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Virtual and real-life ostracism and its impact on a subsequent acute stressor

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ABSTRACT

While ostracism constitutes a social stressor with negative effects on physical and mental health, social inclusion seems to increase resilience. This may be true not only for face-to-face settings, but also for computer-mediated interactions. Hence, this study examined the differences between ostracism and social inclusion in real-life or Virtual Reality (VR) regarding self-reported stress, neuroendocrine and cardiovascular reactivity in a subsequent real-life socio-evaluative task. 84 females were randomly assigned to a 3 (agency: face-to-face/human controlled VR-avatar/computer VR-agent) x 2 (inclusion status: inclusion/exclusion) between-subject design using a Cyberball paradigm. Subsequently, they were exposed to a real-life Trier Social Stress Test (TSST). Results indicate that the experience of ostracism constitutes a threat to fundamental social needs independent of agency. Excluded participants showed cardiovascular reactivity during TSST; also face-to-face and avatar excluded individuals had elevated salivary cortisol levels. Included participants reported more perceived social support during Cyberball and showed a blunted cortisol response to the TSST. These results suggest that face-to-face and avatar-related ostracism provokes responses in the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS). Furthermore, they reveal that social inclusion may act as a stress-protector as it alters HPA- and SNS-related stress responsiveness to subsequent stressors.

1. Introduction

Ostracism in the form of social exclusion, rejection or isolation has been shown to constitute a threat to fundamental human needs [1] and a painful experience [2] which exerts both short- and long-term detrimental effects on physical and mental health (e.g., [3]). Social connectedness and social support, in turn, have been considered a potential stress buffering mechanism [4, 5] with protective effects on several health-related variables such as life expectancy (e.g., [6]), fitness (e.g., [7]), and psychological well-being (e.g., [8]). Accordingly, experiences of social connectedness and disconnectedness are hypothesized to affect the two main human stress axes, the hypothalamus-pituitary-adrenal (HPA) axis and the Sympathetic-Adrenal-Medullary (SAM) axis in a contrary manner [9]. Both axes are instigated from the hypothalamus and help the organism adapt to environmental demands such as social stressors by eliciting according cognitions and behaviors (e.g., the fight-or-flight response, tend-and-befriend response, see [10]). SAM reactivity includes immediate changes in the sympathetic nervous system (SNS) such as the release of catecholamines and increased cardiovascular responses. The reactivity of physiological arousal by SNS can be measured using heart rate as a sympathetic and a subset of heart rate variability as a parasympathetic marker. HPA, in turn, reacts slower than SAM, and encompasses the release of the hormone cortisol, what is an inhibiting feedback mechanism for the HPA. Cortisol is an end-product of the HPA axis and plays a crucial role regarding the stress reactivity and restoring homeostasis. Usually it is measured in saliva or blood. The reactivity of both axes to stress is associated with physical and mental health (see [11] for an overview).

Past studies which used the Cyberball Paradigm [12]—a computer

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based ball tossing game designed to induce experiences of social exclusion in laboratory settings—reported significantly altered cortisol levels (e.g., [13–16]) and blood pressure [15] in individuals who were excluded from the Cyberball game. Most notably, a blunted cortisol response to social exclusion was reported by some authors [17,18]. Contrary to this, other evidence suggests no impact of social exclusion on cortisol (e.g., [19–22]). In sum, data on the exact psychophysiological impact of ostracism experiences are still contradictory, and more research is warranted to shed light on the according mechanisms. Also, a heightened focus has been put on cortisol reactivity in the context of ostracism experiences, the SNS response (HR and HRV), in turn, has rather been underrepresented.

Furthermore, most studies have focused on acute stress reactions, and only little research exists on the prolonged impact of social exclusion experiences on subsequent stress coping. Among the small number of studies, Weik et al. [23–25] found that a prior ostracism experience is linked to a suppressed cortisol reactivity in excluded individuals during a public speaking task in front of a TV camera. Typically, socio-evaluative stressors like a public speaking scenario are assumed to provoke increased HPA- and SNS-activity [58]. This is particularly true for a paradigm traditionally used in social stress research, the Trier Social Stress Test (TSST; [26]). It consists of a standardized job interview and an arithmetic task in front of a jury and has been shown to induce significant levels of stress due to the perceived uncontrollability of the situation (Kudielka et al., 2004). Williamson et al. [27], for instance, who used the TSST following the Cyberball exclusion scenario reported no changes in SNS reactivity to the TSST in those individuals who had been included in the game. Unlike most prior research, the authors attributed the decrease in systolic and diastolic blood pressure as well as HR to the inclusion manipulation, and hence concluded that this may be explained by stress-reducing effects of perceived social support [28–30]. Overall, the scarcity of empirical evidence as well as the heterogeneous findings regarding not only acute HPA and SNS responses to social exclusion but also prolonged stress reactivity to other forms of social stress (e.g., [24,25,27]), warrant further research.

