Supporting Information

Samuni et al. 10.1073/pnas.1616812114

SI Methods

Urine Sample Collection and Analysis. During focal follows, we systematically collected every urine sample possible of the focal subjects from leaf litter using a plastic pipette. We then transferred the urine (200 to 1,000 μL) into a cryovial containing 100 μL 0.5 N H_3PO_4 (19) to prevent hormone degradation, using a 1-mL Eppendorf pipette. We kept the samples cool by storing them in a thermos can with frozen cold packs until arrival at the camp, where we stored all samples in liquid nitrogen (within 12 h of collection). Samples were then shipped frozen on dry ice to the Laboratory of Endocrinology at the Max Planck Institute for Evolutionary Anthropology, where we stored them at −80 °C until analysis. Before the analysis, we assigned urine samples according to the behavior that occurred within the 15 to 60 min time window of oxytocin excretion (19, 43). Samples were excluded if other social behaviors occurred within the excretion window to ensure greater interpretation accuracy of the results in relation to the specific control and social events.

Accordingly, $n = 19$ of the samples collected in the context of intergroup conflict that overlapped with other behaviors that might influence urinary oxytocin levels ($n = 15$ overlapped with hunting behavior and $n = 4$ overlapped with food sharing) were thus not included in the analysis. Similarly, in the context of control with affiliation, $n = 14$ samples were not included in the analysis due to overlap with play behavior.

Sample extraction and analysis followed the protocol used by Crockford et al. (19), incorporating minor changes. Accordingly, we thawed samples while keeping them cool using an IsoPack (0 °C; Eppendorf), vortexed them for 10 s, and centrifuged for 1 min at 214.55 × *g*. We performed a solid-phase extraction with Chromabond HR-X SPE cartridges (1 mL, 30 mg). First, we conditioned the cartridges with 1 mL 100% methanol followed by 1 mL distilled HPLC water. Then, thawed urine samples were diluted 1:2 using 0.1% trifluoroacetic acid (TFA) and loaded onto the cartridge. We continued to wash the cartridge with 1 mL 10% (vol/vol) acetonitrile (ACN) containing 1% TFA in water, and eluted using 1 mL 80% (vol/vol) ACN. Extracted samples were then evaporated with an air stream at 50 °C, reconstituted with 300 μL 100% ethanol, and vortexed for 10 s. Samples were left at 4 °C for 60 min and evaporated again using the same procedure. Once dried, the samples were reconstituted in 250 μL of the assay buffer supplied in the commercially available enzyme immunoassay kit (Assay Designs; 901-153A-0001). We again vortexed the samples for 10 s and then centrifuged for 1 min at 9391 × *g*. Samples were added as 100-μL duplicates to the assay, following the instructions of the assay provider.

The assay standard curve ranged from 15.62 to 1,000 pg/mL, and assay sensitivity was 15 pg/mL. Oxytocin validations of parallelism and accuracy were conducted and appeared satisfactory (19). Interassay coefficients of variation of low- (50 pg/mL) and high- (250 pg/mL) value quality controls were 19.1 and 7.6% ($n = 44$), respectively, whereas intraassay coefficients of variation of low- (50 pg/mL) and high- (250 pg/mL) value quality controls were 12.9 and 8.9%, respectively.

For cases that produced results outside of the linear range of the oxytocin standard curve, we repeated the extraction and analysis, applying less volume. Overall, we excluded 23 cases for which remeasurement produced results outside of the linear range or for which no material was left over for remeasurement.

Statistical Analysis. We fitted a Poisson generalized linear mixed model (GLMM) with log link function (24) to investigate the effect of the type of event (intergroup conflict or control) on the number of times each adult individual left the subgroup. In the model, we included group identity and proximity to border areas as control predictors. Border proximity was expressed as the mean value of proximity to the border throughout the course of each period. We included the duration from an individual's first arrival in the subgroup to the end of the event (log-transformed) as an offset term. Identities of subjects that were present in the subgroup and event identities were included as random effects. To keep type I error rate at the nominal 5%, we included random slopes (46, 47) for both proximity and period type within-subject. The R script formula for the defection model: glmer(number of times each adult individual left ~ event type + *z*-transformed border proximity + group + offset(log(duration)) + (1 | event identity) + (1 + dummy-coded event type 2 + dummy-coded event type 3 + *z*-transformed border proximity || subject identity), family = Poisson).

