Dopamine-Modulated Aversive Emotion Processing Fails in Alcohol-Dependent Patients

Abstract

Introduction: Negative mood states after alcohol detoxification may enhance the relapse risk. As recently shown in healthy volunteers, dopamine storage capacity ($V_d$) in the left amygdala was positively correlated with functional activation in the left amygdala and anterior cingulate cortex (ACC) during an emotional task; high functional connectivity between the amygdala and the ACC, a region important for emotion regulation, was associated with low trait anxiety. Based on these findings, we now tested whether detoxified alcohol-dependent patients have a disrupted functional modulation of the anterior cingulate cortex activation in response to aversive stimuli by amygdala dopamine. Furthermore, we asked whether disrupted functional coupling between amygdala and ACC during aversive processing is related to trait anxiety.

Methods: We used combined 6-[18F]-fluoro-L-DOPA positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and Spielberger’s state-trait anxiety questionnaire (STAI) in 11 male detoxified alcohol-dependent patients compared to 13 matched healthy controls.

Results: Unlike healthy controls, patients showed no significant correlation between our PET metric for dopamine storage capacity (FDOPA $V_d$), in left amygdala and activation in left ACC. Moreover, the functional connectivity between amygdala and ACC during processing of aversive emotional stimuli was reduced in patients. Voxel-based morphometry did not reveal any discernible group differences in amygdala volume.

Discussion: These results suggest that dopamine-modulated corticolimbic circuit function is important for responding to emotional information such that apparent functional deficits in this neumodulatory circuitry may contribute to trait anxiety in alcohol-dependent patients.

Introduction

Negative mood states frequently occur in alcohol-dependent patients and contribute to the long-term risk of relapse [1]. To date, most studies on negative mood states in alcohol-dependent patients have concentrated on dysfunction of serotonergic neurotransmission and its impact on the processing of emotionally negative stimuli in prefrontal and limbic brain areas [2,3]. The processing of emotional cues is dependent on the concerted activity among subdivisions of a corticolimbic system, which is characterized by top-down control of the amygdala by, e.g., the anterior cingulate cortex (ACC), and other cortical brain regions associated with emotion regulation [4,5]. In a recent multimodal imaging study using positron emission tomography (PET) with 6-[18F]-fluoro-L-DOPA (FDOPA) in conjunction with fMRI, we found that dopamine storage capacity in the amygdala correlates with functional activation of the amygdala and dorsal anterior cingulate (dACC) by an emotional task, and that high functional connectivity between the amygdala and the dACC is associated with low levels of trait anxiety, supporting the hypothesis that the ACC contributes to the regulation of negative mood states [4]. Alcohol acutely alters dopamine transmission in the human brain, and long-term alcohol abuse leads to neuroadaptive changes [6]. The mesolimbic dopamine system is also activated by exposure to stress, and alcohol withdrawal-associated alterations in dopamine release correlate with craving and emotional irritability [6–8]. The aim of this study was to test our hypothesis that dopaminergic modulation of
the dACC during the processing of aversive stimuli is disrupted in detoxified alcohol-dependent patients. Furthermore, we assessed whether a disrupted functional connectivity between the ACC and amygdala contributes to trait anxiety, which is a measure of attentional sensitivity to environmental threats. In an extension of our study of healthy volunteers, we employed functional magnetic resonance imaging (fMRI) to assess brain activation elicited by emotionally negative vs. neutral visual stimuli, in conjunction with FDOPA-PET to measure dopamine storage capacity in detoxified patients suffering from alcohol dependence [4]. We also performed voxel-based morphometry (VBM) to assess whether group differences in molecular and functional imaging results might be confounded by amygdala atrophy [9].

