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Oxytocin reduces amygdala activity, increases social interactions and reduces anxiety-like behavior irrespective of NMDAR antagonism

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Abstract

Standard dopamine therapies for schizophrenia are not efficacious for negative symptoms of the disease, including asociality. This reduced social behavior may be due to glutamatergic dysfunction within the amygdala leading to increased fear and social anxiety. Several studies have demonstrated the pro-social effects of oxytocin in schizophrenia patients. Therefore, this study evaluates the effect of sub-chronic oxytocin on electroencephalographic (EEG) activity in amygdala of mice during performance of the three chamber social choice and open field tests following acute ketamine as a model of glutamatergic dysfunction. Oxytocin did not restore social deficits introduced by ketamine, but did significantly increase sociality in comparison to the control group. Ketamine had no effect on time spent in the center during the open field trials, while oxytocin increased overall center time across all groups, suggesting a reduction in anxiety. Amygdala activity was consistent across all drug groups during social and nonsocial behavioral trials. However, oxytocin reduced overall amygdala EEG power during the two behavioral tasks. Alternatively, ketamine did not significantly affect EEG power throughout the tasks. Decreased EEG power in the amygdala, as caused by oxytocin, may be related to both reduced anxiety and increased social behaviors. Data suggest that separate pro-social and social anxiety pathways may mediate social preference.

Keywords

Oxytocin; Electroencephalography; Amygdala; N-Methyl D-Aspartate Receptor; Social

Introduction

Schizophrenia is a psychiatric disorder characterized by hallucinations, delusions, and disorganized thought, as well as a host of deficits that are collectively known as negative symptoms. Its etiology remains unknown and comorbid conditions include affective and anxiety disorders, which can contribute to difficulty retaining relationships or employment. Schizophrenia is thought to arise from the dysfunction of several neurotransmitter systems,

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including both dopamine and glutamate. Current treatment of schizophrenia is limited to dopamine antagonists, which effectively reverse positive symptoms, but do little to relieve negative symptoms such as social impairment and blunted affect (Lehman et al., 2004).

The amygdala is thought to play a crucial role in emotional and social behavior in both animal and humans (Medina, Repa, Mauk, & LeDoux, 2002; Phelps & LeDoux, 2005). This structure can be divided into several nuclei: lateral, basal, accessory basal, and central (LeDoux, 2000; Pitkanen, Savander, & LeDoux, 1997). The lateral nucleus integrates incoming sensory information from cortical and thalamic inputs, although sensory afferents terminate in other nuclei as well, and projects to the central nucleus to trigger an anxiety response (Kirsch et al., 2005; Marazziti et al., 2014; Rosenfeld, Lieberman, & Jarskog, 2011; Sah, Faber, Lopez De Armentia, & Power, 2003). These efferent projections include the lateral hypothalamus and brain stem nuclei to modulate an autonomic response as well as the periaqueductal grey and ventral tegmental area to affect behavioral responses (Davis, 1992; LeDoux, Iwata, Cicchetti, & Reis, 1988).

Over-activation of the amygdaloid system may be responsible for an exaggerated fear response as well as social inhibition in multiple diseases. Blood-oxygen-level dependent functional magnetic resonance imaging (BOLD fMRI) data suggest that even within healthy subjects, those with an inhibited social temperament have stronger amygdala activation while viewing the faces of strangers (Beaton et al., 2008; Blackford, Avery, Cowan, Shelton, & Zald, 2011). Patients with social anxiety show increased amygdala activation, possibly due to reduced connectivity to cortical areas and disinhibition of the amygdala (Blackford et al., 2014). Other studies of patients with anxiety disorders have found evidence of this altered amygdala connectivity, specifically in youth (Hamm et al., 2014; Swartz, Phan, Angstadt, Fitzgerald, & Monk, 2014). Schizophrenia patients also show altered amygdala activity during social tasks and facial recognition studies, with a higher activation to neutral faces and lower activation to fearful ones as compared to controls (Mier et al., 2014; Morris et al., 1996; Mukherjee et al., 2013; Solfanelli et al., 2013). This has led to the hypothesis that dysfunction of the amygdala is closely related to the social impairments seen in schizophrenia patients due to inappropriate activation to normally non-fearful social stimuli.

