



## Pre-experience of social exclusion suppresses cortisol response to psychosocial stress in women but not in men

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### ABSTRACT

Lack of social support and social exclusion is associated with adverse effects for mental and physical health. Additionally, women appear to be more vulnerable to social triggers of health disturbances. The hypothalamus–pituitary–adrenocortical-axis (HPA axis) might play a key role in this context as it has been shown both to relate to psychosocial conditions and health outcomes and to respond differentially depending on gender. In a previous experiment we found no effects of exclusion alone (operationalized via Cyberball) on cortisol secretion. Here we examine the effects of a social exclusion pre-experience on psychological and cortisol responses to a public speaking stressor. Subjects (33 m, 34 f) were randomly assigned to social exclusion (SE) or one of two control conditions (exclusion attributed to technical default (TD) and social inclusion (SI)). Afterwards salivary cortisol and psychological responses to a public speaking paradigm were assessed. Exclusion pre-treatment does not affect psychological responses to public speaking stress though with respect to cortisol significant. Cyberball by gender and Cyberball by gender by time interactions are found. SE-women show a blunted cortisol stress response to public speaking while cortisol responses of SE-men fall between SI-men and TD-men. Pre-experience of social exclusion leads to a blunted cortisol response to stress in women but not in men. This factor might contribute to the higher vulnerability to social triggers of health disturbances observed in women.

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### Introduction

Becoming involved in social relationships is a fundamental human motivation. Social support has several protective effects; it ameliorates the psychological impact of stressful situations and is associated with positive health outcomes (House et al., 1988). Lack of support and social exclusion, on the other hand, is often observed for instance in depression (Hawthorne, 2008), anxiety disorders (Torgrud et al., 2004), fibromyalgia (Montoya et al., 2004), chronic fatigue syndrome (Prins et al., 2004), cardiovascular disease (Sorkin et al., 2002), cancers and HIV/AIDS (Reynolds and Kaplan, 1990; Leserman et al., 2000). Gender differences are reported in this context, but findings remain inconclusive. Some studies indicate that the beneficial impact of support on health is more pronounced in men than in women (House et al., 1982), whereas others state the opposite (Denton et al., 2004; Berkman and Syme, 1979). However, women seem to be more vulnerable to social triggers of health disturbances (Denton et al., 2004; Troisi, 2001).

Mechanisms mediating between the degree of social integration and physical and mental health are only partially understood. Particularly with regard to physiological mediators, little is known

to date. Remarkably, however, many of the aforementioned diseases are also characterized by dysregulation of the HPA axis (Leserman et al., 2000; Burke et al., 2005; Furlan et al., 2001; Wingenfeld et al., 2008; Van Den Eede et al., 2007, 2008; Koertge et al., 2002; Ehler et al., 2001; McEwen, 1998). Thus, the question arises whether the degree of social integration interferes with HPA-axis regulation.

The HPA-axis is activated by psychosocial stress resulting in increased release of cortisol from the adrenal glands, with women tending to show a dampened cortisol response due to basal biological regulatory mechanisms (Kajantie and Phillips, 2006; Hellhammer et al., 2009). A few studies indicate that salivary cortisol responses to stress can be reduced by social support (Heinrichs et al., 2003; Ditzen et al., 2007; Ditzen et al., 2008; Kirschbaum et al., 1995). Support by their male partners enhances the cortisol release of women, while men, on the other hand, show a dampened cortisol response when supported by their female mates (Kirschbaum et al., 1995). Data on cardiovascular stress responses suggests that this effect might be attributed rather to the gender of the supporting person than to that of the person being supported. Social support provided by women but not by men reduces cardiovascular stress responses in both women and men (Glynn et al., 1999). Considerable research is still needed with respect to gender differences in the physiological response to social support. Even less is known about the effects of social exclusion or ostracism. In particular, effects of social exclusion on the HPA response to a subsequent stressor have not yet been analysed. Studies

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assessing direct effects of social exclusion on salivary cortisol (Zwolinski, 2008; Blackhart et al., 2007; Stroud et al., 2002) are rare as well. The results of these studies are equivocal, which, at least in part, might be due to the exclusion paradigm that includes a direct social interaction hardly to standardize.

Indeed, experimental research on exclusion faces the problem of how to induce a standardized exclusion condition. “Cyberball,” a virtual ball game, represents one possibility of inducing social exclusion in a highly standardized manner (Williams et al., 2000). In this paradigm, participants are made to believe that they are playing with three other participants (which in fact are computer generated). During the game the degree of social inclusion (i.e., how often they receive the ball from the other participants) is manipulated: “included” participants receive the ball regularly throughout the game while “excluded” participants receive no further ball after the first throws. Previous research on this paradigm indicates that “excluded” participants not only perceive themselves as being excluded but also suffer from lower self-esteem compared to the included participants (Williams et al., 2000). Furthermore, fMRI studies have shown that the dorsal anterior cingulate cortex, a region also activated while experiencing physical pain, is activated during exclusion (Eisenberger et al., 2003).

