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Oxytocin Enhances an Amygdala Circuit Associated With Negative Symptoms in Schizophrenia: A Single-Dose, Placebo-Controlled, Crossover, Randomized Control Trial

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Negative symptoms are core contributors to vocational and social deficits in schizophrenia (SZ). Available antipsychotic medications typically fail to reduce these symptoms. The neurohormone oxytocin (OT) is a promising treatment for negative symptoms, given its role in complex social behaviors mediated by the amygdala. In sample 1, we used a double-blind, placebo-controlled, crossover design to test the effects of a single dose of intranasal OT on amygdala resting-state functional connectivity (rsFC) in SZ (*n* = 22) and healthy controls (HC, *n* = 24) using a whole-brain corrected approach: we identified regions for which OT modulated SZ amygdala rsFC, assessed whether OT-modulated circuits were abnormal in SZ relative to HC on placebo, and evaluated whether connectivity on placebo and OT-induced connectivity changes correlated with baseline negative symptoms in SZ. Given our modest sample size, we used a second SZ (*n* = 183) and HC (*n* = 178) sample to replicate any symptom correlations. In sample 1, OT increased rsFC between the amygdala and left middle temporal gyrus, superior temporal sulcus, and angular gyrus (MTG/STS/AngG) in SZ compared to HC. Further, SZ had hypo-connectivity in this circuit compared to HC on placebo. More severe negative symptoms correlated with less amygdala-to-left-MTG/STS/AngG connectivity on placebo and with greater OT-induced connectivity increases. In sample 2, we replicated the correlation between amygdala-left-MTG/STS/AngG hypo-connectivity and negative symptoms, finding a specific association with expressive negative symptoms. These data suggest intranasal OT can normalize functional connectivity in an amygdala-to-left-MTG/STS/AngG circuit that contributes to negative symptoms in SZ.

Keywords: resting-state/functionalconnectivity/temporal lobe/expressive negative symptoms

Introduction

Schizophrenia (SZ) is a chronic and debilitating illness comprising positive, negative, and disorganized symptoms.[1] Negative symptoms constitute the absence or loss of normal function observed among individuals from the same culture.[2,3] Negative symptoms include deficits in motivation (*avolition*), experience of pleasure (*anhedonia*), social interaction or desire for close relationships (*asociality*), and affective expression (*alogia*, *blunted affect*). Further, negative symptoms profoundly contribute to the vocational and social functioning impairments observed in SZ.[4–8] While available antipsychotic medications can improve positive symptoms, including delusions and hallucinations,[9] they typically do not alleviate negative symptoms.[10,11] Given stagnant pharmaceutical development for SZ,[12,13] novel treatments for negative symptoms are desperately needed.

The hypothalamic neuropeptide oxytocin (OT) is a promising treatment for negative symptoms. The OT system helps regulate complex social behaviors,[14] and OT

system dysfunction is implicated in SZ pathophysiology.[15] In SZ, OT most consistently ameliorates deficits in higher-level social cognitive processes like mentalizing.[16] Early findings bolstered OT's potential to reduce negative symptoms in SZ.[17–21] Although recent meta-analyses of human clinical trials did not find OT improved negative symptoms,[22,23] heterogeneity in age, sex, other medications, OT dosage and administration chronicity, and early attachment experiences may moderate OT effects.[15] An incomplete understanding of OT pharmacodynamics and pharmacokinetics may also contribute to inconsistent findings.[24] Nonetheless, OT is fundamental to mammalian social behavior and remains a vital area of investigation for disorders with social deficits like SZ.[25]

