Oxytocin Receptor Polymorphism Decreases Midline Neural Activations to Social Stimuli in Anorexia Nervosa

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Oxytocin is a neurotransmitter related to both feeding and social behavior; anorexia nervosa is a psychiatric illness defined by reduced food intake, weight loss, and problems in social perceptions. Oxytocin receptor single nucleotide polymorphisms rs2254298 or rs53576 and neural responses to social stimuli were evaluated in adult women with or recovered from anorexia nervosa using functional magnetic resonance imaging. Carriers of the A allele for OXTR rs2254298 (2 AA and 10 AG) showed significantly reduced activation of portions of the posterior cingulate cortex and medial prefrontal cortex for social stimuli as well as greater negative connectivity between the posterior cingulate and the occipital lobe relative to the GG subjects for rs2254298. Differences in the other OXTR SNP, rs53576, did not result in detectable neural differences in either whole brain or region of interest analyses. Development of a mechanistic, biological model of how social behavior is impacted by mental illness requires linking genes to functional brain activations in disease. This pilot study suggests that in anorexia nervosa, differences related to OXTR SNP rs2254298 may alter neural responses to social stimuli and disrupt the engagement and disengagement of the default mode network.

Keywords: social cognition, fMRI, eating disorders, neuroimaging, self-perception, depression, anxiety, endophenotypes

INTRODUCTION

Anorexia nervosa (AN) is an illness that includes altered perceptions related to body-image and self-esteem in concert with an inability to maintain a healthy body weight. Differences in social behaviors are observed in AN, such as impaired recognition of the emotional content in faces, and reduced mentalization (Oldershaw et al., 2011; Tchanturia et al., 2011; Lang et al., 2016). Neural differences in regions important for social cognitive processing, such as the precuneus, temporoparietal junctions, medial prefrontal cortex, and inferior frontal gyri, have also been reported in this disease (McAdams and Krawczyk, 2011, 2014; Schulte-Ruther et al., 2012; McAdams et al., 2015, 2016), but it remains unclear how genetic differences might impact social brain function in AN.

Starvation is an environmental stressor present in all patients with AN, and it impacts many biological pathways, with effects on both reproduction and metabolism. The oxytocin system promotes social behaviors in mammals, including reproduction, pair-bonding, and maternal-infant attachment (Feldman et al., 2016). The down-regulation of oxytocin systems in response
to the environmental stress associated with starvation may be beneficial as social behaviors are disadvantageous under such conditions. In AN, hypermethylation of the oxytocin receptor has been observed (Kim et al., 2014a; Booij et al., 2015), and reduced oxytocin levels were found in cerebral spinal fluid (Frank et al., 2000).

Polymorphisms of the oxytocin receptor gene (OXTR) in healthy populations have been related to differences in personality characteristics, including neuroticism, self-esteem, anxiety, and autistic traits (Feldman et al., 2016). Differences in these personality traits have also been observed in AN (Cervera et al., 2003; Godart et al., 2003; Anckarsater et al., 2012). Both restrictive and purging eating behaviors have been associated with oxytocin receptor variants in a community sample, with the GG rs53576 genotype associated with both binge-eating and purging behaviors, and the AG/AA rs2253298 genotype associated with lifetime restrictive eating behaviors (Miccoli et al., 2017). Administration of oxytocin to patients with AN is now being explored as a potential treatment (Kim et al., 2014b, c, 2015). In healthy populations, brain structure and function have been shown to differ based on both OXT and OXTR polymorphisms (Inoue et al., 2010; Tost et al., 2010, 2011; Furman et al., 2011; Love et al., 2012; Chang et al., 2014). By examining oxytocin receptor polymorphisms in concert with brain function in women with AN, we evaluate how molecular changes in this pathway impact the neural circuits involved in social perception.

In a pilot study using the Social Attribution Task in 3T fMRI, we found differences in neural responses to social stimuli in 17 women recently diagnosed with AN and 17 healthy control women (McAdams and Krawczyk, 2011). Recently, we found that two polymorphisms of the OXTR, rs53576 and rs2254298, were related to the severity of eating disorder symptoms (Acevedo et al., 2015). Both OXTR rs53576 (minor allele frequency 0.39; range 0.19 to 0.65) and rs2254298 (minor allele frequency 0.21; range 0.10–0.34) are common intron variants whose frequency varies in different populations although the molecular processes impacted by these minor alleles is unknown (Genomes Project Consortium Auton et al., 2015). This study explores how gene environment can interact by considering social brain function in the context of a common genetic polymorphism using a disease model defined by the presence of the environmental stressor of starvation. The goal of the study was to assess whether single nucleotide polymorphisms (SNP) of the oxytocin receptor gene (OXTR) could be mechanistically related to regional differences in functional neural activity in response to social stimuli in women with AN.