Additionally, methodological concerns have been expressed about the artificial nature of the traditional Cyberball paradigm (e.g., [24,31]). This has led to the development of potentially more ecologically valid alternatives in sensorially enriched virtual environments (VE) (see [32–34]). Past work suggests that the experience of being excluded from a virtual reality (VR)-based ball-tossing game [32,35] or socially supported by a virtual character [30] seems to approximate experiences observed in comparable “real” interactions. But despite this work, there is still a lack of research to HPA and SNS reactivity regarding virtual ostracism or inclusion compared to real-life interactions. A particular challenge of this setup lies in the fact that virtual characters have to be distinguished with regards to their agency, i.e. whether they are controlled by another human being (avatar) or whether they represent a computer algorithm (agent) [36]. While both seem to cause the same immediate reaction (i.e., they both pose the same threat to human needs in the context of social exclusion, e.g., [35]), the delayed reaction to avatars and agents differs. While it has been hypothesized that the observed differences may be due to different attribution mechanisms [35, 37], research is still in its infancy and lacks knowledge about the influence of virtual and real-life ostracism and inclusion on subsequent stressors.

Therefore, this research sets out to gain a better integrative understanding about differences between ostracism and social inclusion regarding alterations of HPA and SNS reactivity and investigates prolonged effects on HPA and SNS reactivity and recovery in a subsequent socio-evaluative task (TSST). Moreover, this study uses a novel ecologically valid paradigm (VR) to induce ostracism or social inclusion and investigates the influence of agency during ostracism or social inclusion (comparing: face-to-face, VR-avatar, VR-agent) on a subsequent stress response. Additionally, we controlled for the impact of fundamental social needs, perceived social support and affective cognitions (aggressive behavior, tendency to escape from the situation, emotional numbing) at baseline levels and after each stressor task.

2. Method

2.1. Sample

Participants were recruited through advertisements at the University of Vienna and selected via an online screening questionnaire. Only females between 19 and 30 years were included to control for sex specific differences in stress reactivity. The sample consisted of 84 women (age: $M = 23.06$, $SD=2.10$, range: 20–29 years). All participants were screened using semi-structured interview for childhood trauma or past experiences of mobbing and only included in the study if they scored negative in both measures. None of the participants reported to smoke or have used other drugs for the last 12 months, or medication for the last 2 weeks before they were invited to the study. On the day of the assessment, all participants were abstinent from alcohol, caffeine or sports. Power analysis using G*Power [57] with $\alpha=0.05$ and $1\text{-}\beta=0.80$ indicated that our study was sufficiently powered. For our 2×3 ANOVAs (manipulation checks, see below), we were expecting large effect sizes (Cohen’s f of 0.40) for main effects (c.f. [38]), which requires $N = 64$. For our repeated-measures ANOVAs, we were expecting medium effect sizes (interactions of $f = 0.25$, correlation among measurements $r = 0.50$), which requires $N = 54$. Furthermore, the sample size of 84 is within the recommended range of level 2 grouping variables to establish accurate estimates in multilevel-modeling [39].

2.2. Procedure

The current study was conducted according to the Declaration of Helsinki, and thus a comprehensive informed consent was obtained from all participants prior to their voluntary participation. All participants were allowed to withdraw from the experiment at any time. All participants volunteered in exchange for course credit. The experimental procedure took place between 13:00–16:00 in temperature-controlled (23 °C) rooms.

