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Intranasal oxytocin in rhesus monkeys alters brain networks that detect social salience and reward

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Abstract

In primates, resting state functional neuroimaging (rsfMRI) has identified several large-scale, intrinsic brain networks, including the salience network (SN), which is involved in detecting stimulus salience. Intranasal oxytocin (IN-OT) has been shown to modulate the salience and rewarding quality of social stimuli in mammals and numerous studies have shown that it can affect the functional connectivity between brain regions. Less is known, however, about how these effects unfold over time following IN-OT administration. This study used rsfMRI in anesthetized rhesus macaques to track temporal changes in the functional connectivity between brain regions involved in the SN, including the anterior cingulate cortex (ACC), anterior insula (AI), amygdala (amy), and ventral striatum (vstr), lasting three hours after IN-OT or Placebo (saline) administration. We found significant temporal changes in the functional connectivity between all regions associated with treatment condition. IN-OT increased the functional connectivity between AI_vstr, ACC_amy (right hemisphere), ACC_vstr (left hemisphere), and amy_vstr (right hemisphere), but reduced the functional connectivity between ACC_AI, and the AI_amygdala. These results suggest that IN-OT may dampen salience detection in rhesus monkeys, consistent with previous findings of reduced social vigilance, while enhancing the connectivity between the SN and regions involved in processing reward.

Keywords

intranasal oxytocin; salience network; functional connectivity; fMRI; reward; rhesus macaque

Studies demonstrating the effects of IN-OT on social behavior in humans are growing exponentially, increasing excitement among researchers that the neuropeptide might confer therapeutic benefits and improve social behavior in individuals with psychiatric conditions characterized by social impairments, including anxiety disorders, schizophrenia, depression,

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personality disorders, and autism (Bakermans-Kranenburg & van IJzendoorn, 2013; Heinrichs & Domes, 2008; Insel, 2010; Modi & Young, 2012). Although it remains unclear how IN-OT modulates social behavior in primates and whether the effects are purely centrally mediated, one of the main hypotheses to emerge is that IN-OT influences the salience of social stimuli and their rewarding quality (Bartz et al., 2011; Leng & Ludwig, 2016; Piva & Chang, 2018). This is supported by studies showing that IN-OT increases the salience of the eyes in faces (Gamer et al., 2010), the salience of emotional stimuli (Domes et al., 2013), and the rewarding quality of social stimuli (Hu et al., 2015; Hurlemann et al., 2010). Moreover, there is a known interaction between the oxytocinergic and dopaminergic systems, providing a direct mechanism by which social salience and social reward may be co-influenced (Skuse & Gallagher, 2009). In nonhuman primates, OT receptors have been found in brain regions involved in detecting the salience of visual stimuli and regulating attention to them, including the superior colliculus, pulvinar, and nucleus basalis of Meynert (Freeman et al., 2014). Although only a few studies have focused on the effects of IN-OT on social behavior and cognition in nonhuman primates, they have broadly replicated many of the findings from human IN-OT studies, including increased attention to the eyes in faces (Ebitz et al., 2013; Dal Monte et al., 2014), enhanced socially-reinforced learning (Parr, 2014), and reduced attention to negative, threatening or potential dangerous stimuli (Chang et al., 2013; Parr et al., 2013). Together, these findings support the general idea that IN-OT influences prosocial behavior by increasing the salience and rewarding quality of mild or moderately arousing social stimuli, while reducing vigilance towards highly arousing, negative stimuli.

In humans and macaques, the brain is organized into several large-scale, intrinsic brain networks that are involved in a variety of processes including the control of attention (Corbetta & Schulman, 2002; Seeley et al., 2007), task control (Dosenbach et al., 2006), visual and auditory perception (Vincent et al., 2007), somatomotor function (Biswal et al., 1997), and the default mode network (Raichle et al., 2001). These networks are identified in the resting state and do not depend on the performance of specific tasks. One such network, the salience network (SN), is primarily responsible for detecting and responding to the salience of encountered stimuli. The SN is primarily anchored by two cortical regions, the anterior insula (AI) and the dorsal anterior cingulate cortex (ACC), (Menon, 2015; Seeley et al., 2007). The AI is primarily involved in the detection and affective integration of salient, multisensory information from the environment and from interoceptive and visceral awareness and is, thus, important in a wide variety of cognitive tasks and affective processes (Craig, 2009; Menon & Uddin, 2010). In contrast, the ACC is more involved with determining how to respond to salient information and coordinating with motor systems important for the regulation of emotional processes (Menon, 2015; Rushworth, 2008). In addition to these two core SN regions, several subcortical structures provide context-specific information about emotion and reward-based cues (Delgado, 2007). In particular, information exchange with the amygdala provides contextual information about threat and negative affective stimuli, while connectivity with the ventral striatum supports information exchange about reward and motivational value. Therefore, the SN is involved broadly in detecting and selectively allocating attention to both positive and negative, emotionally salient stimuli and mobilizing behavioral responses to them. Salient stimuli are interpreted in

a context-dependent manner and may include things that are unusual or unexpected, socially-meaningful or rewarding, self-relevant, and/or emotionally engaging (Menon, 2015). Impairments of SN function are associated with a variety of neuropsychiatric and neurodevelopmental disorders, including attention-deficit hyperactivity disorder, autism, schizophrenia, and major depression (Di Martino et al., 2008; Fair et al., 2010; Hamilton et al., 2013; Seeley et al., 2009; Uddin et al., 2013; White et al., 2010). Interestingly, functional connectivity between the ACC and amygdala has been specifically implicated in social inhibition, such that greater connectivity is associated with reduced social inhibition (Blackford et al., 2014).

