Oxytocin promotes prosocial behavior and related neural responses in infant macaques at-risk for compromised social development

Fabrizia Festante 1, Holly Rayson 1, Annika Paukner, Stefano S. K. Kaburu, Giulia Toschi, Nathan A. Fox, and Pier Francesco Ferrari

1 These authors contributed equally

Corresponding author: Pier Francesco Ferrari, pierfrancesco.ferrari@isc.cnrs.fr

Supplementary Information (SI)

1. SI Methods

1.1. Subjects and housing conditions

Infants were separated from their mothers on the day of birth, and subsequently raised in a nursery following the protocol reported by Simpson et al. (2016). For the first 14 days, the infants were kept in an incubator (51 × 38 × 43 cm) with a surrogate mother, terry cloths, and various toys. On day 15, infants were moved to a larger cage. From day 37, infants were given physical access to same aged-peers, to facilitate socialization, following the protocol previously described by Simpson et al. (2016)

1.2. Saliva collection procedure

Saliva samples were collected one hour after oxytocin/saline nebulization, but prior to the EEG assessment, on both testing days, for the analysis of infant oxytocin and cortisol levels. Flavored dental cotton rope pretreated with sugar-sweetened fruit drink crystals was placed in the infant’s mouth, and the infant was then allowed to chew on the rope for approximately 10 minutes (i.e. until the rope was saturated or until the infant refused the rope). The saliva was then extracted and stored in a freezer (−78 °C) until processing.

1.3. Saliva processing and analyses

Infant saliva samples were processed and analyzed at the University of Massachusetts Amherst following the protocol reported by Simpson et al. (2014). The samples were analyzed in duplicate using a sensitive and specific oxytocin enzyme immunoassay (Enzo Life Sciences, Inc., NY, USA). The assay procedure followed that which is recommended by the manufacturer. The intra-assay coefficient of variation (CV) was 3.47% for the 2014 cohort and 7.05% for the 2015 cohort. The inter-assay CV was 10.13 % for the 2014 cohort and 9.19% for the 2015 cohort.
Saliva samples were assayed for cortisol in duplicate using an enzyme immunoassay (Salimetrics, PA, USA) and followed the procedure recommended by the manufacturer. The intra-assay CV was 6.67% for the 2014 cohort and 4.32% for the 2015 cohort, while the inter-assay CV was 2.81% for the 2014 cohort and 4.71% for the 2015 cohort.

### 1.4 Oxytocin administration for EEG experiment

During nebulization, infants were cradled in the arms of a trained experimenter, and a small nebulization mask was gently held over the infant’s nose and mouth. Aerosolized oxytocin or sterile saline solution was administered for seven minutes, and the EEG experiment was conducted one hour after nebulization. A one-hour delay was selected because previous studies indicate both changes in infant macaque behavior and peripheral (saliva) oxytocin levels 60 minutes post aerosolized oxytocin delivery (Simpson et al., 2014).

Two EEG test sessions (oxytocin/saline) were conducted on consecutive days or the next business day. All experimenters were blind to treatment (i.e., oxytocin or saline) at the time of testing and data analysis. Identities of the solutions, labeled as A and B by an assistant not present for the testing sessions, were not revealed until all analyses were completed.

### 1.5. EEG preprocessing

EEG data were exported and analyzed using the EEGLAB v13.3.2 toolbox (Delorme and Makeig, 2004). Data were bandpass filtered at 2-35 Hz, and artefact-contaminated regions of the continuous signal were interpolated using the automated ASR approach available in EEGLAB (Mullen et al., 2013). 1-second epochs were extracted from the stimulus presentation periods and static (i.e. baseline) periods. Noisy epochs were excluded based on visual inspection, if they contained previously marked periods of inattention/movement (based on the video coding), or if more than 15% of channels exceeded +/- 250µV. To compare the power during stimulus periods relative to baseline periods, we computed event related spectra for each condition using built-in EEGLAB procedures. Time-frequency decompositions were computed with a fast Fourier transform using a 1-second Hanning window with 50% overlap in 1Hz bins from 2-30Hz. To make our results comparable with those of other studies, the log spectral power was converted to absolute power and averaged across the frequency bins of interest.

In addition to alpha/mu suppression during observation, we attempted to analyze suppression when infants performed LS or TP themselves, but there were not enough clean epochs after pre-processing of the EEG data. Therefore, we did not include any analyses focused on execution.
1.6. Statistical analysis

A mixed model framework was used for statistical analysis using R (v3.6.3; (R Development Core Team, 2020)) and the lme4 v1.1.21 (Bates et al., 2015), nlme v3.1.144 (Pinheiro et al., 2020), lsmeans v2.30.0 (Lenth, 2016), and car v3.0.6 (Fox and Weisberg, 2019) packages. Residuals of each model were checked for normality and homogeneity (linear mixed models) or the data for overdispersion (Poisson generalized linear mixed models). If the former assumption was not met, weights were added to the model to compensate for heterogeneity of variance (Pinheiro and Bates, 2000), and if data were overdispersed, quasi-poisson models were utilized. The Kenward-Roger approximation was used to estimate degrees of freedom for non-weighted models, and for weighted models, by the grouping level of each factor in the model (Pinheiro and Bates, 2000).

2. SI Results

2.1 Oxytocin administration

To ensure the administration of oxytocin was successful, a linear mixed model was run with treatment (oxytocin/saline) as a fixed effect, and subject-specific intercepts as a random effect. Oxytocin levels (pg/mL) were log transformed for this analysis due to non-normally distributed residuals. A significant main effect of treatment was revealed \( F(1, 17) = 337.92, p < 0.0001 \), confirming that oxytocin levels were higher in the oxytocin treatment \( (n = 18; M = 9.34, SD = 0.68) \) than in the saline treatment \( (n = 18; M = 5.79, SD = 0.86) \). See Fig. S1.
Fig. S1: Saliva oxytocin levels after nebulization. Box plots show saliva oxytocin levels in the oxytocin and saline treatment one hour after nebulization and before EEG acquisition.