Social cognitive deficits in SZ may present as negative symptoms.[25] The amygdala is a likely route through which OT may impact social behavior and in turn, negative symptoms.[25,26] It is a hub for social processing given its anatomical connections to key social brain regions like the prefrontal cortex and superior temporal cortex,[27] and its interactions with reward regions like the nucleus accumbens.[28,29] The amygdala is rich with OT receptors,[30] and neuroimaging studies suggest OT modulates activity in the amygdala,[14] as well as activity in frontal, temporal, and other subcortical structures[31] (involved in social cognition[32]). The amotivational/anhedonic features of negative symptoms can be conceptualized as aberrant frontal-subcortical processes, and have been linked with emotional processing abnormalities supported by the amygdala (given its subcortical connections).[33] Additionally, aberrant amygdala reactivity in response to emotional versus neutral stimuli[34–36] may underlie blunted emotional expression,[33] and abnormal amygdala activity, volume, and task-based connectivity correlates with blunted affect.[37–39] OT administration is, therefore, a potential treatment for SZ-related amygdala dysfunction and/or dysconnectivity that contributes to the emotional and reward deficits underlying negative symptoms.[33,38]

Here we used functional magnetic resonance imaging (fMRI) to assess OT-related effects on amygdala resting-state functional connectivity (rsFC). rsFC measures the correlation between the blood oxygen level-dependent (BOLD) signal time-series of seed and target brain regions in the absence of task,[40] and may, therefore, reflect the functional communication between brain areas.[41] This feature is particularly important in light of the hypothesis that SZ symptoms emerge from cerebral connectivity alterations.[42–44] rsFC approaches also avoid many potential confounds related to attention, comprehension, or effort[45] that can arise in task-based fMRI studies of clinical disorders accompanied by significant cognitive deficits like SZ. Studies of OT's effects on rsFC have primarily focused on connectivity between the amygdala and other brain areas. Converging evidence finds that OT impacts social processes by modulating connectivity between social and emotional brain areas (eg, amygdala, precuneus).[46] In healthy males, OT increases amygdala-prefrontal coupling[47] and decreases amygdala-precuneus connectivity[48]; additionally, OT can normalize amygdala-prefrontal hypo-connectivity in men with social anxiety compared to controls.[49] No study to date has investigated OT-related rsFC changes in SZ.

We employed a double-blind placebo-controlled design to test whether a single intranasal OT administration modulated amygdala rsFC differentially for individuals with SZ compared to healthy controls (HC). We aimed to (1) identify brain regions for which OT-modulated amygdala rsFC in SZ using whole-brain corrected analyses, (2) assess if amygdala-connected circuits modulated by OT were abnormal in SZ relative to HC on placebo, and (3) evaluate whether amygdala connectivity on placebo and OT-induced connectivity changes correlated with baseline negative symptom severity in patients. Given our modest sample size, and mixed results from prior trials,[22,23] we tested whether regions with aberrant rsFC that changed following OT treatment in our initial sample (sample 1) were related to negative symptoms in a large, independent SZ sample (sample 2).

Methods

Subjects

Sample 1. Male outpatients with a SZ spectrum disorder (n = 22; mean age = 35.50 years) and HC (n = 24; mean age = 28.08 years) were recruited from the San Francisco area from late 2014 through early 2017 (supplementary table S1 contains additional demographic details). SZ met diagnostic criteria for either SZ (n = 12) or schizoaffective disorder (n = 10) based on the Structured Clinical Interview for DSM-IV,[50] and had no hospitalizations or medication changes for at least 1 week prior to study enrollment and until study completion. For additional enrollment and exclusion criteria see supplementary materials. We also note that SZ were older than HC (t_{44} = 2.81, P = .01, d = 0.82).

Baseline negative symptom severity was determined using the Positive and Negative Syndrome Scale (PANSS),[51] which yields a Negative score by summing items 8–14 (mean = 12.18; supplementary table S1); scores were log-transformed to improve normality. To enhance the quality of our assessments, all assessments were video-taped, and 2 trained research assistants were present for most assessments. If only one assessor was available at the time of the session, a second assessor completed ratings based on the video. In either case, final ratings were determined via consensus.