These large-scale, intrinsic brain networks are identified in their resting state using an imaging technique called resting state functional connectivity MRI (rsfMRI). RsfMRI is a relatively new and rapidly evolving neuroimaging technique that utilizes the fMRI BOLD time series to measure correlations in low frequency (0.01 – 0.1 Hz), high-amplitude patterns of neuronal activity between specific brain regions (Biswal et al., 1997; van den Heuvel & Hulshoff Pol, 2010; Power et al., 2011; Yeo et al., 2011). Studies have shown that the connectivity of these networks in the resting state correlates with that seen during task-based activity (Biswal et al., 1997; Seeley et al., 2007; Smith et al., 2009), and persists during sleep and anesthesia (Smyser et al., 2010). This has led researchers to propose that the network activity reflects a history of co-activation between functionally interconnected brain regions (Fair et al., 2007; Katz & Shatz, 1996; Miranda-Dominguez et al., 2014; Power et al., 2011; Vincent et al., 2007). By continuing to transmit information across functionally connected regions at rest, these neurons keep the brain in a semi-ready state, perhaps improving the reaction time to become engaged when needed (van den Heuvel & Hulshoff Pol, 2010). The development of resting state functional neuroimaging is an extremely important finding for comparative studies as it provides a relatively non-invasive and accessible method for studying brain function in anesthetized animals.

Several studies have now shown that the functional connectivity between brain regions is affected by IN-OT in both task-based and resting state conditions (Wigton et al., 2015). Dodhia & colleagues (2014) reported increased resting state functional connectivity between the amygdala and ACC, and the amygdala and medial prefrontal cortex (mPFC) in individuals with generalized social anxiety disorder (GAD). A similar task-based study reported that IN-OT increased the functional connectivity between the amygdala and the insula, and the amygdala and ACC in participants with GAD when processing fearful faces (Gorka et al., 2015), thus “normalizing” the decreased functional connectivity typically seen between these regions in GAD versus control individuals. Using a whole brain analysis, Riem and colleagues (2012) showed an overall decrease in amygdala activation after IN-OT administration when female participants listened to infant laughter relative to control sounds, but an increase in the functional connectivity between the amygdala and the orbito-frontal cortex, and the amygdala and ACC. Sripada and colleagues (2013) showed an increase in resting state functional connectivity between the amygdala and ACC, and the amygdala and medial prefrontal cortex (mPFC) after IN-OT compared to Placebo treatment. Wittforh-Schardt and colleagues (2012) found that IN-OT influenced the salience of children’s faces. Functional connectivity between reward regions was reduced after IN-OT compared to Placebo treatment when fathers viewed either their own child (arousing) or an unfamiliar

child (novel), compared to images of less salient, familiar children. Kovacs and colleagues (2015) used rsfMRI to examine the functional connectivity between the amygdala and other brain regions in individuals who were self-prescribing IN-OT to improve their mood, social ability, and sexual desire, compared to control participants who were not using OT. Therefore, this study did not examine the effects of acute IN-OT administration, but rather the cumulative effects in individuals who had a history of IN-OT use. They administered two behavioral assessments, the Reading the Mind in the Eyes Test and the National Institutes of Health's Adult Social Relationship scale. Despite finding no differences in the behavioral measures, they reported significant increases in the functional connectivity between the right amygdala and the dorsal ACC in the IN-OT users compared to controls, which was positively correlated with their history of cumulative use (Kovacs & Keri, 2015). Finally, Hu & colleagues (2015) reported increased functional connectivity between the amygdala and insula, and the amygdala and caudate during a socially-reinforced learning task in which faces served as the reinforcing stimuli. This study is interesting because the participants were Chinese and, unlike studies in Caucasians, the Placebo condition was characterized by decreased performance after social compared to nonsocial reinforcement. However, IN-OT reversed this and resulted in enhanced learning in the social compared to nonsocial conditions, consistent with previous studies in both humans and monkeys (Hurlemann et al., 2010; Parr, 2014). The aforementioned studies focused primarily on the connectivity between the amygdala and the rest of the brain due to its well-established role in processing emotionally-salient stimuli. What is notable, however, is that many of the regions that were found to be modulated by IN-OT, including the amygdala, are involved in the SN.

