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The salivary testosterone response to a chance-determined contest is associated with face-gazing behaviours in athletic women

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ABSTRACT

Human gaze is an important indicator of dominant and submissive behaviours related to positioning in a social hierarchy. This study investigated face gazing after a chance-determined contest and its linkage to salivary testosterone (sal-T) reactivity in athletic women. Twenty-six women athletes completed a coin-toss game on days 7 (D7), 14 (D14) and 21 (D21) of a single menstrual cycle. The game was played against an unknown opponent of similar age with the winner congratulated and rewarded with all coins. Gazing towards an opponent's head or face was timed after each contest (over 2 min) from video footage. Salivary T (sal-T) was assessed before and 15 min after these contests. The sal-T residuals increased after winning and decreased after losing on D7, D14 and D21 ($p < 0.05$). Gaze times were longer after a loss ($M = 7.8$ s) than a win ($M = 3.1$ s) across all days. Regression analyses revealed that the sal-T residuals and contest outcome interacted to predict gaze time. Upon deconstruction we found that, when losing a contest, a larger sal-T response (i.e., smaller decline) predicted a longer gaze ($\beta = 1.71$, $p = 0.004$), but no association was evident when winning ($\beta = -0.06$, $p = 0.821$), and these slope patterns differed ($p = 0.011$). In conclusion, winning a contest by chance increased sal-T responsivity and decreased opponent gaze across the menstrual cycle. A positive relationship between individual sal-T reactivity and gaze duration was observed, but only when losing. These preliminary results support suggestions that women's T may help modulate post-competition behaviours (e.g., face gazing) possibly to achieve social cohesion and cooperation.

1. Introduction

Human gaze is an important indicator of dominant and submissive behaviours related to position within a social hierarchy (Mazur and Booth, 1998). In primates and humans, submissive individuals often avert their gaze from dominant conspecifics (Terburg and van Honk, 2013). However, in naturally-cycling women, a longer opponent gaze was observed after losing (not winning) a laboratory contest (Sharp and Hamilton, 2017). This might be explained by women's choices to affiliate with others under stress (Taylor et al., 2000) and achieved via directed gaze vigilance. Indeed, some suggest that testosterone (T), a hormone linked to social status, may motivate women's post-competition behaviours towards social cohesion (Casto and Edwards, 2016). In the earlier report (Sharp and Hamilton, 2017), the win-loss difference was eradicated among oral contraceptive (OC) users, perhaps arising from a reduction in T availability due to OC's (Cobey et al., 2013).

Menstrual variation in salivary T (sal-T) adds to these complexities, as a possible signal for competitive behaviours (Crewther and Cook, 2018) that are relevant to contest appraisal, T reactivity and human gaze. Previous work implies that athletic women might have higher basal and responsive sal-T than the general population (Bermon and Garnier, 2017; Cook et al., 2018), thereby potentially amplifying T responsiveness under these conditions and subsequent gaze behaviours. As such, this population provides an ecological model for verifying the impact of natural T variation on face gazing after a contest. Therefore, we examined the gaze behaviours of athletic women after a chance-related contest and the impact of sal-T with repeated testing across the menstrual cycle. We broadly hypothesised that gaze time would vary by contest outcome and menstrual day, corresponding to changes in sal-T levels.

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2. Materials and methods

2.1. Participants

Twenty-six women athletes from different sports (i.e., netball, soccer, tough rugby, rugby and hockey) were recruited. The cohort characteristics were as follows; age (M = 22.0, SD = 1.8 years), height (M = 1.72, SD = 0.04 m), body mass (M = 69.5, SD = 4.6 kg), and body mass index (M = 23.4, SD = 1.1 kg/m^2). Participants had been hormone-based contraceptive free for at least six months. They reported a mean menstrual cycle length of 29.3 days (SD = 1.4) across three consecutive cycles with no irregularities and were not taking any other medications, drugs or doping agents. The cohort reported training 6.6 h (SD = 2.2) a week before this study. No physical activity was performed 24 h before testing and no intense exercise (or competition) within 48 h. Each participant signed an informed consent form. Ethical approval was given by a local university Human Research Ethics Committee.

2.2. Experimental design

A coin-toss game was performed on day seven (D7), 14 (D14) and 21 (D21) of a single menstrual cycle, but starting day was randomised across subjects. This assignment method is often used to identify discrete periods that differ in T availability (Cobey et al., 2013; Cook et al., 2018; Maner and McNulty, 2013), as a natural model of T variation for testing research hypotheses. Day one was the self-reported start of menses. Each athlete played an opponent from a pool of similar-aged women unknown to them. The opponents were rotated to ensure a different dyad on each testing day. Both players had three or six turns (each) where they selected a heads or tails on their own throws. The winner (best of 3 or best of 6 if needed) was congratulated and given the six coins in view of the loser to magnify status disparity. The investigator then left the room on the pretext of collecting forms. Both players sat in sight of each other (~2.2 m distance) and were asked not to communicate in anyway. Gaze behaviour was recorded for 2 min using a video camcorder (Canon LEGRIA HFR806). The files were manually coded to determine the length of time (to the nearest 0.1 s) participants spent looking directly at the head or face of their opponent. Data coding was performed by two observers (> 96% agreement) and averaged for analysis.