In a previous study (Zöller et al., 2010) we analysed the immediate effects of Cyberball exclusion on cortisol secretion and psychological parameters in women. While exclusion increased depression and anger no effects on cortisol were found. In the present study we used the Cyberball paradigm to assess the effects of a social exclusion pre-experience on HPA stress responsiveness to a standard laboratory stressor in men and in women.

## Materials and methods

### Participants and ethics

Participants were healthy students between 18 and 35 years, recruited by advertisement on the University campus and received a monetary compensation (15€) for participation. Exclusion criteria were acute or chronic infections, acute allergy, diseases of the adrenal gland, regular use of any medication, gravidity, and actual or past mental illness. Detailed flows of participants and final samples according to the CONSORT criteria are displayed in the Supplemental information. The final sample consists of 33 men and 32 women who were equally distributed across groups. All participants provided informed, written consent. The study was approved by the local Ethics Committee and was found to conform to the guidelines of the World Health Organization (Declaration of Helsinki).

### Experimental conditions

#### Independent variable: experimental variation of social exclusion

Experimental variation of social exclusion (ostracism) was done via the Cyberball paradigm (Williams et al., 2000). The participant is made to believe that he or she is connected to three other players (actually computer generated), one same sex, two opposite sex, whose photographs and names are displayed on the computer screen. Players are asked to throw a ball per mouse-click to each of the others. Every thrower is free to decide who receives the ball next. The ball is thrown 60 times. Three conditions were run: social exclusion (SE: after having received the ball three times, the participant does not receive it any more), technical default (TD; control condition 1: game identical to ostracism, but at the end of the game a pop-up window indicates that network problems precluded other players from addressing the participant), social inclusion (SI; control condition 2: the participant receives an average of every fourth ball).

To obtain the photographs for the three computer generated players, 40 students (20 of each sex) from other universities were photographed and rated by 10 male and 10 female students respectively, according to their attractiveness. For each sex two photographs with medium attractiveness ratings were selected and used for the experimental sessions.

*Randomization and blinding.* Subjects were randomly assigned to the experimental conditions. An equal number of cards containing the respective condition were put in sealed opaque envelopes prior to study onset. Envelopes were shuffled immediately prior to each experiment and a person not involved in data assessment and not in contact to participants drew an envelope and put the respective experimental settings at the Cyberball game. Experimenters in direct contact to the subjects were blinded until the end of an experimental session, when subjects were debriefed. Experimenters for the stress session differed from those during Cyberball and the other sessions. To keep participants blind with respect to hypotheses they were told the purpose of the study would be to examine the effects of mental visualisation on the endocrine system (see also Supplemental information).

### Dependent variables

*Cortisol response.* To assess effects on free cortisol saliva samples were taken every 15 min by means of Salivettes® (Sarstedt, Rommelsdorf, Germany) throughout the experiments and stored at  $-20^{\circ}\text{C}$  until analysis. Salivary cortisol is considered the most valid parameter of HPA activation in psychoendocrinological studies (Hellhammer et al., 2009). After defreezing saliva samples were centrifuged at  $1700\times g$  for 3 min. All samples were visually untainted. A competitive luminescence immunoassay (IBL International®, Hamburg, Germany) was used to assess cortisol concentrations. Alterations in cortisol concentrations throughout the experiment were assessed as primary endocrine outcome variable.

*Psychological response.* Subjective mood during the respective experimental section was assessed via the German short version of the Profile of Mood States (POMS) questionnaire (Sacham, 1983; Biehl and Landauer, 1975; McNair et al., 1971) with 35 items (seven-point-response scale) loading on four scales (depression, anger, fatigue, and vigour). The assessment took place immediately after Cyberball and after public speaking, respectively. Anger and depression immediately after public speaking stress were analysed as primary psychological outcome parameters. Anger and depression after Cyberball were examined to validate the psychological effectiveness of the exclusion paradigm.

### Control variables

Perceived social support (Fydrich et al., 2007) and Locus of control (Levenson, 1972) were assessed by means of standardised questionnaires (internal consistencies between  $\alpha=0.81$  and  $\alpha=0.98$ ) one week prior to the experiments to prove whether groups were comparable with respect to these parameters. Furthermore, baseline cortisol and oral contraceptive intake in women were assessed as further control variables.

### Manipulation checks

To assess the effectiveness of the Cyberball manipulations, standardized interviews followed at the end of each experiment. Participants were asked to describe any feelings and ideas they had regarding the Cyberball game. Socially excluded subjects were furthermore asked to explain why they have been excluded. Most of them stated they could not find any explanation or attributed it to their own character (e.g. “I am not attractive enough”). Only five

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