Because of IN-OT's reported effects in enhancing the salience of social stimuli and their rewarding qualities (Bartz et al., 2011), and affecting the functional connectivity of brain regions involved in socio-emotional processing, this study focused on the effects of acute IN-OT administration on temporal changes in the functional connectivity between the core and extended SN regions (AI, ACC, amygdala and ventral striatum). Based on the results of previous studies, we hypothesized that IN-OT would generally reduce social vigilance (Ebitz et al., 2013; Parr et al., 2013), thus decreasing the functional connectivity between ACC and AI. Because of its prosocial effects, we hypothesized that IN-OT will enhance connectivity between the ACC and amygdala, thus reducing social inhibition (Blackford et al., 2014). Because IN-OT has also been shown to enhance the rewarding quality of social stimuli in both humans and macaques (Hu et al., 2015; Hurlemann et al., 2010; Parr, 2014), we predicted that it would enhance the functional connectivity between the ventral striatum and other core regions within the SN.

Methods

Subjects:

Four adult male rhesus monkeys, approximately eight years of age, were the subjects of this study. These subjects were born into large social groups at the Yerkes National Primate Research Center's field station where they were mother-reared until they were approximately three years of age. After this time, they were socially housed in a same-sex, peer group with six other monkeys, consistent with colony management procedures. When

they were four years of age, they were moved to the Yerkes Primate Center's main campus where they were pair-housed with another member of their peer group in the same colony room. Two of these individuals had participated previously in several studies involving the acute effects of IN-OT on social cognition using non-invasive, computerized tasks. It is estimated that their prior exposure to IN-OT included approximately 15 treatments over a 3 year period. Subjects were OT-free for several months prior to this study and had no prior imaging procedures.

Procedure:

Prior to scanning, subjects were anesthetized in their home cage using telazol (4–5 mg/kg) and brought to the Yerkes Neuroimaging Center located onsite. There, they were placed supine on the scanner table and their head was secured in an 8-channel, high resolution knee coil. They were then intubated and isoflurane gas (1–2% continuous inhalation) was used to maintain anesthesia during the scan. After this, researchers administered the intranasal compound (OT or saline/Placebo) in aerosol form using a pediatric nebulizer. Because of the tracheal intubation, the typical nasal cycle, e.g., the exchange of air through inhalation and exhalation, was suppressed. Therefore, to ensure that the aerosol penetrated the nasal cavity past the nasal valve, researchers “pressurized” the aerosol by piping it into a fine gauge tube with a syringe tip. This created a fine stream of air rather than a passive mist. To deliver the dose, the syringe tip was then inserted into the monkey's nasal passage (left side), causing the stream of air to enter the nasal cavity and circulate around the highly vascularized nasal mucosal and into the nasal turbinates where the sinus openings, one proposed route of nose-to-brain access, are located (Djupestrand, 2013). Additionally, both the olfactory and trigeminal nerves innervate the nasal mucosa and contain perivascular spaces which act as lymphatic pathways between the nose and CNS, providing another route for the transfer of small molecules into the brain (Hanson & Frey, 2008; Johnson et al., 2010). The circular penetrance of the aerosol through the nasal passage was verified by noting the exit of the aerosol through the other nostril as monitored by the presence of condensation on a hand held mirror. Although not using this exact method, the combination of more powered aerosol delivery in addition to the closure of the soft palate has been shown to more effectively distribute aerosol throughout the extended nasal passages and may represent a more viable way to deliver compounds to the CNS than passive breathing or sniffing (Djupestrand, 2013).

The OT was prepared from oxytocin acetate salt (Sigma #06379) at a concentration of 48IU (1.71 µg/IU) in a 2ml volume of saline and administered in aerosol form over a four minute period using a pediatric nebulizer (Pacifica elite, www.drivemedical.com) (Modi et al., 2014). Given the broad range of doses and administration routes used in previous animal and human studies, no attempt was made to correct the dose for each subjects' body weight. However, the weight of the four subjects only ranged between 12.2 and 13.6 kg at the time of the study. The Placebo was sterile saline and 2ml was aerosolized for four minutes. The order of IN-OT/IN-P administration was counterbalanced across subjects and research staff were blind to the treatment condition. Immediately after the aerosol administration, the scan began with the acquisition of the localizer sequence. The time between the end of aerosol administration and the start of the rsfMRI procedure averaged 7:24 minutes (range 6:01 – 9:20 minutes) for the IN-OT condition and 7:28 minutes (range 6:20 – 8:52 minutes) for the

IN-P condition. At the completion of the scan, subjects were extubated and returned to their home cage and recovered by veterinary and research staff. All procedures were approved by the Institutional Animal Care and Use Committee of Emory University and were in compliance with regulations governing the ethical treatment of animals.

Neuroimaging Data Acquisition:

MRI sequences were acquired in a single session using a 3T Magnetom Trio Tim scanner (Siemens Medical Solutions, Malvern, PA) and an 8-channel phase array coil. The combined sequence included a T1- MRI scan for anatomical registration purposes, and four ~15 minute resting state fMRI (T2*-weighted) scans (referred to as 'RUNS') to measure temporal changes in blood-oxygen-level dependent (BOLD) signal change. The T1-weighted structural MRI scans were acquired using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) parallel image sequence (TR/TE = 2600/3.46msec, FoV: 116mm, voxel size: 0.5mm³ isotropic, 8 averages, GRPPA, R=2) and lasted approximately 15 minutes.

