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State, not trait, neuroendocrine function predicts costly reactive aggression in men after social exclusion and inclusion

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ABSTRACT

Social exclusion increases aggressive behaviour, and the possible neuroendocrine underpinnings of the effect are largely unknown. Here, we examined the extent to which testosterone and cortisol responses to social exclusion would predict subsequent reactive aggression. Men were randomly assigned to a social exclusion (SE) or inclusion (SI) condition of 'Cyberball', a computer ball-toss game. Aggression was then measured using the Point Subtraction Aggression Paradigm (PSAP). Saliva was collected at three points for the measurement of testosterone and cortisol. Regression analyses indicated that testosterone concentrations 10-min into the PSAP (controlling for pre- and post-Cyberball testosterone) were positively correlated with aggressive behaviour, irrespective of SI/SE. Post hoc analyses for the conditions separately, however, suggested the relationship was stronger for SI men ($R^2_{change} = 13.3\%$, $F_{1,\ 29} = 5.28$, $p = 0.03$) than for SE men ($R^2_{change} = 1.8\%$, $F_{1,\ 26} = 0.49$, $p = 0.49$). Aggressive behaviour was also positively correlated with cortisol concentrations 10-min into the PSAP (controlling for pre- and post-Cyberball cortisol) irrespective of SE/SI. When both hormones were included in the regression model, the interaction of baseline 'Cortisol' × 'Testosterone' × 'Experimental Group' approached significance ($R^2_{change} = 5.4\%$, $F_{1,\ 55} = 3.53$, $p = 0.07$), but no significant effects were observed in either group alone. The findings add to evidence that individual differences in state neuroendocrine function map onto variability in human social behaviour.

1. Introduction

Psychologists from a broad array of theoretical perspectives concur on the importance of social relationships for humans, with affiliation considered a basic human drive (Baumeister and Leary, 1995; Blackhart et al., 2009). There is much evidence that the quality of a person's social bonds impacts physical and mental health (Aldabe et al., 2010; Wilkinson and Marmot, 2003). Although there are evolutionary benefits to social inclusion, social exclusion has been cast as an adaptive behaviour as well, whereby others who are costly to one's inclusive fitness are avoided (Leary et al., 2006). Social exclusion also can be viewed as an act of aggression using the standard definition of aggressive behaviour, which is behaviour intended to harm or injure another, and with the type of harm or injury physical, psychological, social, or financial (Baron and Richardson, 1994; Leary et al., 2006). Social exclusion is thus a form of relational aggression through the harm it does to social relationships (Murray-Close et al., 2010).

Exclusion induces a hostile mindset (DeWall et al., 2009; Romero-Canyas et al., 2010; Twenge et al., 2001), which may underlie the many reports of increased aggressive behaviour in the excluded from studies using both non-experimental (e.g., Downey et al., 2000; Murray-Close et al., 2010; Pellegrini et al., 2007) and experimental approaches (Wesselmann et al., 2010) (reviewed in Leary et al., 2006). Aggression after social exclusion in laboratory studies usually fits the definition of reactive aggression rather than proactive aggression (Ayduk et al., 2008; Buckley et al., 2004; DeWall and Bushman, 2009; Kirkpatrick et al., 2002; Twenge et al., 2001; Warburton et al., 2006; Wesselmann et al., 2010). Reactive aggression is typically a defensive, retaliatory response to perceived or actual provocation that is characterized by anger and high physiological arousal, whereas proactive aggression does not involve provocation, is aimed at gaining resources (e.g., money, territory, social status, and mating opportunities), and does not typically involve physiological arousal (Crick and Dodge, 1996; Dodge and Coie, 1987). In the laboratory studies of aggression after exclu-

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sion, the aggressive behaviour was not costly to the individual; the greatest cost to the individual in the experimental setting was the loss of social status from the exclusion itself, with aggressive behaviour occurring when there was little opportunity to regain status by other means. For example, in many of the studies, the excluded aggressor acted under conditions of anonymity without any likelihood of meeting the target. When there is the possibility of influencing the rejector(s), however, the excluded individual instead may act ingratiatingly despite a hostile mindset (reviewed in Maner et al., 2007; Romero-Canyas et al., 2010), and there is some evidence of increased affiliative or prosocial behaviour in the excluded individual towards others than the rejector(s) (Gross, 2009; Maner et al., 2010). Thus, situational factors appear to moderate the behaviour of the excluded, with aggressive behaviour after exclusion more likely to occur under conditions of anonymity and when there is no expectation of interaction with the rejector.

That aggressive behaviour after exclusion has been found towards others and in the absence of the instigator of the threat to status suggests that aggression may be a behaviour pattern that occurs in response to a variety of threat contexts depending on the extent to which automatic, emotional processing is activated rather than, or relative to, deliberative processing (Anderson and Bushman, 2002; Berkowitz, 2008; Richetin and Richardson, 2008; Todorov and Bargh, 2002). Recent evidence suggests impulse control and cost-benefit analysis operate in parallel as determinants of aggressive behaviour (e.g., Archer et al., 2010; Archer and Southhall, 2009). Thus, social exclusion may diminish impulse control (Twenge and Baumeister, 2005), but also may require low cost conditions for aggressive behaviour to be expressed.