The use of oxytocin, on the other hand, has been shown to increase sociability in both humans and preclinical models. Oxytocin is a nine amino acid peptide synthesized in the supraorbital and paraventricular nuclei of the hypothalamus and released into both peripheral and central circulation (Marazziti et al., 2014; Rosenfeld et al., 2011). Chronic and acute oxytocin administration has been shown to affect social behavior in multiple rodent models, increasing social interaction time and enhancing social memory (Ferguson, Aldag, Insel, & Young, 2001; Guzman et al., 2013; Lee, Brady, Shapiro, Dorsa, & Koenig, 2005; Teng et al.) In addition to social effects, oxytocin has shown anxiolytic and stress protective properties in rodents at low doses (Bale, Davis, Auger, Dorsa, & McCarthy, 2001; Blume et al., 2008; McCarthy, McDonald, Brooks, & Goldman, 1996; Peters, Slattery, Uschold-Schmidt, Reber, & Neumann, 2014). In patients, oxytocin has also been used successfully to treat social deficits in autism, schizophrenia, and social anxiety disorder (Feifel et al., 2010; Guastella et al., 2010; Pedersen et al., 2011).

The mechanism of action of oxytocin in pro-social behavior may be largely mediated by a reduction of amygdala activity, decreasing activation of downstream anxiety centers. Oxytocin receptor density is high in the amygdala and oxytocin receptor binding is thought to mediate GABAergic transmission in the central and lateral nuclei (Huber, Veinante, & Stoop, 2005; Rosenfeld et al., 2011). Specifically, bath application of oxytocin facilitates the development of GABAergic synapses among *in vitro* hypothalamic cells (Theodosis et al., 2006). These inhibitory mechanisms may be responsible for the attenuation of amygdala activity seen in clinical settings (Kirsch et al., 2005; Labuschagne et al., 2010).

Ketamine, an NMDA receptor antagonist, can be used to induce changes in glutamatergic function in mouse models and produce social deficits and electroencephalographic changes similar to those seen in schizophrenia (Connolly et al., 2004; Ehrlichman et al., 2009; Featherstone et al., 2012; Kocsis, Brown, McCarley, & Hajos, 2013). Therefore, the current study evaluates the effect of a combination of ketamine and oxytocin treatment in mice on behavior and amygdala EEG recordings during a social task and an anxiety task in order to elucidate the mechanisms by which the two treatments affect social behavior. We hypothesized that ketamine would reduce social interaction during a three-chamber social test with a concurrent increase in amygdala activation, indicating a fear response during the social task. Likewise, we expected ketamine to reduce time spent in the center of an open field chamber with a similar increase in amygdala activation. Alternatively, oxytocin was expected to increase social interaction time and time spent in the center in both ketamine and vehicle treated mice, accompanied by decreased amygdala activation.

Methodology

Figure 1 depicts the experimental timeline.

Animals

Adolescent male C57BL/6J mice (4 weeks of age) and juvenile male A/J mice (3 weeks of age) were purchased from Jackson Laboratories (Bar Harbor, ME) for use as test mice and non-threatening targets respectively. Mice were group housed prior to electrode implantation and singly-housed thereafter in ventilated cages with ad libitum food, water, and a 12-hour light/dark cycle (light on at 7:00am). All procedures were performed according to the University Laboratory Animal Resources guidelines, were approved by the Institutional Animal Care and Use Committee, and were conducted during the light phase between 8:30 and 17:00 hours.

Implantation of electrode into peri-amygdala region

At 5 weeks of age, C57BL/6J mice were anesthetized (1% isoflurane) and a tripolar electrode (PlasticOne Inc., Roanoke, VA, USA) cut to 5, 5, 1 mm was placed into the right amygdala region. The positive and negative electrodes were placed in the amygdala region at 2.1 and 3.1 mm lateral, 2.0 mm posterior and 4.8 mm ventral to bregma (Figure 2). Grounded electrodes were placed at the cortical surface 2.0 mm posterior, 1.1 mm lateral, and 0.8 mm ventral to bregma. Electrodes were secured to the skull using ethyl cyanoacrylate (Loctite, Henkel KGaA, Duesseldorf, Germany) and dental cement (Ortho Jet,

Lang Dental, Wheeling IL, USA). Animals were allowed seven days to recover in singly-housed cages before undergoing social interaction or open field EEG recording.