Sample 2. Clinically stable male and female SZ outpatients (n = 183, mean age = 38.73 years, 75% male) were recruited from 7 sites as part of the Functional Biomedical Informatics Research Network (FBIRN) study (supplementary table S1; supplementary materials); here SZ included SZ but not schizoaffective disorder. Enrollment

and exclusion criteria are found in reference.[52] The 2 SZ samples did not differ by age ($P = .21$) or chlorpromazine equivalents (CPZeq; $P = .38$), but sample 2 patients had longer illness durations ($t_{199} = -2.38$, $P = .02$, $d = -0.62$).

Negative symptoms were assessed using the PANSS and the Scale for the Assessment of Negative Symptoms (SANS).[53] PANSS Negative scores were computed equivalently to sample 1 (mean = 14.51), and SANS Negative scores were computed by summing all items (mean = 29.81; supplementary table S1). PANSS and SANS scores were log and square root-transformed, respectively, to improve normality (correlation between transformed PANSS and SANS Negative scores: $r_{179} = .80$, $P < .001$, with a comparable result using a Spearman correlation on untransformed symptom scores: $r_{179} = .79$, $P < .001$); there was also a trend-level mean difference between the PANSS Negative scores across samples 1 and 2 ($t_{201} = -1.92$, $P = .06$, $d = -0.45$) but no difference in their variances ($F_{21,180} = 0.80$, $P = .56$). Group trainings led by experienced clinical raters were used to standardize raters across sites before any data were collected.[54] Trainings involved rating videotapes from several patients and comparing ratings with expert assessments.

Drug Procedure and Randomization

Sample 1. We used a randomized, double-blind, placebo-controlled, crossover design (supplementary figure S1). OT and placebo days were separated by at least 2 weeks. Staff administered a single OT (40 IU, Wellspring Pharmacy, Berkeley) or placebo dose via nasal spray ~90 minutes before the resting-state fMRI scan[21] (supplementary materials contains drug order and blinding information). OT protocols typically include a 30-minute delay after administration before assessment,[15] and acute effects should last at least 90 minutes.[55–57] Subjects could not differentiate between the conditions, with SZ correctly guessing whether they received OT or placebo 61% of the time ($\chi^2 = 1.29$, $P = .26$), and HC 63% ($\chi^2 = 2.13$, $P = .14$).

Sample 2. Subjects did not receive OT or placebo.

Neuroimaging Acquisition and Image Processing

Sample 1. Each subject was scanned twice, once on OT and once on placebo. Resting scans were acquired on a 3T Siemens Skyra scanner with a 32-channel head coil; image parameter details are found in supplementary materials. Data were pre-processed using FEAT v.5.0.9 in FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT/).[58] Initial steps included brain extraction, motion correction, and slice-timing correction. We then conducted additional steps to reduce motion confounds using the artifact detection tools toolbox (ART; http://www.nitrc.org/projects/artifact_detect/) and aCompCor[59] (see supplementary materials for details). Following ART and aCompCor, we used FEAT for high-pass temporal filtering (100 seconds[60]) and spatial smoothing (6 mm kernel).

For each subject's first-level model, we entered the ART and aCompCor noise parameters as nuisance regressors along with the amygdala seed's time-series (described below) to generate whole-brain connectivity maps.

Sample 2. rsFC data collection and pre-processing details were reported previously.[52] We implemented the same denoising procedures in both samples.

Data Analysis

Sample 1. An amygdala seed was defined using FSL's Harvard-Oxford Subcortical Structural Atlas probabilistic maps; we used a bilateral mask given no a priori laterality hypotheses. To ensure our seed was maximally representative of amygdala activity, we applied a stringent 80% probability threshold to the probabilistic amygdala mask.[61] We checked for comparable temporal signal-to-noise (tSNR) ratios across groups (group $t_{44} = -0.11$, $P = .92$; drug $t_{45} = -0.50$, $P = .62$; supplementary materials).