In our investigations of reactive aggression using modified versions of the Point Subtraction Aggression Paradigm (PSAP, originally designed by Cherek, 1981), reactive aggressive responses are made at a cost to earning financial reward (Carré and McCormick, 2008; Carré et al., 2009). Nevertheless, the extent of aggressive behaviour changes with cost of the behaviour, with more aggressive responses evident when there is less financial cost (Carré et al., 2010), suggesting an influence of cost-benefit analysis. We thus hypothesized that social exclusion would increase aggressive behaviour in the PSAP, in keeping with the evidence that social exclusion diminishes impulse control and impairs decision-making (Rilling et al., 2008; Twenge and Baumeister, 2005). Furthermore, there is evidence that social exclusion increased the desire for money and the distress over its loss in participants (Zhou et al., 2009), and thus excluded individuals may be more prone to retaliate to an opponent's stealing of their points. We have argued that there is greater intrinsic reward of the aggressive behaviour under conditions in which it is most costly, which is supported by higher ratings of enjoyment of the PSAP in conditions of high cost of aggression and a positive correlation between aggressive responses and enjoyment of the PSAP only in conditions of high cost of aggression (Carré et al., 2010). Thus, we also tested whether social exclusion would strengthen these relationships.

The investigation of social exclusion and costly aggressive behaviour also provided us with the opportunity to investigate individual differences and the context-specificity of the relationships between endocrine function and aggression. There is substantial evidence for a rise in testosterone in men in specific contexts, most notably competitive situations and sexual encounters (see reviews by Archer, 2006; Booth et al., 2006; van Anders and Watson, 2006). The Biosocial Model of Status posits that testosterone promotes dominance behaviour aimed at preserving status, and thus testosterone concentrations in men are highly sensitive to changes in status (Mazur and Booth, 1998). Based on the latter, one would predict that social exclusion, because of loss of status, decreases testosterone concentrations in men. Many studies, however, have failed to find winner/loser differences in testosterone responses (Carré et al., 2009; Mehta and Josephs, 2006; Schultheiss et al., 2005; van der Meij et al., 2010), and the one study of testosterone concentrations after social exclusion found a decrease in testosterone after inclusion and no change after exclusion (DeSoto et al., 2009). Nevertheless, individual differences in testosterone responses predicted subsequent competitive behaviour more so for losers than for winners (Carré et al., 2009; Mehta and Josephs, 2006). Thus, individual differences in testosterone responses may contribute to the expression of aggressive behaviour to a greater extent after social exclusion than after inclusion.

Behaviour after social exclusion may involve neuroendocrine factors other than testosterone. One study reported a drop in progesterone (a hormone considered relevant for affiliative behaviour) after social exclusion among participants high in social anxiety (Maner et al., 2010). There also is some evidence for a rise in cortisol concentrations after social exclusion, but when found, the effect is small and limited to subgroups of participants (reviewed in Zoller et al., 2010). There has been one investigation of endocrine function after social exclusion with respect to subsequent aggressive behaviour: Ford and colleagues found that those individuals who increased cortisol concentrations after exclusion were more derogatory towards a partner (Ford and Collins, 2010). This result fits the relationship that has been reported between cortisol and aggressive behaviour, with aggressive behaviour associated with low baseline cortisol (reviewed in Poustka et al., 2010; van Goozen et al., 2007) and high cortisol reactivity (e.g., Gerra et al., 2001; Lopez-Duran et al., 2009).

Cortisol also has been reported to moderate the relationship between testosterone and aggression, whereby a relationship between the testosterone and aggressive behaviour is found only among those with low cortisol concentrations (Dabbs et al., 1991; Popma et al., 2007). Cortisol also was found to moderate the change in testosterone in men after winning or losing a competition (Mehta et al., 2008), and the interaction of cortisol and testosterone predicted dominance (Mehta and Josephs, 2010). Van Honk and colleagues have proposed that a combination of low cortisol and high testosterone concentrations are hallmarks of a predisposition to aggression (Terburg et al., 2009; van Honk et al., 2010). Nevertheless, the extent to which baseline concentrations of either testosterone or cortisol or their reactivity or their interaction best predict aggressive behaviour is still unknown. Thus, the present experiment investigated the extent to which baseline and changes in testosterone and cortisol could explain individual differences in costly aggressive behaviour after social exclusion compared to after social inclusion.

2. Methods

2.1. Participants

Undergraduate men were recruited from Brock University ($n = 78$, all self-identified as Caucasian). Four participants taking prescription medications (e.g., SSRIs, glucocorticoids, Ritalin) were removed from the analyses, resulting in a sample of 74 men (mean age = 19.96, S.D. = 2.43).

2.2. Procedure

Arrival. (See Fig. 1 for a timeline of the experimental procedures.) Participants were tested individually within the hours of 12:00 and 18:00 h to minimize diurnal variation in hormone concentrations. On arrival, each participant completed consent forms, a demographic questionnaire, and posed for a photograph (headshot while posed in a neutral facial expression). Next (about 15 min after arrival), the participant provided a 1–2 mL saliva sample (baseline sample) while the experimenter loaded the photograph into the computer program. Participants were told that they would first play an online ball toss game (Cyberball) that would allow them to interact with three other participants before the main experimental task.