Drugs

Oxytocin (Sigma Aldrich, St. Louis, MO) was dissolved in saline at a final dose concentration of 1 mg/10mL/kg of body weight. Mice were injected with either oxytocin or vehicle four times prior to social testing over the course of 9 days (day 1, 3, 6, and 9) according to the methods in Teng et. al in order to minimize the stress of injections yet maintain a physiological effect of oxytocin (Teng et al.). The injection schedule began immediately after the recovery period. Ketamine hydrochloride (JHP Pharmaceuticals, Rochester, MI) was diluted in saline injection volume of 10 mL/kg of body weight for a final dose of 20 mg/kg. Ketamine or vehicle was administered 15 minutes before the three-chamber social test or open field test. Mice were divided into four treatment groups: chronic vehicle followed by acute vehicle prior to testing (VV), chronic vehicle followed by acute ketamine (VK), chronic oxytocin followed by acute vehicle (OV), and chronic oxytocin followed by acute ketamine (OK). Mice underwent either an open field test or social interaction test.

Three-chamber social test and EEG recording

The social choice test was performed as previously described (Moy et al., 2013). Seven week- old male C57BL/6J test mice and three week-old male A/J target mice were used. The social testing apparatus was a three-chambered black Plexiglas box with no top or floor. An absorption mat was placed below the apparatus. The center chamber was 12×26×50 cm and the end chambers were each 19×26×50 cm (length/width/height). There were 10-cm wide openings from the center chamber into the two end chambers. The two end chambers of each box contained a white Plexiglas cylinder (8 cm diameter, 13 cm high) in the center with multiple small holes (1.5 cm diameter) over the entire surface to allow visual, olfactory, auditory, and limited tactile contact between the target and the test mouse. The activity of the test mice was video-recorded through cameras located 60 cm above each box and connected to a computer. Behavioral testing occurred between 10:00 AM and 4:00 PM in a room dimly lit (3–5 lux) by red light. Test mice were connected to a cable for EEG recording and given 1 hour to acclimate to the cable before entering the test environment. Target mice were allowed to acclimate to the test room for at least 1 hour. Test mice explored the chamber containing empty cylinders for 10 minutes during the habituation phase and returned their home cage while both cylinders were cleaned with 70% ethanol. Each cylinder contained a target mouse (social side) or a toy mouse (non-social side) during the 10 minute social choice test. The social side was randomly assigned. All environments were thoroughly cleaned with 70% ethanol between trials and mats were replaced. Figure 1 depicts the experimental timeline, with the test representing the timing of the social interaction trials.

Each entry into either the social or non-social chamber was marked on the EEG recording (A = enter social side; Q = leave social side; L = enter non-social side; P = leave non-social side) using Spike2 software and video recordings. Subjects with unstable EEGs or incorrect electrode placement were excluded from analysis. To analyze EEG data at the point of a

social or non- social choice, time-frequency analysis was performed at each of the 'A' (enter social) and 'L' (enter non-social) EEG markers using MATLAB and EEGLAB software. All data of time-frequency analysis were expressed in dB ($\log_{10}V^2$). To analyze the interaction phases, all EEG data was divided into social EEG or non-social EEG epochs according to the keyboard markers and an FFT analysis was performed with Spike2 software to compute power spectra. The data were expressed as $\log_{10}V^2$. The time spent in each chamber and number of entries into each chamber was analyzed by a blind experimenter using MATLAB.

Open Field test and EEG recording

The open field apparatus was a four walled Plexiglas box that measured 70 cm $\times$ 55 cm $\times$ 15cm (length/width/height) with white walls and no top. The center region of the chamber (36 cm $\times$ 33 cm) was marked with tape underneath the floor of the box. A bright light (100 lux) was placed under the center region of the box. The activity of the test mice was video-recorded through cameras located 55 cm above the box and connected to a computer. Behavioral testing occurred between 10:00 AM and 4:00 PM in a room dimly lit (3–5 lux) by red light. Mice were connected to a cable for EEG recording and given 1 hour to acclimate to the cable before entering the test environment. Each mouse explored the box for 10 minutes during a habituation phase and returned their home cage while the box was cleaned with 70% ethanol. A final 10 minute test was recorded following cleaning. All environments were thoroughly cleaned with 70% ethanol between trials. Figure 1 depicts the experimental timeline, with the test representing the timing of the open field trials.

Each entry into the center was marked as previously described using Spike2 software and video recordings (A = into center/out of periphery and Q = out of center/into periphery). To analyze the center and periphery EEG data, all EEG data were divided into epochs according to the keyboard markers and FFT analysis was performed using Spike2 software to compute power spectra. All data was expressed as $\log_{10}V^2$. EEG data was not analyzed at the choice time point due to the brevity of the center epochs. Time spent in each region and number of center-periphery crossings was analyzed by a blind experimenter using MATLAB.

