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Oxytocin enhances gaze-following responses to videos of natural social behavior in adult male rhesus monkeys

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Abstract

Gaze following is a basic building block of social behavior that has been observed in multiple species, including primates. The absence of gaze following is associated with abnormal development of social cognition, such as in autism spectrum disorders (ASD). Some social deficits in ASD, including the failure to look at eyes and the inability to recognize facial expressions, are ameliorated by intranasal administration of oxytocin (IN-OT). Here we tested the hypothesis that IN-OT might enhance social processes that require active engagement with a social partner, such as gaze following. Alternatively, IN-OT may only enhance the perceptual salience of the eyes, and may not modify behavioral responses to social signals. To test this hypothesis, we presented four monkeys with videos of conspecifics displaying natural behaviors. Each video was viewed multiple times before and after the monkeys received intranasally either 50 IU of OT or saline. We found that despite a gradual decrease in attention to the repeated viewing of the same videos (habituation), IN-OT consistently increased the frequency of gaze following saccades. Further analysis confirmed that these behaviors did not occur randomly, but rather predictably in response to the same segments of the videos. These findings suggest that in response to more naturalistic social stimuli IN-OT enhances the propensity to *interact* with a social partner rather than merely elevating the perceptual salience of the eyes. In light of these findings, gaze following may serve as a metric for pro-social effects of oxytocin that target *social action* more than *social perception*.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

Author name	Category 1			Category 2	Category 3
	conception and design of study	Acquisition of data (laboratory or clinical)	Data analysis and/or interpretation	Drafting of manuscript and/or critical revision	Approval of final version of manuscript
P.T. Putnam	✓	✓	✓	✓	✓
J.M. Roman	✓	✓	✓	✓	✓
P.E. Zimmerman		✓	✓		✓
K.M. Gothard	✓		✓	✓	✓

Keywords

Oxytocin; Gaze following; Rhesus; Social; Natural behavior; Primate

1. Introduction

Gaze following is an expression of social engagement, a behavior that indicates an explicit interest in where others are looking. There is an important distinction between gaze following and joint attention. Joint attention implies the presence of a target object fixated by both social partners. One individual might direct the attention of a social partner to that object (pointing with the eyes), but ultimately it is the object that captures the gaze of both partners (Emery et al., 1997). In contrast gaze following does not require the presence of the object, therefore it is not simply the object but rather, the gaze direction of the social partner that causes the gaze following saccade (Mosher et al., 2011; Shepherd, 2010). Gaze following is not unique to humans; it has been described in multiple social species, ranging from birds to humans as reviewed by Shepherd (2010). Deficits of gaze following are among the symptoms of autism spectrum disorders (ASD) (Dawson et al., 1998). The neuromodulator oxytocin (OT) has been shown to improve social deficits in both ASD and other disorders accompanied by social deficits (Guastella et al., 2010, 2010; Hollander et al., 2002, 2007; Yatawara et al., 2015; Young and Barrett, 2015). The social behaviors controlled by OT include parental care (Rilling and Young, 2014), social bonding (Donaldson and Young, 2008; Johnson and Young, 2015; Nagasawa et al., 2015), social recognition (Ferguson et al., 2001; Skuse et al., 2014), and increased empathy (Burkett et al., 2016). When administered intranasally, OT increases the time that rhesus monkeys spend viewing the eyes of conspecifics in static images (Dal Monte et al., 2014a; Ebitz et al., 2013) and communicative gesturing in rhesus infants (Simpson et al., 2014). OT also enhances socially reinforced learning (Parr, 2014), and increases reward allocation to a partner monkey (Chang et al., 2012). These results in non-human primates replicate similar findings in humans (Guastella et al., 2008; Hurlemann et al., 2010). A large portion of the human studies focused on the effect of OT on allocating attention to salient social stimuli, such as the eyes (Andari et al., 2010; Auyeung et al., 2015; Guastella et al., 2008), but these studies address only one component of social interactions, that of social perception. By comparison, gaze following is an elemental form of social interaction.

We have previously shown that videos which depict the natural behaviors of monkeys reliably elicit gaze following from viewer monkeys (Mosher et al., 2011). We hypothesized that in addition to enhancing attention to the eyes, OT might also enhance the active social engagement of the recipient manifested through increased frequency of gaze following. To test this hypothesis we presented videos of unfamiliar monkeys (henceforth stimulus monkeys) to four adult male rhesus monkeys before and after intranasal administration of OT or saline.