Seed-based connectivity was implemented in FEAT. The average amygdala seed time-series was extracted from pre-smoothed images. First-level connectivity analyses generated voxel-wise beta maps separately for each subject and drug condition, reflecting the correlation between the amygdala seed's time-series and the time-series of every voxel in the brain. A second-level fixed-effects analysis produced within-subject OT minus placebo contrasts maps of the first-level betas. Finally, a higher-level analysis tested between-group differences in these contrast maps, effectively testing the Group x Drug interaction. Because SZ were significantly older than HC, we controlled for age (mean-centered) in the higher-level analysis. Resulting t-maps were transformed to z-maps and warped into MNI standard space. We applied a voxel-wise height threshold of $z > 2.8$ and a corrected-cluster significance threshold of $P < .05$, 2-tailed.

Mean connectivity values (betas) from significant cluster(s) were extracted for each subject and drug condition. We unpacked the Group × Drug interaction for the aforementioned cluster(s) in a mixed-effects model using the lme4 package in R[62]: *Connectivity* ~ *Group* x *Drug* + (1|*Subject*), where *Group* (HC, SZ) was treated as a between-subjects fixed effect, *Drug* (OT, placebo) as a within-subjects fixed effect, and *Subject* as a random effect with a compound symmetric covariance structure. To parse the interaction effects for accurate interpretation, we conducted 4 follow-up tests to determine whether SZ had aberrant connectivity compared to HC on placebo and OT, and whether OT induced significant connectivity changes relative to placebo for both SZ and HC.

Lastly, we interrogated relations between negative symptoms and connectivity within SZ from the cluster(s) showing significant Group × Drug interactions. We

correlated baseline negative symptoms with connectivity on placebo and OT-induced connectivity change values for the same cluster(s). OT-induced connectivity change values were calculated as connectivity on OT minus connectivity on placebo. We report uncorrected and Bonferroni-adjusted *P*-values for the 4 tests and the 2 correlations.

Sample 2. Using the same amygdala seed from sample 1, we generated voxel-wise whole-brain connectivity maps for each subject, expressed as Fisher *r*-to-*z* transformed correlation maps (analyzed using SPM 8, see ref.[52] for details). We interrogated Group × Drug cluster(s) identified in sample 1 for correlations with SZ negative symptom severity.

Results

OT Increases Amygdala-to-Temporal Connectivity for SZ (Sample 1)

Higher-level analysis revealed a left-lateralized cluster including voxels from the middle temporal gyrus, superior temporal sulcus, and angular gyrus (MTG/STS/AngG; *z*-max = 3.78, number of voxels = 456, $P = .001$), which corresponded to Brodmann areas BA19, BA21, BA37, and BA39 (figure 1); this was the only cluster to survive our whole-brain corrected threshold. A mixed-effects model using extracted mean values from this cluster clarified the Group × Drug interaction (figure 1): specifically, OT increased connectivity between amygdala and left MTG/STS/AngG in SZ ($t_{44} = -4.77$, $P < .001$, $P_{adj} < .001$, $d = 1.00$; supplementary figure S2), and modestly decreased connectivity in HC ($t_{44} = 2.51$, $P = .02$, $P_{adj} = .06$, $d = -0.60$), though this effect was not significant after removal of an outlier with connectivity > 3 SDs above the HC mean ($t_{43} = 1.91$, $P = .06$, $P_{adj} = .25$). Additional follow-up analysis revealed SZ had amygdala-to-left-MTG/STS/AngG hypo-connectivity compared to HC on placebo ($t_{44} = 3.49$, $P = .001$, $P_{adj} = .004$, $d = -0.92$), and more similar connectivity to HC on OT ($t_{44} = -2.05$, $P = .05$, $P_{adj} = .19$, $d = 0.69$). We also note that the Group x Drug interaction remained significant after removing 1 outlier point with connectivity > 3 SDs above the mean ($F_{1,43} = 24.47$, $P < .001$), and when accounting for framewise displacement ($F_{1,41} = 28.51$, $P < .001$).

