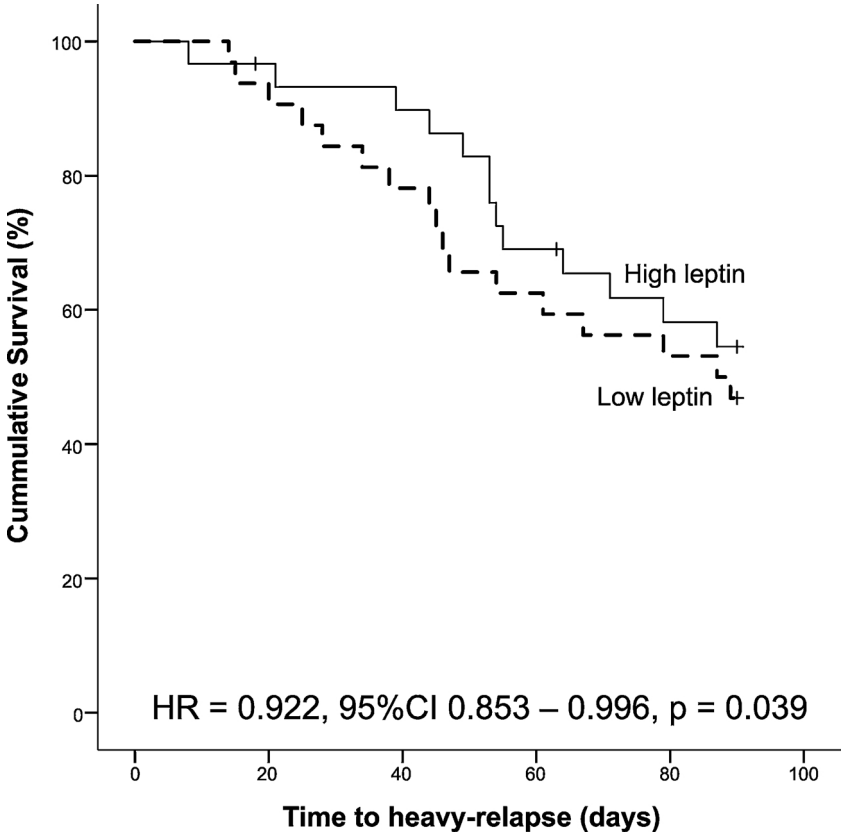

Fig. 3. Estimated survival curves illustrating the association between leptin plasma values and time to first heavy-relapse. For illustration purposes, survival curves are plotted separately for high (> median) and low (< median) leptin values. HR = hazard ratio, CI = confidence interval.

acute withdrawal had a higher risk to relapse to drinking. A persisting increase in leptin levels during abstinence might result in leptin resistance. In line, animal studies reported that leptin application resulted in leptin receptor desensitization in multiple brain areas including the PFC (Del Rio et al., 2016) and a coincident upregulation of dopamine-related genes. A resulting increased dopaminergic tone and lower responsivity to leptin signals might explain increased relapse risk in patients who demonstrate persistent increase in leptin levels during prolonged abstinence. The current study extends previous findings by demonstrating a significant association between leptin and time to first heavy-relapse in alcohol dependent patients during protracted abstinence.

Further, we show a positive association between acylated ghrelin and cue-induced brain response in the left and right insula, and a positive correlation to alcohol craving dynamics during the scanning session. In line with our finding, intravenous administration of ghrelin to healthy volunteers during an fMRI food-cue task, increased brain response in the amygdala, orbitofrontal cortex, insula, and striatum (Malik et al., 2008). Additionally, Farokhnia and colleagues found that an intravenous administration of acylated ghrelin increased alcohol-related signaling in the amygdala and modulated food-related signaling in the medial orbitofrontal cortex and nucleus accumbens in alcohol dependent heavy-drinking individuals during fMRI (Farokhnia et al., 2017). These findings provide further support for an association between BOLD fMRI response and ghrelin levels.

Moreover, clinical studies found that higher ghrelin plasma levels are associated with increased craving in patients suffering from an alcohol dependence (for a review see (Koopmann et al., 2016), although there are some divergent results (Kraus et al., 2005), which did not find such an association. The differences in clinical results concerning the role of leptin and ghrelin in the context of alcohol dependence might possibly be due to the small sample sizes of former clinical studies, very specific study samples, different times (i.e. when the tests were performed in relation to drinking stop), different methods used for determining abstinence and lastly different instruments used to assess craving. Another aspect which must be kept in mind when interpreting the existing clinical results is that in all clinical studies appetite regulating peptides were peripherally analyzed and the relation to concentrations of the hormones in the central nervous system has yet to be fully understood.