We then investigated whether chimpanzee in-group behavior during and before hostile intergroup conflicts engaged the oxytocinergic system. All urine samples associated with intergroup conflicts were collected from individuals that participated in the intergroup conflict. We fitted LMMs (24) with Gaussian error structure and identity link function, and log-transformed the response variable, urinary oxytocin levels (pg/mg creatinine). Our test predictor for the models was the type of events sampled (event model, $n = 468$: control with and without affiliation, control with coordination, and intergroup conflict with and without affiliation; anticipation model, $n = 52$: control with affiliation and preborder patrol with affiliation). To control for factors that might influence hormone levels, we included in each model subgroup size as well as individuals' sex and rank. We also included proximity to border areas by assigning each urine sample a value according to the minimum polygon in which it was excreted. This provided the relative distance from the border areas of the territory where intergroup encounters are more likely, to evaluate potential risk and its possible effect on oxytocin excretion. The anticipation model included samples from a single chimpanzee group (East) because no preborder patrol with affiliation samples were attained for South group, hence the reduction in sample size from $n = 100$ samples to $n = 38$ for control with affiliation. Therefore, we included group identity (i.e., East or South) as a control predictor only in the event model. We also included duration of affiliative contact as a control predictor in the anticipation model (affiliation duration) to control for its effects on urinary oxytocin levels (26). We initially included an interaction between our test predictor (event type) and sex in the event model; however, because it did not reveal a significant effect ($P = 0.580$), we excluded this variable and reran the model. Event and subject identity were included as random effects to control for having several samples from the same events and subjects. Furthermore, we included random slopes (46, 47) for the test predictors (i.e., event or anticipation) as well as for subgroup size, rank, and proximity within-subject. For the anticipation model, we also included the random slope of affiliation duration within-subject. We did not include the correlation among the random slopes and random intercepts in any of the fitted models (46). Furthermore, we investigated the effect of affiliation with or without intergroup conflict, and coordination, by conducting a post hoc analysis for the event model. This was done by dummy coding the test predictor and subsequently changing the reference categories (Tables S5, S6, and S7).

Before fitting the models, we checked all predictors and the response for their distribution and, as a consequence, log-transformed urinary oxytocin levels to achieve a more symmetrical distribution. We then proceeded by *z* transforming the covariates of subgroup size, rank, proximity, and affiliation duration to a mean of zero and an SD of one (49). Visual inspection of qqplots and residuals plotted against fitted values did not reveal obvious deviations from the assumptions of normally distributed and homogeneous residuals.

The R script formula for the event model: lmer(log-transformed urinary oxytocin ~ event + *z*-transformed border proximity + *z*-transformed subgroup size + group identity + subject sex + *z*-transformed subject rank + (1 + dummy-coded event 2 + dummy-coded event 3 + dummy-coded event 4 + dummy-coded event 5 + z-transformed border proximity + z-transformed subgroup size + z-transformed subject rank||subject identity) + (1|event identity).

The R script formula for the anticipation model: lmer(log-transformed urinary oxytocin ~ event + *z*-transformed border proximity + *z*-transformed subgroup size + subject sex + *z*-transformed subject rank + *z*-transformed affiliation duration + (1 + dummy-coded event 2 + z-transformed border proximity + z-transformed subgroup size + z-transformed subject rank + z-transformed affiliation duration||subject identity) + (1|event identity).

Moreover, to determine how similar contexts influenced urinary oxytocin levels, we fitted three additional models: (*i*) the type of intergroup conflict (i.e., control versus border patrol or intergroup encounter; intergroup conflict type model; Fig. S1 and Table S2), (*ii*) a reduced event model excluding intergroup conflict samples of border patrols (Fig. S2 and Table S4), and (*iii*) pre versus during intergroup conflict affiliation ("persistence" model; Fig. S3 and Table S9). We fitted LMMs (24) with Gaussian error structure and identity link function, with the response being log-transformed urinary oxytocin levels (pg/mg creatinine). We included subgroup size, proximity to border areas, and individuals' sex and rank as control predictors in both models. We included group identity (i.e., East or South) as a control predictor only in the intergroup conflict type model and in the reduced event model, including samples from individuals of both groups. Event and subject identity were included as random effects to control for having several samples from the same events and subjects. Furthermore, to keep type I error rate at the nominal 5%, we included random slopes (46, 47) for the two test predictors, as well as for subgroup size, rank, and proximity within-subject. We did not include the correlation among the random slopes and the respective random intercept in any of the fitted models (46).

