The effect of oxytocin on pupil response to naturalistic dynamic facial expressions

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Abstract

The neuropeptide oxytocin (OT) has been found to play an important role in a variety of social behaviours and social cognition in particular. The social salience hypothesis of OT suggests that OT shifts attention towards socially relevant stimuli, which offers an explanation for improvements on social cognition measures following OT administration. Pupil dilation occurs with increasing attentional resource allocation and previous research has found that OT administration led to an increase in pupil diameter in response to social stimuli relative to placebo (PL), thereby suggesting increased social attention. The current study aimed to investigate the effects of OT on pupillary responses to more naturalistic social stimuli in a larger sample. Ninety-four male participants took part in the double-blind, placebo controlled, mixed-design study, in which they self-administered either an OT or PL nasal spray before viewing naturalistic dynamic facial expressions of emotion (happy, sad, fear and anger). Contrary to prediction, there was no effect of OT administration on pupil diameter. The results are discussed in light of the social salience hypothesis and with reference to the methodological differences between studies.

Introduction

The neuropeptide oxytocin (OT) has been implicated in wide variety of complex social behaviours, such as the ability to discriminate between ingroup and outgroup members (De Dreu & Kret, 2016), and allocate resources accordingly (Daughters et al., 2017). In particular, researchers have focused on the effects of OT administration on social cognition, finding a reliable effect of improving facial emotion recognition (Leppanen et al., 2017; Shahrestani et al., 2013). The social salience hypothesis of OT (Bartz et al., 2011; Shamay-Tsoory & Abu-Akel, 2016) seeks to explain why OT is implicated in such a variety of behaviours, proposing that OT increases awareness of socially relevant stimuli. The social salience hypothesis therefore suggests that OT alters visual attention.

Pupillometry, the measurement of pupil diameter over time, has been shown to be a sensitive measure of attentional resource allocation (Laeng et al., 2012). Pupil diameter is determined by the iris dilator muscle, controlled by the sympathetic branch of the autonomic nervous system, and the iris constrictor muscle, innervated by the parasympathetic branch. Pupil dilation has been observed in response to stimulus-specific and task-related increases in cognitive demand (Kahneman & Beatty, 1966; Laeng et al., 2012), as well as emotionally arousing stimuli (Bradley et al., 2017; Snowden et al., 2016), thereby supporting the idea that pupil diameter reflects the degree of attention given to a stimulus. Measuring changes in pupil diameter after OT administration therefore offers a way to empirically test whether OT alters social attention, as suggested by the social salience hypothesis.

Several pupillometry studies have reported supportive evidence for the social salience hypothesis, finding that intranasal OT led to larger pupil dilation in response to facial expressions, consistent with the view that OT increases attentional resource allocation during the processing of social stimuli (Leknes et al., 2013; Prehn et al., 2013). Leknes et al. (2013)

administered 40IU of OT intranasally to 39 (20 females) participants before showing them photographs of neutral faces, and implicit and explicit happy and angry facial expressions. Participants were asked to rate how happy/angry/attractive/friendly each face was. OT led to increased pupil diameter in response to images of facial expressions of emotion, regardless of whether the stimuli depicted angry or happy faces, suggesting an overall increase in pupil diameter, rather than a valence-specific effect. Research has shown, however, that static photographs, in addition to being unrepresentative of the dynamic nature of real-life facial expressions, lead to reduced autonomic arousal – including pupil dilation – compared to dynamic faces (Alves, 2013; Burley et al., 2017), which may in turn underestimate the effects of OT on autonomic arousal to emotional stimuli.

Prehn et al. (2013) presented dynamic facial stimuli to 47 male participants and similarly found that (24IU intranasal) OT enhanced pupil dilation in response to facial expressions of emotion. Participants were asked to observe neutral facial expressions that gradually morphed at 5% intensity increments over 16 seconds into a target emotion (happy, sad, fear and anger). Participants were asked to click a button when they could identify the facial expression. Follow-up analysis revealed that the effect of OT on pupil diameter was only significant for happy facial expressions. Given the small sample size, and the correspondingly low statistical power, it is difficult to interpret this happy-specific effect of OT. Interestingly, pupil dilation was indexed during an early epoch (0.80 – 3.20 seconds) in order to maximise the available data; however, because the stimuli reached 100% intensity after 16 seconds, the intensity of the facial expressions of emotion was low (5 – 20%) during the analysis window. Indeed, it is likely that the emotion being expressed was not identifiable during this early analysis window, given that mean emotion recognition for the sample (the point at which the trial was discontinued by the participant) occurred after the analysis window, at 7.07 seconds. Therefore, these findings cannot be generalised to dynamic facial expressions at a high

emotional intensity. It would therefore be beneficial to explore the effects of OT on pupil dilation in response to dynamic facial expressions that unfold more quickly, which would be more reflective of naturally occurring facial expressions.