Cyberball. Participants were assigned randomly to the social inclusion or social exclusion condition of Cyberball, a widely used, reliable experimental procedure for simulating the experience of social exclusion (Williams, 2007; Williams et al., 2000; Williams and Jarvis, 2006). To increase the believability of the task, the participant's

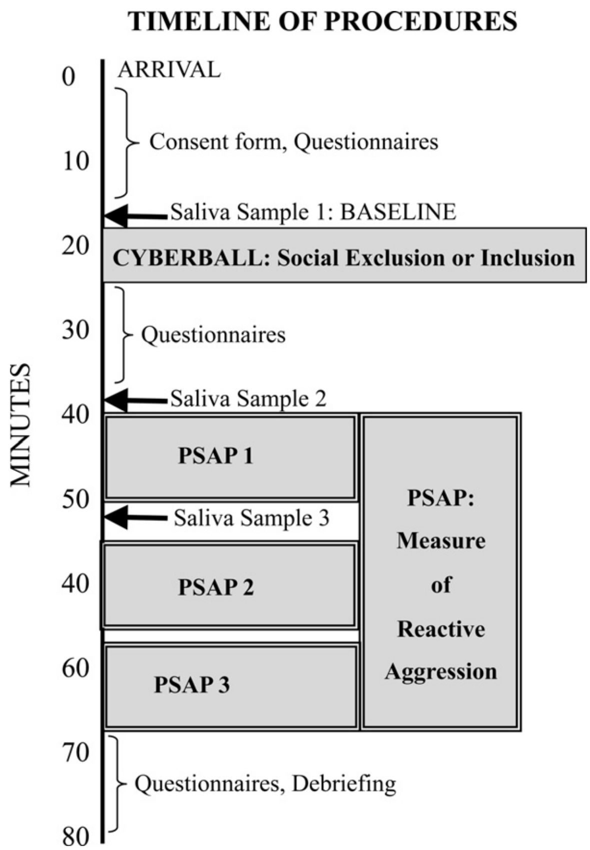

Fig. 1. Timeline of the experimental procedures.

photograph appeared at the bottom of the screen and the photographs of the other three fictitious participants appeared at the top left, center, and right of the screen. The photographs for the fictitious participants were of three Caucasian men obtained from another experiment. The participant was asked whether he recognized any of the other men participating in the study to increase the believability that other men were participating in the experiment. None of the participants reported recognizing any of the other fictitious participants.

Participants were told that hitting button 1 of a standard keypad would pass the ball to the participant located at the top left hand corner of the screen, button 2 would pass the ball to the participant located at the top center of the screen, and button 3 would pass the ball to the participant located at the top right hand corner of the screen. Cyberball was programmed such that 100 ball tosses occurred in a session that lasted approximately seven minutes. Socially excluded participants received seven ball tosses, after which they were excluded from ball tosses. In the social inclusion condition, participants received approximately 25 ball tosses over the session. At the end of Cyberball, a feedback screen appeared on which was written "Percentage of ball tosses received during Cyberball". The screen also displayed the four photographs of participants and indicated the percent of ball tosses received by each, with 10% appearing below the photograph of socially excluded participants and 40% below that of socially included participants. Another statement indicated: "For the next task, you will be paired with participant 3". For socially excluded participants, the screen showed that participant 3 received 30% of ball tosses and for socially included participants, participant 3 had received 20% of ball tosses. Thus, participants were paired with an individual who received 20% more (excluded condition) or 20% fewer (included condition) passes than them.

Next, the participant completed a short questionnaire as a manipulation check (e.g., "I enjoyed playing Cyberball," "I received a fair share of passes during Cyberball," "I felt somewhat angry during the Cyberball task"; 10 point Likert scale from 1 = definitely true to 10 = definitely false). To increase the belief that participant 3 was a real person, the participant completed a brief questionnaire for exchange with the fictitious participant 3 (e.g., music preferences, hobbies, career goals) under the pretext of allowing them to know each other better before the next task. Based on the possibility that socially excluded individuals may act prosocially or ingratiatingly when they expect to interact again with the excluder (Maner et al., 2010), responses on the questionnaire were scored. To capture participants' embellishments (considered as affiliative) or brevity on the questionnaires, the category code, overstatement (OVRST), was used based on the Lasswell Value Dictionary Categories (Stone, 1965, described in Weber, 1990): OVRSTs are words used to clarify or go beyond the minimum required to answer a question. The number of OVRSTs on the questionnaire was tallied by an investigator blinded to whether the participant had been included or excluded. A completed version of the questionnaire, ostensibly written by participant 3, was given to each participant to read. The participant then provided a second 1–2 mL saliva sample and was given a detailed description of the Point Subtraction Aggression Paradigm (PSAP).

Point Subtraction Aggression Paradigm (PSAP): The PSAP is a computer game designed by Cherek (1981), and is a well-validated measure of aggressive behaviour (Cherek and Lane, 1999a,b; Lieving et al., 2008). The task involves three possible button presses and an opponent (fictitious; participant 3 in this experiment). Presses of button "1" earns points exchangeable for money, presses of button "2" deducts points from the opponent, and presses of button "3" protects points for a short interval from the opponent. Participants were told the main goal of the task was to gain as many points as possible, the more points earned, the more money they would make. They were told they had been assigned randomly to the experimental condition whereby the points that they steal would not be added to their point counter, whereas participant 3 would be able to keep any points stolen. Aggressive behaviour in the PSAP is defined as the number of times a participant steals points (button "2" presses). Because participants do not get to keep the points they steal and because stealing points actually comes at the expense of gaining points, the aggression is considered reactive aggression to the provocation of having had their points stolen.