2. Methods

2.1. Subjects

Behavioral data was collected from four adult male rhesus macaques (*Macaca mulatta*): G, Z, U, and R. At the time of the study the ages of all animals were between 6 and 12 years old. Monkey R was pair housed with a monkey not participating in the study, while all other monkeys were housed in double cages in the same room with visual access to all other monkeys in the colony. All experiments were performed in compliance with the guidelines of the National Institute of Health for the use of primates in research and were approved by Institutional Animal Care and Use Committee at the University of Arizona. For accurate eye tracking each monkey was fitted with a three-point head fixation device attached, under isoflurane anesthesia, to the skull. Subject monkeys were seated in custom built primate chairs with their eye positioned 57 cm from a LCD monitor spanning 37 × 28 ° of visual angle (dva). Videos and static images subtended 26 × 18 dva. The videos contained 299 frames shown at 30 frames pers second. Neurobehavioral Systems Presentation software (Albany, CA) was used for experimental control and presentation of the videos. Prior to each experimental session monkeys were calibrated by fixating on a nine point calibration grid within an error of ±1 dva. Eye position was recorded using an infrared camera at 240 Hz (ISCAN Inc., Woburn, MA) and collected as an analog signal using a CED Power 1401 data acquisition system and Spike 2 software (Cambridge Electronic Devices, UK).

2.2. Task

Each subject monkey participated in 10 experimental sessions (5 OT and 5 saline inhalations). Each session contained a set of 12 unique videos, viewed 7 times in blocks. The duration of each video was 10 s; in each video an unfamiliar monkey (stimulus monkey) displayed threatening, neutral, or appeasing gestures, postures, and facial expressions 1–3 times. Sets of 12 videos were randomly allocated to OT and saline sessions. The sequence of 12 videos in each block was randomized, and videos were separated by 4–12 s inter-trial intervals. Two of the four monkeys (U and R) viewed in addition to the videos, static frames extracted from the videos shown in that session. These images were displayed for 3 s each. Monkeys were free to view, or not, the stimuli; however monkeys U and R were required to initiate the start of stimulus presentation by fixating on a center spot for 250 ms and were rewarded after the stimulus presentation for this correct fixation. Monkeys were not otherwise rewarded or incentivized to view the stimuli. Throughout the experiments the monkeys were in head fixation.

2.3. Gaze following saccades

Gaze follows were scored manually using previously established criteria (Mosher et al., 2011). These criteria were: [1] the viewer monkey had to first fixate on the eyes of the stimulus monkey; [2] the saccade immediately following this fixation coincided in direction with the stimulus monkey's line of sight (± 30°); and [3] the gaze following saccade did not terminate on an object or on the body (to prevent confusion with joint attention). Manual scoring of gaze following saccades was done blindly, where the scorer was unaware of the treatment, session, or the order in which the videos were viewed in a session.

2.4. Treatment

We administered either 50 IU Oxytocin (Santa Cruz Biotechnology, Dallas, TX) in 2 ml of saline or 2 ml of saline vehicle using a pediatric nebulizer (Pari GmbH, DE). This dose has been used previously used for rhesus monkeys (Dal Monte et al., 2014b; Parr et al., 2013). Treatment began immediately after the first 3 blocks of videos (pre-treatment videos) were viewed. Nebulization was performed over a ten-minute period, followed by ten minutes post-nebulization resting period. Monkeys remained in head fixation during the nebulization period. Thus, the first post-treatment block of videos was shown 20 min after the start of nebulization. This way the expected period of rise in OT levels (20–45 min) coincided with the post-treatment viewing period (Chang et al., 2012; Dal Monte et al., 2014b). All experimenters were blind to the treatment.