We fitted all models in R [version 3.3.0 (42)] using the functions lmer and glmer of the R package lme4 (45) and derived variance inflation factor (VIF) values using the function vif of the R package car (50), applied to a standard linear model lacking the random effects. We determined model stability for all models by excluding subjects and event identities one at a time. We then compared the estimates derived for these data with those derived for the full dataset. This indicated no influential subjects or event identities to exist. We derived confidence intervals by means of parametric bootstraps (function bootMer of the package lme4). VIFs did not reveal collinearity problems, as indicated by the largest value being <4 (51) (event model 2.59; anticipation model 2.43; defection model 1.98; intergroup conflict type model 2.74; persistence model 2.03; reduced event model 2.72).

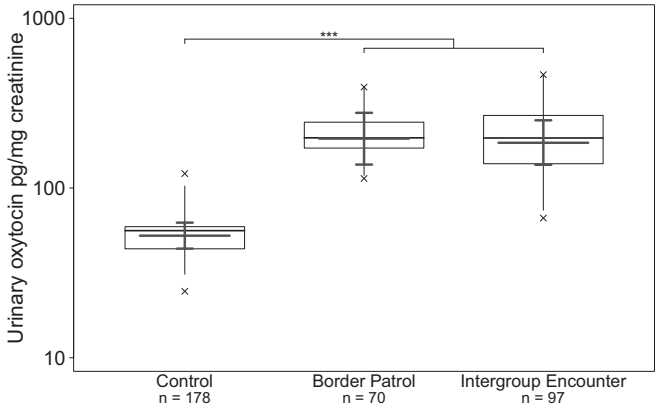

Fig. S1. Effects of the type of intergroup conflict on urinary oxytocin levels in wild chimpanzees in East and South groups (n = 345 samples, 16 subjects, 194 events). Shown are medians (thin horizontal lines), quartiles (boxes), percentiles (2.5 and 97.5%; vertical lines), minimum and maximum (laying crosses), as well as the fitted model and its 95% confidence intervals (thick lines). ***$P < 0.001$.

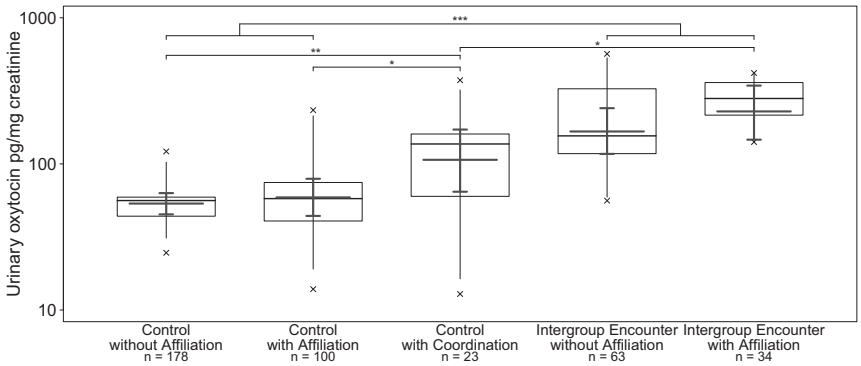

Fig. S2. Effects of intergroup encounters with and without in-group affiliation on urinary oxytocin levels in wild chimpanzees in East and South groups (n = 398 samples, 20 subjects, 282 events). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

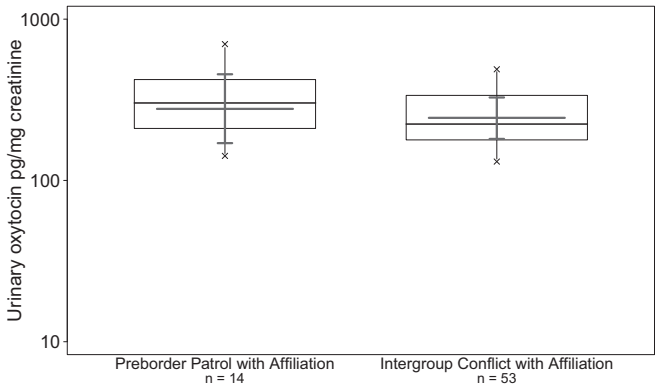

Fig. S3. Effects of intergroup conflict anticipation and participation on log-transformed urinary oxytocin levels in wild chimpanzees of East group (n = 67 samples, 7 subjects, 29 events). Shown are medians (thin horizontal lines), quartiles (boxes), percentiles (2.5 and 97.5%; vertical lines), minimum and maximum (laying crosses), as well as the fitted model and its 95% confidence intervals (thick lines).