Statistics

Each mouse was given a social score (social time/total time spent in both the social and non-social chamber) or a center score (time spent in center/total time in open field chamber). The number of entries into either chamber or number of center-periphery crossings was recorded for each group. The effect of the drug administration on behavior was analyzed with Statistica software using a one-way ANOVA with Fisher LSD post-hoc test for multiple comparisons and a factorial ANOVA to determine the effect of each individual treatment.

For the EEG interaction phase, analysis was completed using a one-way ANOVA for each wave type (theta: 4–8 Hz, alpha: 8–12 Hz, beta: 12–30 Hz, gamma: 30–80 Hz, high gamma: 80–120 Hz) using Statistica software. The differences between the social and non-social power were computed as a difference score (social – nonsocial power) and analyzed by factorial ANOVA to determine significant increases and decreases between chambers among drug groups. Likewise, the differences in center and periphery power were computed as a difference (center power - periphery power). In the social choice phase analysis,

comparisons between social and non-social choices were analyzed using MATLAB/EEGLAB software in the window 500 ms before and 500 ms after a choice. The power values for each frequency in the time window were extracted using EEGLAB software and analyzed by ANOVA with Statistica software. Thirteen subjects were excluded from EEG analysis due to insufficient quality of EEG data due to technical and mechanical issues. These were also excluded from behavioral analyses.

Results

Effects of drug treatment on EEG during Behavior

A total of 68 mice had evaluable data for amygdala EEG and behavioral tests with $n=29$ for open field and $n=39$ for social interactions.

Across all cohorts, oxytocin treatment was associated with a significant decrease in EEG power in the gamma ($F_{1,64} = 4.799$, $p = 0.032$) and ($F_{1,64} = 4.947$, $p = 0.030$) ranges during the behavioral tasks (Figure 3, Factorial ANOVA). There was no significant main effect of oxytocin for theta, alpha and beta frequencies (Supplemental Table 1).

Ketamine treatment did not have any significant effect on EEG power in any frequency range (Figure 3 and Supplemental Table 2, Factorial ANOVA).

There was no significant interaction between drug effects and task, indicating that the overall effect on amygdala activity was not modified by overall performance of either the open field or social interaction behaviors (Supplemental Table 3).

Three-Chamber Social Test Behavior

Oxytocin produced a significant increase in social score regardless of ketamine treatment ($F_{1,35}=4.300$, $p = 0.046$, Factorial ANOVA). Ketamine produced a significant social deficit as measured by social score regardless of oxytocin treatment regimen ($F_{1,35} = 15.237$, $p = 0.000$, Factorial ANOVA). There was no significant interaction between oxytocin and ketamine on social score ($F_{1,35} = 0.778$, $p = 0.384$, Factorial ANOVA). Specifically, there was no significant reduction for social interaction score in mice treated with both oxytocin and ketamine relative to controls (Figure 4, $MS = 0.018$, $df = 35$, $p = 0.198$, Factorial ANOVA with Fisher LSD), suggesting that oxytocin attenuated the detrimental effects of ketamine. However, there were no significant differences between social scores of the VK and OK groups (Figure 4, $MS = 0.018$, $df = 35$, $p = 0.411$, Factorial ANOVA with Fisher LSD), indicating that oxytocin administration did not fully reverse the deficit among mice that received ketamine. Interestingly, oxytocin treatment alone was associated with significantly increased social scores in comparison to control mice (Figure 4, $MS = 0.018$, $df = 35$, $p = 0.041$, Factorial ANOVA with Fisher LSD). Mice in the OK group had a significantly lower social score as compared to those in the OV group (Figure 4, $MS = 0.018$, $df = 35$, $p = 0.002$, Factorial ANOVA with Fisher LSD).

Open Field Behavior

There was a significant effect of oxytocin on the fraction of time spent in the center of the open field across both ketamine and saline groups (Figure 5a, $F_{1,25} = 5.854$, $p = 0.023$,

Factorial ANOVA). Ketamine treatment had no significant effect on the center score (Figure 5a, $F_{1, 25} = 0.058$, $p = 0.811$, Factorial ANOVA). There was no significant interaction among the two drug treatments on center score ($F_{1, 25} = 0.000$, $p = 0.998$, Factorial ANOVA). However, oxytocin treated mice showed significantly more crossings between the center and periphery regions ($F_{1, 25} = 7.387$, $p = 0.012$, Factorial ANOVA) while ketamine treatment had no effect on the number of center-periphery crossings (Figure 5b, $F_{1, 25} = 0.881$, $p = 0.357$, Factorial ANOVA). There was no significant interaction between ketamine and oxytocin treatment for either center score or number of crossings (Figures 5a and 5b, $F_{1, 25} = 0.006$, $p = 0.941$, Factorial ANOVA).