We did not detect within-group OT-induced changes for SZ or HC outside of the left MTG/STS/AngG area; although a post hoc analysis using a reduced voxel-wise height threshold revealed connectivity increases in similar right temporal and occipital areas (supplementary figure S3; supplementary materials). Significant effects were not explained by drug order, and we observed similar patterns when seeding from the left or right amygdala, separately (supplementary figure S4; supplementary materials).

Amygdala-to-Temporal Hypo-connectivity is Associated With More Severe Negative Symptoms (Sample 1)

More severe PANSS negative symptoms correlated with less amygdala-to-left-MTG/STS/AngG connectivity on placebo ($r_{20} = -.56$, $P = .006$, $P_{adj} = .01$; figure 2), and greater amygdala-to-left-MTG/STS/AngG connectivity

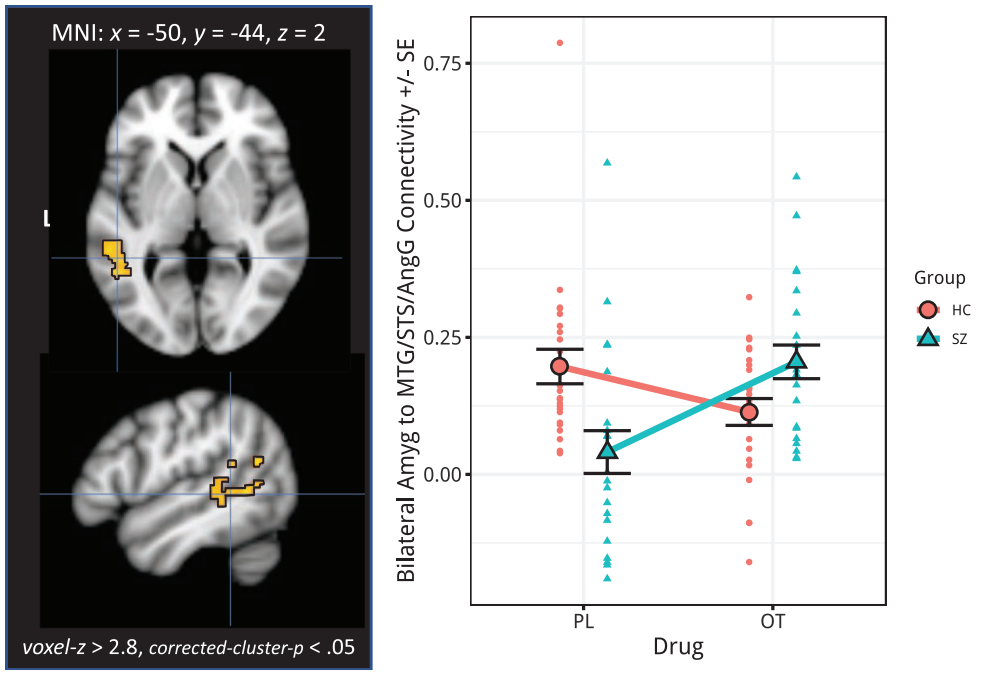

Fig. 1. A Group × Drug interaction revealed connectivity changes between bilateral amygdala and a left temporal cortex cluster (*left*) in sample 1. SZ had increased connectivity in this area following OT administration (*right*). Points represent mean *t*-to-*z* values. Overall means and standard errors are overlaid on raw data. *Amyg*, amygdala; *MTG*, medial temporal gyrus; *STS*, superior temporal sulcus; *AngG*, angular gyrus; *PL*, placebo; *OT*, oxytocin; *HC*, healthy controls; *SZ*, schizophrenia subjects.