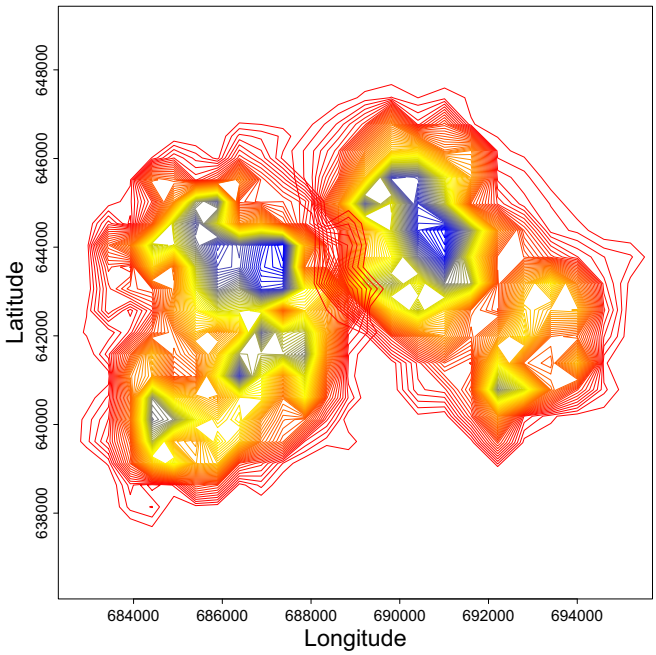

Fig. S4. Kernel density estimate constructing polygons representing the percentage of home-range use in South (west of longitude 688500) and East (east of longitude 688500) groups. The polygons range from 5 to 99, with 5 (blue) representing the very core of the home range and 99 (red) being the border areas.

Table S1. Defection model: Effect of intergroup conflict on subgroup cohesion

Term	Estimate	SE	CI_{lower}	CI_{upper}	χ^{2}*	*P*
Intercept	−0.475	0.241	−0.962	−0.026	–	–
Intergroup conflict[†]	**−1.732**	**0.446**	**−2.586**	**−0.809**	**13.484**	**<0.001**
Group[‡,§]	**−1.358**	**0.671**	**−3.113**	**−0.311**	**4.700**	**0.030**
Proximity[¶]	0.184	0.201	−0.190	0.587	0.829	0.363

Statistically significant results ($P \leq 0.05$) appear in bold.
*Degrees of freedom are 1.
[†],[‡],χ^{2} and *P* values refer to comparison with the reference categories [†]control and [‡]East group.
[§]Group identity was associated with large uncertainty due to the small sample size of South group.
[¶]*z*-transformed, mean ± SD of the original variables: 72.57 ± 24.19 (range 5 to 99).

Table S2. Intergroup conflict type model: Effect of the type of intergroup conflict, namely border patrol or intergroup encounter, on urinary oxytocin levels, log-transformed

Term	Estimate	SE	χ^{2}*	*P*
Intercept	4.399	0.214	–	–
Test predictor				
Border patrol[†]	**1.321**	**0.221**	**22.227**	**<0.001**
Intergroup encounter[†]	**1.261**	**0.189**	**37.522**	**<0.001**
Control predictors				
Group[‡]	**−0.358**	**0.134**	**7.014**	**0.008**
Sex[§]	−0.359	0.209	2.788	0.095
Proximity[¶]	−0.058	0.068	0.714	0.398
Subgroup size[#]	0.077	0.054	2.010	0.156
Rank[‖]	0.086	0.099	0.614	0.433

Statistically significant results ($P \leq 0.05$) appear in bold.

*Degrees of freedom are 1.

[†,‡,§]χ^{2} and *P* values refer to comparison with the reference categories [†]control, [‡]East group, and [§]female.

[¶,#,‖]*z*-transformed, mean ± SD of the original variables: [¶]65.86 ± 29.35 (range 5 to 99), [#]11.45 ± 5.96, and [‖]0.61 ± 0.24 (range 0 to 1, with 1 being the highest social rank).

Table S3. Post hoc intergroup conflict type model: Post hoc analysis of the effect of the type of intergroup conflict, namely border patrol or intergroup encounter, on urinary oxytocin levels, log-transformed

Term	Estimate	SE	χ^{2}*	*P*
Intercept	5.660	0.248	–	–
Test predictor				
Control[†]	**−1.261**	**0.189**	**39.372**	**<0.001**
Intergroup Encounter[†]	0.060	0.192	0.097	0.756
Control predictors				
Group[‡]	**−0.358**	**0.134**	**7.014**	**0.008**
Sex[§]	−0.359	0.209	2.788	0.095
Proximity[¶]	−0.058	0.068	0.714	0.398
Subgroup size[#]	0.077	0.054	2.010	0.156
Rank[‖]	0.086	0.099	0.614	0.433

Statistically significant results ($P \leq 0.05$) appear in bold.