EEG during Social Test

There were no significant differences in EEG power between the social and non-social interaction phases among any of the treatment groups (Supplemental Table 4 and Figure 6, factorial ANOVA).

The induced power results during the choice phase (500 ms before and after a social or non-social choice) are detailed below, separated by frequency range.

Theta: There was a significant increase in induced theta power during a non-social choice relative to a social choice across the pre/post choice condition and all drug conditions (RMANOVA, $F_{1, 33} = 8.170$, $p = 0.007$). Additionally, there was a significant interaction of chamber choice and pre/post choice condition (RMANOVA, $F_{1, 33} = 9.380$, $p = 0.004$). Specifically, there was no significant difference between social and non-social choice prior to chamber entry (Figure 7, RMANOVA with Fisher LSD, $MS = 3.65$, $df = 33$, $p = 0.940$), but there was significantly lower induced theta power following a social entry relative to a non-social chamber entry (Figure 7, RMANOVA with Fisher LSD, $MS = 3.65$, $df = 33$, $p = 0.000$). There was also an increase in nonsocial power following a choice as compared to nonsocial power prior to a choice (Figure 7, RMANOVA with Fisher LSD, $MS = 3.65$, $df = 33$, $p = 0.009$). Power following a nonsocial choice was significantly higher than power prior to a social choice (Figure 7, RMANOVA with Fisher LSD, $MS = 3.65$, $df = 33$, $p = 0.011$). However, there were no significant effects of oxytocin, ketamine, their combination or timing (pre/post choice) on induced theta power (Supplemental Table 5).

Alpha: There was a significant increase in induced alpha power during a non-social choice relative to a social choice across pre/post choice condition and all drug conditions (RMANOVA, $F_{1, 33} = 8.150$, $p = 0.007$). Additionally, there was a significant interaction of chamber choice and pre/post choice condition (RMANOVA, $F_{1, 33} = 9.070$, $p = 0.005$). Specifically, there was no significant difference between a social and non-social choice prior to chamber entry (Figure 7, RMAOVA with Fisher LSD, $MS = 3.61$, $df = 33$, $p = 0.790$), but there was significantly lower induced alpha power following a social relative to a non-social chamber entry (Figure 7, RMANOVA with Fisher LSD, $MS = 3.61$, $df = 33$, $p = 0.000$). There was also an increase in nonsocial power following a choice as compared to nonsocial power prior to a choice (Figure 7, RMANOVA with Fisher LSD, $MS = 3.61$, $df = 33$, $p = 0.031$) and a decrease in social power following a choice as compared to social power before a choice (Figure 7, RMANOVA with Fisher LSD, $MS = 3.61$, $df = 33$, $p = 0.041$). Power following a nonsocial choice was significantly higher than power prior to a social choice

(Figure 7, RMANOVA with Fisher LSD, $MS = 3.61$, $df = 33$, $p = 0.017$). Power following a social choice was significantly lower than power prior to a nonsocial choice (Figure 7, RMANOVA with Fisher LSD, $MS = 3.61$, $df = 33$, $p = 0.022$). However, there were no significant effects of oxytocin, ketamine, their combination or timing (pre/post) on induced alpha power (Supplemental Table 5).

Beta: There was a significant increase in induced beta power during a non-social choice relative to a social choice across pre/post choice condition and all drug conditions (RMANOVA, $F_{1,33} = 6.020$, $p = 0.012$). Additionally, there was a significant interaction of chamber choice and pre/post choice condition (RMANOVA, $F_{1,33} = 8.380$, $p = 0.007$). Specifically, there was no significant difference between social and non-social choice prior to chamber entry (RMANOVA with Fisher LSD, $MS = 3.46$, $df = 33$, $p = 0.960$), but there was significantly lower induced beta power following a social relative to a non-social chamber entry (Figure 7, RMANOVA with Fisher LSD, $MS = 3.46$, $df = 33$, $p = 0.000$). Power decreased significantly following a social choice as compared to prior to the choice (Figure 7, RMANOVA with Fisher LSD, $MS = 3.46$, $df = 33$, $p = 0.001$). Power following a social choice was significantly lower than power prior to a nonsocial choice (Figure 7, RMANOVA with Fisher LSD, $MS = 3.46$, $df = 33$, $p = 0.001$). However, there were no significant effects of oxytocin, ketamine, their combination or timing (pre/post) on induced beta power (Supplemental Table 5).

