

No Evidence of Interactive Effects of Oxytocin and Naltrexone on Social Gaze in Autism: A Naturalistic Eye Tracking Study

Raimund Buehler

`raimund.buehler@univie.ac.at`

University of Vienna

Ulrich Ansorge

University of Vienna

Matthäus Willeit

MedUni Wien

Giorgia Silani

University of Vienna

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Abstract

Rationale: Social gaze, particularly eye contact, is commonly reduced in autism spectrum disorder (ASD), potentially reflecting alterations in the oxytocin and opioid systems. Interactive effects of these systems on social gaze have been reported in animal models.

Objectives: We tested whether social gaze during a naturalistic face-to-face conversation is modulated by oxytocin, naltrexone, or their combined administration, and whether these effects differ between adults with ASD and neurotypical controls.

Methods: Seventy-three adults (34 ASD, 39 controls) completed four double-blind sessions receiving naltrexone (50 mg), oxytocin (10 IU), their combination, or placebo. Across a total of 248 sessions, eye-tracking data was collected to capture gaze patterns towards facial regions. Salivary cortisol was assessed as a secondary physiological measure of hypothalamic–pituitary–adrenal (HPA) axis activity.

Results: Individuals with ASD showed atypical gaze patterns relative to controls, characterized by increased gaze to the background, especially among female participants. Contrary to our hypotheses, we found no main effects of medication and no interactions between medication, diagnostic group, or Sex. However, naltrexone reliably increased cortisol levels across groups, indicating engagement of the HPA axis.

Conclusions: We found no evidence that oxytocin, naltrexone, or their combination modulated social gaze during face-to-face interaction. Exploratory findings suggest that gaze patterns in ASD may differ between male and female participants, highlighting the importance of considering heterogeneity in social attention research.

Background

Humans rely on a wide range of verbal and nonverbal signals to initiate, interpret, and sustain social interactions. Among these signals, eye contact is particularly important because it communicates attention, emotional engagement, and readiness for interaction (Kleinke 1986). The eye region also conveys information about another person's affective state and intentions, making gaze allocation a central component of social cognition. A substantial body of research indicates that autism spectrum disorder (ASD) is associated with atypical patterns of social attention, including reduced fixations on the eye region of faces (Chevallier et al. 2015; Chita-Tegmark 2016; Murias et al. 2018; Nayar et al. 2022). Atypical social gaze in ASD has, in turn, been linked to differences in several neurobiological systems involved in social salience, reward, and stress regulation (Alvares et al. 2017; Pellissier et al. 2018).

One neurochemical system that has long been implicated in social motivation and attachment is the endogenous opioid system. Early accounts have emphasized the role of endogenous opioids in reinforcing affiliative and bonding behavior, with social interactions assumed to stimulate opioid release (Panksepp 1979). In animals, endogenous opioids have been associated with attachment, separation

distress, and the rewarding properties of social interaction (Machin and Dunbar 2011; Pellissier et al. 2018). In humans, opioids have similarly been implicated in social reward, social connection, and responses to social stimuli, i.e. faces (Chelnokova et al. 2016; Løseth et al. 2024; Nummenmaa et al. 2016; Pellissier et al. 2018; Troisi et al. 2011; Way et al. 2009). Collectively, these findings suggest that endogenous opioids may contribute to the hedonic and motivational value assigned to social interactions.

Pharmacological blockade of opioid receptors has frequently been used to investigate this proposed function (Chelnokova et al. 2016; Machin and Dunbar 2011). For example, naltrexone, a non-selective opioid receptor antagonist that preferentially blocks μ -receptors (Trøstheim et al. 2023), has widely been used in human research to examine the opioid system's role in social reward and affiliative behavior. Early theoretical accounts have proposed that elevated endogenous opioid activity could contribute to behavioral characteristics of autism, including reduced social responsiveness and diminished motivation to seek social contact (Bouvard et al. 1995; Panksepp 1979). However, despite some evidence indicating benefit from low-dose naltrexone in autistic individuals with elevated β -endorphin levels, clinical trials have generally reported limited efficacy. Consequently, current clinical evidence to support its use remains insufficient; for a review, see Roy et al. (2015).

Moreover, the mechanisms through which endogenous opioids regulate social behavior remain incompletely understood, and findings concerning the behavioral effects of naltrexone are conflicting (Putnam and Chang 2022b). Specifically, it is not fully understood whether naltrexone *increases* affiliative behavior by enhancing social seeking or conversely, reduces affiliation by attenuating the rewarding properties of social interaction. In some studies, naltrexone has been associated with increased grooming in non-human primates (Fabre-Nys et al. 1982; Keverne et al. 1989) and heightened attention to emotional facial expressions in humans (Wardle et al. 2016). Other studies, however, have reported *reduced* grooming in primates (Martel et al. 1993), as well as diminished social attention and social connection in humans (Chelnokova et al. 2016; Inagaki et al. 2016). These apparently opposing effects suggest that the effects of endogenous opioids on social behavior cannot be described by a simple linear relationship. Instead, recent models propose that opioidergic influences on social behavior are state- and context-dependent, reflecting a dynamic balance between baseline μ -opioid receptor activity, motivational state, social context, and individual differences in social motivation or neurodevelopmental characteristics (Løseth et al. 2014; Pellissier et al. 2018).

Apart from the endogenous opioid system, considerable attention has been directed toward oxytocin as a neuromodulator of social behavior. Oxytocin is a nonapeptide synthesized in the hypothalamus, from which it is released both centrally in the brain and peripherally into systemic circulation via the posterior pituitary. It has been implicated in a broad range of processes relevant to social functioning, including attachment, threat processing, and the allocation of attention to socially salient cues (Young and Barrett 2015). Oxytocin has therefore become a major focus of ASD research (Alvares et al. 2017). For example, atypical peripheral oxytocin levels have been reported in autistic children (Modahl et al. 1998) and variations in the oxytocin receptor gene have been associated with social-communicative impairments

and autism-related traits (Gregory et al. 2009; LoParo and Waldman 2015). Several studies have also reported increased gaze toward socially informative facial regions, including the eyes, in autistic participants following oxytocin administration (Andari et al. 2010; Auyeung et al. 2015; Kanat et al. 2017; Le et al. 2020). However, the broader oxytocin literature is characterized by substantial heterogeneity and studies with larger sample sizes have often failed to replicate previously reported prosocial effects (Guastella et al. 2015; Sikich et al. 2021). Meta-analyses suggest the oxytocin literature remains generally mixed (Peled-Avron et al. 2020); for a review, see Quintana et al. (2021).