Material and Methods

Subjects and diagnostic screening instruments

We measured differences in amygdala and dACC activity during exposure of the subjects to aversive vs. neutral emotional pictures using blood oxygen level dependence (BOLD) fMRI in 11 alcohol-dependent men with a mean age of 41.9 years [standard deviation (SD) =7, range 32–57 years; all right-handed, medication free]. Patients with a mean of 8.8 years of diagnosed alcohol addiction (SD =9.1) and a mean of 930 kg (SD =846) of total consumed alcohol to the time of the imaging study. Lifetime alcohol consumption was estimated with the lifetime drinking history questionnaire (LDH) [10]. Mean body mass index (BMI) was 25 (SD =3); 9 of 11 subjects were current smokers, consuming a mean of 25 (SD =6) cigarettes per day, and reporting a mean score of 4 (SD =2.5) in the Fagerstrom questionnaire [11]. Smokers stopped smoking for at least 1 h before imaging. No subject reported craving for cigarettes or withdrawal symptoms before or while images were recorded. 6 of 11 subjects were singles, 1 unmarried subject lived in a steady relationship, and 3 subjects lived with their married partner. 5 of 11 subjects graduated after 9 years of school, 3 subjects had 10 years of school, and 2 were university graduates. All but one subject had regular full-time employment. One subject did not give information about family, education and occupational status. Personality assessment with the TCI showed a mean value in the dimensions “novelty seeking” =55 (SD =10), “harm avoidance” = 43 (SD =9) and “reward dependence” =51 (SD =12) (for overview see Table 1). Subjects with other psychiatric disorders according to DSM IV were excluded through the structured clinical interview (SCID) axis I and II [13,14]. Thus, alcohol-dependence was the only diagnosed disorder (SCID I). All smoking subjects had refrained from smoking for at least 1 h before imaging sessions; no subject reported craving for cigarettes or nicotine withdrawal symptoms before or during the imaging sessions. All patients were alcohol abstinent for a minimum of 2 and a maximum of 4 weeks. However, patients were only included after clinical withdrawal symptoms had fully ceased, as attested by an experienced medical doctor. Trait anxiety was measured with Spielberger’s state-trait anxiety questionnaire (STAI) [15]. Onset age of alcohol dependence was recorded according to DSM IV criteria. Abuse of drugs other than alcohol or nicotine was excluded with clinical interviews and urine tests.

The study was approved by the local Ethics Committee according to the Declaration of Helsinki and written informed consent was obtained from all participants after the procedures had been explained. Data for all subjects was acquired within 1½ years. All used methods of data acquisition and analysis as well as hard- and software were identical throughout the complete study.

Imaging data in the healthy volunteers were published in Kienast et al. [4]. The PET and fMRI protocol as well as data processing followed a standard procedure (for detailed information see [4,16,17]). The fMRI paradigm followed a well evaluated procedure as well (for detailed information see [4]).

PET protocol

Using FDOPA-PET, we calculated the steady-state distribution volume of its decarboxylated metabolite [18F]-fluorodopamine (Vd; mL g⁻¹) in the amygdala. All subjects were given carbodopa (100 mg p.o.) less than 1 h prior to PET scanning. Subjects reclined on the scanning bed with their eyes closed and their head comfortably immobilized within the aperture of the Siemens ECAT EXACT PET scanner operating in 3-D mode. Dynamic emission recordings consisted of 28 frames (4 × 1 min, 3×2 min,
3×3 min, 15×5 min, 3×10 min), which were initiated upon intravenous administration of 194 MBq FDOPA. Serial arterial blood samples were collected and the fractions of FDOPA and O-methyl-[18F]-fluoro-L-DOPA (OMFD) in selected plasma extracts were measured by reversed phase. A dual input model was fitted to a cerebellum time-activity curve (TAC) in order to calculate the brain OMFD curve, which then was subtracted voxel-wise from the entire dynamic recording [16]. We next calculated the steady-state cerebral binding of FDOPA together with the decarboxylated metabolites (Vb, mL g⁻¹) in amygdala and ventral striatum [16] regions of interests (ROIs) which were derived from the WFU-Pickatlas SPM toolbox [18] using the AAL atlas [19]. The FDOPA steady-state parameter Vb represents the composite of free FDOPA in brain together with the vesicular pool of the decarboxylated metabolite [18F]-fluorodopamine, along with the diffusible acidic metabolites of [18F]-fluorodopamine. FDOPA Vb can be interpreted as an index of the capacity for vesicular storage of endogenous dopamine, reflecting the “state of readiness” for local impulse-dependent dopamine release [16].