2.5. Analysis

For analyses of time spent looking at the videos/static images and at the eyes, viewing behavior in the 4 post-treatment trials was normalized to the 3 pre-treatment baseline trials. Viewing behavior was quantified by plotting the eye position of the viewer monkey over each frame of the stimulus video or over the static image. Viewing time of the eyes and mouth were quantified using regions of interest (ROIs) drawn on each frame around the eyes and mouth. Unless otherwise noted we used two-way ANOVA for statistical comparisons, with factors *treatment* and *monkey*. For comparisons of viewing time the normalized viewing time per each post-treatment video viewing was used as the dependent variable. For gaze following comparisons, ANOVA models used as the dependent variable the difference in gaze following frequency (post-treatment frequency – pre-treatment baseline frequency) calculated separately for each session. Peak saccade speeds above a 1000 dva/s threshold were discarded from analysis, as this was indicative of eye tracking errors. Saccades were manually identified using custom MATLAB software (Mathworks, Natick, MA). Saccades to, from, and outside of the eye region were automatically identified based on the previously drawn ROIs around the eyes. Gaze following saccade velocities were calculated individually for a subset (n = 202) of randomly selected of gaze following saccades, which were manually verified. Co-occurrence of gaze following across sessions and monkeys were examined using permutation tests, which compared the observed rates of gaze following co-occurrences to a hypothetical distribution (n = 10,000) where gaze following saccades were distributed randomly. This was considered to be below or above chance if it was respectively inferior or superior to a confidence interval set at $p < 0.05$ (two-tailed test).

3. Results

3.1. Gaze following reliability

Replicating previous reports, gaze following saccades occurred relatively infrequently, in response to 11.85% (min = 3.94%, max = 20.67%) of video presentations (Mosher et al., 2011). We extracted 465 gaze following saccades from scanpaths made during the viewing of 3924 videos. Although the scanpaths were highly variable across viewings of the same video, gaze following saccades occurred with remarkable predictability. Gaze following saccades tended to occur in response to the same segment of a video during successive presentations of the video to a given viewer monkey, and also across different viewers (Fig.

1). To determine whether gaze following saccades were indeed prompted by specific behaviors of the stimulus monkey, we compared the observed co-occurrences of gaze following saccades (both between viewers and within viewers) to a computed distribution of random occurrences of gaze following saccades (see supplementary methods). We found that gaze following saccades were significantly more likely to occur in response to the same segment of the videos whether we considered repeated viewings by the same viewer (Permutation test, two-tailed, $p < 0.001$), or gaze follows from multiple viewers (Permutation test, two-tailed, $p < 0.001$). Thus, while relatively rare, gaze following saccades occurred reliably in response to a specific stimulus, i.e., the behavior of the stimulus monkey.

3.2. Effect of intranasal OT on gaze following

Confirming our hypothesis, inhalation of a nebulized solution of 50 UI OT increased the frequency of gaze following saccades (significant effect of treatment, $F(1,1) = 25.0$, $p < 0.001$, $\eta_p^2 = 0.44$, $d = 1.77$) compared to saline inhalation. No main effect of monkey ($F(1,3) = 0.96$, $p = 0.42$) and no significant interaction between monkey and treatment ($F(1,3) = 0.98$, $p = 0.41$) was observed. Fig. 2 illustrates the increase in the number of gaze following saccades before and after treatment separately for each viewer monkey. IN-OT produced an increase in gaze following frequency compared to the pre-treatment baseline ($F(1,1) = 7.56$, $p < 0.05$), in contrast to saline inhalation, which produced a significant decrease in the frequency of gaze following saccades ANOVA, ($F(1,1) = 65.92$, $p < 0.001$), which we interpret to be due to habituation. Because some stimulus monkeys were more active and socially engaging than others, the viewers' interest in the videos also showed broad variation. The effect of OT was quantified, therefore, as pre/post-treatment paired comparisons. This approach eliminated the variability induced by the content of the videos and by monkeys' declining interest in repeated viewings of the same videos. The variation in the number of pre-saline and pre-oxytocin gaze following saccades is due to random assignment of videos sets different content to saline or OT experiments. Individual preferences of the viewers for the video monkeys might have also contributed to variation of gaze following saccades for different videos. The reduction in gaze following frequency post-saline inhalation is likely due to habituation to the 7 successive presentations of the video sets. Indeed repeated presentation of the same videos in the current and in previous studies led to a gradual reduction in attention to the videos (Mosher et al., 2011). To ascertain that the increase of gaze following frequency post-OT inhalation could not be accounted for by the unique videos set viewed under each treatment, we compared the observed difference between OT and saline treatment to a computed distribution of the same data randomly assigned between treatments. We found that the observed difference between the two treatments was significantly higher than chance (Permutation test, two-tailed, $p < 0.001$). Interestingly, after IN-OT the monkeys tended to respond with gaze-following saccades to segments of the video that did not elicit gaze following saccades prior to IN-OT. Although the facial expression of the stimulus monkeys was not predictive of the number of gaze following saccades before IN-OT, we found that the increase in gaze following saccades after IN-OT was driven primarily by videos with neutral content (Supplemental Fig. S1).