In the version of the PSAP used here, participants had to press button "1" 50 consecutive times to earn a point, button "2" 10 consecutive times to steal points, and button "3" 10 consecutive times to protect their points by initiating a provocation-free interval. When a provocation-free interval was initiated, the computer program did not provoke participants for a minimum of 45 s after which the random point subtractions would continue to occur every 6–45 s. Once participants selected one of the three response options, they were committed to this option until they completed the fixed ratio of button presses, after which they were free to select any other option. The PSAP consisted of three 10-min sessions with 3 min intervals between sessions. After the first session, participants provided a third 1–2 mL saliva sample. To gauge suspicion as to the reality of the opponent, after the PSAP participants completed a short questionnaire in which they were asked about their impressions of their opponent. Three in the excluded condition and one in the included condition were suspicious. The removal of these participants did not affect statistical analyses, and they are kept in the analyses described here. Participants also rated their enjoyment of the PSAP game on a 5-point Likert scale ranging from −2 very inaccurate to +2 very accurate. Last, participants were given $10 at the conclusion of the experiment irrespective of the points earned on the PSAP and were fully debriefed about the procedures and aims of the experiment.

In keeping with our previous research (Carré et al., 2009, 2010; Carré and McCormick, 2008), the average aggressive responses across the three PSAP sessions was used for statistical analyses. The test–retest reliability of the PSAP is not known, but aggressive responses across the three sessions are highly correlated, and were so for the sample of men here in both groups of men (r ranged from 0.61 to 0.85, all $p < 0.0001$, two-tailed). We also tallied the number of aggressive responses participants made in the first 45 s of the PSAP before the first provocation by the fictional opponent, but only in a subset of the sample ($n = 21$ Excluded men and $n = 25$ Included men) because of a technical problem preventing us from separating the number of responses pre-and post-provocation for the remaining participants. To compare aggressive responses before and after provocation, the average number of aggressive responses made during session 1 of the PSAP after the first provocation was averaged and divided by the number of 45-s intervals (12.33).

2.3. Saliva collection procedure and salivary testosterone and cortisol assays

Saliva samples were collected in polystyrene culture tubes and were stored at −20 °C until assayed using commercial enzyme immunoassay kits (DRG International, Inc.). Saliva samples were measured in duplicate and on the same day. Briefly, frozen samples were first warmed to room temperature and then centrifuged (3000 rpm) for 15 min. Duplicate 100 μL aliquots of saliva were assayed according to the instructions of the kits. Optical densities were determined using a Bio-tek Synergy plate reader at 450 nm. The mean intra-assay and inter-assay coefficients of variation are less than 10%. The stability of testosterone and cortisol concentrations over time has been reported to be $r \geq 0.65$ from samples obtained over a two week period (Liening et al., 2010).

2.4. Statistical analyses

Independent sample *t*-tests were used to test for group differences in responses to the post-Cyberball questionnaires. Mixed factor ANOVAs were used to examine the neuroendocrine measures as a function of experimental group. The pre- versus post-provocation aggressive responses in the PSAP and effect of experimental group was analyzed by Wilcoxon signed ranks tests and Mann–Whitney *U* tests, and, for consistency with other analyses, also by mixed factor ANOVA.

Hierarchical multiple regression analyses were used to investigate the extent to which testosterone, cortisol, and/or their interactions influenced mean aggressive behaviour in the three sessions of the PSAP. We used the regressor variable method in which earlier samples are controlled for in a regression model, such that the residuals at time 2 and time 3 are change scores. To minimize multicollinearity, all predictor variables were standardized prior to analyses and their product terms were computed using these standardized variables. Also, data were screened for univariate or multivariate outliers, influential cases, and violations of the assumptions of independence, homoscedasticity, or normality. The standardized DFBETA diagnostic for an observation is the standardized difference in the parameter estimate due to deleting an observation, and it is used to assess the effect of an individual observation on each estimated parameter of the fitted model. Because individual cases with large DFBETAS have a disproportionate influence on the regression coefficients (especially in small samples) (Cohen et al., 2003), these cases were removed from the analyses. We considered DFBETAS values greater than 0.90 and more than twice that of any other participant to be large. The final sample for statistical analyses was reduced therefore to $n = 63$: Three participants were removed as outliers on at least one measure, two were removed because of missing data from saliva samples, and another six were removed because they were influential cases (large DFBETAS) on either the hierarchical regression with testosterone values as predictors of aggression, cortisol values as predictors of aggression, or the baseline testosterone by cortisol interaction as a predictor of aggression. A significance level of $p = 0.05$, two-tailed was used for all analyses.

3. Results

3.1. Social exclusion manipulation check

Social inclusion and Social exclusion participants differed in the extent to which they agreed with several statements (all $ps < 0.0001$), with socially excluded participants indicating they felt less included, felt less connected with other participants, and were less likely to perceive the ball tosses as fair than were socially included participants. The two groups did not differ in their feeling of anger ($p = 0.11$) or in their desire to play Cyberball again ($p = 0.65$), and the increased enjoyment of Cyberball stated by the socially included compared to the Excluded missed statistical significance ($p = 0.06$). Men in the social inclusion group made more overstatements on the questionnaire for exchange with the fictional participant than did men in the social exclusion group ($p = 0.03$).