Resting state functional MRI (rsfMRI) BOLD images were acquired using a T2*-weighted gradient-echo echoplanar imaging (EPI) sequence (4 runs of 400 volumes each, TR/TE = 2400/25msec, voxel size: 1.5mm³ isotropic). The rsfMRI sequence was run before the T1 MRI scan (which lasted about 30 min) to ensure that measurement of functional connectivity began as soon as possible after intranasal dosing. An additional 20-volume, reverse-phase encoding scan was also acquired for unwarping distortions in the EPI scans using previously validated methods (Andersson et al. 2003).

Image Preprocessing:

T1-weighted images underwent bias correction and nonlinear registration to the F99 atlas using ANTS (Avants et al., 2009), and image segmentation using FSL (Jenkinson et al., 2012). Linear transformations were computed and inverted in order to use T1-weighted image segmentations to extract BOLD signal from white matter and cerebrospinal fluid (CSF). In addition, FSL's tools were applied for BOLD preprocessing following Power and colleagues (2012), including slice timing correction, motion correction, calculation of motion parameters, EPI unwarping, regression of nuisance variables (including 6 motion parameters and white matter, CSF, and global BOLD signals), bandpass temporal filtering at 0.009–0.08 Hz, mean-centering, and spatial smoothing with a Gaussianized kernel at 1 voxel FWHM. Motion in this dataset was very low, with no volumes showing framewise displacement > 0.3 mm. Linear BOLD-to-T1 and nonlinear T1-to-template transforms were inverted and concatenated in order to apply template-space regions of interest to native-space BOLD data. Centroids of the 3mm-radius ROIs in F99-space are listed in Table 1. Time series were extracted from these ROIs in the native-space, processed BOLD data.

Data Analysis:

The time series correlations were extracted for each run (15 minute resting state imaging sequence) between each of the four seed regions using a sliding window procedure where the BOLD values were correlated across bins of 10 samples and advanced by increments of one, resulting in 1559 correlations from the original 1600 values (Allen et al., 2014; Chang

& Glover, 2010). The functional connectivity time series correlations were analyzed using mixed models ANOVAs in SPSS (v.24) where treatment condition (IN-OT vs IN-P) and hemisphere (Left vs Right) were fixed factors and subject and time (runs 1–4) were modelled as random factors (Field, 2013). Because each TR was 2.4 s, the 400 samples in each run equated to 16 minutes and combined with the initial localizer scan time, each run ended approximately 23 min, 39 min, 55 min and 71 min post-treatment time. Time was also modelled using linear, quadratic, and cubic functions. The primary interests were any significant interactions involving both treatment condition and time, in addition to main effects of treatment condition. Both of these analyses used an unstructured covariance matrix and p-values were adjusted to $p < 0.001$ as a precaution against Type I error and the performance of multiple comparisons.

Results

Results from the time series correlation analyses revealed significant findings, summarized in Figure 1, and detailed by each pair of regions below. Supplementary Figure S1 presents a schematic of the results summary.

ACC_AI:

For the functional connectivity between the ACC and AI, there was a significant linear treatment condition $\times$ hemisphere $\times$ time interaction, $F(1,24936) = 10.2$, $p = 0.001$. In the left hemisphere, this revealed a significant treatment condition $\times$ time interaction, $F(1,12464) = 59.09$, $p < 0.001$, that was quadratic in nature whereas this interaction was significantly linear in the right hemisphere, $F(1,12464) = 53.12$, $p < 0.001$. In both hemispheres, IN-OT significantly decreased the functional connectivity between these two regions compared to IN-P (see Figure 2a).

To further understand these treatment condition $\times$ time interactions, time effects were analyzed for each treatment condition in each hemisphere separately. In the left hemisphere, there was no significant effect of time after IN-P treatment, whereas there was a quadratic effect of time after IN-OT treatment, $F(1,6228) = 75.29$, $p < 0.001$. Mean correlations were significantly different between the two treatment groups at time points 2: $t(3118) = 3.93$, $p < 0.001$ and 3: $t(3118) = 8.93$, $p < 0.001$. In the right hemisphere, there were significant effects of time for both treatment conditions. This effect was cubic after IN-P treatment, $F(1,6228) = 11.84$, $p = 0.001$, and quadratic after IN-OT treatment, $F(1,6228) = 26.13$, $p < 0.001$. Mean correlations were significantly different between the two treatment groups at all time points except 2, 1: $t(3118) = 3.92$, $p < 0.001$; 3: $t(3118) = 9.2$, $p < 0.001$ and 4: $t(3118) = 4.28$, $p < 0.001$.

There were also significant main effects of treatment condition in each hemisphere; Left: $F(1,12464) = 28.38$, $p < 0.001$ and Right: $F(1,12464) = 30.88$, $p < 0.001$, where IN-OT decreased the functional connectivity between ACC and AI compared to IN-P (Figure 2b). To address issues of individual variability, supplementary Figure S2 shows the effects of IN-OT versus IN-P on the mean correlations between ACC and AI in each individual subject and the group as a whole.