3.3. Effect of intranasal OT on attention to videos

OT inhalation also increased the viewer's overall attention to the videos as measured by the total time the monkeys spent viewing the videos (Fig. 3A/B) significant effect of treatment ($F(1,1) = 15.41$, $p < 0.001$) and no significant interaction between monkey and treatment ($F(1,3) = 1.79$, $p = 0.15$, with an effect size of $\eta_p^2 = 0.01$). As the small effect sizes indicate, this outcome, albeit statistically significant, was less prominent than the large increase in the number of the gaze following saccades. To confirm this result was not due to the different videos seen under each treatment we performed a second statistical test which compared the observed changes in viewing after treatment to those randomly shuffled post-treatment pre-treatment session (Permutation test, two-tailed, $p < 0.001$). Nevertheless, we explored the possibility that the increase in the number of gaze following saccades was due to the overall increase of attention to the videos. A lack of correlation between the number of gaze following saccades and the time spent viewing the movie eliminated this possibility. This lack of correlation was true for videos shown before the treatment (Spearman rank correlation, $R = 0.204$, $p > 0.05$), post-saline inhalation (Spearman rank correlation, $R = 0.038$, $p > 0.05$) and post-oxytocin inhalation (Spearman rank correlation, $R = 0.037$, $p > 0.05$). Thus, the increase in the number of gaze following saccades did not result from a global enhancement of attention to the videos.

3.4. Effect of intranasal OT on time spent viewing eyes

As previous studies have shown that IN-OT increased the time monkeys spend looking at the eyes of conspecifics, we attempted to replicate these findings. We required monkeys R and U (the other two monkeys were not available at the time of this test) to look at single frames from the same videos presented as static images for a duration of 3 s. We found that OT inhalation increased the time spent viewing the eyes in static images (Fig. 3D, significant main effect of treatment $F(1, 1) = 5.39$, $p < 0.05$, $\eta_p^2 = 0.01$) as reported by previous studies. Surprisingly, when viewing the videos the time spent looking at the eyes was not significantly increased (Fig. 3C, $F(1,1) = 0.05$, $p > 0.05$). The differences in eye looking in response to static images and videos might be explained by multiple factors. While in static images the gaze direction of the stimulus gaze is constant, it changes during the videos. Thus we determined whether OT affected the duration of mutual gaze (Supplemental Fig. S2A) and found no significant difference compared to saline inhalation (no significant main effect of treatment $F(1,1) = 1.8$, $p > 0.05$). Furthermore, no difference between treatments was found for viewing the mouth (Supplemental Fig. 3B, no significant main effect of treatment $F(1,1) = 0.68$, $p > 0.05$). We conclude, therefore, that the effect of OT on looking at the eyes depends on the static or dynamic nature of the social stimuli.

3.5. Effect of intranasal OT on saccade ballistics

Finally, we examined the possibility that OT influenced the ballistic parameters of saccades. This analysis was justified by previous reports on changes in saccade parameters induced by social stimuli (Xu-Wilson et al., 2009). We compared the peak velocities of saccades: (1) into the eye region, (2) away from the eye region, and (3) outside of the eye region, both before and after IN-OT or saline. We found that independently of treatment, saccades into the eye region had higher peak velocities (377 dva/s ± 3.79) than other types of distance-