3.2. Salivary testosterone and cortisol

A mixed-factor ANOVA on salivary testosterone concentrations found no main effect of time ($p = 0.18$), experimental group ($p = 0.52$), or time × experimental group interaction ($p = 1.00$). A mixed-factor ANOVA on salivary cortisol concentrations found cortisol concentrations to decrease across sampling time points ($F_{2,122} = 42.39$, $p < 0.0001$) (see Table 1). The effect of experimental group ($p = 0.77$), and the interaction of time × experimental group interaction ($p = 0.93$) were not significant. Within hormone correlations of samples at the various time points were all significant (all $r \geq 0.75$, $p < 0.0001$), and there was no association between cortisol and testosterone concentrations for any comparison (all <0.18 and >−0.02).

3.3. PSAP measures

The two groups did not differ in number of aggressive responses, in the number of points earned, or in their enjoyment of the PSAP (see Table 1). For both socially excluded and socially included men, there was a negative correlation between points earned and aggressive responses (together, $r = -0.58$; socially excluded $r = -0.51$; socially included $r = -0.63$; all $p < 0.004$). Enjoyment of the PSAP and aggressive responses were positively correlated (together, $r = 0.24$, $p = 0.07$; socially excluded, $r = 0.42$, $p = 0.02$; socially included, $r = 0.09$, $p = 0.67$) (see Fig. 2).

For the sample of men for which we were able to determine aggressive responses before the first provocation, there were no differences between the groups in aggressive behaviour either before or after the provocation (both $p > 0.50$) (see Table 1). Aggressive behaviour increased after provocation in the socially included men only ($p = 0.009$; $p = 0.59$ for socially excluded men). No main effect or interaction was significant using mixed factor ANOVA (all $p > 0.23$).

3.4. PSAP aggression and testosterone reactivity

We first tested whether the relationship we previously found (Carré et al., 2009, 2010) between change in testosterone, and not baseline testosterone, and aggressive behaviour is evident after social exclusion. A hierarchical multiple linear regression analysis was used to investigate the extent to which changes in testosterone concentrations predicted aggressive behaviour on the PSAP. For this analysis, experimental group (dummy coded as 1 = included, 0 = excluded) and baseline testosterone concentrations (T1) were

Table 1
Descriptive statistics for men in the social inclusion and exclusion conditions.

	Social inclusion			Social exclusion		
	N	Mean	S.D.	*N*	Mean	S.D.
Post-Cyberball questionnaire[a]						
Felt included[***]	33	3.0	2.18	30	8.25	1.81
Made connection[***]	33	6.37	2.34	30	8.78	1.79
Would play again	33	4.87	3.05	30	5.83	3.30
Enjoyed Cyberball	33	5.68	2.65	30	7.31	2.25
Received a fair share[***]	33	2.45	2.25	30	9.14	1.69
Felt angry	33	7.92	2.38	30	7.42	2.70
Overstatements on questionnaire for exchange[*]	33	9.16	7.17	30	5.77	4.29
Testosterone (pg/mL)						
Time 1 (baseline)	33	96.98	37.94	30	91.26	37.85
Time 2 (after Cyberball)	33	99.59	29.64	30	93.77	30.17
Time 3 (during PSAP)	33	102.24	47.79	30	96.53	5.41
Cortisol (ng/mL)						
Time 1 (baseline)	33	7.27	1.97	30	7.37	3.14
Time 2 (after Cyberball)	33	6.52	1.75	30	6.77	2.56
Time 3 (during PSAP)	33	5.69	1.36	30	5.78	1.91
PSAP						
Pre-provocation aggressive responses (session 1, first 45 s)	25	12.04	15.9	21	13.52	16.0
Post-provocation aggressive responses (session 1) in average 45 s	25	20.1	16.4	21	16.3	12.2
Average aggression across sessions	33	216.8	168.5	30	186.7	144.4
Enjoyment of PSAP[b]	33	0.77	1.18	30	0.62	1.08

[a] Lower scores (1–10 scale) indicate greater agreement with statement.
[b] Higher scores (−2 to +2 scale) indicate greater agreement with statement.
[*] Group difference of $p < 0.05$.
[***] Group difference of $p < 0.0001$.

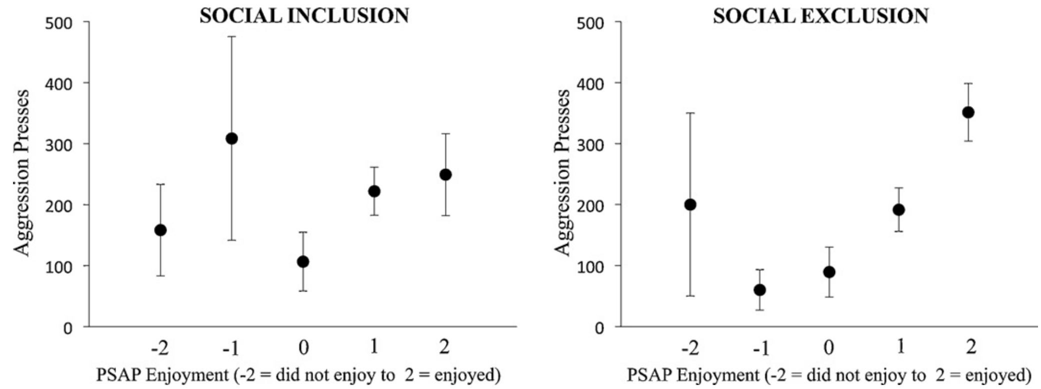

Fig. 2. Mean (±S.E.M.) of aggression button presses on the PSAP for men based on their rating of enjoyment of the PSAP. Enjoyment of the PSAP and aggressive responses were positively correlated for socially excluded men ($r = 0.42$, $p = 0.02$) but not socially included men ($r = 0.09$, $p = 0.67$) men.

entered on Step 1; experimental group × T1 was entered on Step 2; post-Cyberball testosterone concentrations (T2) was entered on Step 3; experimental group × T2 interaction was entered on Step 4; testosterone concentrations 10 min into the PSAP (T3) was entered on Step 5; and experimental group × T3 interaction was entered on Step 6. To increase statistical power, regressions were also conducted for each group separately.