Taken together, findings from both the opioid and the oxytocin literature have yielded inconsistent and, in some cases, opposing results (Putnam and Chang 2022a; b). One possible explanation is that social behavior is unlikely to be governed by any single neurochemical system in isolation. Accordingly, researchers have increasingly emphasized the need to investigate interactions between neuromodulatory systems rather than focusing exclusively on the effects of individual pathways (Fan et al. 2020). Such an interaction account is biologically plausible as oxytocinergic and opioidergic signaling converge within neural circuits implicated in social reward, attachment, and salience (Loth and Donaldson 2021). Animal work on pair bonding suggests that oxytocin signaling in the nucleus accumbens contributes to the formation of social attachments (Ross et al. 2009), while endogenous opioid signaling in striatal and accumbens subregions contributes to the rewarding and hedonic properties of affiliative interaction (Burkett et al. 2011; Resendez et al. 2013). Importantly, these systems do not appear to act independently. Oxytocin receptor activation has been shown to influence the expression of genes associated with opioid signaling (Young et al. 2001), whereas endogenous opioids can inhibit oxytocin release (Bicknell and Leng 1982; Douglas et al. 2000; Vandesande and Dierickx 1975). These reciprocal influences raise the possibility that the behavioral consequences of manipulating one system may depend partly on the state of the other.

Within the domain of social attention, the two systems may influence social gaze through partially overlapping but distinct mechanisms: Oxytocin may modulate the salience of social cues, while opioid signaling may regulate their rewarding value. Pharmacologically targeting both systems could therefore produce effects that differ from those observed when either system is manipulated alone. Notably, one study combining exogenous administration of oxytocin and naloxone reported an increase in fixations on the eye region of conspecifics in non-human primates (Dal Monte et al. 2017). This suggests that, at least in animal models, a combined administration may be more effective for increasing social attention than targeting either system in isolation (Fan et al. 2020; Putnam and Chang 2022a; b). Whether a comparable interaction can be observed in humans, particularly in ASD, remains unclear.

In addition to their roles in social motivation and attention, both oxytocin and opioid signaling have been associated with regulation of the hypothalamic–pituitary–adrenal (HPA) axis. Oxytocin administration has been linked to altered cortisol responses under social stress, whereas opioid receptor antagonism can disinhibit HPA-axis activity and increase cortisol secretion (Heinrichs et al. 2003; King et al. 2002). This is particularly relevant because HPA-axis function has been implicated in social gaze (Vatheuer et al. 2021) and altered patterns of cortisol regulation and stress responsivity have been reported in ASD

(Corbett et al. 2012; Corbett et al. 2010). Assessing cortisol alongside gaze behavior may therefore help to clarify whether the effects of oxytocin and naltrexone on social attention are accompanied by changes in HPA axis activity.

The present study therefore aimed to investigate the separate and combined effects of oxytocin and naltrexone on social attention during a face-to-face interaction. We conducted a within-subject, double-blind, placebo-controlled pharmacological study involving neurotypical participants and participants with ASD. Our primary outcome was attention to the eye region of an interaction partner. Cortisol levels were examined as a secondary outcome.

We hypothesized that participants in the ASD group would allocate less attention to the eye region compared to the neurotypical control group (Cañigüeral and Hamilton 2019; Chevallier et al. 2015; Frazier et al. 2017). Based on previous research outlined above (Auyeung et al. 2015; Kanat et al. 2017; Le et al. 2020), we further expected that oxytocin would increase attention to the eye region compared to placebo across groups. In line with eye tracking results in animals (Dal Monte et al. 2017), we expected naltrexone to increase social attention across groups. We additionally predicted that the combined administration of oxytocin and naltrexone would exert a stronger effect on social attention than either drug administered in isolation (Dal Monte et al. 2017). Finally, based on previous evidence that opioid antagonists disinhibit HPA axis activity, we expected conditions containing naltrexone to increase cortisol levels relative to conditions without naltrexone (King et al. 2002). As the task was not designed to elicit social stress, no a priori hypothesis on the effect of oxytocin on cortisol response was put forward. Analyses and hypotheses were preregistered before data collection (OSF: <https://osf.io/c3mkg>). Deviations from the preregistration are described in the Statistical Analysis section.

Methods

Sample

All participants gave written informed consent before participation. Experimental procedures were carried out in accordance with the Declaration of Helsinki and experimental protocols were approved by the ethics committee of the Medical University of Vienna (EK-1393/2017). Neurotypical controls (CTRL) were recruited via the University of Vienna's participant management platform. Participants with ASD were invited via social media and outreach to specialized institutions. ASD participants were required to hand in confirmation of diagnosis by a licensed psychiatrist or psychotherapist. For the control group, inclusion criteria required participants to be over 18 years old, fluent in German, with no vision impairment, no history of psychiatric or neurological disorders (or medication for such conditions), no traumatic head injury, no drug or alcohol addiction, and no contraindication for oxytocin or naltrexone. The same criteria applied to ASD participants, although ASD participants were not excluded for comorbid disorders and medication use, as both are very common in the ASD population. Type of disorder and medication intake are reported in the Supplementary Material, section 1.1, "Sample". For

final analyses, data from a sample of $N = 73$ (ASD = 34; CTRL = 39) participants across 248 sessions were included. Gender was self-reported as male, female, or non-binary. Because only one participant identified as non-binary, analyses involving Sex were restricted to male and female participants; for consistency, we refer to this variable as Sex throughout. Further demographic and questionnaire data from the 73 participants included in the analysis are summarized in Table 1. A participant flow diagram (adapted from CONSORT guidelines) and full recruitment details are provided in the Supplementary Material, Figure S1 and sections 1.1–1.2, “Sample” and “Demographics and Questionnaires”.

Experimental Procedure

Each participant completed four sessions, each lasting approximately 2.5 – 3 hrs. The order of medication conditions was randomized. After providing informed consent, a urine test was conducted to screen for opioid use and pregnancy. If tests were negative, the session continued with the pharmacological administration (see below). Saliva samples were collected at multiple time points to measure cortisol levels. During each session, participants took part in a 7–10 min semi-structured interview with a same-sex experimenter, while gaze was recorded using a mobile eye-tracking headset. The same experimenter was assigned to each participant across sessions to ensure consistency. Figure 1 illustrates the design and timing of measures. Details are provided in the Supplementary Material, sections 1.3–1.4, “Eye-Tracking Interview” and “Interview Questions”.

Eye-Tracking Equipment and Procedure

Eye movements were recorded with a mobile head-mounted eye tracker (Pupil Labs Core, Berlin, Germany). A single-marker calibration was performed before each session, with a printed calibration marker held at eye level in front of the experimenter’s face. Calibration accuracy was comparable across groups (ASD: $M = 1.67^\circ$, $SD = 0.47^\circ$; CTRL: $M = 1.79^\circ$, $SD = 0.58^\circ$), $t(220.77) = -1.79$, $p = .075$. To assess whether calibration quality influenced the results, we conducted a sensitivity analysis excluding the 10 sessions with the poorest calibration quality; this analysis supported the robustness of the main findings and is reported in the Supplementary Material, section 2.2.1, “Eye Tracking Accuracy”. Fixations were extracted with the manufacturer’s algorithm, blinks and low-confidence samples (below 0.8) were excluded. Areas of interest (eyes, mouth, face, background) were defined automatically using a convolutional neural network-based face detection algorithm. Further details on the eye-tracking setup, preprocessing, and AOI detection are provided in the Supplementary Material, sections 1.5–1.6, “Eye Tracking” and “Data Preprocessing and Face Detection” and in our previous publication (Buehler et al. 2024).