METHODS

Participants

A total of 49 female participants (age range 18–47 years) were enrolled and participated in the study from 2009 to 2015. All participants were recruited from the Dallas, TX area, and provided written informed consent to participate in three different protocols approved by the UT Southwestern Institutional Review Board. Only participants able to complete in both an imaging study and the genetic study were included. Two protocols with the same neuroimaging task were conducted sequentially in time: study 1, 14 subjects included here that enrolled in 2009–2011 (McAdams and Krawczyk, 2011), and study 2, 35 subjects included here that enrolled 2012–2015 (McAdams et al., 2015); a third protocol involving the collection of the DNA began in 2010 (Acevedo et al., 2015).

All subjects were interviewed at first visit using the Structured Clinical Interview for DSM-IV disorders (SCID-RV) to confirm history of or current symptoms of AN in these participants. For enrollment in the first and second imaging protocols, different clinical criteria were utilized to determine the degree of recovery from AN. Both studies still required subjects to have a stable or increasing weight for at least 2 months prior to the scan to minimize the chances of neural differences caused by acute effects of starvation or dehydration (McAdams and Krawczyk, 2011; McAdams et al., 2015). Demographic information was also obtained at first interview. Diagnoses of anxiety disorders and subjects with a history of major depression were permissible, but subjects currently meeting criteria for major depression as well as those with current or past substance dependence, current or past bipolar disorders, and current or past psychotic disorders were not eligible for imaging studies. Participants were screened for MRI compatibility. Body mass index was measured the day of the MRI scan.

Assessments

Three clinician-based measures assessed depression, anxiety, and eating disorder symptoms. The Quick Inventory of Depressive Symptomatology (QIDS-CR, Rush et al., 2003) is a 16 question inventory of depressive symptoms. The Structured Interview Guide for the Hamilton Anxiety Scale (SIGH-A, Shear et al., 2001) assessed anxiety symptoms in fourteen categories. The Yale Brown Cornell Eating Disorder Survey (YBC-EDS, Marez et al., 1994; Sunday et al., 1995) is a self-report checklist followed by a 22-statement clinician-administered ED inventory about eating preoccupations and rituals.

Each participant also completed a self-report packet. The Young-Brown Obsessive-Compulsive Symptoms (Y-BOCS, Woody et al., 1995) provided an overall estimate of obsessions and compulsions, including those unrelated to the ED. The 26-item Eating Attitudes Test (EAT-26, Berland et al., 1986) provided a second measure of ED behaviors with subscales for dieting behavior (EAT-D), bulimia behaviors (EAT-B), and oral control behaviors (EAT-O). The 34-item Body Shape Questionnaire (BSQ, Rosen et al., 1996) provided a measure of ED symptomatology related to shape and weight concerns.

DNA Processing

Blood samples were collected from patients and submitted to McDermott Center for Human Growth & Development Human Genetics Clinical Laboratory, located at UT Southwestern Medical Center in Dallas, TX, for processing and isolation of DNA. Samples were quantified using nanodrop and diluted in 96 well plates. The plated samples were then sent for SNP analysis at McDermott Center for Human Growth & Development DNA Sanger Sequencing Core located at UT Southwestern Medical.
中心在达拉斯，美国。两种催产素受体SNP都被预先制作并采购自Applied Biosystems生物技术：rs53576/C和rs2254298/G。

功能性磁共振成像（fMRI）任务

受试者观看视频显示简单形状变化在两种条件下，每个条件下的3秒画面（图1；Schultz et al., 2003; McAdams and Krawczyk, 2011）。在视觉空间条件，这个画面是“挡板车：等重量吗?”和在社会归因条件，这个画面是“人们：朋友吗?”。总共16段视频被拍摄，每段视频为每种条件。每个视频片段分别显示一个圆，一个三角和一个正方形，然后在画面中间的一个白色盒子里移动，这个盒子只能看不掉出来。形状在两种条件下移动不同。小形状在盒子里互相碰撞偶尔在盒子中移动。在“人”条件下，受试者考虑这个视频是否代表友谊。在“挡板车”条件下，受试者判断这些形状的重量大小。