Testosterone concentrations 10 min into the PSAP (T3), controlling for pre and post-Cyberball testosterone (T1 and T2, respectively), were positively correlated with aggression responses ($R^2_{change} = 6.5\%$, $F_{1,\ 56} = 4.39$, $p = 0.04$). Post hoc analyses suggested this association was driven primarily by men in the social inclusion condition. For socially excluded men, T3 was not associated with aggression responses ($R^2_{change} = 1.8\%$, $F_{1,\ 26} = 0.49$, $p = 0.49$), whereas T3 was positively correlated with aggression responses for socially included men ($R^2_{change} = 13.3\%$, $F_{1,\ 29} = 5.28$, $p = 0.03$) (see Fig. 3).

3.5. PSAP aggression and cortisol reactivity

We tested next whether the relationship others have reported between baseline cortisol (reviewed in Poustka et al., 2010; van Goozen et al., 2007) or change in cortisol (e.g., Ford and Collins, 2010; Gerra et al., 2001; Lopez-Duran et al., 2009) and aggressive behaviour is evident after social exclusion. The same analyses as for testosterone were repeated to examine the extent to which changes in cortisol concentrations would be associated with aggression responses. Cortisol concentrations 10 min into the PSAP (C3), controlling for pre and post-Cyberball cortisol (C1 and C2,

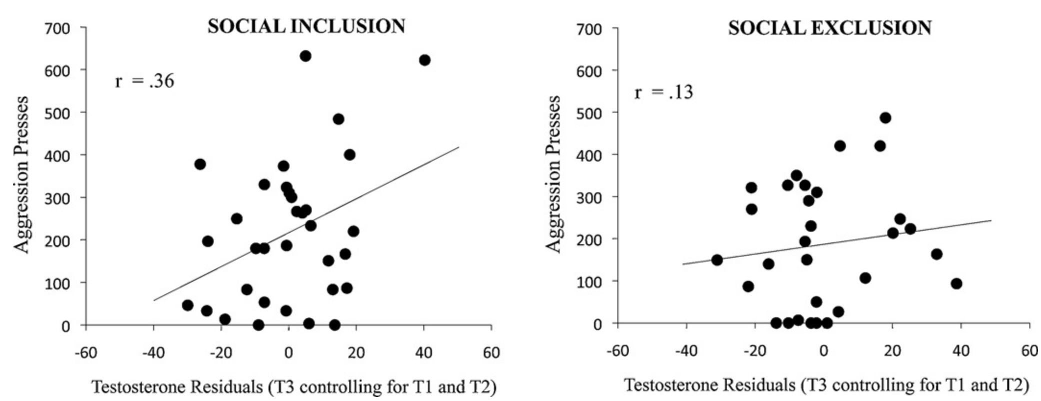

Fig. 3. Testosterone concentrations at T3 (controlling for T1 and T2) were positively correlated with aggression, irrespective of group. When analyses are conducted for the groups separately, the relationship is significant only for men in the social inclusion group.

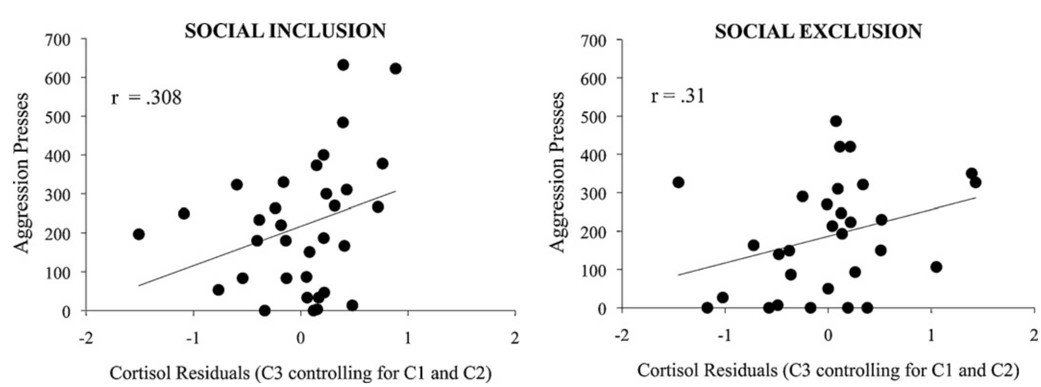

Fig. 4. Cortisol concentrations at C3 (controlling for C1 and C2) were positively correlated with aggression, irrespective of experimental group.