Gamma: There was a significant reduction in induced gamma power in the post entry relative to pre entry condition (Figure 7, RMANOVA, $F_{1,33} = 7.410$, $p = 0.010$). Unlike the lower frequency ranges, there was no significant effect of chamber choice (social vs nonsocial) on induced gamma power (RMANOVA, $F_{1,33} = 0.401$, $p = 0.530$). Additionally, there were no significant effects of oxytocin, ketamine, their combination on induced gamma power (Supplemental Table 5).

High Gamma: Similar to the gamma frequency, there was a significant reduction in induced high gamma power in the post entry relative to pre entry condition (Figure 7, RMANOVA, $F_{1,33} = 7.560$, $p = 0.0010$). Also similar to gamma power, there was no significant effect of chamber choice (social vs nonsocial) on induced high gamma power (RMANOVA, $F_{1,33} = 0.410$, $p = 0.560$). Additionally, there were no significant effects of oxytocin, ketamine, their combination on induced theta power (Supplemental Table 5).

EEG during Open Field Test

There were no significant main effects of zone on induced power in any frequency range for oxytocin, ketamine and their combination ($p > 0.05$ for all comparisons). There were also no significant main effects of drug treatments (oxytocin, ketamine, oxytocin/ketamine, $p > 0.050$ for all comparisons, Supplemental Table 6). Additionally, there were no significant interactions between oxytocin, ketamine nor their combination on power within any frequency or zone (Figure 8).

Discussion

Ketamine treatment was associated with a significant decrease in social interactions, consistent with previous data demonstrating the effects of NMDA receptor antagonists (Lee et al., 2005; Morales & Spear, 2014; Silvestre, Nadal, Pallares, & Ferre, 1997). Oxytocin did not restore this deficit, contrary to studies in which social deficits were ameliorated by either chronic or acute oxytocin administration (Ferguson et al., 2001; Lee et al., 2005; Teng et al.). However, among mice that did not receive ketamine, oxytocin treatment was associated with a significant increase in social interaction time. Similarly, oxytocin treatment was also associated with significantly more time in the center of an open field, suggesting a reduction in anxiety. Ketamine had no effect on this measure. Consistent with this pattern, oxytocin also significantly increased the number of crossings from center to periphery while ketamine had no significant effect. Taken together, these data do support a potential role for oxytocin as a potential therapeutic agent for social deficits, perhaps by reducing elevated social anxiety.

Two distinct patterns of changes in EEG power were observed in the low frequency ranges and high frequency ranges during the choice phase of the social interaction task. In the theta, alpha, and beta (low frequency) ranges, there was a consistent increase in induced power following a non-social choice as compared to the power following a social choice, though there was no difference in power prior to a choice. In the gamma and high gamma (high frequency) ranges, EEG power decreased significantly after a choice, regardless of whether it was social or non-social.

Previous studies suggest that low frequency power may be representative of the activity resulting from regional connectivity while high frequency power illustrates local activity (Nir et al., 2007; Siegel, Donner, & Engel, 2012; Wang, Saalman, Pinsk, Arcaro, & Kastner, 2012). Therefore, changes in theta, alpha, and beta activity following a social-nonsocial choice may reflect an overall decrease in afferent and efferent signaling between amygdala and other brain regions following a social choice. Alternatively, the reduction in high frequency power after a decision suggests that the local activity within the amygdala is related to making a choice, rather than the subsequent experience during interactions. As such, alterations in amygdala activity that have been reported in schizophrenia may be more relevant to the anticipatory phase of a social interaction than the subjective experience during it.

Examination of the effect of oxytocin and ketamine on amygdala activity throughout both tasks reveals that oxytocin treatment was associated with a significant decrease in EEG power in high frequency ranges. Ketamine did not have an effect on amygdala activity in any frequency range. There were no significant differences in power between the two tasks, indicating that this effect was consistent across both the three chamber social test and the open field test. These data suggest that oxytocin may be reducing local circuit activity within amygdala, without altering long distance connectivity to other brain regions, as would be reflected in lower frequency bands. Furthermore, during the choice phase of the social interaction test, amygdala activity was similar prior to a social or nonsocial choice, regardless of drug treatment. Alternatively, low frequency amygdala activity was

significantly lower after a social than a nonsocial choice. EEG power significantly decreased in the high frequency range, following a choice but this decrease was not specific to the social or nonsocial chamber. These data suggest that there is an increase in local amygdala activity during the choice phase, regardless of which decision the animal made.