Variable		ASD		CTRL			
		<i>N</i>		<i>N</i>		χ^2	<i>p</i>
Self-reported gender	Male	13		24		3.51	.061
	Female	20		15			
	Non-Binary	1		0			
		<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>t</i>	<i>p</i>
Age		34.06	13.37	29.00	10.48	1.78	.080
AQ - Autism Spectrum Quotient		24.31	5.90	9.18	4.41	11.36	< .001
<i>AQ - Communication and Reciprocity</i>		7.55	1.80	3.47	1.80	8.97	< .001
<i>AQ - Social Interaction and Spontaneity</i>		8.55	2.57	3.06	2.50	8.56	< .001
<i>AQ - Imagination</i>		8.21	2.62	2.65	1.67	9.84	< .001
<i>AQ - Social Scales</i>		16.10	3.88	6.53	3.65	10.04	< .001
BDI-A - Beck Depression Inventory		6.93	2.94	5.70	2.29	1.81	.075
Verbal IQ (MWT)		112.59	17.15	107.67	10.70	1.45	.154
IRI - Interpersonal Reactivity Index		61.26	12.57	70.71	10.80	-3.24	.002
<i>IRI - Empathetic Concern</i>		17.45	5.30	19.50	5.09	-1.59	.118
<i>IRI - Fantasy</i>		14.32	5.42	20.12	3.88	-4.91	< .001
<i>IRI - Personal Distress</i>		14.42	4.99	12.21	4.91	1.80	.077
<i>IRI - Perspective Taking</i>		15.06	5.40	18.88	4.93	-2.97	.004
SIAS - Social Interaction Anxiety Scale		51.90	11.07	25.59	14.03	8.37	< .001
STAI-t - State-Trait Anxiety Inventory (trait)		49.10	5.57	45.49	4.55	2.84	.006
PANAS - Positive and Negative Affect Schedule							

	<i>Negative Affect</i>	Session 1	1.62	0.44	1.40	0.33	2.39	.020
		Session 2	1.57	0.43	1.38	0.30	1.93	.059
		Session 3	1.42	0.44	1.43	0.51	-0.08	.933
		Session 4	1.54	0.50	1.38	0.42	1.31	.196
	<i>Positive Affect</i>	Session 1	2.78	0.59	3.26	0.56	-3.55	< .001
		Session 2	2.64	0.68	3.21	0.69	-3.23	.002
		Session 3	2.62	0.86	3.04	0.77	-1.93	.059
		Session 4	2.75	0.85	2.88	0.75	-0.63	.528
Sympathy Ratings	Interesting		4.78	1.46	5.14	1.47	0.88	0.381
	Good		5.84	1.12	6.21	0.82	1.98	0.051
	Kind		6.22	0.94	6.46	0.75	1.53	0.129
	Open-Minded		5.86	1.22	5.97	1.13	0.19	0.847

Table 1 - Demographic and questionnaire data. *T*- and *p*-values were obtained through standard *t*-tests, except for sympathy ratings, which were obtained using a linear mixed model with random intercepts by subject, as data was collected each session (within-subject). Questionnaires and subscales marked in bold highlight significant differences between groups.

Pharmacological Procedure

The study followed a double-blind, placebo-controlled crossover design. In each session, participants received both a pill and a nebulizer solution, containing either active medication or placebo. Participants completed four sessions in a within-subject, randomized order, corresponding to the four medication conditions: naltrexone and oxytocin (BOTH), naltrexone and placebo nebulizer (NAL), oxytocin and placebo pill (OXT), and placebo pill and placebo nebulizer (PLA). Naltrexone (50 mg tablet; Dependex, Austria) was administered orally 50 min before testing. This dose is commonly used in human opioid-antagonism studies and is expected to produce >90% central μ -opioid receptor blockade that is

sustained for several hours (Lee et al. 1988; Trøstheim et al. 2023). The timing was chosen because oral naltrexone is rapidly absorbed, with peak plasma concentrations typically reached within approximately 1 h after dosing (Gonzalez and Brogden 1988).

Oxytocin (10 IU; Syntocinon, Switzerland) was administered intranasally via nebulizer (Pari 128 G1000 Sinus 2, Pari, Germany) 20 min before testing. This interval is consistent with human pharmacokinetic studies showing that intranasal oxytocin produces peak peripheral concentrations within approximately 15–30 min after administration (Gossen et al. 2012). The nebulizer allowed controlled administration of a defined volume to the nasal cavity and reduced variability related to manual spray application (Guastella et al. 2013). This route is supported by evidence that nebulized intranasal oxytocin increases central oxytocin concentrations in non-human primates (Dal Monte et al. 2014). Human work comparing nebulized and standard spray administration indicates comparable pharmacokinetic profiles for the same dose and demonstrates central effects (Martins et al. 2020). More broadly, intranasal oxytocin research suggests that lower doses can be sufficient to modulate social-cognitive behavior and neural responses and may in some cases produce stronger effects than higher doses (Martins et al. 2022; Quintana et al. 2016; Quintana et al. 2015). We therefore selected a 10 IU dose to engage central oxytocinergic mechanisms while limiting the likelihood of nonspecific effects associated with higher doses, including potential cross-activation of vasopressin receptors (Neumann and Landgraf 2012; Quintana et al. 2021).

Matching placebo pills containing mannitol filler and isotonic saline solution (0.9% NaCl) were administered in placebo conditions. To minimize potential carryover effects, we aimed for an interval of 2 weeks between sessions. However, some variation between testing dates could not be avoided. The mean interval between session dates was $M = 15.8$ days, $SD = 7.44$ days. Additional sensitivity analyses examined session order and potential carryover effects across testing sessions; these analyses did not indicate carryover effects and are reported in the Supplementary Material, section 2.2.4, “Session Order and Carryover Effects”. Extended information on drug administration, dose rationale, and blinding checks is provided in the Supplementary Material, section 1.7, “Pharmacological Procedure”.

Importantly, although the study involved administration of medications, it was not designed as a clinical trial and did not investigate treatment efficacy. Instead, it constitutes a basic experimental investigation of the neurobiological mechanisms underlying social attention. As this is, to our knowledge, the first investigation of a combined application of oxytocin and naltrexone in humans, we also included a neurotypical control group.

Cortisol

Saliva samples for cortisol measurement were collected at three timepoints: First, at the beginning of the session (baseline; T1), second, during the estimated peak of plasma medication levels (T2), shortly before testing, and third, at the end of the experiment (T3). IBL SaliCaps (IBL International GmbH, Hamburg, Germany) were used to collect samples according to the manufacturer’s protocol. Cortisol

levels were analyzed by Dresden LabService (Dresden, Germany) using a standard chemiluminescence immunoassay, which demonstrated high sensitivity and low inter- and intra-assay variability. Detailed collection and assay procedures are provided in the Supplementary Material, section 1.8, “Cortisol Samples”.