Prior studies have also shown that the posterior insula is a location where interoceptive and exteroceptive information from multiple modalities converge. In addition, it was shown that the insula itself is tightly connected with the VTA, the amygdala and the anterior cingulate cortex, forming a “salience detection” network (Chang et al., 2013). In line with this proposition, multiple imaging studies revealed activation of the insula during drug urges (for review see (Droutman et al., 2015). In addition, the insula activation – as in the current study – positively correlated with drug urges, while insula lesions resulted in dramatically reduced craving (Naqvi and Bechara, 2009; Naqvi et al., 2007). Our findings further corroborate recent studies of our workgroup that showed a positive correlation between ghrelin levels and activation in mesolimbic brain areas, such as the ventral striatum, caudate, putamen and, importantly, bilateral insulae (Koopmann et al., 2018). Additionally, the reported data confirms and extends the hypothesis that the effect of ghrelin on alcohol craving is mediated by modulation of mesolimbic cue-reactivity by showing a positive association between insula activation and an increase in desire to consume alcohol during the cue-reactivity experiment. This is compatible with the hypothesis that increased plasma ghrelin levels sensitize reactions to alcohol cues by means of mesolimbic cue-reactivity and alcohol craving. Existing evidence implicated an involvement of leptin and ghrelin pathways in modifying cue-induced alcohol craving (Haass-Koffler et al., 2015; Leggio et al., 2014) and preclinical studies have

already implicated that GHS-R1a antagonists reduce alcohol intake in mice and alcohol preference in mice and prairie voles (Gomez and Ryabinin, 2014; Stevenson et al., 2015, 2016). Importantly, a recent study in heavy drinking individuals provided preliminary evidence that the GHS-R1a inverse agonist PF-5190457 is capable of reducing cue-induced alcohol craving in heavy drinking subjects (Lee et al., 2018). This adds further support to the role of ghrelin and leptin and their respective receptors in the pathophysiology of alcohol cue-induced craving. In addition, as cue-induced alcohol craving represents a risk factor for relapse and heavy drinking, GHS-R1a inverse agonists might be a promising novel treatment for alcohol dependence. Still, further research is needed to test the efficacy of such novel treatment approaches.

4.1. Strengths and limitations

Strengths of the current study are the implementation of a well characterized clinical sample that took no other psychotropic drugs. In addition, the whole-brain analyses of imaging data allowed the detection of significant brain activation without constraints to predefined ROIs. In addition, the consideration of covariates that were indicated by previous research to affect ghrelin or leptin levels (e.g. smoking, gender) facilitates the determination of effects specific to the respective variable. Analyses of clinical data and hormone levels did not consider smoking or FTND values as covariates, because initial analyses indicated no significant differences in hormone levels between smokers and non-smokers and the inclusion of additional covariates would have reduced the explained variance and power of our analyses. In addition, ADS scores were not considered in the analyses as near zero correlations to hormone levels were indicated in our analyses. While this should be considered as potential limitation of the current study, we suggest that our approach represents a reasonable trade-off between internal validity and sufficient power. The current study implemented a set of well-validated and established psychometric scales (e.g. OCDS) and questionnaires (e.g. TLFB). It should be noted that other psychometric tools (e.g. AUDIT) are available for assessing symptoms of alcohol dependence that were not included in the current study, in order not to overburden the participants. We choose to select this specific set of questionnaires because it has been used and validated in previous multi-center studies, in order to facilitate comparability of results. It should be noted that other adipocyte hormones, which take part in appetite regulation, were not measured in the framework of the current study. We focused on the investigation of leptin and ghrelin whose effect on endophenotypes of alcohol dependence (e.g. craving) has been established by multiple recent studies (Haass-Koffler et al., 2015; Leggio et al., 2014). While this should be considered as potential limitation of this work, we suggest that our approach represents a reasonable trade-off, as this study should be a proof of concept of the role of leptin and ghrelin in this context. On the basis of these results future studies should include other adipocyte hormones in their analyses. Presented results are based on regression and correlation analyses. Therefore, data does not allow causal interpretations. However, presented results are in line with and extend the existing literature.

4.2. Conclusion

We are the first to show a significant negative association between leptin plasma levels and alcohol cue-induced brain response in the striatum, as well as lower OCDS scores in alcohol dependent patients. In addition, we demonstrate a significant association between higher leptin levels and longer time to first heavy-relapse. Moreover, we replicate the positive association between acylated ghrelin and increased cue-induced activation in mesolimbic areas, as well as craving for alcohol. To conclude, our results further elucidate the neurobiological effects and interaction between leptin and acylated ghrelin on mesolimbic cue-reactivity, craving and relapse risk.

Conflict of interest

All authors state that they have no conflicts of interests except for the above-mentioned funding.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.psyneuen.2018.09.026.

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