FMRI protocol
Affective stimuli: We presented 18 different negative and 18 different neutral pictures from the international affective picture system (IAPS) to each participant in a randomized event-related design over 750 ms [20–22] in order to evoke emotional reaction with individual control for valence [23]. Valence was rated on a scale extending from “1 = unhappy” to “9 = happy”; neutral pictures were rated 5.8 (SD = 1.1) and negative pictures 2.6 (SD = 2.4). Arousal was rated on a scale from “1 = low” to “9 = high”; neutral pictures were rated 2.7 (SD = 1.4) and negative pictures 5.4 (SD = 2.9). We have previously shown these stimuli to elicit significant FMRI activation in the left prefrontal cortex and ACC, and in the left amygdala of healthy controls [20, 21]. FMRI data were used to calculate the time course of the BOLD signal changes by introducing a random jitter between inter-trial interval and acquisition time, resulting in an equal distribution of data points after each single stimulus. During the inter-trial interval a fixation cross was presented as a fixation condition.

Data acquisition and analysis
For FMRI recordings we used a 1.5 T clinical whole-body scanner for FMRI recordings (Magnetom VISION; Siemens, Erlangen, Germany) equipped with a standard quadrature head coil and shimming filter. For fMRI, 24 slices of 4 mm thickness and 1 mm gap were acquired every 3.3 s. A standard EPI sequence was used with a 3×3×5 mm voxel size, TR= 3300 ms, TE= 66 ms, α= 90° with an in-plane resolution of 64×64 pixels (FOV 220 mm). A morphological 3D T1-weighted magnetization prepared rapid gradient echo (MPRAGE) image data set (1×1×1 mm³ voxel size, FOV 256 mm, 162 slices, TR= 11.4 ms, TE= 4.4 ms, α= 12°) covering the whole head was acquired for anatomical reference. For functional imaging, motion exceeding 1 voxel was an exclusion criterion; altogether, 2 healthy controls and 1 alcohol-dependent patient were excluded due to motion artefacts during fMRI or PET scanning. Finally the evaluable group size for healthy controls was n = 13 and alcoholics was n = 11. Statistical parametric mapping (SPM5) was used for data analysis (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) [24]. The structural image was spatially normalized to a standard template and smoothed. For statistical analysis of the FMRI imaging data, the negative and neutral condition were modeled within the context of the general linear model as explanatory variables after convolution with the hemodynamic response function (HRF) as well as their first temporal derivative on a voxel-by-voxel basis. To detect BOLD response differences elicited by averse stimuli, the contrast images for the contrast ‘negative minus neutral pictures’ of all subjects were included in a second level random effect analysis using a 2-sample t-test. The relationship between FDOPA trapping (Vb) in left amygdala and the BOLD activation revealed by the contrast between negative vs. neutral stimuli was assessed in a separate SPM analysis with Vb as an explanatory variable without further regressors. Correction for multiple comparisons was performed using small volume correction with an amygdala mask from the WFU-Pickatlas SPM toolbox [18] with the AAL atlas [19]. Significance level was always set to p < 0.05 family-wise error (FWE)-corrected for the amygdala region of interest (ROI). All other activations are reported at p < 0.005 uncorrected with a cluster threshold of 5 voxels.

Psychophysiological interaction
To analyze functional connectivity between the left amygdala and other brain regions during affective processing, we used psychophysiological interactions (PPI) as implemented in SPMS [25]. A significant PPI occurs if there is a significant change in the regression coefficient between the 2 compared task-specific conditions (e.g., negative vs. neutral pictures). To create the PPI term, the individual time series of the left amygdala were extracted from a 5 mm sphere around the group peak activation for negative vs. neutral pictures at x = −18, y = −6, z = −18, and deconvolved within a Bayesian framework to generate the neuronal signal for the seed region [26]. The PPI was then defined as the element-by-element product of the neuronal time series, and a vector coding for affectively negative and neutral pictures. To assess areas where this interaction term shows significant effects, single subject statistics were computed which also included as covariates of no interest the psychological and physiological time courses from which the interaction term was derived [27]. These SPM models included all task conditions from the previous single subject statistic with its temporal derivative, the time series of the seed region (physiological variable), and the reconvolved interaction term (psychophysiological variable).