In contrast, however, two recent studies found either no effect of OT or the opposite effect on pupil dilation (Quintana et al., 2019; Wynn et al., 2019). Quintana et al. (2019) investigated the effect of two different doses of OT (8IU and 24IU) and two different administration techniques (intranasal and intravenous) on pupil diameter in 16 male participants in a crossover design. Participants were shown the same static photographs of angry, happy and neutral facial expressions of emotion as used by Leknes et al. (2013) as well as geometric shapes, and asked participants to answer similar questions (how angry/happy/trustworthy the face was, and what colour the shapes were). Conversely, they found that all OT conditions were associated with smaller pupil sizes compared to the placebo condition, and no difference in pupil size between emotional expressions or between emotional expressions and shapes.

Finally, Wynn et al. (2019) administered eight different doses of intranasal OT to 47 (16 female) outpatients with schizophrenia (six patients per drug dose). Participants were shown photographs of happy, fear, and neutral facial expressions and scrambled images. After each image, participants were asked to identify which of the four stimulus categories they had seen. There was no effect of OT on pupil size, and no effect of category. Moreover, it should be noted, that Wynn et al. (2019) studied a sample of adults with schizophrenia, meaning that the findings may not be generalizable to neurotypical adults.

Given the mixed findings to date, it is unclear whether OT leads to a reliable effect on pupil diameter in response to facial expressions of emotion. The present study investigated the effect of OT administration on pupil diameter in response to viewing naturalistic dynamic facial expressions of emotion. We recruited a larger sample size relative to previous studies

with a view to replicating the finding that OT administration increases pupil size. We also sought to extend the existing literature by including more emotional expressions and examining whether the predicted main effect of OT would be moderated by emotion.

Material and Methods

Participants and Ethics

One hundred and four male undergraduates[1] took part in the double-blind, placebo-controlled, randomised study that was approved by the School of Psychology Ethics Committee at Cardiff University. Due to technical issues, 10 participants were not able to complete the task with the eye-tracker. This was due to eye-tracking data on the preceding task taking too long to save and therefore, in order to avoid delays in testing and ensure that all tasks were completed under peak OT concentrations, 10 participants were instructed to complete the current task without eye-tracking. Ultimately 94 participants were included in the analyses (M_{age} = 19.65, SE = 1.71; 47 participants in each drug condition). Some participants were recruited via an online participation system and were awarded course credits for taking part; others were recruited via an email advertisement and were paid £20 for taking part. Participants completed a medical screening form during a face-to-face session before taking part in the study. Participants were not allowed to take part if they had a history of cardiovascular disease, neurological disorders, or a history of severe allergic reactions. All participant forms were assessed by a medical professional before being cleared to take part. On the day, participants were asked to avoid alcohol consumption 24 hours prior to their session, and to avoid smoking and caffeine intake in the 2 hours before the session. Testing was carried out between 09:00-20:00, because participants were therefore asked to attend the study around mealtimes, they

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[1] Female participants were excluded from taking part due to ethical and logistical difficulties regarding administration of OT to women (e.g., pregnancy and menstruation).

were not asked to avoid food 2 hours before, however, if participants had consumed food immediately before the study, they were asked to rinse their mouths with water before starting the session. Although the evidence regarding diurnal rhythms in natural concentrations of OT is unclear (Amico et al., 1983), because the present study does not report OT concentrations, and because exogenous administration of OT increases natural OT concentrations by several fold (suggesting that any baseline differences in natural levels would have a very small effect relative to this large increase) sessions were not restricted to a specific time of day. Finally, all participants provided a signed statement of health before leaving the testing facility.

Pupillometry Task

Participants viewed 32 videos of dynamic facial expressions from the Amsterdam Dynamic Facial Expression Set (ADFES; Van Der Schalk et al., 2011), a well-validated stimulus set of short video clips showing neutral faces developing over time into one of nine discrete emotions. For the purposes of the present study, participants were shown video clips of four different North-European actors (two males, two females), facing forward, depicting four emotions: happy, sad, fear, and anger. The original video clips were edited to be 4000 ms long. The onset of the target emotion begins at approximately 1000 ms and reaches full intensity at 3000 ms. Participants were shown each display eight times (twice for each actor). The stimuli were presented in two pseudorandomised blocks of 16 trials (the blocks were pseudorandomised to ensure that participants did not see multiple versions of the same expression after another). The order of blocks was randomised. The dynamic faces were presented in their original colour, as in Burley et al. (2017), in order to maintain the naturalistic character of the stimuli and because the videos did not vary noticeably in luminance across emotions. Furthermore, our primary interest was in the effect of OT on pupil diameter and all participants viewed the same stimuli regardless of drug condition.