Statistical analysis

The primary dependent variable was relative fixation duration, defined as the proportion of total fixation time allocated to each area of interest (AOI; eyes, mouth, face, background) within each session. The analysis was conducted using beta regression models. As a sensitivity analysis, we also analyzed fixation rates, defined as fixation counts normalized by interview length. This analysis is reported in the Supplementary Material, section 2.2.5, “Fixation Rate Outcome”.

The main model included Group (ASD, CTRL), AOI, Medication (BOTH, NAL, OXT, PLA), Sex, and Session as fixed effects. Our preregistered hypotheses concerned group differences across AOIs (Group × AOI) and effects of medication on gaze (Medication × AOI), OSF: <https://osf.io/c3mkg>. Analyses involving Sex, the time course of gaze, dimensional analyses on autistic traits / social anxiety, and cortisol were not preregistered and are therefore reported as exploratory. Model selection compared several fixed-effect variants (including interactions with medication and Sex). The model with the best fit according to AIC was selected as the main model. The final model formula was:

Relative fixation duration ~ Group × AOI × Medication + Group × AOI × Sex + Session

Due to the repeated sessions, we also evaluated a model including a participant-level random intercept. However, as each participant contributed only one value per session and AOI combination, this random-effects model produced a singular fit, estimated negligible participant-level variance, and showed poorer fit than the corresponding fixed-effects model (AIC = -1012.59 vs. -1014.59). We therefore retained the more parsimonious fixed-effects beta regression model for the main analysis. Omnibus tests for fixed effects were based on Wald chi-square tests and likelihood-ratio tests for model comparisons, as implemented for beta regressions in the glmmTMB package in R. Follow-up contrasts were estimated using emmeans with pairwise comparisons reported as z-tests and p-values.

To examine whether group differences were stable across the interview, we also analyzed relative fixation duration across normalized interview progress. For this, the interview was divided into non-overlapping 10%-wide progress bins, and separate beta mixed models were fitted for the eye and background regions (Figure 2B). Fixed effects were aligned with the main model above and included Group, Medication, Progress Bin, Session, and Sex. To account for repeated observations within participants and sessions, random-effect structures were selected by AIC: the eye model included random intercepts for participant and individual interview session, whereas the background model included a random intercept for participant and uncorrelated random slopes for Medication within

participants. Omnibus interaction tests and follow-up contrasts were conducted in line with the main analysis.

Dimensional analyses on autistic traits (AQ) and social anxiety (SIAS) were conducted by replacing the categorical group predictor with dimensional predictors. These analyses were intended to test whether dimensional variation replicated the categorical pattern observed in the main model. For cortisol, raw levels were log-transformed, and LMMs assessed effects of group, medication, timepoint, and their interactions, with repeated samples nested within participant and session. Exploratory models also tested whether cortisol reactivity was associated with gaze to the eyes, reported in the Supplementary Material, section 2.1, “Cortisol”.

All statistical analyses were performed in R (R Core Team 2019). Linear mixed models were fitted using lmerTest (Kuznetsova et al. 2017), and beta-regression mixed models for relative fixation duration were fitted using glmmTMB (Brooks et al. 2017). For LMMs, omnibus tests were obtained using Satterthwaite-approximated F-tests (Kuznetsova et al. 2017). For glmmTMB models, omnibus effects were evaluated using Wald χ^2 tests via the car package (Fox and Weisberg 2018). Estimated marginal means and pairwise contrasts were computed using emmeans, with correction for multiple comparisons where appropriate (e.g. with the default Tukey method) (Lenth 2022). Model diagnostics were inspected using DHARMA (Hartig 2016) and performance (Lüdtke et al. 2021). Figures were created using ggplot2 (Wickham 2016). Details on model selection and diagnostics, sensitivity analyses for eye-tracking accuracy, medication use, comorbid diagnoses, menstrual-cycle phase and session order / carryover effects are reported in the Supplementary Material, section 1.9 “Statistical Analysis: Eye Tracking” and 2.2, “Sensitivity Analyses”.

Results

Main Model – Group and Medication Differences

Likelihood-ratio tests of the main model revealed a significant effect of AOI, $\chi^2(3) = 97.14$, $p < .001$, indicating that fixation allocation differed across regions of interest. Pairwise AOI contrasts confirmed greater fixation allocation to the background than to the eyes, $z = 6.33$, $p < .001$, and greater fixation allocation to the eyes than to the face and mouth, $z_s \geq 5.74$, $p_s < .001$.

Importantly, the Group $\times$ AOI interaction was significant, $\chi^2(3) = 16.40$, $p < .001$ and was further characterized by a significant Group $\times$ AOI $\times$ Sex interaction, $\chi^2(3) = 21.39$, $p < .001$. Because Sex was not preregistered, Sex-related effects are reported as exploratory. Follow-up contrasts showed that, among females, ASD participants allocated less gaze to the eyes than CTRL participants, $z = -3.00$, $p = .003$, and more to the background, $z = 4.10$, $p < .001$. No significant group differences for the eyes or background were observed among males (Figure 2A). When averaging across Sex and medication, the group contrast was significant for the background, $z = 2.53$, $p = .011$, but not for the eyes, $z = -1.80$, $p = .073$. There was no evidence for a main effect of medication, $\chi^2(3) = 0.60$, $p = .896$, or for interactions of

medication with group, $\chi^2(3) = 0.04$, $p = .998$, AOI, $\chi^2(9) = 4.30$, $p = .891$, or the three-way interaction, $\chi^2(9) = 6.14$, $p = .726$. Because none of these medication effects reached significance, we did not decompose them further. Descriptive medication-stratified distributions for the full sample are reported in Figure 2C.

Time-Course Analysis (exploratory)

The time-course analysis was performed to assess whether the group differences observed in the main model were stable across the interview or confined to specific interview segments. For fixation allocation to the eyes, there was no main effect of group, $\chi^2(1) = 0.71$, $p = .398$, and no evidence that group differences varied by medication, $\chi^2(3) = 3.30$, $p = .348$, or interview progress, $\chi^2(9) = 12.91$, $p = .167$. The Group $\times$ Sex $\times$ Progress Bin interaction was significant, $\chi^2(9) = 18.82$, $p = .027$, but follow-up contrasts did not identify ASD versus CTRL differences within individual bins after stratifying by Sex. Thus, the eye-region time course did not provide evidence for temporally localized group differences.

For fixation allocation to the background, ASD participants showed greater allocation than CTRL participants overall, $\chi^2(1) = 6.16$, $p = .013$. There was no evidence that this group difference varied by medication, $\chi^2(3) = 3.52$, $p = .318$, or interview progress, $\chi^2(9) = 10.45$, $p = .315$. The Group $\times$ Sex $\times$ Progress Bin interaction was significant, $\chi^2(9) = 20.81$, $p = .014$, consistent with the female-driven pattern observed in the main model. Exploratory bin-wise contrasts suggested that greater background fixation allocation in female ASD participants was visible across multiple interview segments, whereas no corresponding pattern was observed among males (Figure 2B).

