Oleoyl ethanalamide and Human Neural Responses to Food Stimuli in Obesity

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IMPORTANCE Obesity has emerged as a leading health threat but its biological basis remains insufficiently known, hampering the search for novel treatments. Here, we study oleoyl ethanalamide, a naturally occurring lipid that has been clearly implicated in weight regulation in animals. However, its role for weight regulation and obesity in humans is still unclear.

OBJECTIVE To investigate associations between plasma oleoyl ethanalamide levels and body mass index (BMI, calculated as weight in kilograms divided by height in meters squared) and functional magnetic resonance imaging response to food stimuli in obese patients and matched control participants.

DESIGN, SETTING, AND PARTICIPANTS Case-control study of 21 obese patients and 24 matched control participants. Obesity was defined as having a BMI of at least 30. The mean age of participants was 40.8 years and BMIs ranged from 18.2 to 47.5.

MAIN OUTCOMES AND MEASURES Interactions between plasma oleoyl ethanalamide levels and obesity on BMI and functional magnetic resonance imaging response to food stimuli.

RESULTS Associations between oleoyl ethanalamide and BMI differed significantly depending on whether individuals were obese or not (P = .02). In obese individuals, oleoyl ethanalamide showed a trend toward a positive correlation with BMI (P = .06, p = 0.42), while this relationship was inverse for nonobese control participants (P = .07, p = −0.34). Similarly, we found significant interactions between oleoyl ethanalamide levels and obesity on food-related brain activation in cortical areas associated with reward processing and interoceptive signaling (P = .009). Specifically, nonobese individuals with higher oleoyl ethanalamide levels had higher insular brain activity (P < .001, p = 0.70); again, the relationship trended to be inverse for obese patients (P = .11, p = −0.36). These effects were not associated with plasma levels of leptin and anandamide, suggesting an independent role of oleoyl ethanalamide in hunger-associated interoceptive signaling. Analysis of food craving during the functional magnetic resonance imaging task suggested that the identified brain areas may be involved in suppressing food-liking reactions in nonobese individuals.

CONCLUSIONS AND RELEVANCE This study suggests that oleoyl ethanalamide-mediated signaling plays an important role for hedonic regulation of food craving and obesity in humans and thus may be a valuable target for developing novel antiobesity drugs.

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Obesity has reached a prevalence of 31% in the United States and has emerged as a leading health threat and major risk factor for type 2 diabetes mellitus, cardiovascular disease, and hypertension. It has recently been classified as a disease by the American Medical Association to direct public attention and research funding toward obesity and improve current therapy options. Despite the significant increase in prevalence during the last 40 years, there is still progressive weight gain in the United States, Western Europe, and other industrialized nations. At the same time, current obesity treatments lack sufficient efficacy and are complicated by high relapse rates. Overeating has been associated repeatedly with addiction, both at the behavioral and the biological levels. Despite some controversy, there is growing consensus that both conditions share common neural underpinnings.

The biological mechanisms that modulate hunger and feeding behavior involve the satiety molecule, oleoylethanolamide, a naturally occurring lipid and agonist of peroxisome proliferator-activated receptor alpha (PPAR-α) that plays an important role for the absorption, storage, and use of dietary fat. Oleoylethanolamide is expressed in astrocytes, neurons, and cells of the small intestine, where levels are modulated by feeding status. More specifically, intestinal oleoylethanolamide mobilization is induced selectively by dietary fat using dietary oleic acid as a substrate, which suggests that oleoylethanolamide acts as a sensor linking fat ingestion to satiety. Consistent with this, oleoylethanolamide administration has been shown to reduce food intake and body weight and increase across-meal satiety in rodents. Because food intake is not affected by the injection of oleoylethanolamide into rat brain vesicles and anorexic oleoylethanolamide actions are prevented by the removal of peripheral sensory fibers, oleoylethanolamide action on feeding regulation is thought to be induced peripherally. Although it has been suggested that the modulation of lipid metabolism plays a role for the anorexic effects of oleoylethanolamide, available evidence supports oleoylethanolamide action as independent of several gut hormones involved in the regulation of food intake, such as ghrelin, peptide YY, glucagon-like peptide 1, apolipoprotein A-I, and cholecystokinin, as well as corticosterone, leptin, and insulin.