Psychophysiological and clinical correlations
Correlations with the state-trait anxiety questionnaire (STAI-Trait) were computed with PASW Statistics 18 (SPSS, Chicago, IL) by extracting the parameter estimates from the peak voxel of the group difference and using Pearson correlations. To test for group differences in the association between STAI and connectivity measures, an interaction term was computed by multiplying the STAI values with a group factor (healthy controls coded with 1 and alcohol-dependent patients coded as 2). This interaction term was used as a predictor in a linear regression analysis with SPSS.

Voxel-based morphometry (VBM)
Before pre-processing, all structural images were checked for artefacts and reoriented for the center point on the anterior commissure. The pre-processing procedure followed the optimized approach of VBM developed by Good and colleagues [28], using an isotropic 8-mm FWHM (full width at half maximum) Gaussian smoothing kernel [28].
For a comparison of grey matter volumes between alcohol-dependent and healthy subjects, a 2-sample t-test was performed with SPM5. Age and whole brain volumes as confounding covariates were accounted for in the analysis.

**Results**

**Group differences in fMRI and PET results**

Presentation of aversive vs. neutral stimuli elicited a significant BOLD response in the left amygdala of all subjects (x = −18, y = −6, z = −18; t = 3.20; p = 0.021, FWE-corrected for volume of interest) with no significant group difference. For group differences outside the amygdala see Table 2. Amygdala [18F]-fluorodopamine storage capacity (FDOPA Vd) also showed no group difference between alcohol-dependent patients and controls (left amygdala: t = 0.09, p > 0.9; right amygdala: t = 0.31, p > 0.7). Voxel-based morphometry did not reveal alterations in amygdala structure.

There was no significant association between years of diagnosed alcohol addiction or alcohol quantities and FDOPA Vd of left or right amygdala (p > 0.5) as well as STAI trait (p > 0.5) nor between duration of abstinence and FDOPA Vd of left or right amygdala (r = 0.5, p > 0.07), STAI trait (p > 0.05) as well as left amygdala fMRI BOLD effect (p > 0.3). There was also no significant association between the number of cigarettes smoked per day and Vd of left or right (p > 0.4) amygdala, STAI-trait (p > 0.1) as well as left amygdala fMRI BOLD effect (p > 0.05) calculated over all subjects. There were also no significant effects for each group calculated separately.

As expected, the mean magnitude of FDOPA Vd values in the amygdala for each group were substantially lower compared to FDOPA Vd values in the reference region ventral striatum, which is also known as a major region for dopamine storage (alcohol-dependent patients: left amygdala mean FDOPA Vd = 1.17 mL g⁻¹, SD = 0.24, right amygdala mean FDOPA Vd = 1.10 mL g⁻¹, SD = 0.32 and left ventral striatum mean FDOPA Vd = 3.29 mL g⁻¹, SD = 1.20, right ventral striatum mean FDOPA Vd = 2.91 mL g⁻¹, SD = 0.94; healthy controls: left amygdala mean FDOPA Vd = 1.18 mL g⁻¹, SD = 0.19, right amygdala mean FDOPA Vd = 1.35 mL g⁻¹, SD = 0.28 and left ventral striatum mean FDOPA Vd = 2.97 mL g⁻¹, SD = 0.62, right ventral striatum mean Vd = 2.93 mL g⁻¹, SD = 0.59). There was no group difference in FDOPA Vd in the ventral striatum, which was chosen as control region to amygdala (left ventral striatum: t = 0.82, p = 0.43; right ventral striatum: t = 0.05, p = 0.96; Fig. 1).

Correlation between dopamine synthesis capacity in the amygdala and functional amygdala activation

Because of our previous observation of a lateralized correlation between dopamine synthesis capacity and functional brain activation in the left amygdala of the healthy group [4], we tested the association between FDOPA Vd of the left amygdala and BOLD activation in the left amygdala of the group of patients by conducting a

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Whole brain results for group differences between healthy controls and alcohol-dependent patients (AUD) reported at p &lt; 0.005 uncorrected with a cluster extend of more than 5 voxels.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A) Group differences fMRI negative minus neutral pictures</strong></td>
<td><strong>Region</strong></td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>cuneus L</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>cuneus R</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>posterior cingulate L</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>precuneus L</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>precuneus R</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>caudate head L</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>caudate head R</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>cuneus L</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>middle occipital gyrus L</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>lingual gyrus R</td>
</tr>
</tbody>
</table>