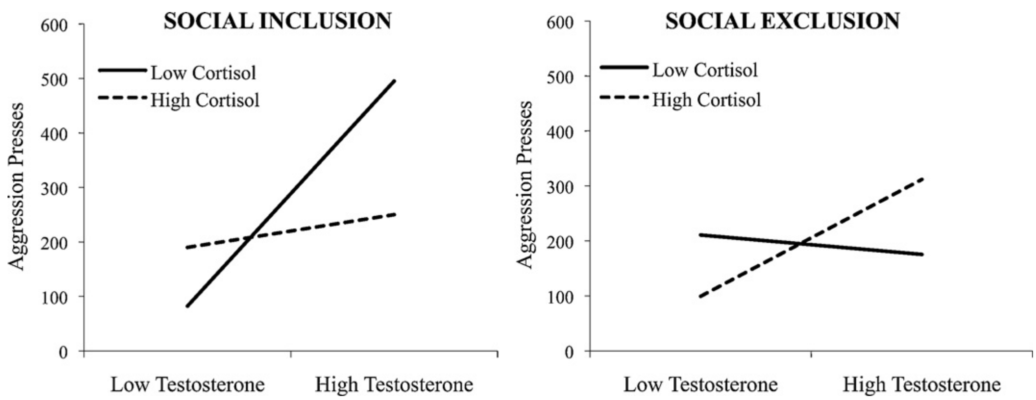

Fig. 5. Baseline testosterone × cortisol × experimental group interaction did not meet statistical significance for the sample ($p = 0.07$). The testosterone × cortisol interaction was not significant for either group separately (socially included: $p = 0.14$; socially excluded: $p = 0.29$). Baseline testosterone concentrations are plotted for men with low baseline cortisol concentrations (<1 S.D. below the mean) and high baseline cortisol concentrations (>1 S.D. above the mean) for socially included and socially excluded men.

respectively) were positively correlated with aggressive behaviour ($R^2_{change} = 9.2\%$, $F_{1,\ 56} = 5.89$, $p = 0.02$). Although the relationship only approached significance in each group when analyzed separately (for Excluded, $p = 0.10$, for Included, $p = 0.09$), the R^2_{change} was of similar magnitude for both groups as when combined (for Excluded, $R^2_{change} = 9.9\%$, for Included, $R^2_{change} = 9.5\%$) (see Fig. 4).

3.6. PSAP aggression and testosterone × cortisol interaction

Last, we tested whether baseline cortisol and testosterone concentrations interact in predicting aggressive behaviour. To test for this interaction, experimental group, baseline testosterone (T1) and cortisol concentrations (C1) were entered on Step 1; experimental group × T1, experimental group × C1, and T1 × C1 interactions were entered on Step 2; and the T1 × C1 × experimental group interaction was entered on Step 3. When baseline testosterone and cortisol concentrations were entered together into a regression analysis, the three-way interaction missed statistical significance ($R^2_{change} = 5.4\%$, $F_{1,\ 55} = 3.53$, $p = 0.07$), and T1, C1, and T1 by C1 interaction were not significant in either group alone (all $p > 0.14$) (see Fig. 5).

4. Discussion

Our main hypothesis that social exclusion would strengthen the relationships between neuroendocrine function and aggressive behaviour was not supported. Men who were socially excluded did not differ from men who were included in aggressive behaviour on the PSAP, and the neuroendocrine predictors of aggressive behaviour were similar for the two groups of men. The primary findings are, first, that a change in testosterone concentrations is positively associated with reactive aggressive behaviour, although the relationship may be attenuated by social exclusion; second, that a change in cortisol concentrations is positively associated with reactive aggressive behaviour irrespective of social inclusion or exclusion; and third, that the interaction of baseline testosterone and cortisol did not predict reactive aggressive behaviour in either group, and thus state-related changes in endocrine function best predicted aggressive responses.

4.1. The social exclusion manipulation

The manipulation of social exclusion was effective, with responses on the questionnaire consistent with reports of the efficacy of Cyberball at inducing feelings of exclusion (reviewed in Williams, 2007). Compared to the responses of participants in the social inclusion condition, after social exclusion, participants felt less of a connection with the other players, felt less included, and thought the number of passes they received was unfair. We did not find increased anger after social exclusion, which is in contrast to other reports using Cyberball (e.g., Hawkley et al., 2010; Zoller et al., 2010), but may reflect that the negative emotional state induced by Cyberball was found to dissipate within minutes (Hawkley et al., 2010). Further, a recent meta-analysis involving a variety of manipulations of social exclusion found the emotional state after rejection is neutral whereas inclusion and control conditions have more positive emotional states (Blackhart et al., 2009). Other evidence suggests a hostile mindset may better represent the emotional state induced by exclusion (DeWall et al., 2009; Romero-Canyas et al., 2010; Twenge et al., 2001). The brevity in the responses provided by excluded compared to included participants on the questionnaire for exchange with the other Cyberball players is consistent with a hostile mindset and with reports that people withdraw from others after exclusion (e.g., Abecassis et al., 2002; Maner et al., 2010; Molden et al., 2009).

4.2. Social exclusion and aggressive responses

We found no difference in aggressive responses between excluded and included men. One possibility for the discrepancy between our findings and the numerous reports of increased aggressive behaviour after social exclusion is that aggression was costly in our experiment and not costly in other studies (Aydin et al., 2010; Ayduk et al., 2008; DeWall et al., 2009; Kirkpatrick et al., 2002; Twenge et al., 2001; Warburton et al., 2006; Wesselmann et al., 2010). Thus, any increased desire for retaliation in excluded individuals may be offset by an increased motivation for the extrinsic reward. Further, both excluded and included groups received provocation during the PSAP, which may minimize the differences in aggressive behaviour.