Studies in rodents suggest that central oleoylethanolamide effects are mediated through a parasympathetic feedback mechanism that involves the PPAR-α-dependent modulation of neural signaling in the hypothalamus and higher-order areas of the reward circuitry. Oleoylethanolamide modulates motivation for self-administration of intra-gastric feeding, possibly through normalization of PPAR-α-dependent vagal feedback to the brain. This supports oleoylethanolamide’s homeostatic function for regulating dietary fat intake via vagal-nigro-striatal pathways and suggests a role of the molecule in regulating reward-associated neural processes.

Despite consistent reports of PPAR-α activation and oleoylethanolamide effects in animal models, little is known about their influence on the neural regulation of weight gain and satiety in humans. Here, we explored whether plasma oleoylethanolamide is associated with differential brain responses to food cues in obese individuals. To assess the specificity of our findings for oleoylethanolamide, we additionally measured plasma leptin and anandamide levels. This research may substantially contribute to our understanding of how endogenous activation of PPAR-α is linked to alterations of food-related reward processes in obesity and may therefore aid in the identification of valuable new targets for antiobesity drug development.

Methods

The study population, as well as the functional magnetic resonance imaging (fMRI) task, data acquisition, and preprocessing methods, have been described in more detail in a previous study, where we demonstrated associations between food cue-induced activation in human reward pathways and plasma levels of leptin.

Patients and Control Participants

The study was approved by the Ethics Committee II of Heidelberg University and written informed consent was obtained from all participants. Inclusion criteria for obese patients included the following: between 18 and 65 years of age; a body mass index (BMI [calculated as weight in kilograms divided by height in meters squared]) of 30 or higher (accepted threshold for obesity by the World Health Organization); a waist circumference of less than 150 cm (to fit into the scanner); no history or current diagnosis of psychiatric, neurological, neoplastic, or untreated endocrine illnesses (other than nicotine addiction); no current intake of psychoactive or antiobesity medications; and no history of surgical interventions in the gastrointestinal system (eg, gastric banding) or contraindications to fMRI scanning. Fifty individuals recruited between 2008 and 2010 (24 patients and 26 control participants) were eligible for analysis and included in the study. Fatty acid ethanolamides could not be measured in 5 individuals (3 patients and 2 control participants), resulting in a study population of 21 obese right-handed patients (15 women and 6 men) and 24 healthy, age-matched and sex-matched nonobese, right-handed control participants (16 women and 8 men). Five obese participants and 7 control participants were active smokers.

Laboratory and Mass Spectrometry Analyses

Blood samples were taken immediately prior to scanning (6 hours after a standardized breakfast), anticoagulated with sodium ethylenediaminetetraacetic acid (1 mg/mL of whole blood), and stored at −80°C. Plasma leptin concentrations were measured using a human leptin radioimmunoassay kit (Millipore; detection limit, 0.5 ng/mL).

The fatty acid ethanolamides, oleoylethanolamide and anandamide, were extracted by liquid-liquid extraction from plasma. Proteins were precipitated using an equal volume of cold acetone (~−20°C) containing deuterium-labeled oleoylethanolamide (10 pmol) and anandamide (2.5 pmol) standards followed by centrifugation (10 minutes at 3200g). Supernatants were collected and residual acetone was evaporated un-
der nitrogen. Oleoylethanolamide and anandamide were extracted with chloroform/methanol (2 to 1 vol/vol) and the organic phase was concentrated before the liquid chromatography/tandem mass spectrometry analysis.