**B) Left Amygdala Vd by fMRI BOLD interaction (negative minus neutral pictures)**

<table>
<thead>
<tr>
<th>Region</th>
<th>BA</th>
<th>cluster</th>
<th>T</th>
<th>p(unc)</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls &gt; AUD</td>
<td>dACC/medial frontal Gy. R</td>
<td>6</td>
<td>196</td>
<td>5.01</td>
<td>&lt;0.001</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>inferior parietal lobule L</td>
<td>40</td>
<td>98</td>
<td>3.67</td>
<td>0.001</td>
<td>−51</td>
<td>−36</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>dACC/middle frontal gy. L</td>
<td>6</td>
<td>77</td>
<td>3.39</td>
<td>0.001</td>
<td>−27</td>
<td>−6</td>
</tr>
</tbody>
</table>

**C) Group differences connectivity analysis from left amygdala (2-sample t-test PPI)**

<table>
<thead>
<tr>
<th>Region</th>
<th>BA</th>
<th>cluster</th>
<th>T</th>
<th>p(unc)</th>
<th>X</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls &gt; AUD</td>
<td>dorsal ACC R</td>
<td>32/6</td>
<td>28</td>
<td>3.58</td>
<td>0.001</td>
<td>15</td>
<td>−15</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>dorsal ACC L</td>
<td>32</td>
<td>6</td>
<td>3.16</td>
<td>0.002</td>
<td>−12</td>
<td>9</td>
</tr>
<tr>
<td>Controls &gt; AUD</td>
<td>cuneus R</td>
<td>18</td>
<td>8</td>
<td>3.15</td>
<td>0.002</td>
<td>18</td>
<td>−99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region</th>
<th>BA</th>
<th>cluster</th>
<th>T</th>
<th>p(unc)</th>
<th>X</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUD &gt; control</td>
<td>medial frontal gyrus L</td>
<td>10</td>
<td>15</td>
<td>3.75</td>
<td>0.001</td>
<td>−12</td>
<td>51</td>
</tr>
</tbody>
</table>

*BA = Brodmann area; L = left; R = right; x y z = MNI coordinates; Vd = index of the capacity for vesicular storage of endogenous dopamine; dACC = dorsal Anterior Cingulate Cortex; Gy. = Gyrus.
linear regression with SPSS. Here, the left amygdala FDOPA V_d was the dependent variable, entering the amygdala BOLD activation revealed by the contrast negative compared to neutral stimuli, which was extracted at the peak coordinate from the main effect of healthy controls and alcohol-dependent patients together (x = −18, y = −6, z = −18). The linear regression analysis was controlled for age (because of its known influence on FDOPA V_d) [16], group, and anxiety scores (measured with the STAI). In all subjects, with correction for the effects of age (β = −0.446, p < 0.05) the magnitude of the left amygdala FDOPA V_d was significantly and positively correlated with BOLD activation in the left amygdala (β = 0.453, p < 0.05), while group differences and anxiety effects did not reach significance (group: p = 0.76; anxiety p = 0.18). To test the specificity of our results, we used regression analyses of the left and right ventral striatum FDOPA V_d as a dependent variable and left and right amygdala BOLD main effect in negative vs. neutral stimuli, group, age and STAI as independent variables, which did not show significant effects (all p > 0.2).

Correlation between dopamine synthesis capacity in the amygdala and functional dACC activation

As reported previously, in the healthy control group [4], FDOPA V_d in the left amygdala was positively correlated with BOLD activation in the left dACC in a whole brain analysis for the contrast ‘negative minus neutral pictures’ and including the amygdala FDOPA V_d as a covariate (x = −12, y = −12, z = 39; t = 4.09; p = 0.001 uncorrected). However, no such correlation was observed in alcohol-dependent patients. A significant group by covariate (FDOPA V_d in left amygdala) interaction was observed (x = −18, y = −6, z = 51; t = 3.36; p = 0.002 uncorrected) using a 2 sample t-test with FDOPA V_d as covariate in SPM (Fig. 2a–c), confirming a significant group difference between controls and patients in the correlation between amygdala FDOPA V_d and functional activation of the dACC by aversive vs. neutral pictures. Furthermore, left amygdala V_d correlated also with right dACC and inferior parietal lobule (see Table 2b).
Including number of cigarettes smoked per day as an additional covariate of no interest did not alter this finding ($x = -18$, $y = -6$, $z = 51$; $t = 3.24$; $p = 0.002$ uncorrected) of a group by covariate (FDOPA $V_d$) interaction.