*Degrees of freedom are 1.

[†,‡,§]χ^{2} and *P* values refer to comparison with the reference categories [†]border patrol, [‡]East group, and [§]female.

[¶,#,‖]*z*-transformed.

Table S4. Reduced event model: Effect of intergroup encounters and in-group affiliation on urinary oxytocin levels, log-transformed

Term	Estimate	SE	CI_{lower}	CI_{upper}	χ^{2}*	*P*
Intercept	4.484	0.224	4.037	4.986	–	–
Test predictor levels						
Control with affiliation[†]	0.097	0.163	−0.241	0.414	0.340	0.560
Control with coordination[†]	**0.689**	**0.249**	**0.198**	**1.200**	**4.902**	**0.027**
Intergroup encounter without affiliation[†]	**1.136**	**0.199**	**0.723**	**1.559**	**28.920**	**<0.001**
Intergroup encounter with affiliation[†]	**1.452**	**0.226**	**0.949**	**1.923**	**23.401**	**<0.001**
Control predictors						
Group[‡]	**−0.349**	**0.131**	**−0.624**	**−0.075**	**6.521**	**0.011**
Sex[§]	**−0.438**	**0.214**	**−0.905**	**−0.015**	**3.943**	**0.047**
Proximity[¶]	−0.054	0.058	−0.173	0.064	0.858	0.354
Subgroup size[#]	0.116	0.058	0.001	0.224	3.715	0.054
Rank[‖]	0.174	0.109	−0.054	0.391	2.351	0.125

Statistically significant results ($P \leq 0.05$) appear in bold.

*Degrees of freedom are 1.

[†,‡,§]χ^{2} and *P* values refer to comparison with the reference categories [†]control without affiliation, [‡]East group, and [§]female.

[¶,#,‖]*z*-transformed, mean ± SD of the original variables: [¶]56.46 ± 28.43 (range 5 to 99), [#]11.21 ± 5.95, and [‖]0.618 ± 0.24 (range 0 to 1, with 1 being the highest social rank).

Table S5. Post hoc event model 1: Post hoc analysis of the effect of intergroup conflict and in-group affiliation on urinary oxytocin levels, log-transformed, with intergroup conflict without affiliation as the reference category

Term	Estimate	SE	χ^{2}*	*P*
Intercept	5.549	0.236	–	–
Test predictor levels				
Control without affiliation[†]	**−1.197**	**0.171**	**43.294**	**<0.001**
Control with affiliation[†]	**−1.075**	**0.208**	**22.343**	**<0.001**
Control with coordination[†]	**−0.499**	**0.245**	**4.085**	**0.043**
Intergroup conflict with affiliation[†]	0.136	0.14	0.933	0.334
Control predictors				
Group[‡]	**−0.319**	**0.127**	**5.916**	**0.015**
Sex[§]	−0.307	0.200	2.292	0.130
Proximity[¶]	−0.035	0.057	0.378	0.538
Subgroup size[#]	0.082	0.049	2.714	0.099
Rank[‖]	0.077	0.100	0.574	0.449

Statistically significant results ($P \leq 0.05$) appear in bold.

*Degrees of freedom are 1.

[†,‡,§]χ^{2} and *P* values refer to comparison with the reference categories [†]intergroup conflict without affiliation, [‡]East group, and [§]female.

[¶,#,‖]*z*-transformed.

Table S6. Post hoc event model 2: Post hoc analysis of the effect of intergroup conflict and in-group affiliation on urinary oxytocin levels, log-transformed, with intergroup conflict with affiliation as the reference category

Term	Estimate	SE	χ^2*	*P*
Intercept	5.677	0.242	–	–
Test predictor levels				
Control without affiliation[†]	**−1.328**	**0.187**	**44.160**	**<0.001**
Control with affiliation[†]	**−1.205**	**0.219**	**25.042**	**<0.001**
Control with coordination[†]	**−0.633**	**0.256**	**5.973**	**0.015**
Intergroup conflict without affiliation[†]	−0.128	0.130	0.968	0.325
Control predictors				
Group[‡]	**−0.321**	**0.126**	**6.110**	**0.013**
Sex[§]	−0.302	0.198	2.274	0.132
Proximity[¶]	−0.037	0.058	0.411	0.521
Subgroup size[#]	0.082	0.049	2.770	0.096
Rank[‖]	0.072	0.098	0.521	0.471

Statistically significant results ($P \leq 0.05$) appear in bold.
*Degrees of freedom are 1.
[†,‡,§]χ^2 and *P* values refer to comparison with the reference categories [†]intergroup conflict with affiliation, [‡]East group, and [§]female.
[¶,#,‖]*z*-transformed.