matched saccades (leaving eye region 304.14 dva/s ± 2.75; outside of eye region 297.28 dva/s ± 1.17, ANOVA with fixed factors saccade type and continuous factor of saccade distance, main effect of saccade type $F(1, 3) = 173.5$, $p < 0.001$), (Supplemental Fig. S3). Interestingly, gaze following saccades, also had a higher peak velocity (369.33 dva/s ± 19.96) than distance-matched saccades (304.16 dva/s ± 2.75) originating at the eye region and terminating elsewhere (ANOVA with factors saccade type and continuous factor of saccade distance, main effect of saccade type $F(1, 1) = 19.56$, $p < 0.001$). These results support the hypothesis put forth by Xu-Wilson et al. (2009) that saccade parameters might be related to the salience or value of fixation targets. OT inhalation, however, had no effect on the peak velocities of any saccade type (ANOVA with fixed factors saccade type and treatment, and continuous factor of saccade distance, no main effect of treatment $F(1, 1) = 3.15$, $p > 0.05$). The mechanism(s) by which the eyes as a fixation target or as the origin of gaze following saccades may modify saccade velocities are unknown; whatever these mechanism may be, inhaled OT does not appear to modify these mechanisms.

4. Discussion

The results presented here confirm our previous findings that gaze following can be reliably elicited by naturalistic, dynamic social stimuli (Mosher et al., 2011) and report the effect of IN-OT in enhancing the frequency of gaze following saccades without increasing looking time at the eyes. The differential effect of OT on looking patterns elicited by static and dynamic social stimuli can be explained by multiple factors. Motion by itself can reflexively alter viewing patterns as the changes in gestures, postures, and facial expressions of the monkey depicted in the video attract the viewer's attention. As the monkey has to distribute its attention to competing elements of the visual scene, less time might be devoted to each element hence the absence of increased eye looking under OT administration. It is also possible that the increased realism of the videos initiate more naturalistic behavioral responses in the viewer monkey, which includes a reduction of staring at the eyes. Indeed, in response to videos, viewers switch from simply staring at the most salient feature (the eyes) to attempting to engage the stimulus monkey in social interactions. This increased engagement of the viewer is manifested in the production and reciprocation of facial expressions and, more rarely, in the production of social calls (coos) (Mosher et al., 2011). With repeated presentation of the same videos the viewer's responses diminish as they learn that their social behaviors have no effect on the perceived social partner. This is the main reason we used a different set of videos for each experiment (whether saline of OT inhalation) and limited the number of viewings of each video to seven.

The observation that gaze following saccades occurred reliably in response to the same video segments suggests that the stimulus monkeys emitted a, yet insufficiently characterized, signal that compelled the viewers to follow their gaze (Fig. 1). For gaze following to occur it was necessary for the head and eyes of the stimulus monkey to be turned from frontal view to semi-profile or full profile. This was not sufficient, however, because head rotations of this type were abundant in the videos, yet of the 244 videos only 27 elicited gaze following saccades in at least one of the 4 viewers. Only 17 videos of these 27 elicited gaze following in more than 1 viewer monkey. These videos most likely contain a more potent triggering signal for gaze following. While identifying exactly what this signal

might be requires further work, the perception of these signals is subject to individual differences as we rarely identified a video segment that elicited reliably gaze following in all four subjects. Importantly, and regardless of the trigger, OT increased the frequency of gaze following saccades and this was independent of its effect on increasing global attention to the videos. Finally, the OT-induced increase in gaze following cannot be explained by an increased attention to the eyes because OT did not increase eye looking in response to the videos. It should be noted that the effect size for gaze following in our study is quite large (d = 1.6) compared to the effect sizes of typical IN-OT studies in human examining effects on behavior (d = 0.3) (Walum et al., 2015). One possible explanation for the strong effect size is that gaze following may be more proximal to the neurobiological mechanisms influenced by OT. In contrast, the effect size for the increase of global attention was rather small, further substantiating the claim that OT influences the interactive aspect of social behavior rather than modify some aspect of social perception. As suggested by Walum et al. (2015), the effect size can be improved by high number of trials for each subject even if the number of subjects is low. This was the case in this study; we tested only four monkeys, but each monkey viewed 840 videos.

Videos depicting neutral expressions triggered more gaze following saccades after IN-OT treatment than videos with appeasing or aggressive behaviors. There is an interesting parallel between these results and an earlier finding indicating that neutral images with averted gaze induce more reliably skin conductance responses than appeasing or aggressive facial expressions (Hollander et al., 2007). As skin conductance is an index of sympathetic arousal it appears that neutral faces with averted gaze, possibly being more ambiguous, prompt both greater arousal and more frequent gaze following saccades due to the viewer’s interest in the where another monkey is looking.