MRI采集

所有视频都是使用3T Philips Achieva MRI扫描仪采集的。功能性图像是在412个条件下采集的，每段视频128s，使用1秒快速梯度回波（EPI）图像序列，该序列对血液氧合水平敏感。表观体积的大小为64x64，像素大小为3.4° × 3.4° × 3 mm。每个序列是在1.5 s的重复时间（TR）和25 ms的回波时间（TE）下，翻转角度为60°。33片轴位图像（3 mm厚，1 mm间隙）被采集。头运动被限制使用泡沫垫。高分辨率MP-RAGE 3D T1加权图像被采集用于解剖定位。每个条件下的功能成像在4个运行中获取。

fMRI任务激活

在统计分析前，处理过程包括将空间论域到MNI标准模板的归一化，以及对已平滑的BOLD信号的归一化。功能性MRI任务数据被分析使用Statistical Parametric Mapping软件（SPM）运行于Matlab 2012，且在SPM和xjView中被查看。一个事件相关设计在17 s内对每个视频进行了分析（事件：人们和挡板车）。一个一般线性模型被用于创建每个事件的对照组。每个对照组被与一个后续的血流动力学反应函数（HRF）在SPM中提供并输入到修改的通用线性模型（GLM）。参数估计值（即，β值）是从这个GLM分析中每个对照组中提取的。结果对每个对照组进行单个受试者的单样本t-检验，并结合创建一个地图。人们—挡板车对照组标识出在社会认知过程中重要的神经区域。人们—挡板车对照组的区域被参与更多的视觉空间分析（阈值：体素-体素p < 0.005，未校正）。

fMRI连接性分析

相关功能连接性被标识使用一般脑成像研究所的工具箱（McLaren et al., 2012）。基于这些区域之间的差异，我们检查了在“人们”和“挡板车”条件下，以人们—挡板车对照组（见结果节）为研究对象的基线种子功能连接性。我们通过使用额外的回归分析来评估人们—挡板车对照组由后部的中间环束（−8，−56，26）和前额皮质（−6，52，−2）与6 mm半径的集群相关。基于功能连接性，我们通过使用额外的回归分析来评估人们—挡板车对照组与定义的种子相关的区域。这些区域通过在默认网络上定位而被选择（Andrews-Hanna et al., 2010）。对于连通性分析，GLM被修改以包括生理学定义的区域，这些区域是由人们和挡板车条件，分别。在该修订后的GLM中，我们计算人们的β值。随后，我们进行了一段两样本t-检验，以评估对照组的体素之间的相关性。接下来，我们对每个条件下的体素对进行两样本t-检验，以评估每个体素对之间的影响。

遗传分析

全脑两样本t-检验在SPM中进行，其中的条件被遗传性分为多种。对于OXTR多态性（阈值：体素-体素p < 0.005，与簇校正的p < 0.05）。

整脑分析

整个脑的两样本t-检验在SPM 8中进行，其中的条件被遗传性分为多种。对于OXTR多态性（阈值：体素-体素p < 0.005，与簇校正的p < 0.05）。为了补充整个脑的分析，参数估计值被分别提取。
subject for individual ROIs from the People—Bumper and Bumper—People contrasts, and exported to SPSS for subsequent t-tests. These t-tests were exploratory, using a standard p < 0.05, without correction for multiple comparisons; effect sizes of any observed differences are reported, as the primary goal is to explore relationships between genetic polymorphisms and brain function.

**Group Comparisons**

Student’s t-tests were used to compare demographic, clinical, and behavioral data, and the beta values were extracted from the ROIs for the two polymorphisms of rs2254298 and rs53576. These analyses were conducted in SPSS software (version 21). Multivariate linear regressions assessed whether individual participants’ clinical symptom scores, including the QIDS, SIGH-A, EAT, BSQ, and BMI, were related to the neural activations within each of the four ROIs, with Bonferroni correction for multiple comparisons (criterion: \( p < 0.05 \)/number of ROIs).