ACC_amygdala:

Functional connectivity between the ACC and amygdala also showed a significant linear treatment condition x hemisphere x time interaction, $F(1,24936) = 18.82$, $p < 0.001$. For the left hemisphere, none of the interactions reached significance, however, there was a significant main effect of time that was cubic in nature, $F(1,12464) = 26.44$, $p < 0.001$, and this was consistent for both treatment conditions, IN-OT: $F(1,6228) = 16.86$, $p < 0.001$, and IN-P: $F(1,6228) = 10.22$, $p = 0.001$. For the right hemisphere, there was a significant linear treatment condition x time interaction, $F(1,12464) = 36.95$, $p < 0.001$ (see Figure 3a). IN-OT compared to IN-P increased the functional connectivity between ACC and amygdala in the right hemisphere (see Figure 3a). However, individually, the effects of time did not reach significance for either treatment condition using the current model. Comparing treatment groups at each individual time point showed significant differences at every time point in the left hemisphere, 1: $t(3118) = 4.44$, $p < 0.001$; 2: $t(3118) = 4.5$, $p < 0.001$; 3: $t(3118) = 3.43$, $p = 0.001$; 4: $t(3118) = 4.57$, $p < 0.001$, and at time points 3: $t(3118) = 5.04$, $p < 0.001$ and 4: $t(3118) = 4.85$, $p < 0.001$ in the right hemisphere.

There were also significant main effects of treatment condition in each hemisphere; Left: $F(1,12464) = 72.97$, $p < 0.001$ and Right: $F(1,12464) = 13.79$, $p < 0.001$, where IN-OT increased the functional connectivity between ACC and amygdala (Figure 3b). Supplementary Figure S3 shows the effects of IN-OT versus IN-P on the mean correlations between ACC and amygdala in each individual subject and the group as a whole.

ACC_vstr:

Functional connectivity between the ACC and ventral striatum also showed a significant linear treatment condition x hemisphere x time interaction, $F(1,24936) = 16.27$, $p < 0.001$. For the left hemisphere, there was a significant quadratic treatment condition x time interaction, $F(1,12464) = 12.96$, $p < 0.001$. Looking only at effects of time for each treatment condition, there was no significant effect of time after IN-P treatment, but a significant quadratic effect of time after IN-OT, $F(1,6228) = 25.41$, $p < 0.001$. In the left hemisphere, there was also a significant main effect of treatment condition, $F(1,12464) = 23.84$, $p < 0.001$, where IN-OT increased the functional connectivity between ACC and ventral striatum compared to IN-P (Figure 4b). Significant differences between the treatment conditions were seen at time point 4: $t(3118) = 5.33$, $p < 0.001$.

In the right hemisphere, there was a significantly linear interaction between treatment condition and time, $F(1,12464) = 18.64$, $p < 0.001$ (see Figure 4a). Again, there was no effect of time after IN-P treatment, and a cubic effect of time after IN-OT treatment failed to reach the strict threshold to reject the null hypothesis, $F(1,6228) = 7.97$, $p = 0.005$. The main effect of treatment condition did not reach significance in the right hemisphere, however, there were significant pair-wise differences between treatment conditions where the mean correlations were significantly greater after IN-OT versus IN-P at time point 1: $t(3118) = 3.29$, $p = 0.001$, and significantly lower correlations after IN-OT versus IN-P at time point 4: $t(3118) = 3.83$, $p < 0.001$. Supplementary Figure S4 shows the effects of IN-OT versus IN-P

on the mean correlations between ACC and ventral striatum in the left hemisphere in each individual subject and the group as a whole.

AI_amygdala:

Functional connectivity between the AI and amygdala showed a significant linear treatment condition x time interaction, $F(1,24936) = 66.22$. Overall, the functional connectivity after IN-P was significantly quadratic and increasing, $F(1,12464) = 10.91$, $p = 0.001$, whereas this change was decreasing and cubic after IN-OT, $F(1,12464) = 20.18$, $p < 0.001$ (see Figure 5a). There was also a significant treatment condition x hemisphere interaction, $F(1,24936) = 15.02$, $p < 0.001$. In the left hemisphere, the main effect of treatment condition was significant, $t(12470) = 6.72$, $p < 0.001$, such that IN-OT reduced the functional connectivity between AI and amygdala compared to the IN-P condition, but this did not reach significance in the right hemisphere (Figure 5b).

Overall, significant differences between treatment conditions were seen at all time points where functional connectivity was significantly greater after IN-OT versus IN-P at time 1: $t(6238) = 4.3$, $p < 0.001$, but significantly lower at time points 2: $t(6238) = 3.24$, $p = 0.001$; 3: $t(6238) = 5.18$, $p < 0.001$; and 4: $t(6238) = 7.09$, $p < 0.001$. Supplementary Figure S5 shows the effects of IN-OT versus IN-P on the mean correlations between AI and amygdala in the left hemisphere in each individual subject and the group as a whole.

AI_vstr:

Functional connectivity between AI and the ventral striatum showed a significant treatment condition x time interaction that was cubic in form, $F(1,24936) = 11.41$, $p = 0.001$ (see Figure 6a). Changes in functional connectivity over time failed to reach significance after IN-P treatment, $F(1,12464) = 6.98$, $p = 0.008$, but was significantly quadratic after IN-OT treatment $F(1,12464) = 16.05$, $p < 0.001$.