The higher peak velocity of gaze following saccades and/or saccades targeting the eyes demonstrate that the eyes represent an object of high interest or intrinsic value (Xu-Wilson et al., 2009). The observation that OT did not change the dynamics of these saccades (despite the presence of OT receptors in the superior colliculus (Freeman et al., 2014)) suggests that the intrinsic value or saliency of eyes as a target of attention remained unchanged by OT inhalation. Alternatively it is possible that gaze following saccades normally have a maximal velocity, which cannot be enhanced by OT inhalation.

Numerous working hypotheses (Bartz et al., 2011) have been proposed to explain the prosocial effects of OT. This hormone might (1) increase the saliency of social cues (Shamay-Tsoory and Abu-Akel, 2016; Young, 2015), (2) reduce vigilance to facilitate social interactions (Ebitz et al., 2013; Parr et al., 2013), or (3) increase motivation for social interaction and for seeking social information (Chevallier et al., 2012; Scott-Van Zeeland et al., 2010). While these alternatives are not mutually exclusive, our results appear to lend support to the idea that OT increases social motivation and/or decreases social inhibition.

The precise neural mechanisms that might account for the effect of OT on gaze following are unknown. Recent findings reporting the distribution of oxytocin receptors (OXTR) in the monkey brain suggest a few possibilities. Unlike in rodents, OT receptors (OXTR) are not found in the primate amygdala, but are instead sparsely clustered in distinct regions

including the nucleus basalis of Meynert (NBM) (Freeman et al., 2014). The cholinergic projections of the NBM to several nodes of the social brain likely play a role in gaze following. These projections target the amygdala (Kitt et al., 1987), a structure heavily implicated in social cognition (Adolphs, 2010). In the amygdala, neural responses to incoming signals are strongly enhanced by cholinergic inputs (Unal et al., 2015). The NBM also provides cholinergic innervation to many cortical regions of the social brain, including the superior temporal sulcus (STS) (Selden et al., 1998), a region involved in face processing, the detection of gaze direction, and gaze following. Indeed, pharmacological inactivation of the posterior STS reliably reduces gaze following (Roy et al., 2014) in rhesus monkeys. The STS shows increased BOLD signals during gaze following in both monkeys (Kamphuis et al., 2009) and humans (Materna et al., 2008), suggesting that this cortical region could be essential to track the gaze and attention of others. It is likely, therefore, that gaze following behaviors rely on neural connections between face- and gaze-selective regions in the STS and the amygdala (Iidaka et al., 2012; Kliemann et al., 2012; Tsao et al., 2003) which might be modulated by OT through the NBM. Finally, neurons in the lateral intraparietal area track the gaze directions of both viewer and stimulus monkeys, which likely contributes to gaze following behaviors (Shepherd et al., 2009). This area is also a target of cholinergic modulation by the NBM. There are several alternative explanations for the present results. OT is known to also bind to vasopressin-1A receptors (AVPR1A), which are amply expressed in the amygdala and the cortex (Young et al., 1999), implying that the behavioral effects seen in this study might be explained independently of OXTR activation (Schorscher-Petcu et al., 2010). Indeed, genetic variation in the AVPR1A in chimpanzees is associated with joint attention (Hopkins et al., 2014). Although IN-OT does increase the CSF levels of OT (Dal Monte et al., 2014b; Modi et al., 2014) we also cannot rule out the possibility that the observed results are due, at least in part, to the peripheral effects of OT (Leng and Ludwig, 2015). Ongoing experiments in which we specifically block peripheral OT receptors, or site specific injections of OT into the amygdala and NBM along with electrophysiological recordings will provide more mechanistic interpretation of the results reported here.

The primary limitation of this study is the small number of subjects (N = 4). This sample size does not compare favorably to well-powered human studies, but is justified by the difficulties to carry out these studies in large cohorts of non-human primates. The effects of IN-OT are oftentimes subtle and many studies reporting behavioral effects of IN-OT are indeed underpowered (Walum et al., 2015). We compensated for the small cohort of subjects by recording large numbers of observations from each subject. While it is encouraging that the results are consistent across subjects, a larger sample size would clearly lead to stronger conclusions. Static images were shown only to two of the four monkeys. The outcome of the static images should not be taken as the main result of this report, these were additional controls carried out only to corroborate a previously published finding that in our paradigm IN-OT also increases viewing the eyes of conspecifics (Dal Monte et al., 2014a). Finally, only male monkeys were used, thus these results may not extend to female monkeys, as there may be a sex-dependent expression of OXTR in the brain (Insel et al., 1993). No control videos without social content were presented to the animals during the experiment,

which prevent us from distinguishing between the role of OT in enhancing attention to all videos or only to videos with social content.