Alternatively, connectivity between the amygdala and distant regions shows a more selective pattern of activation, such that there is a relative suppression of this activity prior to initiating social contact.

Behaviorally-induced changes in amygdala activity were consistent across all treatment groups, indicating that drug effects on behavior were not mediated by local activity as hypothesized. Additionally, while not specific to either the social or nonsocial chamber, we observed a decrease in high frequency activity (gamma and high gamma) in the oxytocin treated mice as compared to the vehicle treated mice over the course of the entire task. Taken together, data indicate that oxytocin reduces the overall local circuit (i.e. high frequency) activity within amygdala throughout a task, altering the bias towards social approach as evidenced by an increased social interaction score.

A similar pattern has been observed in human subjects. Oathes et al. found that increased gamma activity is present in both subjects with and without generalized anxiety disorder (GAD) when presented with an anxiety-producing stimulus (Oathes et al., 2008). Oya et al. found a similar pattern of increased gamma and high gamma activity when subjects were presented with aversive stimuli as compared to neutral and pleasant stimuli (Oya, Kawasaki, Howard, & Adolphs, 2002). Our data supports the hypothesis that oxytocin affects social interaction by decreasing activation of the amygdala, as evidenced by decreased gamma and high gamma power, and its downstream anxiety pathways (Kirsch et al., 2005; Labuschagne et al., 2010; Marazziti et al., 2014). It remains unclear if the initial disinhibition of the amygdala that oxytocin reduces is due to increased local activity or from aberrant connectivity to other regions, such as the prefrontal cortex or insula (Hamm et al., 2014; Swartz et al., 2014).

Neither of the ketamine treated groups showed any significant change in EEG power in any frequency range. Although both the OK and OV group exhibited decreased high frequency activity, only the OV group displayed increased social interaction. This data suggests that ketamine may be affecting social interaction via brain regions other than the amygdala. For example, ketamine may reduce the hedonic value of a social interaction, or may alter the subject's motivation to act on social drive. In conjunction with the finding that oxytocin, but not ketamine, treatment modulates behavior in an anxiety task, these results suggest that oxytocin mediates behavior via anxiolytic properties while ketamine has no detectable effect on anxiety responses.

Clinical studies to date have shown success with both acute and chronic oxytocin treatment in patients with schizophrenia (reviewed in (Macdonald & Feifel, 2012)). The current study suggests that any changes caused by chronic oxytocin treatment may be orthogonal to improvement in social deficits caused by acute ketamine treatment. Interestingly, though a combination of oxytocin and ketamine treatment did not result in a significantly higher social interaction score than ketamine treatment alone, there was no significant difference in

social interaction between the treated and diseased (OK) group and the control (VV) group. This suggests that the oxytocin treatment did partially restore social function to a normal level, though not significant in comparison to the diseased (VK) group.

We do not think that oxytocin's lack of reversal was due to a problem with route of administration or dose, as the treatment was associated with increased social interaction in control mice. This is consistent with previous work that demonstrates oxytocin's ability to affect the central nervous system via intraperitoneal injection (Lundeberg, Uvnas-Moberg, Agren, & Bruzelius, 1994; Ring et al., 2006; Teng et al.). Although ketamine decreases social interaction, it may not produce the same sort of negative symptoms of schizophrenia or may not affect behavior through amygdala activation, as proposed in clinical populations (Ferguson et al., 2000; Mier et al., 2014; Morris et al., 1996; Mukherjee et al., 2013). It is possible that chronic ketamine treatment or developmental reduction of NMDA receptors would yield a pattern of amygdala activation that is more consistent with schizophrenia, both with respect to behavior and electroencephalography, allowing for more accurate evaluation of potential therapeutic interventions for negative symptoms.

The current study suggests that the amygdala modulates social decision-making, as evidenced by its change in activity from before to after a chamber choice. However, amygdala activity was consistent across treatment groups for social and nonsocial behavioral trials. Because behavior varied nonetheless, another component of the amygdala-anxiety circuit may be acting as a final filter for social interaction. Oxytocin's pro-social effects in control mice are mediated by the amygdala pathway, as seen from the overall decreased in high frequency activity, but the final decision will integrate communication with other brain structures.