Both molecules were measured by isotope-dilution liquid chromatography/tandem mass spectrometry (MRM mode) using an Agilent 1200 high-performance liquid chromatography system (Agilent Technologies) coupled with an API 5000 triple quadrupole mass spectrometer (AB Sciex; ESI+ mode). Samples were injected into a 4-μm Synergi Hydro-RP C18 column (150 × 2 mm; Phenomenex) and eluted using methanol/water gradient with 0.1% formic acid (flow rate of 0.5 mL/min). The limits of quantitation were 0.195 pmol/mL and 0.048 pmol/mL for oleoylethanolamide and anandamide, respectively.

fMRI Tasks

Neuroimaging was performed between noon and 3 PM. Participants had a standardized breakfast of 500 kcal (2093 kJ) 6 hours before fMRI scanning during which we presented 18 blocks of food stimuli and 12 blocks of neutral stimuli during a time frame of 18 minutes, as previously described. Food stimuli contained the following 3 categories: salty high calorie, sweet high calorie, and low calorie (both salty and sweet) and were chosen based on their ability to induce food cravings as evaluated by 44 voluntary participants at our institution. Neutral cues were taken from the International Affective Picture Series, avoiding pictures of food-related items.

Each block consisted of 5 stimuli of the same category shown for 4 seconds each. Food cravings were assessed after each block using a visual analog scale ranging from very weak to very strong (values 0 to 100); missing values were replaced by the median craving across all participants and a given participant’s cravings were computed as the median across food blocks. Grosshans et al provided further methods details.

fMRI Acquisition and Processing

Scanning was performed using a 3-T scanner (Siemens Trio). We acquired 453 whole-brain T2*-weighted echo planar images per participant, with the following sequence specifications: repetition time, 2.41 seconds; echo time, 25 milliseconds; flip angle, 80°; 42 sections; section thickness, 2 mm; 1-mm gap; voxel dimensions, 3 × 3 × 3 mm; field of view, 192 × 192 mm; and in-plane resolution, 64 × 64. Grosshans et al provided further method details.

Preprocessing, spatial realignment, and 8-mm gaussian normalization and subsequent statistical analysis of imaging data were performed with standard procedures implemented in the SPM5 software (Wellcome Department of Cognitive Neurology).

Statistical Analysis

Group comparisons of demographic variables were performed using nonparametric Wilcoxon tests and comparisons between 2 continuous variables with Spearman correlation tests. Multiple regression analysis was applied for the analysis of blood parameters and fMRI data. For blood parameters, BMI, age, obesity grouping, sex, and subthreshold depression symptoms were determined using the Beck Depression Inventory and obesity-related 2-way interactions with the aforementioned variables were considered predictors of oleoylethanolamide levels. We focused on obesity-related interactions for statistical power reasons because these interactions appeared most relevant for the objectives of the present study. In addition to multiple regression estimates, we showed non-parametric correlation coefficients to improve readability because our primary interest for blood parameter analysis was the difference in the association of oleoylethanolamide and BMI between obese patients and control participants, we did not perform adjustments for multiple hypothesis testing.

For fMRI analysis, we considered age, oleoylethanolamide levels, obesity grouping, sex, Beck Depression Inventory scores, and all obesity group-related 2-way interactions with the aforementioned variables as predictors of voxel-wise activity contrasts. Because oleoylethanolamide was correlated with age, the 2 variables were orthogonalized and, owing to nonnormality, orthogonalized oleoylethanolamide levels and Beck Depression Inventory scores were ranked prior to analysis. Peak voxel activity contrasts were exported for an additional exploratory multiple regression analysis of molecules other than oleoylethanolamide and this was conducted using R (http://cran.r-project.org/). To account for multiple hypothesis testing in predefined regions of interest, P values were corrected for the family-wise error rate and P values less than .05 were considered significant.

We preselected brain regions of interest based on a meta-analysis investigating reproducibly activated brain areas in response to food vs nonfood stimuli. We used the Automated Anatomical Labeling Atlas to define an anatomical mask that corresponds to the areas reported by this study, comprising the lateral orbitofrontal cortex (left), insula (bilateral), ventral striatum (left), amygdala (left), parahippocampal gyrus (left), precuneus (right), postcentral gyrus (bilateral, Brodmann area 2), occipital lobe (left), and lingual gyrus (bilateral). Similar to the David et al study, we defined the left ventral striatum based on biological landmarks ranging from x = −4 to −10, y = +6 to +18, and z = 0 to −10. We focused our analysis on the high-calorie food vs nonfood contrast to maximize neural activation in response to food cues and consequently increase the power to detect significant interactions.