Despite these limitations, the use of videos as social stimuli added to the growing evidence that natural stimuli, albeit more difficult to control, elicit natural behaviors that are quantifiable and robust. Gaze following is a basic form of social interaction and a fundamental building block of theory of mind, and as such is one step closer to spontaneous, natural social behavior than looking at the eyes. This behavior can be readily elicited and studied in the laboratory because eye movements are relatively easy to record and quantify. As we have shown here the parameters of eye movements in monkeys (including saccade velocity) and humans are highly similar. Given this similarity gaze following can be incorporated in electrophysiological and pharmacological studies in monkeys that will allow us to understand at a circuit level how OT influences social relationships in primates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2016.05.016.

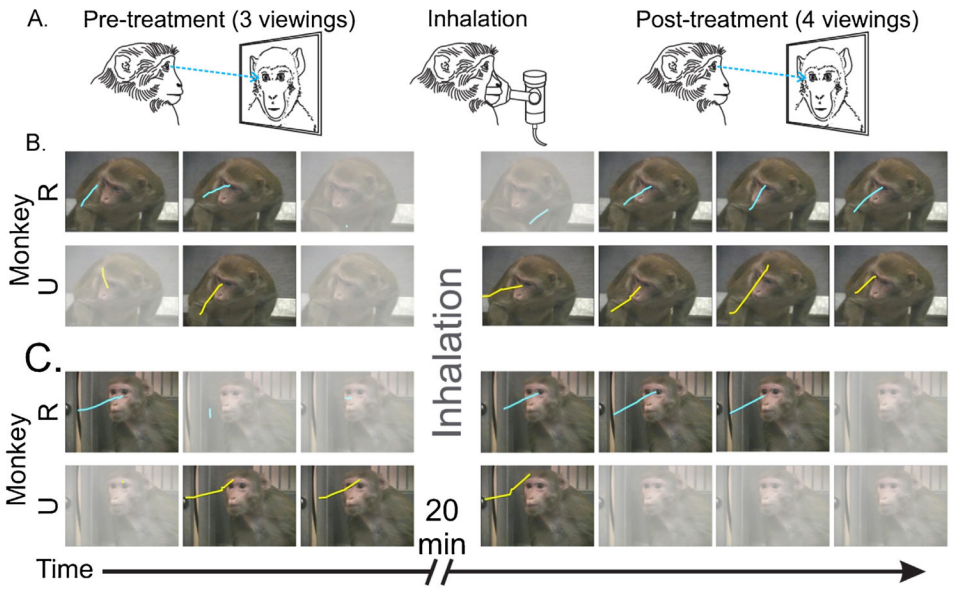

Fig. 1.

Example video frames that reliably elicited gaze following saccades within and across viewers. (A) Monkeys viewed 12 videos, unique to that session, 3 times before inhalation of nebulized oxytocin or saline solutions. Following inhalation monkeys viewed the same videos 4 more times. (B) Example frame from a video that elicited gaze following saccades across multiple presentations. The viewers' gaze following saccade is superimposed on the video frame (cyan line for monkey R, and yellow line for monkey U). (C) A second example that elicited gaze following saccades more reliably in monkey R. Note that the absence of a scanpath indicates that the animal was not looking at the video. White overlay means that the animal did not follow the gaze of the movie monkey on this trial. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

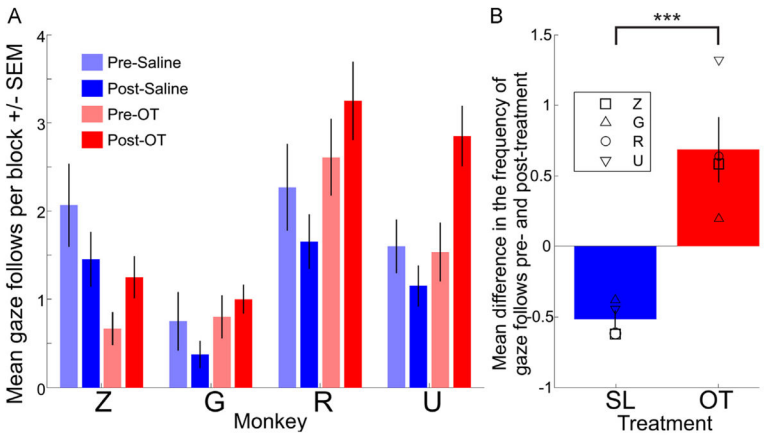

Fig. 2.