All participants were randomly assigned to a 2 (inclusion status: included/excluded) x 3 (agency: face-to-face/avatar/agent) between-subject design for a Cyberball paradigm (see below for different modi of Cyberball) prior to a TSST (Trier Social Stress Test, see [26] for a detailed description). As depicted in Fig. 1, upon arrival to the lab, participants were guided to a waiting room where they were asked to wait alongside two other participants (confederates of the experimenter). All persons in the waiting room wore headphones to prevent unwanted communication between the confederates and the participants. After a waiting period of 10 min, the experimenter entered the room and provided the participant with an instruction about the experimental procedure of the Cyberball and the subsequent TSST depending on the three agency conditions: (1) Face-to-face: participants were told that they had to play a face-to-face ball-tossing game with the other two persons they had met in the waiting room; (2) Avatar: participants were told that they had to play with the other two persons over a computer-mediated interface while they were all in different rooms; (3) Agent: participants were told that they had to play with two computer generated characters in the laboratory, while the other two persons in the waiting room were obviously guided to “another experiment”. Subsequently, participants were guided back to the waiting room and were told that the confederates had to wait in other rooms until all participants went through a job interview task. After 20 min, participants were instructed for the TSST and the 5 min anticipation phase (preparation of the job interview) began. Participants provided saliva samples 20 min after arrival at the lab, 15 min after the Cyberball paradigm, after preparation to the TSST, immediately after the TSST, and 15 as well as 30 min after the TSST. After the TSST participants filled out the remaining questionnaires and waited about 40 min in the waiting room for the final saliva sample. Finally, participants were fully debriefed about the aim of the study.

2.3. Cyberball-manipulation: face-to-face (ftf) and virtual reality (VR)

The original Cyberball paradigm [12] consists of a computer-based ball-tossing game in which three schematic players are arranged in a triangle. One player is the participant who may toss the ball to one of his co-players by clicking on the computer-mouse. An algorithm pre-defines the number of tosses until the participant is automatically excluded from the mutual game and does not receive any more tosses. For the remainder of the time, the two co-players keep tossing the ball to each other, but the participant has no possibility to interact with them or fetch the ball anymore. There is an ongoing debate about the lack of mundane realism of the traditional Cyberball paradigm as it bears only minimal resemblance to face-to-face (ftf)-interactions [31,32,35]. For the current study, we used a VR adaption of this paradigm in both the avatar (group 2) and agent (group 3) conditions. In our study both avatars and agents behave identically according to a specific algorithm throughout the VR experiment. The VR-Cyberball scenario was developed using an open source engine (Ogre3D) for the real-time rendering of the scenes. Textures and graphical surfaces were created with GIMP and C++ was used for source code (see Fig. 2). In the current experiment, participants were donned a Head-Mounted Display (Sony HMZ-T1, Sony, Japan) with an external head tracking device (Track IR5, Natural Point, US) and used a hand-held game controller for tossing the ball.

In addition to the virtual Cyberball conditions, we introduced a ftf version of the ball tossing game. In this adaption, the two co-players (trained confederates) were physically present in the same room without VR mediation. We followed the same procedures as described for the VR adaption of the Cyberball. In all conditions, participants regardless of their assignment to a condition, played one minute with the co-players, receiving about 33.3% of all ball-tosses. After that, excluded participants did not get any ball-toss for the remaining 5 min of the Cyberball game, whereas in the inclusion condition, participants continuously received every third toss throughout the whole game (50:50 from the two co-players). The overall Cyberball game lasted 6 min, and during this time participants were always standing up in an upright position.

2.4. Trier social stress test (TSST)

The TSST [26] is well a known, widely used and validated laboratory paradigm to induce acute stress. The original protocol comprises a 5 min job interview within which participants have to make a case for why they are the best candidates for the job, and a 5 min math task in which 13 has to be mentally subtracted from 1022. Both tasks have to be completed in front of a committee in white lab coats (confederates), and surrounded by environmental elements that provoke the feeling of social evaluation (microphone, video camera). In our study, we instructed all participants to prepare for a job-interview during a waiting period following the Cyberball in a separate room (not including the other two co-players). The TSST was conducted with a male and female confederate who were well trained according to the protocol and blinded regarding the allocation of participants to the groups.