In summary, we found that social interaction deficits caused by ketamine could not be rescued by oxytocin, but among control mice oxytocin treatment increased social interaction. This is contrary to the findings of Lee et al., which demonstrate that acute injection of oxytocin into the central nucleus of the amygdala rescues the social deficits caused by treatment with chronic phencyclidine (PCP), another NMDA receptor antagonist (Lee et al., 2005). These differences may be explained by the mode of administration, as Lee et al. used cannulae to administer both PCP and oxytocin directly to the amygdala. The physiological and behavioral changes induced by PCP and ketamine may also be slightly different, as the two drugs have been shown to have different affinities to receptors other than the NMDA-R (Kapur & Seeman, 2002). Lastly, the present study used chronic oxytocin injections, which may have led to downregulation of oxytocin receptors and decreased potential for social-deficit rescue (Huang et al., 2014).

Oxytocin showed anxiolytic effects on an anxiety task while ketamine caused no effect. This indicates that ketamine and oxytocin may affect social behavior via two distinct pathways. Our data provide evidence that oxytocin mediates this behavioral change by decreasing the anxiety response, coincident with decreased amygdala activation. However, previous work has shown that the reward circuitry of the nucleus accumbens plays a significant role in social behavior (Gunaydin et al., 2014; Young, Lim, Gingrich, & Insel, 2001). As oxytocin receptor density is high in the nucleus accumbens, it is possible that in addition to its

anxiolytic effects in the amygdala, oxytocin may be affecting this reward circuitry. Alternatively, ketamine may be affecting social behavior by inhibiting the reward and motivation associated with social interaction, not by affecting the amygdala as we hypothesized. Further work is needed to separate out effects the anxiety and reward pathways and these drugs on social interactions. Furthermore, the present study suggests that the amygdala may not be the final filter for social decisions, as activity in the region was consistent across treatment groups even though behavior varied.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Full timeline of experiment. Electrodes were implanted and subjects were given 1 week to recover. Subjects were injected with oxytocin or saline 4 times over 9 days. After 24 hours, subjects were habituated to the EEG cable for one hour and injected with either ketamine or saline. They were then given 10 minutes to habituate to the testing apparatus (either open field or 3-chamber social apparatus) and tested for 10 minutes.

Figure 2. Placement of electrode. The positive and negative electrodes were placed 2.1 mm and 3.1 mm ventral, 2.0 mm posterior, and 4.8 mm ventral to the bregma. The ground electrode was placed 1.1 mm lateral, 2.0 mm posterior, and 0.8 mm ventral to the bregma.

Figure 3.

EEG power in each frequency range during behavioral tasks ($n = 16-19/\text{group}$, means + SEM shown). Oxytocin treatment caused a significant decrease in EEG power in the gamma ranges and high gamma ranges (*: $p < 0.050$, factorial ANOVA in each range). Ketamine did not have a significant effect on EEG power in any range.

Figure 4.

Three chamber social interaction test behavioral data (n = 9–10/group, means + SEM shown). Ketamine significantly decreased social scores and oxytocin did not reverse this deficit. Oxytocin increased social interaction score among control mice (*: p < 0.05, **: p < 0.01, ***: p < 0.001, factorial ANOVA with Fisher LSD post-hoc).

Figure 5.

Open field test behavioral data ($n = 6-9/\text{group}$, means + SEM shown). (a) Oxytocin treatment significantly increased time spent in the center of the open field chamber. Ketamine treatment had no effect on the fraction of center time (*: $p < 0.050$, factorial ANOVA). (b) Oxytocin treatment significantly increased the number of crossings between center and periphery of the open field chamber. Ketamine treatment had no effect on this measure (*: $p < 0.050$, factorial ANOVA).

Figure 6.

Three chamber social interaction test EEG data during the interaction phase ($n = 9-10$ /group, means + SEM shown). There were no significant differences in social to nonsocial power differentials in any frequency range between any treatment group (n.s., factorial ANOVA within each range, means + SEM shown).

Figure 7.

Three chamber social interaction test EEG data during the social and nonsocial choice phases ($n = 9-10/\text{group}$, means + SEM shown). Data is shown as collapsed between drug groups because there was no significant effect of drug on the EEG power in any frequency range. (*: $p < 0.050$, **: $p < 0.010$, ***: $p < 0.001$, RMANOVA with Fisher LSD).

Figure 8.

Open field test EEG data ($n = 6-9$ /group, means + SEM shown). Neither oxytocin nor ketamine treatment had a significant effect on EEG power while subjects were in the center or periphery (n.s., factorial ANOVA within each frequency range).