There was also a significant main effect of treatment condition, $F(1,24936) = 32.71$, $p < 0.001$, such that IN-OT increased the functional connectivity between AI and the ventral striatum compared to the IN-P condition (Figure 6b). Differences in functional connectivity between treatment conditions was significant at time points 2: $t(6238) = 5.59$, $p < 0.001$, and 4: $t(6238) = 3.28$, $p = 0.001$. Figure S6 shows the effects of IN-OT versus IN-P on the mean correlations between AI and ventral striatum in each individual subject and the group as a whole.

Amygdala_vstr:

Lastly, functional connectivity between the amygdala and ventral striatum showed a significant linear treatment condition x hemisphere x time interaction, $F(1,24936) = 18.17$, $p < 0.001$. In the left hemisphere, there was a significant treatment condition x time interaction, $F(1,12464) = 14.31$, $p < 0.001$. This effect was driven solely by the IN-OT treatment condition, but this failed to reach the stringent level of significance, $F(1,6228) = 10.04$, $p = 0.002$. There was no effect of time after IN-P. There were, however, significant differences in the functional connectivity between treatment conditions at time point 1:

$t(3118) = 4.77, p < 0.001$; 3: $t(3118) = 3.33, p = 0.001$; and 4: $t(3118) = 3.58, p < 0.001$. There was no significant main effect of treatment condition in the left hemisphere. There was no significant interaction between treatment condition and time in the right hemisphere (see Figure 7a).

In the right hemisphere, there was a significant main effect of treatment condition, $F(1,12464) = 17.91, p < 0.001$, where functional connectivity was greater in the IN-OT compared to IN-P condition, but this failed to reach the adjusted level of significance in the left hemisphere ($p = 0.015$) (see Figure 7b). Significantly greater functional connectivity was shown after IN-OT compared to IN-P at time point 3: $t(3118) = 3.4, p = 0.001$. Figure S7 shows the effects of IN-OT versus IN-P on the mean correlations between amygdala and ventral striatum in each individual subject and the group as a whole.

Discussion

Significant temporal differences were observed between all brain regions after IN-OT compared to IN-P administration. Results showed that IN-OT increased functional connectivity between the ACC and amygdala in the right hemisphere, the AI and ventral striatum, the ACC and ventral striatum in the left hemisphere, and the amygdala and the ventral striatum in the right hemisphere. In contrast, IN-OT decreased functional connectivity between the AI and ACC, the AI and amygdala, the ACC and ventral striatum in the right hemisphere, and the amygdala and ventral striatum in the left hemisphere. These results were relatively consistent across the four subjects tested, although there was some individual variability noted (see Supplementary Material Figures S2-S7). Overall, the present study suggests that IN-OT reduces functional connectivity between the core nodes of the SN, e.g., ACC_AI, AI_amygdala, but not ACC_amygdala, but it enhances the functional connectivity between these core nodes and reward regions, e.g., AI_ventral striatum, ACC_ventral striatum (left hemisphere), and amygdala_ventral striatum (right hemisphere).

The ACC and AI are the core cortical regions in the SN and they are two regions that are frequently activated across a wide variety of cognitive tasks and affective processes. Menon (2015) identified over 400 descriptive features from a meta-analysis of neuroimaging studies that characterize tasks involving these two regions, including pain detection, counting, and anticipation. Thus, the identification of these regions within the SN is best done in the resting state because of their frequent co-activation with task processes. Within the SN, the function of these regions is in the detection and affective integration of salient, multisensory and interoceptive information and determining how to respond through coordination with motor systems. The present study reported decreased functional connectivity between these two regions after IN-OT compared to IN-P treatment. This suggests that IN-OT reduces the effectiveness of the SN to detect and respond to salient stimuli, similar to what has been associated with an ASD symptomatology. One study, for example, reported a positive relationship between the severity of autistic traits and reduced functional connectivity between the ACC and AI (Di Martino et al., 2009). Participants were given the Social Responsiveness Survey which measures autistic traits using a continuous scale where greater scores are associated with higher levels of autistic traits. Results showed a significant negative correlation between SRS scores and functional connectivity between the pregenual

region of the ACC and the AI (Di Martino et al., 2009). This may be indicative of reduced function of the SN in individuals who score high in terms of autistic symptomatology, contributing to their failure to find social stimuli salient or engaging.

The present study also reported reduced functional connectivity between the AI and the amygdala after IN-OT compared to IN-P treatment. Numerous studies using task-based fMRI have shown that IN-OT reduces amygdala activity in response to salient (negative, aversive, and threatening) imagery (Baumgartner et al., 2008; Domes et al., 2007; Kirsch et al., 2005), and IN-OT has been shown to attenuate, or ‘normalize’ hyperactive amygdala activity in patients with GAD (Labuschagne et al., 2010). The amygdala plays an important role in providing negative-valence contextual affective information to the SN, while the AI plays a role in interoceptive awareness, and both structures are involved in the monitoring of salient stimuli. Thus, reduced functional connectivity between the AI and amygdala may attenuate information processing supporting the detection and awareness of threatening or negative-valence stimuli, categories that are of high salience. Stein et al. (2007), for example, reported significant positive coupling between the insula and amygdala during the initial appraisal of negative stimuli. Interestingly, some have noted that the reduced connectivity between AI and amygdala may indicate a diminished capacity for the AI to regulate the amygdala when initiating autonomic responses to negative stimuli, such as threat (Gorka et al., 2015), and previous studies have reported decreased functional connectivity between the amygdala and AI in ASD, similar to that reported above (von dem Hagen et al., 2013). The current findings that IN-OT reduces the functional connectivity between the amygdala and AI suggest that it has an overall effect in dampening emotional awareness and salience detection.