To further consider the effects of weight-restoration on neural function in these regions, the participants were also divided into groups of weight-restored (BMI > 19, \( n = 25 \), \( 6 \) rs2254298A, 19 rs2254298GG) and underweight (BMI < 19, \( n = 24 \), 6 rs2254298A, 18 rs2254298GG). Beta values were extracted for each subject for each of the ROIs with significant differences based on SNP of rs2254298. Analysis of variance was used to determine if effects of weight, genotype, or interactions of these variables could be detected, using Bonferroni correction for multiple comparisons (criterion: \( p < 0.05 \)/the number of ROIs).

**RESULTS**

**Demographic, Clinical, and Behavioral Differences in OXTR SNP**

For rs2254298, there were 12 women who were A carriers (2 AA and 10 AG) and 37 women were GG carriers; this disparate sample size is a limitation of the work. Consistent with our earlier and larger genetic study (Acevedo et al., 2015), the A carriers of rs2254298 showed elevated scores for the Eating Attitudes Test, including the overall EAT, and the subscales for dieting and bulimia (Table 1). No other differences in demographic characteristics of the groups or clinical measures were observed. For rs53576, 27 women were A carriers (5 AA and 21 AG) and 22 women were GG carriers. There were no significant differences related to demographic or clinical assessments when considering rs53576 alone (Supplemental Table 1). This difference from our prior publication may be attributed to the inclusion of 7 individuals that were A carriers for rs2254298 but GG for rs53576 in the GG group, as well as the additional subjects (women with AN that did not participate in the MRI studies) and groups (women with bulimia nervosa and healthy women). No behavioral differences in reaction times or the mean percentage of trials correct responses for either the bumper or the people conditions were observed for the A carriers vs. the GG subjects for either the rs2254298 genotype or the rs53567 genotype using t-tests (criterion of \( P < 0.05 \); Supplemental Table 2).

**Neural Differences Based on Task Conditions**

The task contrasts: People—Bumper and Bumper—People, led to activation of distinct neural circuitry related to social processing and visuospatial analysis (Figure 2). Six regions were activated by the People—Bumper contrast (red) and included the bilateral fusiform gyri, bilateral middle temporal gyrus and inferior frontal gyri, the precuneus, and medial prefrontal cortex. Five regions were activated by the Bumper—People contrast (blue) and included bilateral parietal regions, bilateral middle frontal gyrus, the middle cingulate extending into the dorsal anterior cingulate, and the occipital lobe. Similar results related to activations in this task were previously published, data related to 14 of the participants reported here (McAdams and Krawczyk, 2011). Here, we include additional data from 35 women with AN from the next protocol; this data has not previously been published.

**Neural Activation and Connectivity Differences for rs2254298**

First, in the whole-brain comparison for group differences based on rs2254298, a region of the posterior cingulate cortex extending into the precuneus and a region in medial prefrontal cortex were modulated differently based on SNP for rs2254298 in the People—Bumper contrast (Table 2, Figure 3). Extraction of the \( \beta \) values from this cluster indicated reductions in activation during the People—Bumper contrast for A carriers compared to the GG carriers.

Second, to further examine the impact of this OXTR SNP on task-related neural responses, we extracted \( \beta \) values from all of the regions showing task differences (Figure 2), and compared activation within each region based on the rs2254298 genotype (Table 2). One region from the People—Bumper contrast, the precuneus, showed increased activity in the GG subjects relative to the A carriers and one region from the Bumper—People
FIGURE 2 | Cortical regions differentially activated by task condition. Areas more active during the People condition are shown in red, areas more active during the Bumper condition are shown in blue.

TABLE 2 | Whole-brain two-sample t-test regions related to fMRI task effects and genotype for rs2254298.