A trial consisted of a black screen for 500ms, followed by a white fixation cross on a black background for 500ms before the dynamic facial expression was presented. The cross appeared on the left- or right-hand side (counterbalanced) of the screen to ensure participants fixated away from the face (which was presented centrally) at stimulus-onset. After each video, participants responded to several questions before the next trial began, which allowed time for the pupil to return to baseline diameter before the next trial. Participants were asked: 1) to identify the emotional expression (forced-choice); 2) to rate the intensity of the emotional expression (on a 5-point Likert scale); 3) to rate their affiliative tendency toward the individual in the clip (the Inclusion of Other in the Self Scale, (Aron et al., 1992)). Participants were allowed to complete their responses in their own time. The entire task took approximately 15 minutes to complete. Recognition accuracy will not be discussed in the current paper because 1) the research question seeks to understand the effect of OT on autonomic arousal, and 2) because recognition accuracy was close to ceiling and so yielded little variation to examine in relation to pupillary responses.

Data Acquisition and Analyses

Participants were seated approximately 60-65cm from the screen on which the stimuli were presented and completed a 9-point calibration before beginning the task. If the calibration quality was poor, the calibration process was repeated. Participants did not complete the calibration more than twice.

A portable Tobii X2-60 Hz eye tracker recorded each participant's pupil diameter in response to the dynamic facial expressions presented on a display monitor with a screen resolution of 1920 x 1080. The data were cleaned and analysed following the same procedure as previous studies (Burley et al., 2017; Snowden et al., 2016), using Matlab (MathWorks, version 8.5). Unrealistic pupil size increases or decreases of .375 mm across a 16.67 ms interval were removed. The first data-point following missing data was removed to avoid abnormal

readings. Data were smoothed using a Savitzky-Golay low-pass filter (Savitzky & Golay, 1964) for a span of five readings (83 ms). Based on visual inspection of the pupil response data, these measures were sufficient to remove the majority of outlier and artefacts (Kret & Sjak-Shie, 2019) at the current 60Hz sampling rate and with the high degree of pupil data captured. Pupil size was determined by a mean average across both eyes, although individual pupil diameter was used where data from both eyes were unavailable.

Pupil diameter was recorded in millimetres. Pupil diameter was calculated from 1000-4000 ms in three 1000 ms epochs following the initial pupil constriction for each dynamic facial expression. Although other work has used both larger (Bradley et al., 2008; Henderson et al., 2014) and smaller bins (Kret & Sjak-Shie, 2019), 1000 ms response epochs were chosen to index early through to late pupil reactivity in response to the stimuli consistent with similar studies that have captured emotionally-modulated pupil dilation using a 60Hz sampling rate (Bradley et al., 2017; Snowden et al., 2016). For each participant, pupil data were averaged across individual trials over each time epoch, before an overall average was derived for each facial emotion at each epoch. Data were omitted if there was less than 50% valid data at any stage of this process; missing mean pupil diameter data were imputed with the mean value calculated using a Markov Chain Monte Carlo multiple imputation method, based on 20 imputations (Graham et al., 2007). The mean percentage of successfully recorded pupillometry data was high at 93% (SD = 9.96) and no participants were excluded for failing the overall threshold for determining valid data (50%, Snowden et al., 2016).

The data were analysed using a 2 (Drug: OT/PL) x 4 (Emotion: happy/sad/anger/fear) x 3 (Epoch: 1000-2000ms, 2000-3000ms, 3000-4000ms) mixed-model ANOVA, with Drug as a between-subjects factor, and Emotion and Epoch as within-subjects factors.

Protocol

On arrival, participants provided written informed consent and self-administered either a placebo (PL) nasal spray or 24IU of OT, in line with the guidelines detailed by Guastella et al., (2013), and under the supervision of the experimenter. Immediately after administration, participants completed several questionnaires (the Autism Quotient-Short (AQ-S, Kloosterman et al., 2011) and the State-Trait Anxiety Index (STAI, Spielberger et al., 1989)) (see Table 1 for descriptive data for these questionnaires) and the 2-subscale version of the Wechsler Abbreviated Scale of Intelligence (Wechsler, 1955)[2]. Thirty minutes after administration (Daughters et al., 2015; Gossen et al., 2012), participants completed two social cognition tasks[3] in which they were shown static faces and asked to make social judgements about them. These tasks took approximately 30 minutes to complete. Participants then completed the task in which pupillometry was assessed. Participants completed one remaining task (also assessing social cognition) before being fully debriefed at the end of the study.

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[2] Statistical analysis confirmed that there were no differences in scores across these measures between participants assigned to the PL and OT conditions.

[3] These tasks are not relevant for this paper and will not be discussed further.