Taken together, the time-course models supported the main analysis by showing that increased background fixation allocation in ASD, particularly among females, was relatively stable across the interview. At the same time, they provided no evidence that medication conditions altered group differences in either eye or background fixation allocation across interview progress.

Autistic Traits and Social Anxiety (exploratory)

Replacing the categorical group predictor with dimensional trait measures yielded a broadly similar pattern of results (Figure 3A). In the AQ model, we observed a significant AQ $\times$ AOI interaction, $\chi^2(3) = 11.82$, $p = .008$. Follow-up simple slope analyses indicated that higher AQ scores were associated with greater background fixation allocation, $z = 3.15$, $p = .002$, whereas the corresponding negative association for the eye region was not significant in the full sample, $z = -1.70$, $p = .089$. Consistent with the pattern observed in the main model, this effect of AQ was more pronounced among females: higher AQ scores were associated with lower fixation allocation to the eyes, $z = -2.47$, $p = .013$, and greater background fixation allocation, $z = 3.60$, $p < .001$ (Figure 3A). For SIAS, the corresponding model did not provide strong evidence for AOI-specific gaze effects: the SIAS $\times$ AOI interaction was not significant, $\chi^2(3) = 5.35$, $p = .148$.

AQ and SIAS were strongly correlated in our sample, $r = .86$, 95% CI [0.77, 0.91], $p < .001$ (Figure 3C). Furthermore, AQ scores differed within the ASD group, with autistic females showing higher AQ scores than autistic males, $t(55) = 2.14$, $p = .037$, whereas AQ scores did not differ between male and female controls (see Figure 3C, bottom). A similar pattern was observed for social anxiety: within the ASD group, females reported higher SIAS scores than males, $t(59) = 2.11$, $p = .039$. Thus, the dimensional analysis converged with the categorical main model, suggesting that lower gaze allocation to the eyes and greater background gaze allocation were most evident in females and co-occurred with higher levels of autistic traits and social anxiety in the female ASD subgroup.

Cortisol (exploratory)

Overall, salivary cortisol declined across the session, reflected in a significant main effect of timepoint, $F(2, 476.39) = 69.72$, $p < .001$. However, this pattern was altered by naltrexone, as indicated by a significant main effect of medication $F(3, 173.93) = 8.11$, $p < .001$ and a Medication $\times$ Timepoint interaction, $F(6, 476.46) = 10.49$, $p < .001$. In line with our hypotheses, pooled contrasts showed that sessions involving naltrexone (BOTH, NAL) showed a cortisol increase from T2 to T3, $t(477.64) = 3.45$, $p < .001$, whereas non-naltrexone sessions (PLA, OXT) declined from T2 to T3, $t(474.80) = -6.26$, $p < .001$ (Figure 3B). There was no evidence that the medication effect differed by group, $F(3, 173.93) = 1.90$, $p = .132$, indicating that naltrexone elicited a cortisol response independent of group membership.

Lastly, we also explored the relationship between cortisol reactivity and gaze behavior. Exploratory models initially suggested a Group $\times$ cortisol reactivity interaction for fixation allocation to the eyes, $\chi^2(1) = 9.25$, $p = .002$. However, this effect was not robust to trimming extreme values. The full analysis on cortisol and gaze behavior is reported in the Supplementary Material, section 2.1, "Cortisol". Taken together, these findings indicate that naltrexone reliably increased cortisol, but do not provide robust evidence that cortisol reactivity was related to gaze behavior.

Discussion

The present study investigated the interactive effects of oxytocin and naltrexone on social attention during a face-to-face conversation in neurotypical and ASD participants. Based on evidence that oxytocin and opioid antagonism interactively influence social attention in non-human primates (Dal Monte et al. 2017), we hypothesized that their combined administration would produce a stronger increase in eye-directed gaze than either compound alone. We additionally examined whether these manipulations affected cortisol concentrations, given the involvement of both systems in hypothalamic–pituitary–adrenal (HPA) axis regulation (Heinrichs et al. 2003; King et al. 2002). The main findings were threefold. First, participants with ASD displayed atypical gaze allocation during the face-to-face interaction. Second, these group differences appeared to be driven primarily by female participants in the ASD group. Third, neither oxytocin nor naltrexone, alone or in combination, reliably altered gaze behavior. However, naltrexone produced a robust increase in cortisol levels, consistent with disinhibition of HPA-axis activity

following opioid receptor blockade (King et al. 2002). These findings are discussed in more detail in the following sections.

Group differences in naturalistic gaze behavior

ASD participants showed atypical gaze patterns compared to neurotypical controls, in line with previous research from screen-based settings (Chevallier et al. 2015; Chita-Tegmark 2016; Frazier et al. 2017) and with clinical observations of atypical gaze in ASD (Moriuchi et al. 2017). In the present study, however, the clearest group difference was not a reduction in fixations on the eye region, but an increase in gaze toward the background. This differs from meta-analytic findings from screen-based paradigms, in which group differences are most pronounced for the eye region (Chevallier et al. 2015; Frazier et al. 2017; Guillon et al. 2014).

Several methodological factors may account for this discrepancy. Standardized images and videos do not require participants to manage the reciprocal demands of an ongoing interaction, and looking away from a live partner may serve regulatory or conversational functions that passive viewing cannot capture. For example, gaze during live conversation may be shaped by speech timing, turn-taking, question content, and interpersonal comfort with the experimenter. The group differences observed here were also smaller in magnitude than meta-analytic estimates (Frazier et al. 2017; Guillon et al. 2014). This likely reflects increased variability and reduced statistical power introduced by the naturalistic design. However, the time-course analysis showed that greater background fixation in ASD females was observable across multiple interview segments. Thus, while the naturalistic design likely increased variability, it did not obscure group differences in gaze behavior. Instead, the findings suggest that atypical gaze allocation in ASD can be detected during live interaction, but that its expression may differ from the pattern typically observed in screen-based paradigms. Naturalistic interaction may therefore yield smaller but more ecologically valid estimates of atypical social attention.

Sex-related differences in gaze behavior

In the present sample, group differences were driven by females, who showed lower fixation allocation to the eyes and greater fixation allocation to the background. Our sample also showed a gender imbalance, with more females in the ASD group (a ratio of 0.65:1), diverging from the typically reported male-to-female ratio of 3:1 to 4:1 (Loomes et al. 2017; Zeidan et al. 2022). This likely reflects our recruitment strategy through social media and autism-related online communities, which has been shown to yield samples with more balanced or even reversed sex ratios (Rodgaard et al. 2022). While this limits generalizability, it also allowed us to examine gaze behavior in autistic females, a comparatively understudied group (Lai et al. 2015).