Results

The relationship between demographic variables and obesity status are summarized in the Table. Obese patients and control participants were matched for smoking status (P = .75, Fisher exact test). Plasma oleoylethanolamide and anandamide levels did not differ significantly between patients and control participants (P = .26 and P = .09, respectively, Wilcoxon test). Similarly, neither oleoylethanolamide (P = .19, p = 0.20, Spearman test) nor anandamide (P = .06, p = 0.28) were associated with BMI, although the latter showed a clear trend. Beck Depression Inventory scores were significantly higher in obese patients compared with control participants (P < .001, Wilcoxon test; means [SD] of 8.7 [7.6] vs 2.2 [3.1] in
patients and control participants, respectively) but were not associated with oleoylethanolamide levels ($P = .72, \rho = 0.05$, Spearman test).

The association between BMI and oleoylethanolamide differed depending on whether participants were obese or not (interaction $P = .02$, multiple regression, Figure 1). Multiple regression analysis (considering the same covariates as for all serum investigations, see Methods section) showed a trend toward a negative correlation between BMI and oleoylethanolamide in control participants ($P = .07, \beta = -0.11, \rho = -0.34$) while this correlation was positive for obese individuals ($P = .06, \beta = 0.36, \rho = 0.42$). Oleoylethanolamide was associated with anandamide ($P = .03, \beta = 0.29, \rho = 0.31$) and this correlation did not differ between patients and control participants ($P = .41$, multiple regression). In contrast to oleoylethanolamide, associations between anandamide and BMI did not differ significantly between obesity groups (interaction $P = .94$, multiple regression). Similarly, associations between oleoylethanolamide and leptin did not differ significantly between obese patients and control participants (interaction $P = .59$, multiple regression).

To investigate whether the differential association of oleoylethanolamide and BMI in relation to obesity was mirrored by food cue-induced brain activity, we performed a voxelwise analysis of our neuroimaging data in food cue-related brain regions of interest. Multiple regression analysis identified 2 clusters in the right posterior (Montreal Neurological Institute coordinates: $x = 44, y = -12, z = 0$; expected cluster size, 102 voxels) and anterior (Montreal Neurological Institute coordinates: $x = 44, y = 16, z = -4$, expected cluster size, 210 voxels) insula with significant interactions between oleoylethanolamide and obesity group (family-wise error posterior, $P = .009$; family-wise error anterior, $P = .05$; Figure 2). In both insula clusters, the associations between oleoylethanolamide and brain activity were directionally consistent, significant in control participants and nonsignificant in patients. In control participants, activity correlated positively with oleoylethanolamide levels (posterior: $P < .001, \rho = 0.70$; anterior: $P < .001, \rho = 0.67$) and vice versa in patients, although not significantly (posterior, $P = .11, \rho = 0.36$; anterior, $P = .35, \rho = -0.22$). To evaluate the specificity of findings for oleoylethanolamide, we further assessed effects of BMI, anandamide, and leptin and found no significant interaction between these variables, brain activity, and obesity (minimum $P$ value among insular peak voxels, BMI, anandamide, and leptin = .57).

Finally, we evaluated whether oleoylethanolamide levels and activity of the posterior insula peak voxel were differen-

### Table. Overview of Demographic and Molecule Measurements

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Obese Patients</th>
<th>Control Participants</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>43.1 (12.8)</td>
<td>39.0 (11.6)</td>
<td>.35</td>
</tr>
<tr>
<td>BMI</td>
<td>37.2 (5.7)</td>
<td>22.2 (1.8)</td>
<td>NA</td>
</tr>
<tr>
<td>BDI, total score</td>
<td>8.7 (7.6)</td>
<td>2.2 (3.1)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Smoking, No.</td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Oleoylethanolamide, pmol/mL</td>
<td>5.1 (1.5)</td>
<td>4.8 (2.4)</td>
<td>.26</td>
</tr>
<tr>
<td>Anandamide, pmol/mL</td>
<td>1.0 (1.0)</td>
<td>0.6 (0.3)</td>
<td>.09</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>43.8 (77.7)</td>
<td>7.9 (7.5)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviation: BDI, Beck Depression Inventory; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); NA, not applicable.
Differentially associated with food cravings in obesity. While oleoylethanolamide levels and obesity status showed no significant interaction on food craving status \( (P = .89) \), we found an interaction between food cue–induced insula activity and obesity status \( (P = .04, \text{multiple regression, Figure 3}) \). Patients with higher activity had higher cravings \( (P = .02, \rho = 0.50) \), while no significant relationship was observed for control participants \( (P = .70, \rho = -0.08) \).
Discussion