2.5. Measures

One single item on a 5-point Likert-scale was used to estimate rejection sensitivity ("Do you think others tend to exclude you?"). To assess whether the experimental manipulation of inclusion or exclusion (VR-Cyberball) and of the socio-evaluative stressor was successful, a manipulation check was conducted using 3 items for the VR-Cyberball scenario (Confidence that the interaction was with a computer algorithm: "How did you perceive the interaction in the VR-Ball game?" ranging on a 9-point scale from 1=controlled by a human being to 9=controlled by a computer algorithm, ball tosses: "How many passes did you receive?" in percent, and inclusion status: "Did you feel included/excluded?" on a 5-point Likert-scale). Additionally, the Basic Needs Scale [40] was used to measure perceived ostracism and its impact on fundamental human needs on a 9-point Likert scale (1–do not agree to 9–agree completely) directly after the Cyberball game, example item: "I felt somewhat frustrated during the Cyberball game (α=0.89). Four items on a 4-point Likert-scale were presented as a manipulation check for the TSST ("How evaluated did you feel?" and "How much did you behave like in a real job interview?" ranging from 1=not at all to 4 = very, "How did you perceive this situation?", ranging from "realistic–artificial", and "The interviewer was… very friendly–not friendly at all").

2.6. Self-reported affective cognitions

Participants' affective cognitions regarding their response to the stressor (i.e., aggression, flight response, freezing/emotional numbness or perceived emotional support) were assessed at 3 time points (baseline, post VR-Cyberball and post-TSST) with 4 items on a 5-point Likert scale (1-strongly agree to 5-strongly disagree"): (1) "I am angry", (2) "I want to escape from this situation", (3) "I feel numb" and (4) "I feel supported by others".

2.7. Electrophysiological measures

We used a wireless chest heart rate transmitter and a wrist monitor recorder (Polar RS800TM, Polar Electro, Finland) for the measurement of heart rate (HR) and heart rate variability (HRV). HR served as a marker of stress-related sympathetic activity and was continuously recorded for subsequent 60 s intervals from the baseline measure 5 min before Cyberball until 40 min after TSST. Data were computed using KUBIOS HRV software kit (Biosignal Analysis and Medical Imaging Group, Finland). High HR values indicate more beats per minute (bpm) and high physiological arousal, whereas low HR values reflect less bpm and lower physiological arousal. Additionally, the root mean square of successive difference (rMSSD) for heart rate served as a time-domain measure reflecting a short time parasympathetic measure of HRV. RMSSD was obtained in accordance with the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [41] and constituted a marker for changes in the parasympathetic tone during the experiment. Log-transformed rMSSD values were calculated from the beat-to-beat intervals for four 5 min-periods according to the experimental periods. High log-transformed rMSSD values indicate low physiological arousal, while low values are interpreted as higher physiological arousal. Participants were standing in an upright position during baseline recording and Cyberball. Waiting periods, TSST-baseline, TSST and the rest of the study were conducted in a sitting position.

2.8. Salivary cortisol measurement

Commercial cotton swabs (Salivette®, Sarstedt, Wiener Neudorf, Austria) without any saliva-stimulating additives were used for determination of salivary cortisol concentrations. Participants were thoroughly instructed how to collect their saliva on their own. Thus, participants put the swab into their cheek pouch at designated time points and let the swab saturate with saliva for approximately 60 to 80 s. Afterwards the swabs were replaced into the device container and immediately frozen at −20 °C. For the subsequent analysis, samples were thawed on ice and centrifuged at room temperature at 3000 x g for 15 min to obtain clear saliva. Saliva aliquots (10: l of a 1:10 dilution) were used for the analysis of cortisol. Samples were assayed in duplicates and cortisol concentrations were assessed in a double-antibody biotin-linked enzyme immunoassay (sensitivity of 0.2 ng/ml; [42]).