Moreover, IN-OT decreased functional connectivity between the amygdala and ventral striatum (left hemisphere), perhaps reducing the degree to which negative stimulus information is transmitted from the amygdala to reward regions, although there was no overall effect of treatment condition between these regions and follow-up analyses of the temporal effects failed to reach the strict threshold for significance. These findings are consistent with previous behavioral studies in rhesus monkeys suggesting that IN-OT reduces social vigilance. For example, IN-OT increases the time monkeys spend viewing dominant individuals’ faces, a stimulus that can be threatening and that is normally avoided (Chang et al., 2013), increases attention to the eyes in faces (Ebitz et al., 2013), and reduces attentional biases towards negative, threatening facial expressions (Parr et al., 2013), yet increases socially reinforced learning for both positive and negative social stimuli (Parr, 2014). These data are also consistent with numerous studies in humans that have reported that IN-OT decreases the amygdala’s response to negative stimuli, e.g., negative facial expressions, supporting the hypothesis that IN-OT reduces the aversive quality of social stimuli by damping the response of the amygdala (Baumgartner et al., 2008; Domes et al., 2007; Kirsch et al., 2005). The current data further this hypothesis by suggesting that IN-OT may reduce the salience of highly arousing, negative or aversive images by decreasing amygdala activity and its connectivity within the SN.

The present study also found that IN-OT increased functional connectivity between the amygdala and the other core node of the SN, the ACC. The ACC and amygdala are regions

that have long been reported to play a critical role in emotion processing and emotion regulation. These findings are supported by several studies showing that IN-OT enhances the functional connectivity between the amygdala and ACC in both typical and psychiatric populations (Dodhia et al., 2014; Kovacs & Keri, 2015; Riem et al., 2012; Sripada et al., 2013). Additionally, studies have shown that the ACC and amygdala circuit in humans is involved in social inhibition, such that social inhibition decreases with increased functional connectivity (Blackford et al., 2014). Therefore, IN-OT may function to reduce social inhibition by increasing the connectivity between the amygdala and ACC. This may be an important and necessary step in the ability of IN-OT to facilitate prosocial behavior because it is not enough to reduce the aversive quality of highly salient stimuli, but one must be motivated to socially engage. This may also help to explain previous behavioral findings of greater engagement of monkeys in viewing faces and viewing the faces of dominant (potentially threatening) individuals after IN-OT compared to IN-P treatment (Chang et al., 2013; Ebitz et al., 2013). These results highlight the distinct roles of the ACC and AI within the SN and that IN-OT does not function to broadly reduce the activity of the amygdala.

Finally, significant increases in functional connectivity after IN-OT treatment were found between all regions of the SN (ACC, AI and amygdala) and the ventral striatum, although some of these effects were lateralized. This indicates that IN-OT may function not only to reduce the salience of negative, aversive or potentially threatening images, and decrease social inhibition, but it may function to enhance or alter the rewarding quality of these stimuli. The striatum is not just involved in reward processing but, due to its heterogeneous anatomical and functional connections, it is a convergence area for affective, cognitive and motor processes. The striatum's response to reward increases or decreases with its subjective value and this may be mediated by connectivity with the AI, whereas connectivity between ACC and ventral striatum may be involved in learning about the value of a reward in the absence of explicit feedback (Bhanji & Delgado, 2014).

For all of these regions, there was high consistency in the pattern of change across subjects. The exception was subject number 3, who consistently showed the opposite pattern of change after IN-OT administration. There is no obvious explanation for this as these subjects were approximately the same age, weight and had similar rearing and experimental histories. This difference appears to simply reflect individual variability in the effect of IN-OT on changes in functional connectivity. Individual variation in the neural and behavioral response to oxytocin has been reported in the human literature, with links to varied factors including genetic and physiological traits, differences in life history, and psychological and behavioral traits (reviewed in Guastella and MacLeod, 2012; Bartz et al., 2011). While the sample size of the current study is too small to determine whether any of these factors may have played a role in our results, the results nonetheless suggest that primate research may be able to shed light on the causal mechanisms underlying variability in response to IN-OT.