We previously found that the cost to extrinsic reward that accompanies aggressive behaviour on the PSAP is accompanied by greater intrinsic reward (Carré et al., 2010). The relationship between costly aggression and greater enjoyment of the PSAP approached statistical significance ($p = 0.07$, two-tailed). When each group was examined separately, the relationship between enjoyment of the PSAP and aggression was significant in the socially excluded and not the included ($r = 0.42$, $p = 0.02$ vs $r = 0.09$, $p = 0.62$). The results support our conclusion that costly aggressive behaviour has intrinsic reward value under conditions in which it is retaliatory, possibly as an attempt to regulate another's "unfair" behaviour (Cyberball exclusion or PSAP provocation).

4.3. Social exclusion and endocrine function

Social exclusion had no effect on either cortisol or testosterone concentrations. Many studies have failed to find group differences in endocrine measures after social exclusion or in response to win/loss in competition (Carré et al., 2009; DeSoto et al., 2009; Mehta and Josephs, 2006; Schultheiss et al., 2005; van der Meij et al., 2010; Weik et al., 2010) (and see review by Zoller et al., 2010). Overall, there was a decline in cortisol with time, consistent with the results of another study of Cyberball and cortisol (Zoller et al., 2010), and this decline may involve a reduction in arousal over the duration of a test session (e.g., McCormick et al., 2007) or may reflect the gradual decrease in cortisol that occurs from morning to evening (e.g., Liening et al., 2010). Individual differences in endocrine function, however, were associated with aggressive behaviour after Cyberball.

4.4. Endocrine predictors of aggressive responses in the PSAP

The direction of the relationship between either baseline cortisol or testosterone and aggression in the literature is mixed (reviewed in Archer, 2009; Poustka et al., 2010; Rudolph et al., 2010; van Goozen et al., 2007). Whereas neither baseline testosterone nor baseline cortisol were associated with aggressive behaviour in either group of men, relationships were found with change in testosterone and with change in cortisol (with change defined as specific endocrine concentration during the PSAP while controlling for concentrations before and after Cyberball), depending on the group of men. The timing of the saliva sample, 10 min into the PSAP, suggests that endocrine concentrations at this point reflect events preceding the PSAP because of the time involved between the synthesis of testosterone to its presence in saliva (Riad-Fahmy et al., 1987). Thus the changes in testosterone or cortisol at this point are predictors of aggressive behaviour, rather than the result of the aggressive behaviour.

Consistent with our previous research on testosterone and reactive aggression in the PSAP (Carré et al., 2009, 2010), the change in testosterone was associated positively with aggressive responding in the PSAP. Although the group × change in testosterone interaction was not significant, we examined each group separately based on previous evidence for context-specific associations between testosterone reactivity and competitive/aggressive behaviour. This analysis indicated that the positive correlation between change in testosterone and aggression was significant only in socially included men. Thus, the possibility that social exclusion attenuates the relationship between testosterone and aggression merits further investigation. In a previous study, losers of a competition showed the relationship between a change in testosterone and reactive aggression in the PSAP (Carré et al., 2009). Social exclusion, however, is a more pervasive threat to an individual than is the loss of a competition. In contrast to the results for testosterone, the change in cortisol concentrations was associated positively with aggressive behaviour and similarly in excluded and included men, consistent with some studies of cortisol reactivity (e.g., Gerra et al., 1997; Scarpa et al., 2000) and not others (e.g., Bohnke et al., 2010). If aggressive behaviour in the PSAP is reflective of dominance behaviour, then the present results are consistent with the finding that men with a high need for dominance have a rise in cortisol after a loss (Wirth et al., 2006), but does not explain why the relationship between cortisol and aggressive behaviour was found irrespective of social exclusion, which can be viewed as a threat to dominance. Thus, the rise in cortisol associated with aggressive behaviour may simply reflect heightened arousal.

4.5. The interaction of testosterone and cortisol and aggressive behaviour

We did not have the statistical power to investigate the individual differences in the interaction of testosterone and cortisol reactivity after social exclusion and inclusion. The interaction of baseline testosterone and cortisol suggested a possible three-way interaction ($p = 0.07$), but no relationship emerged as significant in either group alone. Thus, state neuroendocrine function provided better predictors of aggressive behaviour than did trait neuroendocrine function. Van Honk and colleagues have proposed that a combination of low cortisol and high testosterone are hallmarks of a predisposition to aggression (Terburg et al., 2009; van Honk et al., 2010). Inspection of the socially included group in Fig. 5 suggests a larger sample may have provided support for the latter hypothesis, given the trend for a positive relationship between testosterone and aggression only for those with low cortisol. Nevertheless, our results suggest that such a hallmark depends on contextual factors, and echo the suggestion that discrepancies in the literature in studies of endocrine function and aggression have arisen from the lack of consideration of context: "...biological activity may not have a consistent direct effect on aggression but rather may moderate how individuals respond to stressful contexts" (p. 845, Rudolph et al., 2010).

5. Conclusion

In sum, social exclusion did not increase reactive aggression, likely because for all participants, behavioural aggression was occurring in the context of provocation, either as part of the PSAP alone or in concert with social exclusion. The positive association between a rise in testosterone and reactive aggression replicates previous findings. These findings add to a growing body of evidence indicating that individual differences in state and trait neuroendocrine function map onto variability in complex human social behaviour.

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