Table 1. Descriptive data for the sample across personality measures

	Mean (SE)	Range
Autism Quotient-Short		
Social skills	3.00 (0.05)	1.83 – 4.00
Mind-reading	2.92 (0.05)	1.50 – 4.00
State-trait anxiety inventory		
State anxiety	35.86 (0.95)	21 – 73
Trait anxiety	43.55 (1.03)	22 – 65

Results

As can be observed in Figure 1, we observed a pupil constriction at the beginning of each video that reached a nadir at around 1000 ms, before an increase in pupil diameter with emotion modulation emerging from 1500 ms and pupil size levelling out by the end of the video at 4000 ms.
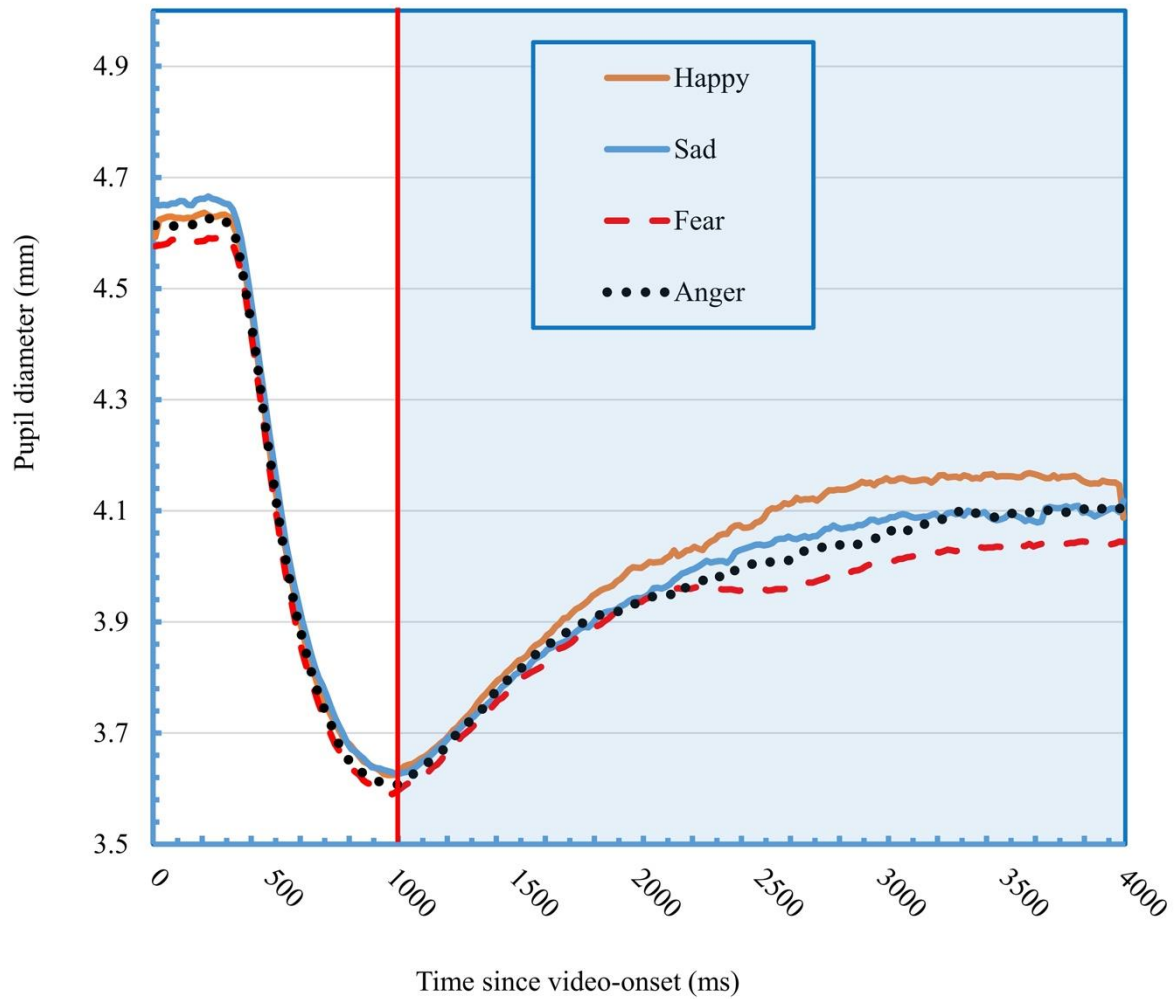
Figure 1. Pupil diameter in response to videos of dynamic facial expressions from stimulus-onset. The blue shaded area indicates the analysis window and the red line indicates the approximate onset of the target expression from a neutral expression.

Table 2 describes the results from the mixed model ANOVA. There was no main effect of Drug. Pupil size was statistically similar between the OT (M = 4.04, SE = .08) and PL (M = 3.90, SE = .08) conditions.