| Condition and Region | Neural ROI Characteristics | Group Comparisons<sup>a</sup> |
|----------------------|---------------------------|-------------------------------|
|                      | Volume (mm<sup>3</sup>)  | Cluster size | Cluster pFWE | Peak Z | MNI Coordinates | GG | GA/AA | T  | p    | Cohen’s d |
| EFFECT OF CONDITION: PEOPLE–BUMPER | | | | | | | | | | | |
| Right Temporal       | 61,184                    | 956           | 0.000        | 52    | −40             | 4  | 1.06 (0.57) | 0.95 (0.21) | 1.00 | 0.32 | n.s.      |
| Left Temporal        | 45,312                    | 708           | 0.000        | 6.49  | −52             | 60 | 0.82 (0.55) | 0.69 (0.33) | 1.00 | 0.33 | n.s.      |
| Medial Prefrontal    | 18,560                    | 290           | 0.000        | 5.48  | 4               | 56 | 0.90 (0.78) | 0.52 (0.70) | 1.61 | 0.12 | n.s.      |
| Precuneus            | 5,440                     | 85            | 0.001        | 4.99  | −52             | 40 | 1.16 (1.11) | 0.48 (0.66) | 2.60 | 0.02 | 0.74      |
| Right Fusiform       | 3,968                     | 62            | 0.008        | 4.73  | 32              | −32| 0.85 (0.86) | 0.49 (0.66) | 1.50 | 0.15 | n.s.      |
| Left Fusiform        | 3,584                     | 56            | 0.013        | 4.65  | −36             | −44| 0.65 (0.56) | 0.36 (0.53) | 1.66 | 0.11 | n.s.      |
| EFFECT OF CONDITION: BUMPER–PEOPLE | | | | | | | | | | | |
| Occipital            | 90,368                    | 1,412         | 0.000        | 7.15  | −84             | 12 | −0.85 (0.80) | −1.21 (0.60) | 1.69 | 0.11 | n.s.      |
| Right Dorsolateral Prefrontal | 6,656 | 104 | 0.000 | 5.28 | 40 | 44 | 8 | −0.46 (0.79) | −0.94 (0.68) | 2.01 | 0.06 | n.s.      |
| Dorsal Anterior Cingulate | 24,576 | 384 | 0.000 | 4.94 | 28 | −12 | 48 | −0.37 (0.59) | −0.85 (0.57) | 2.54 | 0.02 | 0.83      |
| Left dorsolateral Prefrontal | 3,968 | 62 | 0.008 | 4.33 | −28 | 56 | 0 | −0.53 (0.88) | −0.78 (0.70) | 1.00 | 0.33 | n.s.      |
| Right parietal       | 16,320                    | 255           | 0.000        | 4.31  | 24              | −64| −0.41 (0.60) | −0.63 (0.58) | 1.10 | 0.28 | n.s.      |
| EFFECT OF GROUP: rs2254298 GG vs. AG/AA | | | | | | | | | | | |
| Posterior Cingulate  | 5,120                     | 80            | 0.002        | 4.17  | 8               | −44| 0.72 (1.25) | −1.07 (1.64) | 3.47 | 0.003 | 1.23      |
| Medial Prefrontal Cortex | 2,944 | 46 | 0.035 | 3.74 | 8 | 32 | 16 | 0.33 (0.75) | −0.90 (1.29) | 3.13 | 0.008 | 1.17      |

<sup>a</sup> Group comparisons are based on mean(SD) of extracted parameter estimates; shown each group (GG and GA/AA columns). Areas with significant differences are bolded, and significant p values are in red.
contrast, the dorsal anterior cingulate, was more active for Bumper stimuli than People stimuli for the A carriers relative to GG subjects (Table 2, Figure 3).

Third, in the People—Bumper task contrast, the rs2254298A and rs2254298GG groups showed different patterns of connectivity for the posterior cingulate cortex seed. Positive connectivity refers to regions that show a positive correlation in their activation patterns whereas negative connectivity refers to areas that are negatively correlated in their activation patterns. Within group, the rs2254298GG subjects showed positive connectivity between the posterior cingulate cortex seed and bilateral parietal regions, the dorsal anterior cingulate, the middle frontal gyrus, the cerebellum, and the lingual lobe, whereas the rs2254298A subjects showed only negative connectivity between the posterior cingulate cortex seed and the lingual lobe (Supplemental Table 3, Figure 4). In the group comparisons, the rs2254298A group showed greater negative connectivity between the posterior cingulate cortex and the occipital lobe and the cerebellum (Occipital, 51 voxels, 3,264 mm³, MNI −8, −80, −20) relative to the rs2254298GG group (Figure 4). Although there were no statistically significant group differences using the medial prefrontal cortex seed, the rs2254298A carriers showed positive connectivity between this region and the left middle frontal gyrus, and the rs2254298GG carriers showed negative connectivity between the middle temporal gyri, the precuneus, medial frontal gyrus, and the right superior frontal gyrus (Supplemental Table 3).