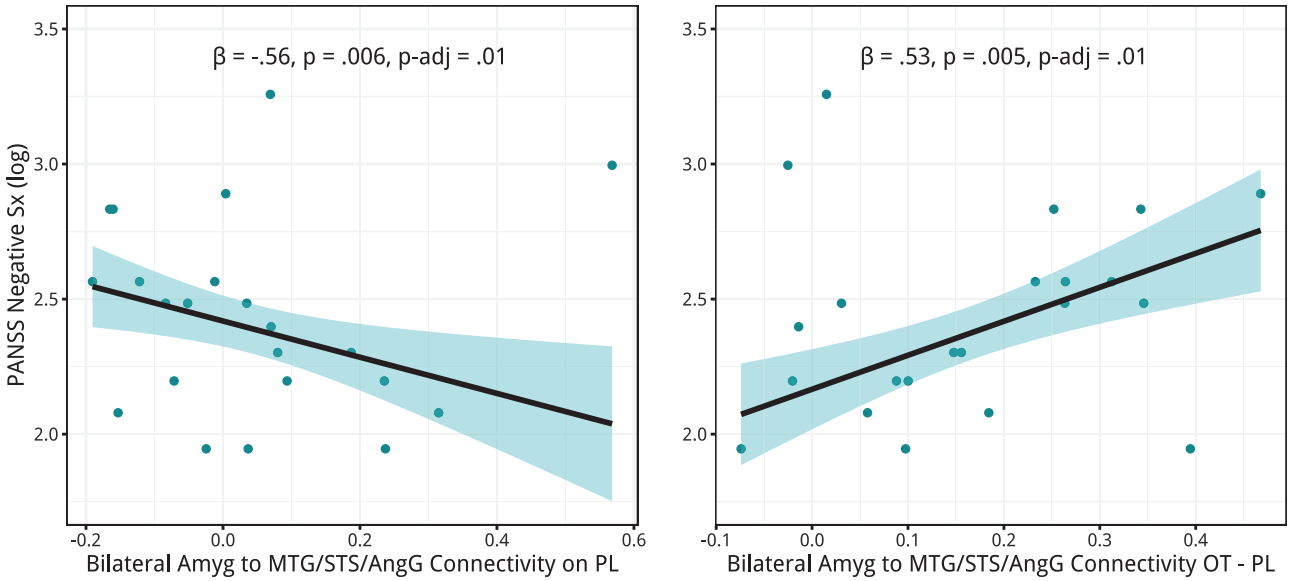

Fig. 2. Correlations between connectivity at placebo and baseline negative symptoms (*left*), and between OT-induced connectivity changes and baseline negative symptoms (*right*) in sample 1. Points represent mean *t*-to-*z* values. Shaded bands represent 95% confidence intervals. *Amyg*, amygdala; *MTG*, medial temporal gyrus; *STS*, superior temporal sulcus; *AngG*, angular gyrus; *Sx*, symptoms; *PL*, placebo; *OT*, oxytocin.

increases following OT (r_{20} = .53, P = .005, P_{adj} = .01; figure 2).

Amygdala-to-Temporal Hypo-connectivity is Associated With More Severe Expressive Negative Symptoms (Sample 2)

Lastly, we correlated amygdala-to-left-MTG/STS/AngG hypo-connectivity with negative symptoms in the SZ replication sample using partial correlations. We controlled for Site, Gender, and covariates for which the SZ samples differed (ie, Illness Duration). We also included Gender because males had higher negative symptoms (PANSS [t_{179} = 2.71, P = .01, d = 0.49] and SANS [t_{179} = 2.15, P = .03, d = 0.39]), and Illness Duration because SZ connectivity was negatively correlated with Illness Duration (β_{181} = −.17, P = .02).

A negative correlation was present for the SANS Negative scores (r_{176} = −.19, P = .01, P_{adj} = .02; figure 3), and nonsignificant (but in the same direction) for PANSS Negative scores (r_{178} = −.11, P = .17, P_{adj} = .34) (Three multivariate outliers removed from the PANSS and SANS analyses). Exploratory analyses further revealed that reduced amygdala-to-left-MTG/STS/AngG connectivity was specifically associated with more severe *expressive* negative symptoms (ie, alogia, flat affect) as compared to *experiential* negative symptoms (ie, amotivation, anhedonia, asociality; supplementary figure S7; supplementary materials).