There was a main effect of Emotion and follow-up pairwise comparisons revealed that pupil size was significantly larger when viewing happy faces (M = 4.02, SE = .06), compared to sad (M = 3.97, SE = .06) and angry faces (M = 3.96, SE = .06), which in turn were larger

than fearful faces ($M = 3.93$, $SE = .06$) (ps < .05). There was also a main effect of Epoch and follow-up pairwise comparisons showed that pupil size increased across epoch (1000 – 2000 ms, $M = 3.80$, $SE = .06$; 2000 – 3000 ms, $M = 4.02$, $SE = .06$; 3000 – 4000 ms, $M = 4.09$, $SE = .06$) and these differences were significant (ps < .001). Finally, there was a significant interaction between Emotion and Epoch. Follow-up t-test pairwise analyses revealed that the pupil was larger in response to happy faces compared to sad, fearful and angry faces across each epoch (ps < .05), although the difference between happy-angry faces only trended towards significance at the 1000 – 2000 ms epoch ($p = .06$). Over both the latter time-points from 2000 – 3000 ms and 3000 – 4000 ms, the pupil was larger in response to sad and angry faces compared to fearful expressions (ps < .05).

All remaining interactions were not significant (see Table 2), however, we include Figure 2 to illustrate a general tendency for pupil diameter to be greater in the OT condition than in the PL condition, and the absence of any indication that this (non-significant) trend was modulated by Emotion or Epoch.[45]

Table 2. Results from a mixed model ANOVA examining the role of oxytocin on pupillary reactivity to dynamic facial expressions.

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[4] A further analysis was carried out on pupil diameter averaged across stimuli presentation (from 1000 – 4000 ms following onset) and the results remained the same regarding the oxytocin effects: there was no significant effect of drug, $F(1, 92) = 1.70$, $p = .20$, $\eta_p^2 = .02$, nor a drug by emotion interaction, $F(2.64, 243.14) = 1.37$, $p = .26$, $\eta_p^2 = .02$.

[5] Given that each dynamic facial expression was viewed twice by participants, we also examined the effects of oxytocin on mean pupil diameter to the first presentation of each face (across 1000 – 4000 ms) and the results remained the same: there was no effect of drug, $F(1, 89) = 1.45$, $p = .23$, $\eta_p^2 = .02$, and no drug by emotion interaction, $F(2.60, 231.09) = 0.37$, $p = .75$, $\eta_p^2 < .01$.

	df	F	p	η_p^2
Drug	1, 92	1.65	.20	.02
Emotion	2.64, 347.59	15.19	**<.001**	.14
Epoch	1.17, 347.59	317.44	**<.001**	.78
Drug x Emotion	2.64, 347.59	1.40	.24	.02
Drug x Epoch	1.17, 347.59	0.14	.75	.002
Emotion x Epoch	3.78, 347.59	7.90	**<.001**	.08
Drug x Emotion x Epoch	3.78, 347.59	0.79	.53	.01