Connectivity analysis between amygdala and dACC and its relation to trait anxiety

Functional coupling between left amygdala and dACC (psychophysiological interaction analysis) was significant only in healthy controls (peak of group difference in dACC: $x = -12$, $y = 9$, $z = 45$, $t = 3.16$, $p = 0.002$ uncorrected, ○ Fig. 2d). Trait anxiety was inversely correlated with the magnitude of this functional connectivity in healthy controls (Pearson's $r = -0.61$, $p = 0.03$, ○ Fig. 2e), but there was no significant correlation in alcohol-dependent patients (Pearson's $r = -0.03$, $p = 0.94$, ○ Fig. 2f). Consistent with the disrupted connectivity between left amygdala and dACC in alcohol-dependent patients, patients were more anxious than controls ($t = 3.1$, $p = 0.005$). To test for group differences in the correlation between amygdala-dACC connectivity and trait anxiety, a group by covariate (i.e., severity of anxiety) interaction was computed (linear regression: $\beta = -0.40$, $p = 0.05$). This indicated that impaired functional amygdala-dACC connectivity in alcohol-dependent patients was associated with higher trait anxiety. Alcohol-dependent patients displayed a higher connectivity from the left amygdala to BA 10 (see ○ Table 2c), but there was no significant correlation between the magnitude of this functional connectivity and trait anxiety in either group (Pearson's correlations between peak voxel of group difference and STAI trait for healthy controls: $p > 0.1$ and alcohol-dependent patients: $p > 0.2$).

Including smoking rate (number of cigarettes smoked per day) into the linear regression analysis revealed no significant effect of smoking status ($p = 0.77$) while the group by covariate (STAI) interaction remained significant ($\beta = -0.54$, $p = 0.01$).

Discussion

We have previously observed that in healthy volunteers, the magnitude of dopamine synthesis capacity (FDOPA $V_d$) in the left amygdala modulates functional activation elicited by aversive stimuli in left amygdala as well as in the left dACC, a brain region associated with emotion control [4,5]. Furthermore, high functional connectivity between the left amygdala and dACC correlated with low trait anxiety in healthy volunteers, suggesting a top-down cortical modulation of limbic processing of aversive emotions [4]. Our data implicate that in alcohol-dependent patients, dopamine synthesis capacity in the left amygdala, although not altered per se, may fail to interact with functional activation of the dACC. Moreover, our data show evidence that the functional connectivity between the amygdala and the dACC may be disrupted in alcohol-dependent patients, who displayed higher levels of trait anxiety compared to healthy controls. Indeed, interaction analysis suggested that impaired functional amygdala-dACC connectivity in alcohol-dependent patients may contribute to their higher levels of trait anxiety. Alcohol dependent patients showed stronger amygdala-BA10 connectivity [20], which may indicate a compensatory mechanism, but which did not correlate with trait anxiety.

Our findings are consistent with dopamine-modulated attribution of salience to negative situations [6], and support the hypothesis that dopamine modulates amygdala and dorsal ACC activation elicited by aversive stimuli. However, our findings also indicate that negative mood states may not be simply associated with the degree of amygdala activation but instead may be specifically strong when there is weak top-down regulation of negative affect due to a reduced functional interplay between limbic and cortical brain areas associated with emotion regulation. Comparable interactions between cortical and limbic brain areas have been reported with respect to serotonergic modulation of amygdala activation in healthy volunteers [20,29]. Again, negative mood states were exacerbated when prefrontal-amygdala connectivity was impaired in patients suffering from major depression, and a high risk for stress-associated negative mood states [30]. Thus, our findings support the hypothesis that negative mood states result from a complex interplay between brain areas associated with emotion representation and regulation [5]. With respect to the regeneration capability of the dopamine system in alcohol-dependent patients, we have to take into account that our findings were observed after 2 weeks of abstinence. Since dopamine function appears to recover during the first weeks of abstinence [31], further studies should assess dopamine function and its interaction with the processing of aversive stimuli at later stages of abstinence.