**Neural Differences Based on rs53576**

No significantly activated clusters were identified in whole-brain comparisons for A carriers vs. GG subjects based on OXTR SNP rs53576. Similarly, there were no significant activation differences in the task-condition ROI analysis using extracted beta values based on being an A carrier or GG subject for SNP rs53576 (Supplemental Table 4).

**Body Mass Index and Genotype**

The participants included both currently underweight and weight-restored individuals with AN. To explore the effects of weight-restoration on social brain function within AN, we conducted two analyses. First, we conducted a full factorial whole-brain analysis examining weight-restoration and carrier status for rs2254298. No differences in neural activations were associated with weight and there were no interactions between weight-recovery status and genotype. Second, we examined whether the parameter estimates, beta values, from each of the four ROIs that differed based on the rs2254298 SNP, showed any significant differences related to weight-recovery using ANOVA (Supplemental Table 5). Three of the regions (precuneus, posterior cingulate cortex, and medial prefrontal cortex) showed effects only for rs2254298 genotype and not for BMI. The fourth region, the dorsal anterior cingulate, was modulated both by BMI \[F_{(1, 45)} = 6.31, p = 0.02\] and genotype \[F_{(1, 45)} = 6.88, df = 1, p = 0.26\], with no interaction between the two factors \[F_{(1, 45)} = 1.33, p = 0.26\] (Supplemental Table 5, Supplemental Figure 1).

**Clinical Symptoms and Regions of Interest**

Multivariate linear regression was used to evaluate whether any of the office-based clinical assessments for depression (QIDS), anxiety (SIGH-A), eating attitudes (EAT), obsessive-compulsive traits (YBOCS), or body shape (BSQ) correlated with neural activations within the four identified ROIs. None of these clinical symptom measures were significantly related to activations in these ROIs.

**DISCUSSION**

This pilot study to explore whether differences in SNPs of OXTR in AN could be related to differences in neural activations to social stimuli was supported for SNP rs2254298, but not SNP rs53576. Neural responses to social stimuli in the medial prefrontal cortex, the dorsal anterior cingulate, the posterior...
cingulate cortex, and the precuneus were reduced in rs2254298A relative to rs2254298GG. These exploratory data were obtained from a small sample in a psychiatric patient population, and limited by unequal group sizes. Nevertheless, AN is ideally suited to examine gene-environment interactions. Specifically, all women with AN have undergone physiological starvation, a clear environmental stressor that may have different effects on people based on genotype. Thus, AN will include some differences in neural functions that are caused by differences in how specific genotypes respond to starvation. Future work to assess the role of these neural pathways and genetic polymorphisms in the context of other psychiatric pathology or examination in less extreme cases of dieting and weight loss will be important to further understand how oxytocin pathways affect human social perception.

The medial prefrontal cortex, posterior cingulate cortex, and precuneus are prominent components of the default mode network (Li et al., 2014) whereas the dorsal anterior cingulate is part of the salience network (Downar et al., 2016). Oxytocin has been proposed to exert its effects via alterations related to salience and motivation toward social stimuli (Love, 2014). Additionally, the posterior cingulate cortex and the occipital lobe showed connectivity differences for the rs2254298A and rs2254298GG groups. In sum, these data suggest that OXTR polymorphism differences may subserve some of the neural differences in social perception in AN, and leads to a hypothesis that oxytocin pathways may impact social behavior by coordinating changes in the default mode network when processing social stimuli. Although this is a small sample, the effect-sizes of all results were moderate to large, suggesting that future work examining OXTR polymorphisms in the brain should include the examination of midline cortical structures.