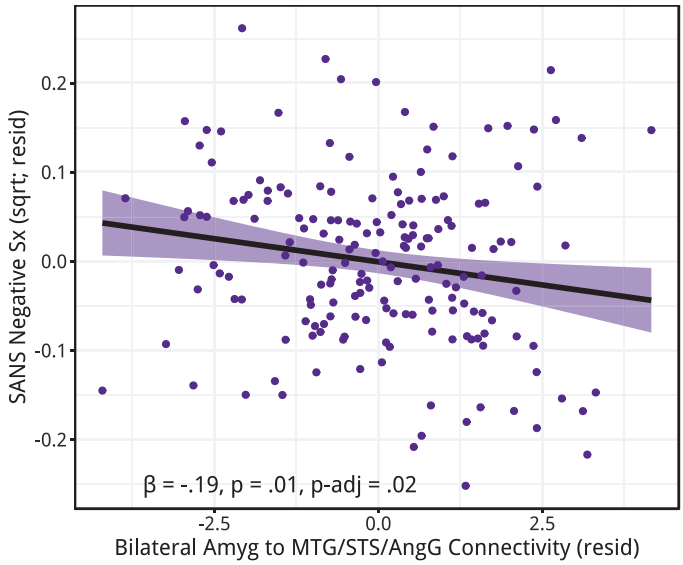

Fig. 3. Correlation between connectivity and SANS negative symptoms in sample 2. Points represent mean *r*-to-*z* values. Shaded band represents 95% confidence interval. *Amyg*, amygdala; *MTG*, medial temporal gyrus; *STS*, superior temporal sulcus; *AngG*, angular gyrus; *Sx*, symptoms; *sqrt*, square root.

Discussion

We found that OT modulates a neural circuit implicated in the negative symptoms of SZ. In sample 1, a single OT administration increased rsFC between the amygdala and left middle temporal gyrus, superior temporal sulcus, and angular gyrus in individuals with SZ, and patients had hypo-connectivity in this circuit compared to HC on placebo. Amygdala-to-left-MTG/STS/AngG hypo-connectivity on placebo and OT-induced connectivity increases were both associated with more severe baseline negative symptoms in patients. In sample 2, we replicated the connectivity and symptom correlation in

a larger, independent sample, showing that amygdala-to-left-MTG/STS/AngG hypo-connectivity was specifically associated with more severe expressive (but not experiential) negative symptoms. These findings have important implications for SZ treatment, as negative symptoms remain an unmet therapeutic target.[63]

Our results revealed that OT increased connectivity between bilateral amygdala and left MTG, STS, and AngG (including the temporoparietal junction, ie, BA39[64]) for individuals with SZ compared to HC. Beyond their role in auditory processing, the temporal lobes aid in social perception,[65] processing emotional faces,[66] and mentalizing.[67] We are not the first to report structural and functional connections between the amygdala and temporal cortex: findings from nonhuman primates indicate that the amygdala shares anatomical connections with most social brain regions,[68] including (direct) afferent projections from the amygdala to the STS.[69] Additionally, a meta-analysis of amygdala functional connectivity in humans noted coactivation of the amygdala and MTG.[70]