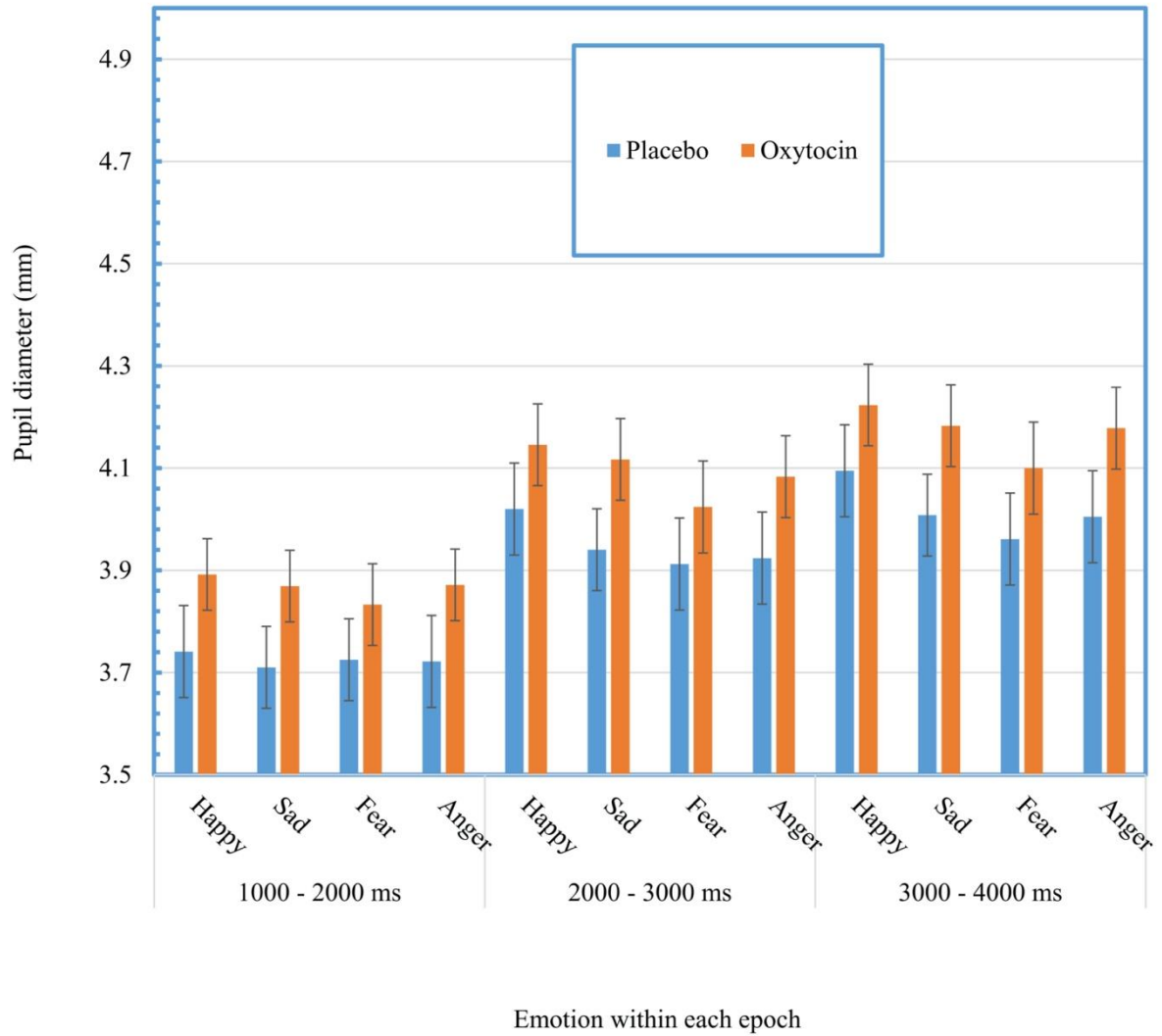Significant effects are highlighted in bold.

Figure 2. Mean pupil diameter in response to facial expressions of emotion in each epoch. Errors bars indicate +/- 1 standard error.

Examination of initial pupil diameter as a potential confounding variable

Given that initial pupil diameter prior to the onset of a visual stimulus can influence the magnitude of change in pupil size in response to that stimulus, we repeated the above analyses controlling for initial pupil diameter. Initial pupil diameter was determined as mean pupil diameter during the period 200 ms prior to facial expression-onset across all trials. There was no difference in initial pupil diameter between the OT and PL conditions, $t(92) = 1.13$, $p = .26$,

and, importantly, the results remained the same when initial pupil diameter was controlled for (i.e., no main effect or interactions involving Drug, ps > .13).

Exploratory analysis of the effect of individual differences

Research suggests that OT exerts stronger effects on individuals with social cognition difficulties (Bartz et al., 2011). In line with this research, we conducted an exploratory analysis assessing the impact of autistic (AQ-S Mind-reading, Kloosterman et al., 2011) and anxious (STAI Trait anxiety, Spielberger et al., 1989) traits on pupillary responses during the task. These sub-dimensions were chosen as they were considered to best reflect individual differences in relation to social cognition for each measure. We repeated the 2 (Drug: OT/PL) x 4 (Emotion: happy/sad/anger/fear) x 3 (Epoch: 1000-2000ms, 2000-3000ms, 3000-4000ms) mixed model ANOVA detailed above and entered STAI Trait and AQ-S Mind-Reading dimensional scores as standardised covariates (ANCOVA) to examine whether anxiety or autism traits influenced the effects of oxytocin on pupillary reactivity. To reflect the exploratory nature of this analysis, p-values were adjusted using False Discovery Rate (FDR) correction to examine Autism/Anxiety group by Drug interactions (Benjamini & Hochberg, 1995).

Table 3 describes the results from this analysis. There was a main effect of STAI Trait on pupillary diameter with increasing scores reflecting decreasing pupillary diameter, but there was no main effect of AQ-S Mind-Reading. We observed no significant interactions between Drug and either STAI Trait nor AQ-S Mind-Reading scores, nor any higher-order interactions (involving Emotion and Epoch), suggesting that autism and anxiety traits did not alter the effect of OT on pupil diameter in response to dynamic facial expressions.