Within the ASD group, females showed higher AQ and SIAS scores than males, which may explain the observed gaze pattern. Eye contact may be experienced as particularly aversive by individuals with elevated social anxiety or autistic traits, and directing gaze toward the background may function as a regulatory strategy. Additionally, a diagnostic bias has been proposed, whereby females may need to

present with more pronounced symptoms to receive a diagnosis (Loomes et al. 2017). Specifically, autistic females show greater internalizing symptoms, including social anxiety (Lai et al. 2015), and autistic traits (Ratto et al. 2018; Schuck et al. 2019). Our findings therefore suggest that ASD-related gaze patterns may differ between male and female participants, though they remain preliminary and require replication in balanced, pre-registered samples.

Absence of medication effects on social gaze

Contrary to our expectations, medication condition did not alter gaze behavior. Neither the main effect nor the group-by-medication interaction was significant in the main model or in the time-course analysis. The absence of an oxytocin effect contrasts with studies reporting increased attention to the eye region following intranasal oxytocin administration (Auyeung et al. 2015; Guastella et al. 2008; Kanat et al. 2017). However, the broader oxytocin literature is highly heterogeneous, and null findings are common (Alvares et al. 2017; Andari et al. 2010; Peled-Avron et al. 2020). Furthermore, oxytocin does not appear to produce a uniform prosocial effect. Instead, its effects depend on the individual, the social context, and the experimental task (Bartz et al. 2011). Our results are therefore consistent with accounts proposing that oxytocin effects are context dependent and may not generalize from controlled stimulus-viewing tasks to spontaneous face-to-face interaction.

Regarding naltrexone, opioid antagonism has been shown to reduce fixations to the eye region of static faces relative to placebo, while a μ -opioid agonist increased it (Chelnokova et al. 2016). We found no corresponding effect in our study. Effects of μ -opioid blockade on human social outcomes are, however, consistently small: a recent meta-analysis of naltrexone studies reported a pooled effect of $g = -0.20$ on self-reported social connectedness (Løseth et al. 2024). Effects of this magnitude may be difficult to detect in face-to-face interactions.

Our central prediction concerned the combination of the two compounds. This hypothesis was based on reports of increased social attention under combined oxytocin and opioid antagonism in non-human primates (Dal Monte et al. 2017). We found no such interaction. However, this should not be interpreted as definitive evidence that oxytocinergic and opioidergic systems do not interact in shaping social attention. Instead, oxytocin dose, administration route, and the experimental situation may all influence pharmacological effects and may have contributed to the observed null effect. Furthermore, null effects may reflect heterogeneity within our sample: sensitivity to oxytocin and naltrexone varies across individuals with factors such as baseline oxytocin levels, opioid receptor density, and polymorphisms in the oxytocin (OXTR) and μ -opioid receptor (OPRM1) genes (Kumsta and Heinrichs 2013; Troisi et al. 2011). Autistic individuals also differ substantially in gaze behavior (Frazier et al. 2017), which was reflected here in the considerable overlap in gaze patterns between groups. Averaged over such variability, individual responses may cancel out, and diagnostic group membership may be too coarse to reliably capture medication responsiveness.

Cortisol response to naltrexone

Naltrexone produced a robust cortisol response, with levels increasing under conditions containing naltrexone across groups, consistent with HPA axis disinhibition under opioid antagonism (King et al. 2002). This offers a useful manipulation check on the null gaze findings. A 50 mg oral dose achieves substantial central μ -opioid receptor occupancy (Lee et al. 1988; Trøstheim et al. 2023), and the cortisol rise confirms that the drug was absorbed and pharmacologically active in each session. The absence of gaze effects is therefore more plausibly attributed to the behavior we measured than to the pharmacological manipulation itself.

Conversely, oxytocin did not affect cortisol levels. Its buffering effects on the HPA axis are most pronounced under acute stress (Cardoso et al. 2014). The lack of oxytocin's effect on cortisol levels in our study may thus reflect the absence of a stress manipulation. Alternatively, the oxytocin dose may have been too low, as discussed below.

Cortisol reactivity has previously been associated with social gaze and social interaction in ASD (Corbett et al. 2010; Schupp et al. 2013; Vatheuer et al. 2021). However, exploratory associations between cortisol reactivity and gaze behavior were not robust in our data (see Supplementary Material, section 2.1, "Cortisol"), suggesting that increased HPA-axis activity following naltrexone did not influence gaze behavior.

Limitations

Several limitations should be considered when interpreting the present findings.

First, although our oxytocin dosage followed established recommendations (Quintana et al. 2016), 10 IU may have been too low. The interactive effect of oxytocin and naltrexone on social attention in macaques was demonstrated with 24 IU (Dal Monte et al. 2017). This is particularly relevant because naturalistic face-to-face interaction introduces greater behavioral variability than screen-based tasks (Cañigueral and Hamilton 2019; Chevallier et al. 2015) and may require a stronger manipulation to produce observable effects. The absence of oxytocin effects or interactions with naltrexone should therefore not be taken as conclusive evidence that these systems do not influence social gaze. Dose-response relationships also differ across species, administration methods, and behavioral contexts (Martins et al. 2022; Quintana et al. 2021; Quintana et al. 2016; Quintana et al. 2015). Further dose-response studies are needed to establish which doses reliably engage social-attentional mechanisms in humans (Leng and Ludwig 2016).

Second, drugs were administered via different routes: oxytocin nasally via a nebulizer, naltrexone orally in pill form. Interactive effects should occur centrally regardless of administration route (Quintana et al. 2021; Trøstheim et al. 2023), but despite efforts to account for differences in uptake and time to peak plasma concentration (Gonzalez and Brogden 1988; Gossen et al. 2012), these factors may have contributed to the absence of medication effects.

Third, our sample consisted of adults. As ASD is a neurodevelopmental condition, medication effects may be attenuated relative to earlier developmental stages (Meredith 2015). Furthermore, adults may have developed compensatory strategies that reduce atypical gaze patterns (Chita-Tegmark 2016; Livingston and Happe 2017).

Fourth, gender was self-reported and, as only one participant identified as non-binary, analyses were restricted to male and female participants. Subgroup sizes were small and the study was not designed to test sex differences, so the female-driven gaze pattern should be considered exploratory and requires replication in larger, balanced samples.

Conclusion

Our results show that ASD was associated with atypical gaze patterns during a naturalistic face-to-face interaction, with group differences driven primarily by female participants with ASD, who also showed higher autistic traits and social anxiety. These findings were exploratory, but they highlight the importance of considering heterogeneity within the ASD phenotype (Loomes et al. 2017; Rodgaard et al. 2022). Despite our initial predictions, we found no evidence that oxytocin, naltrexone, or their combination altered social gaze, consistent with reports that single-dose effects in naturalistic contexts are more subtle than in controlled experimental settings (Andari et al. 2010; Dal Monte et al. 2017; Peled-Avron et al. 2020). Clarifying how oxytocinergic and opioidergic mechanisms contribute to human social attention in naturalistic experiments will likely require dose-response designs and stratification by baseline gaze behavior, autistic traits, or genetic markers (Martins et al. 2022; Putnam and Chang 2022b; Quintana et al. 2015).