Oxytocin systems have been considered in multiple studies as a potential contributor to psychopathology in AN (Table 3). Underweight individuals with AN have lower oxytocin in cerebrospinal fluid levels than controls (Frank et al., 2000). Individuals with AN have lower plasma oxytocin than healthy controls (Monteleone et al., 2016). Overnight oxytocin secretion is reduced acutely in AN (Lawson et al., 2011). Postprandial secretion of oxytocin is decreased in weight-recovered women with AN, while it is increased in individuals with current AN (Lawson et al., 2013). Abnormalities in oxytocin secretion have also been associated with increased severity of disordered eating psychopathology and activation of neural pathways related to food motivation (Lawson et al., 2012). Using a larger sample which included the participants examined here, we previously found that individuals who were A carriers for these two common OXTR SNPs (rs53576 and rs2254298) had elevated severity for eating disorder symptoms (e.g., oral control, eating obsessions, and appearance concerns) if they developed AN; however, this was not the case for individuals with bulimia nervosa (Acevedo et al., 2015). Two studies have found that the OXTR is more highly methylated in individuals with AN than controls (Kim et al., 2014a; Booij et al., 2015), with one study showing that OXTR methylation level was inversely associated with BMI (Kim et al., 2014a). Oxytocin-releasing stimuli cause less increase in oxytocin in individuals with AN than individuals with BN and HC, but this response is normalized after full-weight restoration (Chiodera et al., 1991). Recently, oxytocin administration has been explored as a potential adjunct treatment for AN. In one proof of concept study, intranasal oxytocin administration resulted in significant reductions in attentional biases toward eating-related stimuli and toward fat body parts in individuals with AN, but did not influence juice consumption (Kim et al., 2014b). Here, we found that Eating Attitudes Test scores were significantly higher for rs2254298A carriers compared to rs2244298GG carriers.

Three neuroimaging studies conducted in healthy participants have reported differences in brain volumes for SNP rs2254298. Inoue (Inoue et al., 2010) reported increased amygdala volume

![Figure 4](image-url)
TABLE 3 | Studies examining oxytocin in anorexia nervosa.

| References | N | Study purpose and methods | Results and conclusions |
|------------|---|---------------------------|------------------------|
| Acevedo et al., 2015 | AN = 36, AN-WR = 26, BN = 27, HC = 35 | Examined whether SNPs of the oxytocin receptor gene correlated with AN clinical symptoms | AN-C and rAN with two common SNPs of the OXTR gene (53576 and 2254298) had elevated eating disorder clinical symptom severity |
| Chiodera et al., 1991 | AN = 7, BN = 8, HC = 9 | Measured plasma oxytocin response to two known oxytocin-releasing stimuli (insulin and estrogen) | Oxytocin concentrations increased more in individuals with BN and HC than individuals with AN; after weight restoration, the oxytocin response in AN individuals normalized |
| Demitrack et al., 1990 | AN = 5, BN = 47, HC = 11 | Measures cerebrospinal fluid of women with AN, BN, and HC | Restricting AN (but not underweight, normal weight BN, nor normal-weight women) had lower oxytocin cerebrospinal fluid levels |
| Fetissov et al., 2005 | AN = 12, BN = 42, HC = 41 | Identified autoantibodies reacting with oxytocin; assessed whether autoantibodies correlated with psychological symptoms | Autoantibodies for oxytocin were higher in AN than BN or controls; bulimia score of EDI-2 correlated with oxytocin levels in both AN and BN |
| Frank et al., 2000 | AN-WR = 10, rBN = 23, HC = 17 | Compared cerebrospinal fluid oxytocin in individuals who were weight-recovered from AN-BP, recovered from BN, and HC | Found no significant differences in oxytocin levels in individuals recovered from AN-BP vs. HC and rBN |
| Hoffman et al., 2012 | AN = 20 | Compared oxytocin concentration in blood, saliva, and urine samples in women with AN | Plasma oxytocin and salivary oxytocin concentrations were positively correlated, with lower correlations in individuals with self-induced vomiting |
| Kim et al., 2014b | AN = 31, HC = 33 | Examined intranasal oxytocin (vs. placebo) on attention processing for food, shape, and weight stimuli in AN and HC and also on juice consumption by AN and HC | AN vs. HC showed less attentional biases for both food and weight stimuli with oxytocin; oxytocin did not effect juice consumption in either group |
| Kim et al., 2014c | AN = 31, HC = 33 | Examined intranasal oxytocin (vs. placebo) on attention processing for emotional face stimuli (disgust, anger, happy, neutral) in AN and HC | Oxytocin reduced attentional bias for disgust in both AN and HC. Differences in oxytocin effects for anger varied by group, with AN showing increased vigilance but HC showing decreased vigilance |
| Kim et al., 2014a | AN = 15, HC = 36 | Examined OXTR methylation and illness severity | OXTR is more highly methylated in individuals with AN than HC; methylation inversely associated with BMI |
| Kim et al., 2015 | AN = 35, BN = 34, HC = 33 | Same as 2014c, with BN added. Food intake for 24 h post oxytocin administration measured for all groups. | Oxytocin increased emotion recognition in BN and HC but not AN; decreased calories consumed in BN; no effect in HC or AN |
| Lawson et al., 2011 | AN = 17, HC = 19 | Examined the relationship between oxytocin levels, body composition, and bone mineral density in individuals with AN | Subjects with AN (vs. HC) had lower overnight oxytocin secretion; low oxytocin levels were associated with decreased bone mineral density, fat mass, and leptin levels |
| Lawson et al., 2012 | AN-WR = 9, HC = 13 | Examined the relationship between abnormal oxytocin secretion in AN and anxiety and depression symptoms | Postprandial secretion of oxytocin after eating was increased in AN (vs. HC) and decreased in wrAN (vs. HC); oxytocin secretion is associated with anxiety and depression symptoms in AN and wrAN |
| Lawson et al., 2012 | AN-WR = 9, HC = 13 | Examined the relationship between abnormal oxytocin secretion in AN and eating disorder symptoms and brain activations in a priori regions. | Larger changes in oxytocin in response to feeding was associated with more eating disorder symptomatology and accounted for some of the differences observed in the brain in a priori regions (insula, amygdala, hypothalamus, orbitofrontal cortex) between AN and HC groups. |
| Monteleone et al., 2016 | AN = 23, BN = 27, HC = 19 | Compared oxytocin secretion in AN, BN, and HC and related comparison between oxytocin and personality traits | AN had lower plasma oxytocin than HC; no difference in plasma oxytocin levels for AN-BP and AN-R; no relationship to oxytocin and personality in AN |