Table 3. Results from a mixed model ANCOVA examining whether STAI Trait and AQ-S Mind-Reading scores affected the role of oxytocin on pupillary reactivity to dynamic facial expressions.

	df	F	p	η_p^2
STAI Trait	1, 86	5.53	**.02**	.06
AQ-S Mind-Reading	1, 86	1.13	.29	.01
STAI Trait x Drug	1, 86	0.13	.72	.002
STAI Trait x Emotion	2.67, 317.84	0.34	.78	.004
STAI Trait x Epoch	1.18, 317.84	3.17	.07	.04
AQ-S Mind-Reading x Drug	1, 86	1.07	.30	.01
AQ-S Mind-Reading x Emotion	2.67, 317.84	0.48	.68	.01
AQ-S Mind-Reading x Epoch	1.18, 317.84	.04	.87	<.001
STAI Trait x Drug x Emotion	2.67, 317.84	0.48	.68	.01
STAI Trait x Drug x Epoch	1.18, 317.84	1.13	.30	.01
STAI Trait x Emotion x Epoch	3.70, 317.84	0.84	.49	.01
AQ-S Mind-Reading x Drug x Emotion	2.67, 317.84	0.68	.55	.01
AQ-S Mind-Reading x Drug x Epoch	1.18, 317.84	0.11	.78	.001

AQ-S Mind-Reading x Emotion x Epoch	3.70, 317.84	0.21	.92	.002
STAI Trait x Drug x Emotion x Epoch	3.70, 317.84	.91	.45	.01
AQ-S Mind-Reading x Drug x Emotion x Epoch	3.70, 317.84	1.00	.41	.01

Significant effects are highlighted in bold.

Discussion

We investigated the effect of intranasal OT administration on pupil diameter in response to naturalistic facial expressions of emotion. Contrary to predictions, participants in the OT and PL conditions showed no differences in pupil diameter when viewing dynamic facial expressions. This suggests that in the current sample intranasal OT did not increase visual attention, as reflected in pupil diameter, in response to social stimuli, compared to PL. As such our data do not support the social salience hypothesis of the OT (Bartz et al., 2011; Shamay-Tsoory & Abu-Akel, 2016). However, increased pupil diameter is only one potential mechanism through which increased visual attention can be measured. It is interesting to note, that studies investigating the effects of OT on eye-gaze, another potential mechanism of visual attention, also present contrasting findings (Domes et al., 2007; Guastella et al., 2008; Hubble et al., 2016; Lischke et al., 2012). Thus, increased visual attention may not be the way in which OT affects social cognition, or the way in which OT increases the salience of the social cues in the environment.

We also sought to extend existing literature by including more emotional facial expressions, but there was no evidence that OT had a differential effect on pupil diameter across the four emotions. We also ran an exploratory analysis investigating whether individual differences in autistic and anxiety traits moderated the effect of OT on pupil responses. This analysis revealed that individuals who self-reported higher trait anxiety displayed smaller pupil dilation across the task, but no effect of autistic traits. Moreover, there was no interaction between either of these measures and the effect of OT on pupil responses. This finding contrasts with previous OT literature which suggest that OT induced improvement in social cognition can often be greater for individuals with social cognition difficulties (Bartz et al., 2011). Due to the exploratory nature of this analysis, it is not possible to draw strong conclusions, however, future research may wish to investigate to what extent individual differences influence OT's effect on pupil responses.

The current findings contrast with those of two previous papers (Leknes et al., 2013; Prehn et al., 2013) in which it was found that, relative to PL, OT administration in neurotypical adults led to an increase in pupil diameter when viewing emotional stimuli. However, the current findings are consistent with those of two more recent studies (Quintana et al., 2019; Wynn et al., 2019) that failed to replicate the earlier findings. In an effort to understand the conflicting evidence across these studies, it is important to evaluate the similarities and differences. The first factor worth noting is the nature of the stimulus material used in each study. Three of the previous studies (Leknes et al., 2013; Quintana et al., 2019; Wynn et al., 2019) used static images of facial expressions, compared to dynamic facial expressions used by Prehn et al. (2013) and in the present study. As noted previously, static photographs are associated with less autonomic arousal compared to dynamic faces (Alves, 2013; Burley et al., 2017), which makes it difficult to interpret the effect of OT on autonomic arousal across different types of stimuli.

A related point is that although Prehn et al. (2013) used dynamic facial expressions, their videos were artificially long (16 seconds from neutral to full expression) and the facial expressions were low in intensity at the time when pupillometry data were collected. In the present study we used 4 second videos to be more representative of everyday emotional expressions. This may help to account for the fact that there was a main effect of emotion on pupil diameter in the present study, in contrast to the previous studies (Leknes et al., 2013; Prehn et al., 2013; Quintana et al., 2019; Wynn et al., 2019). That is, pupil diameter varied across individual expressions within the current study; pupil diameter was largest for happy expressions and smallest for fearful expressions (with sad and angry expressions being statistically similar and falling between happy and fear expressions). These results support a motivational perspective where it is proposed that autonomic responses to stimuli reflect the engagement of defensive and appetitive motivational systems – that is, the degree to which these cues 'demand action' - rather than the valence of a stimuli (Bradley et al., 2017). Our results suggest that within the current experimental context happy faces most strongly engaged these fundamental systems, in particular appetitive engagement, consistent with research that has identified pupillometry as a sensitive indicator of responsivity to reward (Chiew & Braver, 2014). Increased arousal in response to positive compared to negative facial stimuli is in contrast to previous pupilometry research using images, where images with aversive content led to the greater pupil dilation, apart from sexualised images (Bradley et al., 2017). This highlights the specificity of individual stimuli to engage motivational systems to varying degrees and highlights the challenge when comparing pupillometry results acorss studies that employed different stimuli types.