Our study shows that oleoylethanolamide is differentially associated with BMI and brain responses to food cues in obese patients and nonobese healthy volunteers. In control participants, oleoylethanolamide showed a trend toward a negative association with BMI, while obese patients had a significant positive correlation. The directionally opposing associations in obese patients and control participants were consistent with a study investigating the effects of oleoylethanolamide administration on self-administered intragastric food intake in mice exposed to prolonged low- and high-fat diets. While oleoylethanolamide inhibited food intake in low-fat-fed mice, it stimulated food intake in high-fat-fed mice, which mirrored the interaction between oleoylethanolamide levels, BMI, and obesity found in humans in the present study. The association between oleoylethanolamide and BMI is further consistent with PPAR-α's known role in fatty acid catabolism and the interaction between oleoylethanolamide levels, BMI, and obesity found in humans in the present study. The positive association between oleoylethanolamide and BMI in obese individuals is further consistent with the previously reported oleoylethanolamide increases in the stomach and duodenum of obese rodents. Because these studies also reported decreased or unchanged oleoylethanolamide levels in the liver, pancreas, and adipose tissue, the present findings may suggest that oleoylethanolamide levels measured here reflect those in the gut, where oleoylethanolamide's main proposed site of action is located. However, it should be noted that compared with oleoylethanolamide, the fibrate class of antihyperlipidemic drugs are weak PPAR-α agonists and are not known to cause satiety or reduce body weight in humans.

The positive association between oleoylethanolamide and BMI in obese individuals is further consistent with the previously reported oleoylethanolamide increases in the stomach and duodenum of obese rodents. Because these studies also reported decreased or unchanged oleoylethanolamide levels in the liver, pancreas, and adipose tissue, the present findings may suggest that oleoylethanolamide levels measured here reflect those in the gut, where oleoylethanolamide's main proposed site of action is located. However, it should be considered that decreased oleoylethanolamide levels have been observed previously in the blood and subcutaneous fat of patients with type 2 diabetes mellitus and that oleoylethanolamide was not significantly altered between groups in the present study. In contrast to oleoylethanolamide, plasma anandamide levels showed a trend toward an increase in obese individuals, which is consistent with previous findings. The results of the present study were also consistent with the well-known orexigenic effects of some members of the endocannabinoid system. Some of these bind to the endocannabinoid receptor CB₁-R, inhibition of which has shown clinical efficacy for antiobesity treatment. However, clinical use of these agents has been terminated owing to the risk of psychiatric adverse effects. Here, we showed that oleoylethanolamide, which specifically acts via PPAR-α and not endocannabinoid receptors, has an endocannabinoid-independent role in obesity.

Using fMRI, we found oleoylethanolamide levels differentially associated with food cue-induced brain activity in the right anterior and posterior insula in obese patients and nonobese individuals. The insula has been associated with a multitude of biological functions including gustatory and gastrointestinal functions and receives (via the thalamus) ascending sensory projections from the base of the tongue and the gut through the vagus nerve. Neuroimaging studies in humans and nonhuman primates have shown that the primary taste cortex is located in the rostral insula adjoining the frontal operculum. In particular, the right anterior insula is a node for integration of interoceptive processing of sensory information (ie, reflecting an individual's gustatory state) and responds to the rewarding properties of food. In line with the results of the present study, caudal insula activity in response to food images has been shown to be related directly to the body's homeostatic state as indexed by peripheral glucose levels. Also, altered insular activation in response to anticipated and actual food consumption has been observed in obese adolescent girls and in response to visual food cues and taste stimuli in patients recovered from anorexia and bulimia nervosa. Specifically, right anterior insula activity in response to sweet-taste stimuli diminished in women recovered from anorexia nervosa and exaggerated in women recovered from bulimia nervosa, relative to control individuals. These studies may suggest that eating disorders are associated with erroneous interoceptive feedback processing where, as in the case of recovered anorexia nervosa, reduced anterior insula signaling could reflect a state of high satiation. Oleoylethanolamide levels are elevated in the cerebrospinal fluid and plasma of individuals recovered from eating disorders, raising the possibility that abnormal interoceptive signaling may be linked to oleoylethanolamide action in these disorders. Given the altered association between oleoylethanolamide and anterior insula activity in obese patients, it is tempting to speculate that differences in interoceptive hunger signaling also play a role in obesity.