Table S7. Post hoc event model 3: Post hoc analysis of the effect of intergroup conflict and in-group affiliation on urinary oxytocin levels, log-transformed, with control with coordination as the reference category

Term	Estimate	SE	χ^2*	*P*
Intercept	5.051	0.283	–	–
Test predictor levels				
Control without affiliation[†]	**−0.698**	**0.222**	**9.517**	**0.002**
Control with affiliation[†]	**−0.576**	**0.255**	**5.000**	**0.025**
Intergroup conflict without affiliation[†]	**0.499**	**0.245**	**4.085**	**0.043**
Intergroup conflict with affiliation[†]	**0.635**	**0.261**	**5.799**	**0.016**
Control predictors				
Group[‡]	**−0.319**	**0.127**	**5.916**	**0.015**
Sex[§]	−0.307	0.200	2.292	0.130
Proximity[¶]	−0.035	0.057	0.378	0.538
Subgroup size[#]	0.082	0.049	2.714	0.099
Rank[‖]	0.077	0.100	0.574	0.449

Statistically significant results ($P \leq 0.05$) appear in bold.
*Degrees of freedom are 1.
[†,‡,§]χ^2 and *P* values refer to comparison with the reference categories [†]control with coordination, [‡]East group, and [§]female.
[¶,#,‖]*z*-transformed.

Table S8. Anticipation model: Effect of intergroup conflict anticipation on urinary oxytocin levels, log-transformed

Term	Estimate	SE	CI_{lower}	CI_{upper}	χ^{2}*	*P*
Intercept	4.369	0.393	3.586	5.157	–	–
Test predictor						
Preborder patrol with affiliation[†]	**1.483**	**0.410**	**0.681**	**2.282**	**11.132**	**<0.001**
Control predictors						
Sex[‡]	−0.418	0.484	−1.418	0.554	0.697	0.404
Proximity[§]	0.261	0.166	−0.081	0.610	2.331	0.127
Subgroup size[¶]	−0.012	0.143	−0.311	0.270	0.007	0.935
Rank[#]	−0.150	0.199	−0.564	0.272	0.528	0.467
Affiliation duration[‖]	0.167	0.172	−0.195	0.526	0.862	0.353

Statistically significant results ($P \leq 0.05$) appear in bold.

*Degrees of freedom are 1.

[†],[‡]χ^{2} and *P* values refer to comparison with the reference categories [†]control with affiliation and [‡]female.

[§],[¶],[#],[‖]*z*-transformed, mean ± SD of the original variables: [§]60.9 ± 25.97 (range 5 to 99), [¶]15.04 ± 5.21, [#]0.628 ± 0.27 (range 0 to 1, with 1 being the highest social rank), and [‖]1357.64 ± 791.9 (s).

Table S9. Persistence model: Effect of intergroup conflict anticipation and participation on urinary oxytocin levels, log-transformed

Term	Estimate	SE	χ^{2}*	*P*
Intercept	5.902	0.427	–	–
Test predictor				
Intergroup conflict with affiliation[†]	−0.128	0.299	0.182	0.670
Control predictors				
Sex[‡]	−0.318	0.424	0.559	0.455
Proximity[§]	0.123	0.127	0.921	0.337
Subgroup size[¶]	0.094	0.116	0.615	0.433
Rank[#]	−0.169	0.138	1.357	0.244

*Degrees of freedom are 1.

[†],[‡]χ^{2} and *P* values refer to comparison with the reference categories [†]preborder patrol with affiliation and [‡]female.

[§],[¶],[#]*z*-transformed, mean ± SD of the original variables: [§]87.55 ± 11.87 (range 5 to 99), [¶]15.71 ± 4.34, and [#]0.64 ± 0.22 (range 0 to 1, with 1 being the highest social rank).

Movie S1. Typical elements of chimpanzees' border patrols and intergroup encounters.

Movie S1

Dataset S1. All data used to fit the defection model

Dataset S1

Dataset S2. All data used to fit the event model

Dataset S2

Dataset S3. All data used to fit the anticipation model

Dataset S3