It is theorized that OT influences the temporal lobes, and in turn social learning and reward processes,[71] via output from the basal ganglia.[72] Numerous whole-brain activation-based studies suggest that OT administration alters temporal lobe activity,[71] and at least 1 seed-based connectivity study found increased amygdala-to-temporal-lobe connectivity following OT[73]; although other studies have reported decreased amygdala-to-temporal connectivity following OT in healthy males,[74] as observed in the current study (see supplementary materials for further discussion). A recent meta-analysis revealed OT administration increases left STS activation during emotion processing, lending support to the hypothesis that OT enhances salience and reward from social stimuli, eg, increased temporal activation may reflect increased attention towards socially relevant information like faces.[46] Greater subcortical (eg, amygdala, striatum) and temporal-lobe (eg, superior temporal cortex, insula) network connectivity following OT in SZ further links OT effects with reward and social cognitive circuitry.[75,76] Moreover, a postmortem study of SZ patients found decreased OT mRNA in the temporal cortex (specifically, BA21), suggesting downregulated expression of the OT receptor in the temporal lobe may contribute to social cognitive deficits or negative symptoms.[77]

Our second finding was that both hypo-connectivity on placebo and OT-induced connectivity increases were associated with worse negative symptoms; that is, patients with higher baseline symptoms had the most connectivity recovery from OT, indicating OT treatment may have a greater impact for more symptomatic patients. Exploratory analyses in the replication sample revealed that lower amygdala-to-left-MTG/STS/AngG connectivity was related to worse expressive deficits, eg, alogia and restricted affect. This dovetails with evidence that the left mid-superior temporal cortex encodes structure-dependent meaning from sentences and predicts affective amygdala responses to semantic information[78]; ie, the left mid-superior temporal cortex encodes information needed to generate an emotional response. Perhaps reduced connectivity in SZ fosters a disconnect between language comprehension and emotional expression. This finding fits with our work showing increased facial expressivity in individuals with SZ following OT,[79] providing a possible mechanism through which OT influences expressive symptoms. Compared to expressive symptoms, connectivity was not correlated with experiential deficits in motivation, which may be more related to alterations in frontal neural activity[80] or connectivity.[81]

Surprisingly, OT did not enhance amygdala-to-prefrontal cortex connectivity among individuals with SZ or HC as found previously.[47,82–84] Given evidence for lower amygdala and prefrontal connectivity in SZ,[85,86] and documented OT-induced connectivity increases for social anxiety,[49] we expected OT to influence amygdala-to-prefrontal connectivity for individuals with SZ. It is possible that we were underpowered to detect this effect, as post hoc analyses using a lower significance threshold revealed OT-induced connectivity increases between the amygdala and the prefrontal cortex, as well as between the amygdala and the precuneus/posterior cingulate cortex in SZ subjects (supplementary figure S6). Dosage differences could also contribute to divergent results (eg, many earlier studies used 24 IU while we used 40 IU), as dosage levels and regimens appear to differentially impact behavior.[15] Similarly, discrepancies in scan times following OT could be important given uncertainty regarding how long it takes OT to reach the brain.[24] Future work is needed to determine optimal dosing and timing for targeting key neural networks.

Limitations

Sample 1 included only men and OT may differentially affect amygdala connectivity for men and women.[82] Hypo-connectivity was associated with total PANSS Negative scores in sample 1 but not 2; though we caution that sample 1 was small, which motivated us to seek replication using a larger sample where confidence intervals are tighter and spurious associations less likely.[87] We also employed a first-generation symptom scale in sample 1, ie, the PANSS, which may not capture the full scope of the negative syndrome compared to some newer scales like the CAINS.[88] The PANSS also does not measure anhedonia or avolition like the SANS. Additionally, we did not assess the reliability of our negative symptom assessments. Because we did not measure symptoms after OT administration, we cannot determine if acutely altering this circuit's rsFC actually improves certain negative symptoms. Lastly, we cannot gauge from the current results whether more chronic OT treatment would yield enduring connectivity changes beyond OT's acute neural effects.

Conclusions

A single OT administration acutely increased rsFC between the amygdala and left temporal lobe in SZ subjects relative to HC. Hypo-connectivity in this circuit correlated with expressive deficits in an independent sample of individuals with SZ, highlighting its role in linking emotional information with facial and/or language expressivity. Future studies should investigate whether OT-induced normalization of this circuit leads to improvement in certain negative symptoms.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

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