Temporal changes in the functional connectivity between all of these brain regions after IN-OT delivery were also observed. Although the intranasal delivery of drugs from nose to brain via the olfactory and trigeminal nerves occurs within minutes of administration, changes over time would be expected given the time required for OT to reach deep brain targets and have an overall effect on the synchronization of neural activity between these

regions (Hanson & Frey, 2008; Johnson et al., 2010). It would have been more ideal to begin scanning just prior to the delivery of each compound to get a true baseline, however, that was not possible given the contexts and parameters of the neuroimaging procedures. In addition, it has been reported that the half-life of OT is approximately 19 minutes in the brain, therefore the dose would be reduced by approximately one half at the beginning of each 16 minute run (Mens et al., 1983). Some temporal changes were observed after IN-P administration. However, this occurred in only four of the twelve analyses performed, including the ACC_AI in the right hemisphere, the ACC_amygdala in the left hemisphere, AI and amygdala, and AI and ventral striatum. This may reflect the continuous fluctuation of correlations between brain regions or other unknown metabolic processes. Nonetheless, significant differences between treatment groups were found for all of these regions, and only one of the twelve analyses failed to show temporal changes after IN-OT administration.

Finally, there has been considerable debate about how IN-OT affects behavior, e.g., central versus peripheral mechanisms, whether the intranasal administration method can actually deliver OT to deep brain targets (Leng & Ludwig, 2016), and where IN-OT may be acting based on the localization of OTXR in the primate brain (Freeman et al., 2014). The present study did not eliminate the contribution of peripheral mechanisms to changes in brain connectivity since IN-OT results in a significant increase in the concentration of OT in plasma (Modi et al., 2014). Quintana and colleagues (2016), however, recently verified that the neural effects of OT in humans were found only after intranasal and not after restricted peripheral administration, supporting a central mechanism of action. In terms of delivery method, our study confirms that IN-OT can significantly affect temporal differences in the functional connectivity between several brain regions, including cortical and subcortical regions, as well as deep brain targets. It remains to be demonstrated where the target of action for IN-OT may be in the nonhuman primate brain since OTXR have not been reported, specifically, in these SN regions. However, OTXR have been found in brain regions involved in detecting the salience of visual stimuli and regulating attention to them, including the superior colliculus, pulvinar, and nucleus basalis of Meynert (Freeman et al., 2014). If the present study included parts of nucleus basalis of Meynert in the ventral striatum seed, then activation of the SN may have been primed by activation of OTXR through this region.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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	ACC	AI	amy	v str
ACC		OT<P	<i>R: OT>P</i>	<i>L: OT>P</i> R: OT<P*
AI			OT<P	<i>OT>P</i>
amy				<i>R: OT>P</i> ^ L: OT<P*
v str				

Figure 1.

Summary of time series correlations (see Supplementary Material, Figure S1). Regions of enhanced IN-OT connectivity are italicized and regions of reduced IN-OT connectivity, compared to IN-P, are in plain text. R=right hemisphere and L = left hemisphere. The asterisk(*) indicates the presence of a temporal change in functional connectivity between treatment conditions, but no significant main effect of treatment. The caret(^) indicates no significant temporal changes between treatment conditions, but a significant main effect of treatment.

Figure 2a.

Temporal changes in the effects of IN-OT versus IN-P on the functional connectivity between ACC and AI in each hemisphere. Asterisks mark the time points where there were significant differences between IN-OT versus IN-P.

Figure 2b.

The main effect of IN-OT versus IN-P on the functional connectivity between ACC and AI in each hemisphere.

Figure 3a.

Temporal changes in the effects of IN-OT versus IN-P on the functional connectivity between ACC and amygdala in each hemisphere. Asterisks mark the time points where there were significant differences between IN-OT and IN-P.

Figure 3b.

Then main effect of IN-OT versus IN-P on the functional connectivity between ACC and amygdala in each hemisphere.

Figure 4a.

Temporal changes in the effects of IN-OT versus IN-P on the functional connectivity between ACC and ventral striatum in each hemisphere. Asterisks mark the time points where there were significant differences between IN-OT and IN-P.

Figure 4b.

The main effect of IN-OT versus IN-P on the functional connectivity between ACC and ventral striatum in each hemisphere.

Figure 5a.

Temporal changes in the effects of IN-OT versus IN-P on the functional connectivity between AI and amygdala. Asterisks mark the time points where there were significant differences between IN-OT and IN-P.

Figure 5b.

The main effect of IN-OT versus IN-P on the functional connectivity between AI and amygdala in each hemisphere.

Figure 6a.

Temporal changes in the effects of IN-OT versus IN-P on the functional connectivity between AI and ventral striatum. Asterisks mark the time points where there were significant differences between IN-OT and IN-P

Figure 6b.

The main effect of IN-OT versus IN-P on the functional connectivity between AI and ventral striatum in each hemisphere.

Figure 7a.

Temporal changes in the effects of IN-OT versus IN-P on the functional connectivity between amygdala and ventral striatum in each hemisphere. Asterisks mark the time points where there were significant differences between IN-OT and IN-P

Figure 7b.

The main effect of IN-OT versus IN-P on the functional connectivity between amygdala and ventral striatum in each hemisphere.

Table 1.

Coordinates for each ROI centroid in F99 space.

Region of Interest	Left Hemisphere			Right Hemisphere		
	x	y	z	x	y	z
Anterior Cingulate Cortex	65	139	76	77	139	76
Anterior Insula	30	110	55	112	110	55
Amygdala	51	110	34	91	110	34
Ventral Striatum	63	120	57	79	120	57