While potential links between the present findings and processes related to food addiction remain speculative, there is a growing body of evidence supporting the importance of insular signaling in addiction and related reward processes. Brain damage to the right or left dorsal posterior insula or the right anterior insula disrupts addiction to cigarette smoking and has subsequently been linked to altered interoceptive signaling perceived as anxiety and tension. The posterior insula provides a cortical image of the physiological condition of the body, whereas the anterior insula has been linked to the mediation of subjective awareness to viscerosensory information. In line with their interacting roles for interoceptive signal integration, we found directionally consistent correlations between food cue–related activity in the anterior, as well as the posterior, insula and oleoylethanolamide levels. Obesity and oleoylethanolamide showed an interaction both on brain activation and food cravings. The directionality of effects within patients suggests that in the identified insula regions, hunger-related interoceptive signals are positively associated with food cue–induced activity and that such association is absent in control participants. Because we
observed positive associations between insular activity and oleoyl-
ethanolamide levels in control participants but not obese patients, it is tempting to speculate that oleoylthanolamide is involved in the suppression of food-liking reactions in control participants and that such processes are altered in obesity. This potential negative feedback between oleoylthanolamide levels and food lik-
ing should be viewed in light of evidence showing a strong re-
duction in postprandial blood levels of oleoylthanolamide lev-
els in nonobese individuals, regardless of whether they are given a palatable or normal meal.35-49 Oleoylthanolamide levels also decrease after a meal in the plasma and several organs of lean rats but increase in the duodenum. These reports speculate that the plasma oleoylthanolamide involved in the suppression of food-liking reactions in control individuals reflects spillover from the duodenum. Conversely, in obese patients where oleoylthano-
lamide levels significantly increased with BMI, such increases might instead lead to desensitization for its ability to suppress food-liking reactions and contribute to hyperphagia. It is in this context that oleoylthanolamide application may have relevance as a pharmacological intervention for treatment of obesity.

The main limitation of the present study was its small sample size and that results have to be further validated. As the present study was of a correlational nature, larger follow-up studies are also required to address the causal relation-
ship between oleoylthanolamide, interoceptive feed-
back control, and food intake in humans. It is of specific interest how oleoylthanolamide mediates obesity-related differ-
ences in brain activation mechanistically and whether regu-
lation of oleoylthanolamide in the human gut mirrors that already observed in rodents. While food intake was controlled for 6 hours prior to fMRI measurements and blood sample col-
lections, future studies should further address dietary hab-
its, possible differences in metabolic rate in obese individu-
als, and longitudinal changes in oleoylthanolamide levels in response to food intake.

Conclusions

This study showed the differential association of plasma oleoyl-
ethanolamide levels with BMI and food cue–induced brain ac-
tivity in obese patients and nonobese individuals. These re-
sults suggested a specific obesity-related neural mechanism that impairs hedonic food regulation and showed that oleoy-
lthanolamide and signaling through PPAR-α receptors play an important role for feeding behavior in humans. Our data furth-
er suggested that these receptors could be valuable targets for development of novel antiobesity drugs targeting PPAR-α, which may not be associated with the psychiatric adverse e-
effects seen for CB, receptor antagonists.

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Critical revision of the manuscript for important intellectual content: All authors.

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Obtained funding: Kiefer, Leweke.

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Study supervision: Grosshans, Vollstädt-Klein, Tost, Meyer-Lindenberg, Leweke.

Conflict of Interest Disclosures: None reported.

Correction: This article was corrected on November 10, 2014, for changes made to the Author Contributions section.

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