2.3. Salivary testosterone

A saliva sample (~1 mL) was taken upon arrival at the lab and 15 min after contest completion. The samples were collected using a passive drool method (Crewther and Cook, 2018; Oliveira et al., 2009) and stored in a −80 °C freezer. All assays were performed in duplicate using commercial enzyme-linked immunoassay kits (Salimetrics LLC, USA) with a calibrator range from 6.1 pg/mL to 600 pg/mL. We eliminated inter-assay variance in sal-T (< 10%) by testing each athletes' samples in the same plate. Participants were asked to refrain from eating food, drinking caffeine, and smoking before (> 90 min) sample collection (Al-Dujaili and Sharp, 2012). To reduce circadian variation, all contests were scheduled between 1100 and 1400 h, with each athlete tested within a smaller (± 30 min) window. To index the sal-T response, we computed residual change scores by regressing post-contest sal-T onto pre-contest sal-T and saving the unstandardised residuals (Carré et al., 2013). The raw pre- and post-contest sal-T data are presented in Fig. 1A.

2.4. Statistical analyses

Data were analysed with the R package lme4 (Bates et al., 2015) using a linear mixed model with random intercepts. First, we employed a two-factor analysis of variance to evaluate menstrual changes (D7, D14, D21) and outcome differences (win, loss) in three variables; pre-contest sal-T levels, sal-T residuals, and gaze time. Paired *t*-tests were used to determine if the sal-T residuals differed from a zero baseline. Where appropriate, post hoc contrasts were performed using the Tukey test. Effect size statistics included eta-squared (η2), Cohen's d (d), and R-squared (R^2). Second, regression analysis was undertaken to investigate the association between sal-T reactivity and gaze behaviour. The sal-T residuals (standardised), contest outcome (0 = loss, 1 = win), and their interaction were entered simultaneously in a model predicting gaze time. Given the small sample size, 95% bootstrap confidence intervals (replications = 1000) were created for each coefficient. Simple slope testing at one SD above and below the mean was used to interpret a significant interaction. The level of significance was set at $p \leq 0.05$ for all tests.

3. Results

Chi-square analysis verified that the respective win-loss frequencies on D7 (15, 11), D14 (15, 11), and D21 (13, 13) did not deviate from expected probabilities, $\chi^2(2, n = 78) = 0.41$, $p = 0.815$. Our analysis of pre-contest sal-T revealed a main effect of menstrual day ($F[2, 72] = 22.24$, $p < 0.001$, $\eta2 = 9.33\%$), but no contest outcome ($F[1, 72] = 1.62$, $p = 0.207$, $\eta2 = 0.34\%$) or interaction effect ($F[2, 72] = 0.29$, $p = 0.746$, $\eta2 = 0.12\%$). Testing for simple effects confirmed the day response ($F[2, 75] = 22.40$, $p < 0.001$, $\eta2 = 9.33\%$) with higher ($p < 0.001$) sal-T on D14 versus D7 ($d = 1.47$) and D21 ($d = 0.89$), but D7 and D21 did not differ ($p = 0.522$). The sal-T residuals (Fig. 1B) increased from baseline in all wins ($p < 0.04$, $d = 0.6$ to 1.1) and decreased across all losses ($p < 0.01$, $d = -1.0$ to -1.6). When testing main effects, sal-T reactivity was influenced by menstrual day ($F[2, 72] = 4.39$, $p = 0.016$, $\eta2 = 4.79\%$), contest outcome ($F[1, 72] = 95.16$, $p < 0.001$, $\eta2 = 51.90\%$), and their interaction ($F[2, 72] = 3.70$, $p = 0.029$, $\eta2 = 4.04\%$). Post-hoc contrasts revealed that all winning sal-T residuals differed from losses on D7, D14 and/or D21 ($p < 0.01$). Gaze time (Fig. 1C) differed by menstrual day ($F[2, 72] = 20.88$, $p < 0.001$, $\eta2 = 4.98\%$) and contest outcome ($F[1, 72] = 406.4$, $p < 0.001$, $\eta2 = 48.49\%$) with no interaction ($F[2, 72] = 1.77$, $p = 0.178$, $\eta2 = 0.42\%$). Follow-up analyses showed no day effect ($F[2, 75] = 2.30$, $p = 0.107$, $\eta2 = 4.98\%$), whilst the outcome effect remained significant ($F[1, 76] = 281.8$, $p < 0.001$, $\eta2 = 50.78\%$) with a longer gaze after a loss (M = 7.8, SD = 2.3 s) than a win (M = 3.1, SD = 2.4 s).

Regression analyses identified the sal-T residuals, contest outcome, and their interplay all as predictors of gaze time (Table 1, $p \leq 0.015$). The R^2 values indicate good model fit, particularly the conditional R^2 (i.e., 86% shared variance) that combines both the fixed and random effects. Decomposing the interaction revealed that, when losing a contest, a larger sal-T response predicted a longer gaze ($\beta = 1.71$, SE = 0.40, $p = 0.004$) in the context of declining sal-T residuals after a loss. Conversely, we found no association between these variables when winning a contest ($\beta = -0.06$, SE = 0.26, $p = 0.821$). The win-loss slope patterns also differed ($p = 0.011$).

4. Discussion

Human gaze is an important social behaviour that often reflects status or positioning within a hierarchy (Mazur and Booth, 1998), analogous to success or failure in a competition. Here we observed a longer opponent gaze among losers of a chance-related contest, relative to winners, and consistently so on D7, D14, and D21. Our findings correspond to non-athletic women who either lost or won a non-physical (Jenga) competition during follicular- and luteal-phase testing around a menstrual cycle (Sharp and Hamilton, 2017). Gazing duration among winners (3.96 s) and losers (7.34 s) in the latter study, which included data on OC users, also closely matched our results (3.1 s, 7.8 s), respectively. This agreement was quite surprising, but the