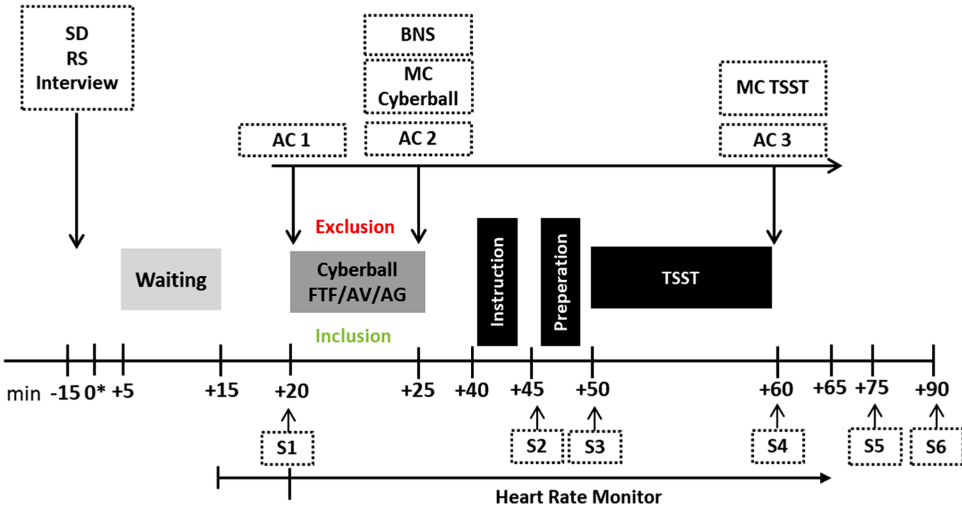

Fig. 1. Overview over experimental procedures and measures.
Note: AC = Affective Cognitions T1-T3, BNS = Basic Needs Scale, Cyberball FTF = face-to-face condition, Cyberball AV = Avatar condition, Cyberball AG = Agent condition, Interview = Semi-structured Interview, MC = Manipulation Check, RS = Rejection Sensitivity, SD = Sociodemographic Variables, *S* = Salivary Cortisol (T1-T6), TSST=Trier Social Stress Test, *arrival for testing were around 15 min. before entering the lab rooms (entering the lab = 0 min).

Duplicate samples with a coefficient of variation >10% were replicated and considered in the analysis only when a coefficient of variation <10% was achieved. If sample volumes fell below the limit needed to run duplicates or sample volumes were generally too low before a coefficient of variation <10% was achieved, the sample was dismissed. Average intra- and inter-assay coefficients of variation were less than 10% and 13%, respectively.

2.9. Data analyses

All statistical analyses were conducted using R version 3.6.1 [43]. 2 × 3 ANOVAs were conducted for the manipulation check, with inclusion status (included/excluded) and agency (ftf/avatar/agent) as between-subject variables and the manipulation check variables as well as the Basic Needs Scale as the outcome variable. Repeated measures ANOVAs with agency and inclusion status as between-subject variable were applied for the outcome variable affective cognitions (4 single-items, 3 times). Bonferroni-corrected Simple effects analyses were applied where interactions were significant.

$$Cortisol\ (\log ng/ml)_{ij} = \gamma_{00} + \gamma_{10}(Reactivity\ Cyberball_{ij}) + \gamma_{01}(Inclusion\ Status_j) + \gamma_{02}(Agency_j) + \gamma_{11}(Reactivity\ Cyberball_{ij}) * (Inclusion\ Status_j) + \gamma_{12}(Reactivity\ Cyberball_{ij}) * (Agency_j) + \gamma_{13}(Reactivity\ Cyberball_{ij}) * (Agency_j) * (Inclusion\ Status_j) + \upsilon_{0j} + e_{ij}$$

We analyzed the salivary cortisol response with multilevel modelling (MLM) to assess differences in cortisol reactivity and cortisol response. MLM was fitted using the lm4 package (Bates et al., 2015) with p-values supplied by the lmerTest package [44]. To examine within- and between-person change in cortisol responses simultaneously, we specified within-person change in level 1 to depict cortisol responses to the TSST and a between-person sub-model in level 2, describing how cortisol responses varied between the groups depending on inclusion status (coded as 0 = Inclusion, 1 = Exclusion) and agency (coded as 0=Face to Face, 1=Avatar, 2=Agent). We examined changes from baseline to 20 min after the Cyberball-paradigm (Reactivity Cyberball), the preparation phase of the TSST to 20 min after the TSST (Reactivity TSST), and the change from 20 min to 40 min after the TSST (Recovery TSST) to determine cortisol levels in response to the Cyberball and the TSST. These level 1 variables were coded as 0 the start of their corresponding trajectories and as 1 (at the peak of reactivity Cyberball); as 1 and 2 (reactivity TSST); and 1 (recovery) at the predefined measurement points. For HR and HRV, MLMs were defined with a time as a level 1 variable and the same between-person sub-models in level 2 as for the cortisol analyses (see above). Separate models were again defined for the reactivity to the Cyberball paradigm and the reactivity to as well as recovery from the TSST.