Abbreviations

AOI – area of interest

AQ – autism spectrum quotient

ASD – autism spectrum disorder

BDI – Beck Depression Inventory

BOTH – naltrexone + oxytocin

CTRL – control group

HPA axis – hypothalamic–pituitary–adrenal axis

IRI – Interpersonal Reactivity Index

LMM – linear mixed model

NAL – naltrexone

OXT – oxytocin

PLA – placebo

SIAS – Social Interaction Anxiety Scale

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Vienna (approval number: EK-1393/2017). All participants provided written informed consent prior to participation, including consent for pharmacological administration and data collection.

Consent for publication

Written informed consent for publication of the image depicted in Figure 1 was obtained from the depicted experimenter.

Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

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Authors' Contributions

RB: Conceptualization; Methodology; Software; Formal analysis; Investigation; Data curation; Writing – original draft; Visualization; Funding acquisition. **UA:** Resources; Supervision; Writing – review & editing.

MW: Resources; Supervision. **GS:** Conceptualization; Supervision; Project administration; Funding acquisition; Writing – review & editing.

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Authors' information – Declaration of AI use

Generative AI was used for language editing to improve clarity and flow, as well as for refining code. It was not used to generate research ideas or draw scientific conclusions.

Clinical trial number: not applicable.

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Figures

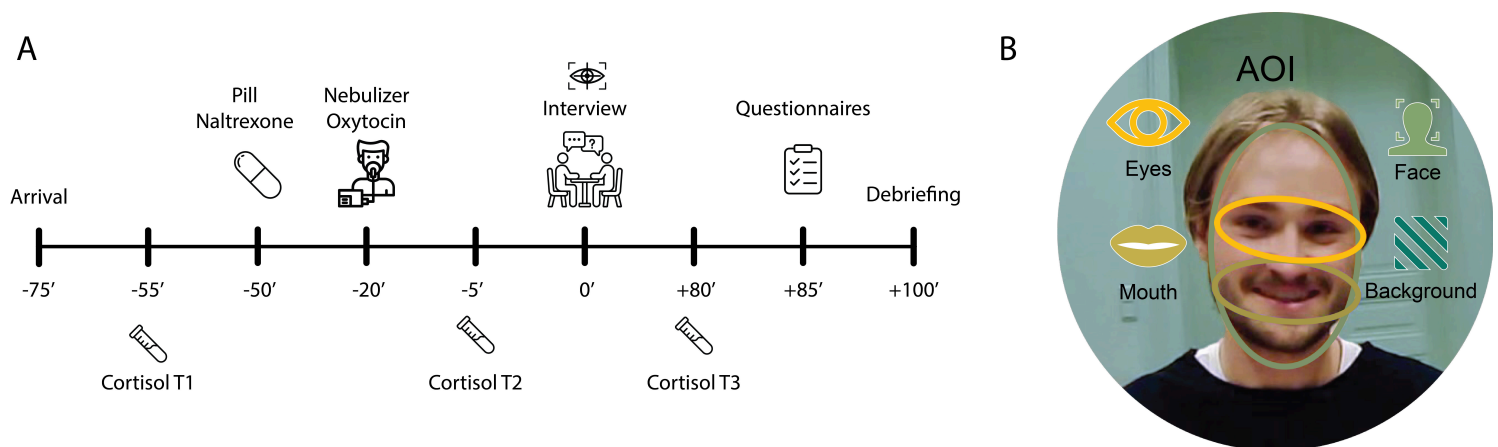


Figure 1

Overview of the experimental design and eye-tracking analysis. A Timeline of the experiment: Participants always received a pill and a nebulizer solution, resulting in four pharmacological conditions: naltrexone and oxytocin (BOTH), naltrexone pill and placebo nebulizer (NAL), placebo pill and oxytocin nebulizer (OXT) or placebo pill and placebo nebulizer (PLA). Conditions were randomized, but note that the order of administration was fixed to account for timing of medication uptake. Following drug administration, participants completed a semi-structured interview while wearing a mobile eye-tracking headset. Cortisol samples were taken at three timepoints. **B** Exemplary frame of an experimenter’s face in the field of view of a participant. Eye-tracking data was analyzed based on predefined areas of interest (AOIs), detected via a custom face detection pipeline: eyes, mouth, face, and background.