Several limitations of our study have to be addressed. First, our FDOPA PET endpoint may not be entirely selective for dopamine innervations of the amygdala. Indeed, about one third of the trapping of decarboxylated [3H]-DOPA metabolites in rat amygdala may be dependent on the integrity of the serotonin innervations [32]. However, the magnitude of FDOPA $V_d$ in amygdala relative to that in striatum, about one third, matches the relative activities of tyrosine hydroxylase in rat amygdala and ventral striatum, suggesting a particular association with catecholamine fibers. Second the sample size was rather limited, owing to the problem to scan medication-free detoxified alcohol-dependent patients during early abstinence. Secondly, we had to exclude 2 healthy controls and 1 patient due to motion artefacts so that our study groups consisted of 13 healthy controls and 11 alcohol-dependent patients. Third, there were more smokers in the group of alcohol-dependent patients than in the healthy control group. However, no significant correlations were observed between the number of cigarettes smoked per day and our outcome parameters: i.e., FDOPA $V_d$ of left or right amygdala, STAI-trait as well as left amygdala fMRI BOLD effect. Also the outcome parameters of the observed group differences with regard to the group by covariate interaction of FDOPA $V_d$ amygdala and BOLD activation in the ACC as well as the group by covariate interaction of severity of anxiety and the amygdala-ACC connectivity remained significant after controlling for smoking status. Therefore, it seems unlikely that the effects observed in this study are merely due to smoking status. Furthermore, we have to caution that our earlier findings of a correlation between FDOPA storage capacity and BOLD signal in healthy controls need to be replicated in an independent sample [4]. Finally, only men were included in our study, and gender differences cannot be ruled out. Altogether, our correlation data do not imply causality per se but suggest that alcohol-dependent men may display reduced dopamine-modulated activation of the dorsal anterior cingulate cortex during processing of aversive cues. This mechanism can interfere with cognitive control of aversive cue processing and thus result in higher levels of trait anxiety. In turn, such higher trait anxiety may interfere with the patient's ability to learn from specific negative consequences of drug intake, which is a hallmark of addiction [6,8].
Acknowledgements

None.

Conflict of Interest

Yes: Dr. Gründer has served as a consultant for Astra Zeneca, Bristol-Myers Squibb, Johnson & Johnson, Otsuka and Pfizer. He has served on the speakers’ bureau of Astra Zeneca, Bristol-Myers Squibb, Eli Lilly, Janssen Cilag, Otsuka, Pfizer, Servier and Wyeth. He has received grant support from Aventis, Bristol-Myers Squibb, Eli Lilly, Johnson & Johnson and Pfizer. All other authors have disclosed financial support and other potential conflicts of interest.

Affiliations
1 Department of Psychiatry and Psychotherapy, Campus Charité Mitte, Charité – Universitätsmedizin Berlin, Berlin, Germany
2 Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany
3 Institute of Medical Psychology, Charité – Universitätsmedizin Berlin, Berlin, Germany
4 Department of Nuclear Medicine, Johannes Gutenberg University of Mainz, Mainz, Germany
5 Section of Systems Neuroscience, Department of Psychiatry and Psychotherapy, Faculty of Medicine Carl Gustav Carus, Technical University Dresden, Dresden, Germany
6 Department of Psychiatry and Psychotherapy, RWTH Aachen University, Aachen, Germany
7 Department of Nuclear Medicine and Research Center for Advanced Science and Technology, Tokyo University, Tokyo, Japan
8 Department of Nuclear Medicine, Ludwig Maximilian University, Campus Gro hadern, Munich, Germany
9 Department of Psychology and Neuroscience, Duke University, Durham, NC, USA
10 Equal contributions

References
12 Cloninger CR, Przybeck T, Svrakic D et al. The temperament and character inventory (TCI): a guide to its development and use. Washington University, St Louis, Missouri: Center for Psychobiology of Personality 1994
30 Friedel E, Schlagenhauf F, Sterzer P et al. 5-HTT genotype effect on prefrontal-amygdala coupling differs between major depression and controls. Psychopharmacology 2009; 205: 261–271