2.2. Cortisol levels
To investigate whether cortisol levels (ug/dL) differed after administration of oxytocin or saline, a linear mixed model was run with treatment (oxytocin/saline) as a fixed effect, and subject-specific intercepts as a random effect. Cortisol was log transformed for this analysis due to non-normally distributed residuals. No differences were revealed (Oxytocin treatment; n = 17, M = -0.69, SD = 0.65: Saline treatment; n = 18, M = -0.73, SD = 0.52).

2.3. EEG analysis: Participants and included epochs
Table S1. Number of participants included in the EEG analyses for each condition and session, as well as the mean (SD) number of usable EEG epochs after data pre-processing.

| Sample size | EEG epochs (SD) |
|-------------|-----------------|
| Lip-smacking (saline) | N = 18 | 27.833 (14.960) |
| Lip-smacking (oxytocin) | N = 18 | 31.222 (8.328) |
| Tongue protrusion (saline) | N = 18 | 22.500 (9.488) |
| Tongue protrusion (oxytocin) | N = 17 | 28.471 (8.464) |
| Disk (saline) | N = 19 | 27.684 (9.995) |
| Disk (oxytocin) | N = 18 | 29.722 (8.930) |

Note, mixed model analyses revealed no significant differences between the number of usable EEG epochs in the different conditions or sessions (all p > 0.05).

2.4. EEG analysis: Alpha power compared to baseline
In the OT treatment, significant differences from baseline occurred in the posterior electrode cluster during observation of LS [t(17) = -3.44, p = 0.003] and TP [t(16) = -2.37, p = 0.031], but not during observation of the disk [p > 0.05]. No significant differences from baseline were found after administration of saline (all p > 0.05). Average differences from baseline in the various experimental conditions and sessions can be found in Table S2.

Table S2. Mean (SD) alpha/mu power in all conditions, sessions, and electrode clusters. Asterisks indicate a significant difference from baseline (% Δ change from baseline).

| Anterior cluster | Posterior cluster |
|------------------|-------------------|
| Lip-smacking (saline) | -4.429 (12.021) | -2.359 (10.488) |
| Lip-smacking (oxytocin) | -2.834 (10.967) | -10.119 (12.496)* |
| Tongue protrusion (saline) | -1.521 (12.447) | -3.852 (13.574) |
### 2.5. Behavioral analysis: Infant attention towards experimental stimuli

Analysis on infant behavior revealed an effect of treatment. On the proportion of time infants spent looking at the experimenter presenting the stimulus in the LS condition only, with more gaze to the experimenter in the OT treatment (n = 18; M = 0.44, SD = 0.16) versus saline (n = 19; M = 0.32, SD = 0.11) treatment (see Fig. S2).

|                  | OT (oxytocin) | Saline |
|------------------|--------------|--------|
| **Tongue protrusion (oxytocin)** | -0.506 (11.652) | -7.029 (12.22)* |
| **Disk (saline)** | -1.757 (14.706) | -3.719 (13.379) |
| **Disk (oxytocin)** | -0.613 (12.007) | -5.025 (10.384) |

#### Fig. S2: Proportion of infant attention towards experimental stimuli

Bar graphs represent the proportion of infant attention toward the stimuli during the disk, LS, and TP conditions, in the saline and oxytocin (OT) sessions. Error bars represent ±1 standard error. Significant differences between sessions are denoted by asterisks (*** p < 0.001).

### 2.6. EEG analysis: Beta band activity

Besides the alpha band, the mu rhythm includes another sub-component falling within the beta frequency range (15-25 Hz, in human adults; 15-19 Hz in human infants)(Hari, 2006; van Elk et al., 2008). Therefore, to test the specificity of our EEG results, we also ran models to investigate power in the beta frequency band (15-17 Hz in infant macaques (Festante et al., 2018)) during observation of LS/TP/disk in the oxytocin and saline treatment (i.e. the same models run to examine alpha/mu activity). No main effects of treatment or interactions between treatment and electrode cluster were
revealed, indicating that the effects of oxytocin revealed in the main analyses were specific to the alpha frequency band. Greater beta suppression in the posterior compared to anterior electrode cluster was revealed in the disk condition \(F(1, 26) = 4.46, p = 0.049\). Power reductions compared to baseline were significant for: OT \(t(13) = -2.17, p = 0.003\) and saline \(t(13) = -3.63, p = 0.003\) in the posterior cluster in the disk condition; and saline \(t(11) = -3.85, p = 0.003\) in the posterior cluster in the LS condition. This is in keeping with some previous human EEG research demonstrating an effect of oxytocin on alpha activity during action observation, but not on beta activity (Festante et al., 2020).

### 2.7. Behavioral analysis: Relationship between cortisol and infant facial gestures

Mixed model analyses revealed a relationship between cortisol levels and infant LS gestures in the LS condition only. Higher levels of cortisol were related to more infant LS in the oxytocin treatment \((n = 16; M = 3.37, SD = 5.55)\), but lower levels of infant LS in the saline treatment \((n = 18; M = 0.33, SD = 0.59)\). This relationship is represented in Fig. S3.

![Fig. S3: Pre-EEG cortisol and frequency of infant facial gestures in the different observation conditions and treatments. Here, a median split of cortisol was performed for illustrative purposes only.](image-url)
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