AN, anorexia nervosa; WR, weight-recovered; BP, binge-purge subtype; R, restricting subtype; BN, bulimia nervosa; rBN, recovered bulimia nervosa; HC, healthy controls; EDI-2, Eating Disorder-Inventory-two; OXTR, oxytocin receptor; SNP, single nucleotide polymorphisms; BMI, body mass index.

in rs2254298A carriers relative to rs2254298GG subjects. Furman (Furman et al., 2011) found increased amygdala volume and decreased total gray matter for rs2254298A carriers. Tost (Tost et al., 2011) found increased gray matter volume in the cingulate and hypothalamus for rs2254298GG subjects, with a more pronounced effect in males. One study recently reported increased activation of the amygdala in response to face stimuli for rs2254298A carriers, which was increased for those participants with early life stress (Marusak et al., 2015). The functional data reported here, reduced modulation within midline cortical structures in response to social stimuli for women with AN that have SNP rs2254298A, extends these studies by providing a deeper understanding of the mechanistic circuitry through which OXTR polymorphisms may modulate cognitive and affective processing.
We propose future work to test a model in which rs2254298A status may lead to heightened reactivity of the amygdala for social stimuli (a genotype trait) which may then impact the long-term activation of regions in both the default mode and salience networks for processing social stimuli in women with AN. Both state (such as starvation-related) and trait (predisposition to this illness) abnormalities may thus impact oxytocin function in individuals with AN. Although we considered whether current body mass index might be related to the genotype and neural differences, these studies were limited by the fact that all participants for the imaging studies were required at baseline to be at a stable or increasing weight. Second, as only 12 subjects carried the A allele of rs2254298, the sample is underpowered to identify interactions related to both genotype and weight, and is a pilot project. Similarly, although these participants were in studies designed to compare neural activations in AN to healthy comparison women, only twenty healthy participants volunteered for both the neuroimaging and genetic studies, and only five were A carriers for rs2254298A; therefore we could not determine if this SNP alters functional neural activations to social stimuli in healthy women. Thus, although the proportion of both cohorts (AN and HC) with the A allele of rs2254298 were similar, its effects on social brain function in the healthy women could not be assessed, and remains an important area for future research. In addition, longitudinal studies examining more women with AN at different stages of weight-recovery, may lead to a better understanding of how genotype and starvation contribute to the differences in social function in AN. Future work should consider examining how oxytocin relates to engagement of the default mode network during social behaviors in a wider range of psychopathological samples.

ETHICS STATEMENT

The three protocols utilized to collect participant data were approved by the University of Texas at Southwestern Medical Center Institutional Review Board. All participants signed a written informed consent to participate in the study.

AUTHOR CONTRIBUTIONS

CM and DK designed the study. SA and CM collected the data. MS, KH, SA, and CM processed and analyzed data. All authors contributed to writing and editing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2018.02183/full#supplementary-material

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