Another potential factor is the dosage of OT administered. The present study and Prehn et al. (2013) used 24IU, which is the most common dosage of intranasal OT in studies of neurotypical adults; Leknes et al. (2013) used a higher dosage of 40IU; Quintana et al. (2019)

used 8IU or 24IU intranasally or 1IU intravenously; finally, Wynn et al. (2019) used 8, 12, 24, 36, 48, 60, 72, or 84IU intranasally. Although Prehn et al. (2013) found an effect of OT on pupil diameter at 24IU, there are now three studies in which there was no effect of OT at 24IU: Quintana et al. (2019), Wynn et al. (2019), and the present study. Thus the weight of evidence suggests that 24IU of OT administered intranasally does not result in a significant increase in pupil diameter, although it is worth keeping in mind that in the current study there was a small but consistent, albeit non-significant, tendency for pupil dilation to be higher in the OT condition.

The final factor that should be taken into account when comparing findings is the sample size achieved in each study. Indeed, the issue of achieving adequate statistical power has become a particularly salient issue of late (Button et al., 2013). Two of the previous studies evaluated different drug dosages and as a result had smaller sample sizes (Wynn et al. (2019) n = 6, between-subjects; Quintana et al. (2019) n = 16, within-subjects) compared to the remaining two studies (Prehn et al. (2013) n = 24 and 23 in the PL and OT condition, respectively, between-subjects; Leknes et al. (2013) n = 39, within-subjects). In the present study, we sought to address the sample size issue by recruiting the largest sample size to date: 94 participants, 47 participants in each drug condition.

These three factors (stimuli, dosage, and sample size) makes it challenging to compare and contrast the findings and indeed may account for the inconsistent findings in the literature. We argue that the current study furthers our understanding of the effects of OT on pupil diameter in response to social stimuli, because we have tried to address some of the limitations associated with these factors. There are, however, some limitations of our study that should also be acknowledged. Although we concluded that there was no effect of OT on pupil size in response to dynamic facial expressions, it is possible that (despite recruiting the largest sample size) with greater statistical power we would have detected a significant effect, as is suggested

in Figure 2. Indeed, although significant findings are often questioned with respect to Type 1 error (Lane et al., 2016; Walum et al., 2016), questioning null findings with respect to Type 2 error is less common. In addition, and due to practical and ethical considerations, we only recruited male participants. Of the two previous papers that recruited mixed gender samples, one (Wynn et al., 2019) did not include gender in their analysis and the other (Leknes et al., 2013) found no effect of gender. Moreover, previous research suggests that there is no effect of gender on pupil diameter or response to emotional stimuli (Bradley et al., 2017). It therefore seems unlikely that gender would moderate the effects observed here. Finally, our undergraduate sample had a mean age of 19 years. It would be interesting for future research to investigate whether these results are generalisable to younger adolescents and children, and whether this effect may change during key periods of social development.

In conclusion, despite recruiting a larger sample and using more naturalistic stimuli, we did not replicate earlier findings that intranasal administration of OT increases pupil diameter in response to viewing facial expressions of emotions. Our results are consistent with those of more recent studies that also failed to find that OT administration increased pupil diameter. In addition, the present study extends previous work, which has typically used two opposite valence emotional expressions, thereby making it difficult to assess whether results are truly emotion specific or rather valence specific, and whether this is moderated by OT. However, OT had no significant effect on pupil diameter regardless of emotional expression, although there were consistent and significant effects of emotion. In addition to contributing to the literature on pupillometry and OT, our findings are discussed in light of the need to carefully evaluate different methodological approaches taken in the literature.

Funding

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Supplemental Material

Table S1. Descriptive data for the sample across personality measures

	Mean (SE)	Range	Median
Autism Quotient-Short			
Social skills	3.00 (0.05)	1.83 – 4.00	3
Mind-reading	2.92 (0.05)	1.50 – 4.00	3
State-trait anxiety inventory			
State anxiety	35.86 (0.95)	21 – 73	35
Trait anxiety	43.55 (1.03)	22 – 65	43

SE, standard error