Oxytocin increased the number of gaze following saccades. (A) The average number of gaze following saccades per block for each monkey shown for: pre-saline blocks (light blue), post-saline blocks (dark blue), pre-oxytocin blocks (light red), and post-oxytocin blocks (dark red). Error bars represent the standard error of the means. (B) Mean difference in gaze follows between pre- and post-treatment blocks pre-treatment per-session means subtracted from post-treatment per-session means for each monkey for saline and OT inhalation. Means for data from each of the 4 monkeys are shown scattered as different symbols for each treatment. Note that the decrease in the frequency of gaze following saccades after saline inhalation reflects the viewer's habituation to the videos. The same habituation is expected for the videos presented during the OT treatment. The increase in the number of gaze following saccades after OT inhalation indicates that OT counteracted the expected habituation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

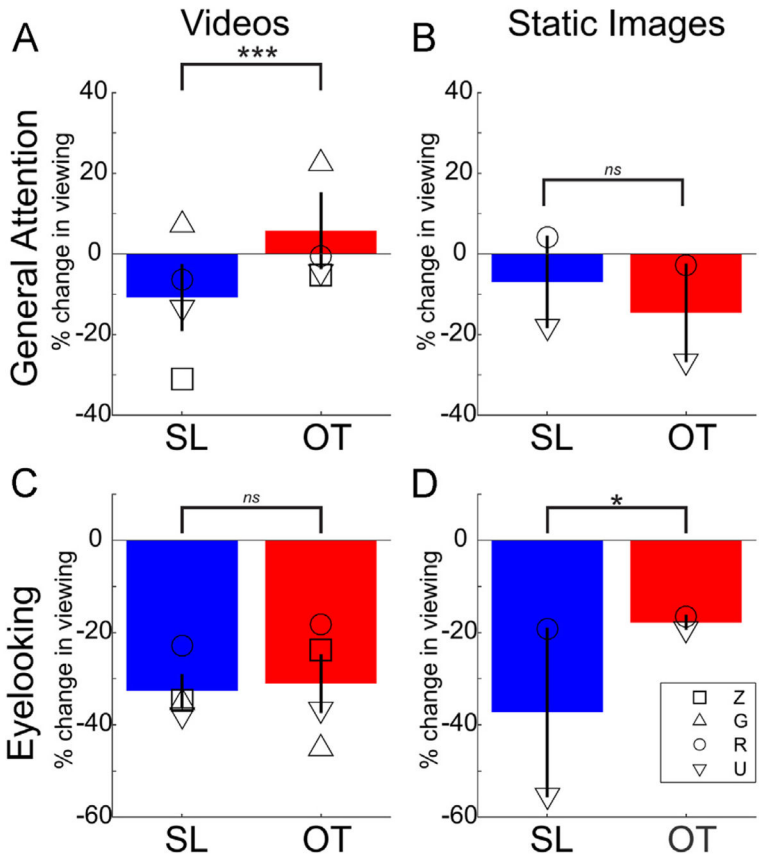

Fig. 3.

OT increased overall looking time at the videos but did not increase time spent looking at the eyes. (A) A gradual reduction in overall attention to the videos was observed during saline inhalation, which was likely due to habituation; OT inhalation significantly counteracted the expected habituation. (B) The same effect was not observed for static frames extracted from the same videos. A and B together show that OT counteracts habituation only for dynamic videos. (C) Similar reduction of time spent looking at the eyes for videos viewed during saline and OT inhalation. (D) When the videos were replaced by static images of the same monkeys, OT counteracted the habituation-induced reduction of eyelooking. C and D together show that OT enhances eyelooking only on static images and not on videos.