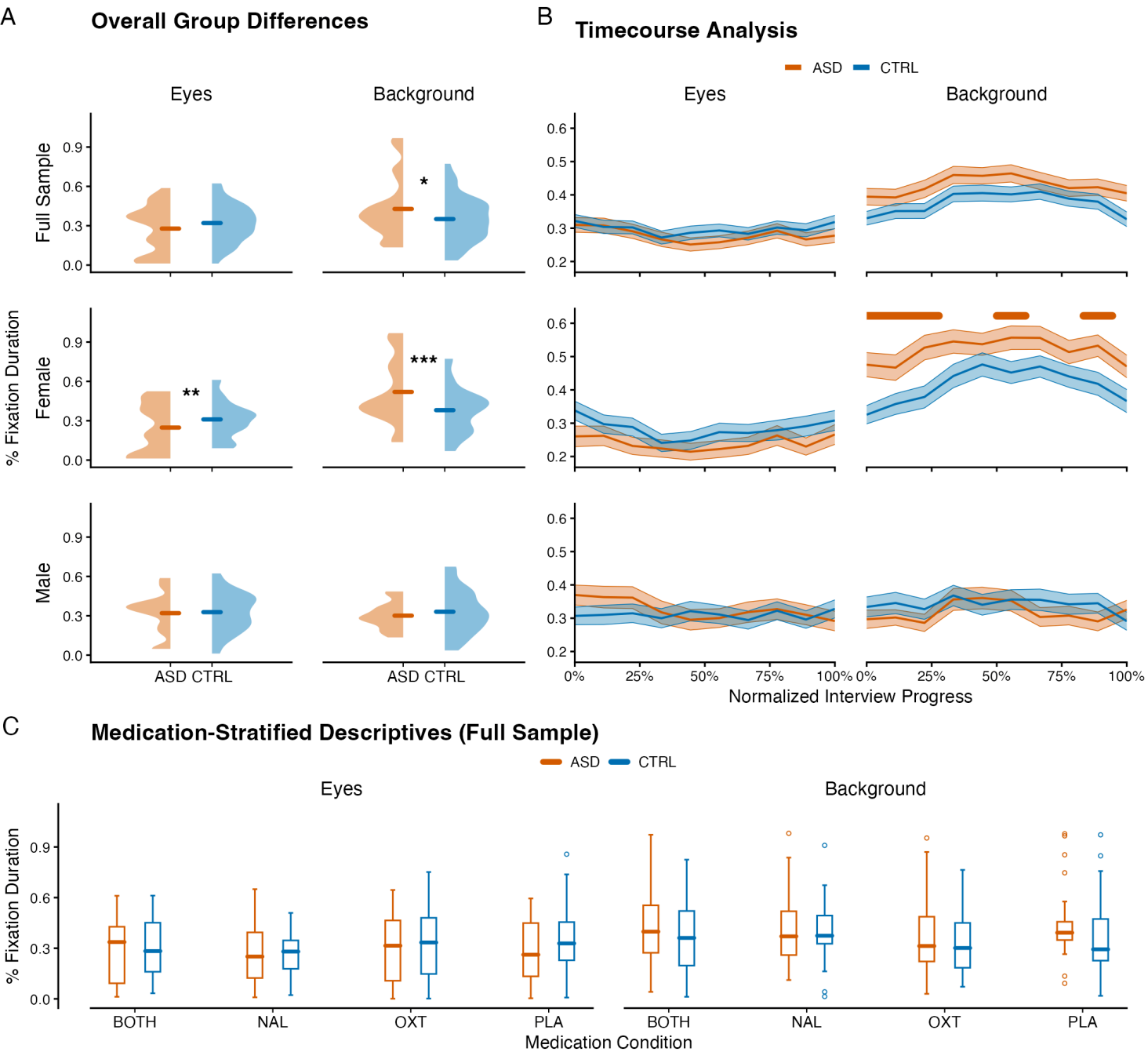


Figure 2

Diagnostic-group differences in fixation-duration allocation across regions, interview progress, and medication conditions. Rows in panels A and B correspond, from top to bottom, to the full sample, females, and males. Orange denotes ASD and blue denotes CTRL. **A** Participant-level distributions of the proportion of fixation duration directed toward the eyes and background, averaged across medication conditions. Half-violin plots show the observed distributions and horizontal marks indicate arithmetic group means. Asterisks indicate ASD–CTRL contrasts estimated from the primary beta-regression model: * $p < .05$, ** $p < .01$, and *** $p < .001$. **B** Fixation-duration time courses across non-overlapping 10%-wide bins of normalized interview progress. Lines show observed means and ribbons show ± 1 SEM. Horizontal bars identify bins with significant ASD–CTRL contrasts following Benjamini–Hochberg FDR correction across the ten bins within each subgroup-by-region series ($q < .05$) **C** Descriptive full-sample distributions stratified by medication condition for the Eyes and Background. No inferential comparisons are shown in panel C, as the medication $\times$ AOI interaction was not significant. BOTH = naltrexone plus oxytocin; NAL = naltrexone plus placebo; OXT = placebo plus oxytocin; PLA = double placebo.

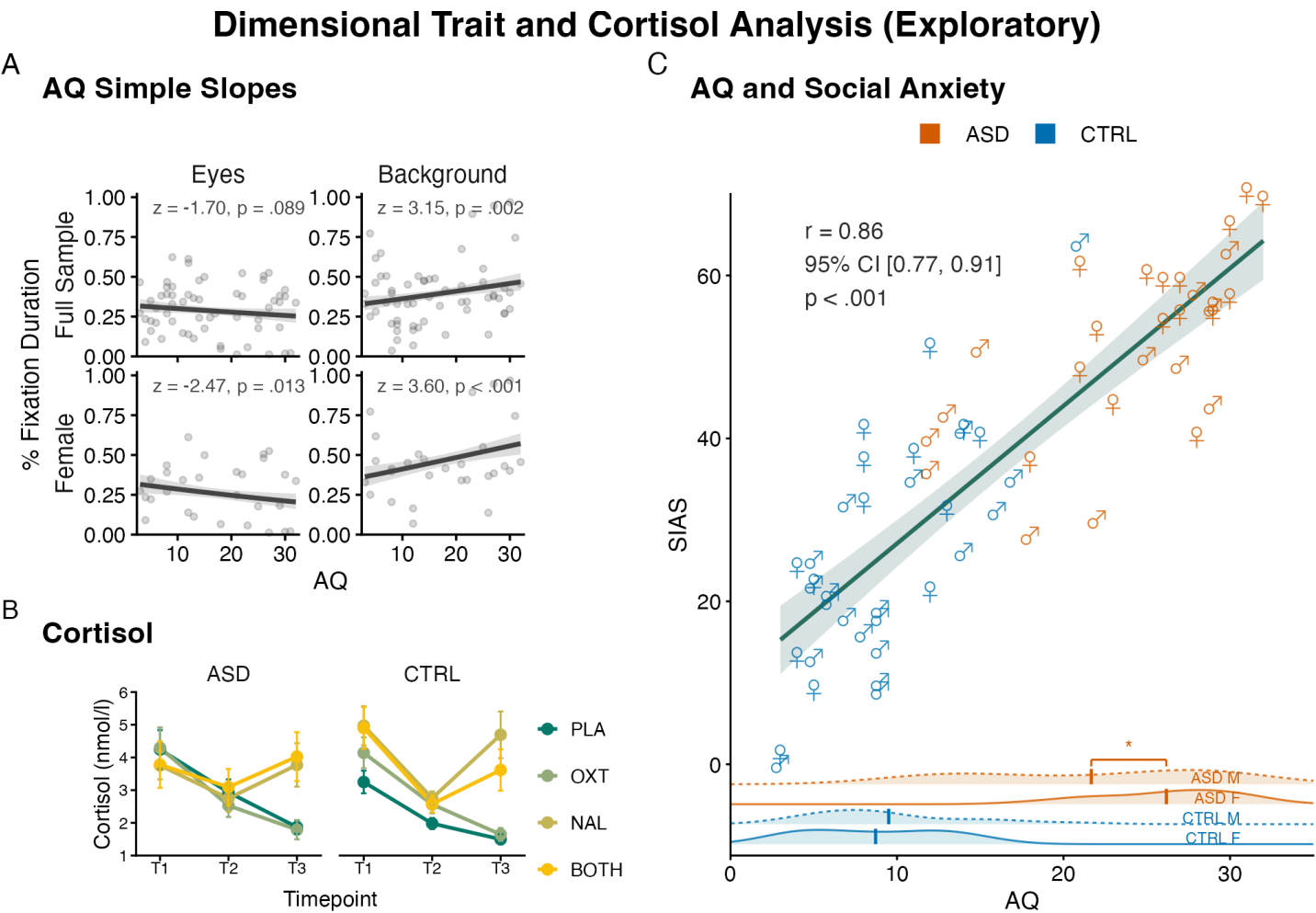


Figure 3

A Associations between autistic traits (AQ) and relative fixation duration for the eyes and background in the full sample and female subsample. **B** Mean cortisol concentrations across timepoints, stratified by group (ASD and CTRL) and medication condition; error bars represent SEM. **C** Correlation between AQ

and Social Interaction Anxiety Scale (SIAS) scores. Marginal densities at the bottom show AQ distributions by group and Sex; vertical marks indicate means. * $p < .05$

Supplementary Files

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