2.10. Salivary cortisol model for Cyberball reactivity

2.11. Salivary cortisol model for tsst reactivity and recovery

$$Cortisol\ (\log ng/ml)_{ij} = \gamma_{00} + \gamma_{10}(Reactivity\ TSST_{ij}) + \gamma_{20}(Recovery\ TSST_{ij}) + \gamma_{01}(Inclusion\ Status_j) + \gamma_{02}(Agency_j) + \gamma_{11}(Reactivity\ TSST_{ij}) * (Inclusion\ Status_j) + \gamma_{21}(Recovery\ TSST_{ij}) * (Inclusion\ Status_j) + \gamma_{12}(Reactivity\ TSST_{ij}) * (Agency_j) + \gamma_{22}(Recovery\ TSST_{ij}) * (Agency_j) + \gamma_{13}(Reactivity\ TSST_{ij}) * (Agency_j) * (Inclusion\ Status_j) + \gamma_{24}(Recovery\ TSST_{ij}) * (Agency_j) * (Inclusion\ Status_j) + \upsilon_{0j} + e_{ij}$$

2.12. Heart rate model for Cyberball reactivity

$$Heart\ Rate\ (bpm)_{ij} = \gamma_{00} + \gamma_{10}(Time_{ij}) + \gamma_{01}(Inclusion\ Status_j) + \gamma_{02}(Agency_j) + \gamma_{11}(Time_{ij}) * (Inclusion\ Status_j) + \gamma_{12}(Time_{ij}) * (Agency_j) + \gamma_{13}(Time_{ij}) * (Agency_j) * (Inclusion\ Status_j) + \upsilon_{0j} + e_{ij}$$

2.13. Heart rate model for TSST reactivity and recovery

$$Heart\ Rate\ (bpm)_{ij} = \gamma_{00} + \gamma_{10}(Time_{ij}) + \gamma_{01}(Inclusion\ Status_j) + \gamma_{02}(Agency_j) + \gamma_{11}(Time_{ij}) * (Inclusion\ Status_j) + \gamma_{12}(Time_{ij}) * (Agency_j) + \gamma_{13}(Time_{ij}) * (Agency_j) * (Inclusion\ Status_j) + \upsilon_{0j} + e_{ij}$$

2.14. Heart rate variability model for Cyberball reactivity

$$Heart\ Rate\ Variability\ (RMSSD)_{ij} = \gamma_{00} + \gamma_{10}(Time_{ij}) + \gamma_{01}(Inclusion\ Status_j) + \gamma_{02}(Agency_j) + \gamma_{11}(Time_{ij}) * (Inclusion\ Status_j) + \gamma_{12}(Time_{ij}) * (Agency_j) + \gamma_{13}(Time_{ij}) * (Agency_j) * (Inclusion\ Status_j) + \upsilon_{0j} + e_{ij}$$

2.15. Heart rate variability model for TSST reactivity and recovery

$$Heart\ Rate\ Variability\ (RMSSD)_{ij} = \gamma_{00} + \gamma_{10}(Time_{ij}) + \gamma_{01}(Inclusion\ Status_j) + \gamma_{02}(Agency_j) + \gamma_{11}(Time_{ij}) * (Inclusion\ Status_j) + \gamma_{12}(Time_{ij}) * (Agency_j) + \gamma_{13}(Time_{ij}) * (Agency_j) * (Inclusion\ Status_j) + \upsilon_{0j} + e_{ij}$$

3. Results

3.1. Manipulation check

See Table 1 for descriptive data and results of all analyses pertaining to the manipulation check variables as well as basic needs scale. At baseline, there was no difference regarding self-reported rejection sensitivity between the conditions. For the confidence that the interaction was with a computer algorithm, a significant interaction was found. Simple effects analyses indicated that participants in both the ftf and the avatar conditions reported significantly lower scores in this item than the agent condition—in both the inclusion and exclusion conditions (all *ps*$<$0.001, expect for ftf vs. avatar in the exclusion condition: $p = 0.021$). Included participants reported they received more ball tosses (measured in percentages) and higher scores in the Basic Needs Scale than excluded participants, irrespective of the agency condition.

Moreover, the manipulation check post TSST showed neither differences between the groups regarding the participants' perception of social evaluation nor regarding the participants' attribution whether they would have behaved or responded differently in a real job interview (no significant main effects or interactions between conditions in both outcomes, see Table 1). Yet, participants in the agent condition rated the interviewers as significantly more friendly ($M = 2.89$, SD=0.994) compared to the other agency conditions (ftf: $M = 1.96$, SD=0.881, avatar: $M = 1.79$, SD=0.833). Participants previously interacting with an agent reported that the subsequent TSST situation felt significantly more artificial to them compared to participants in the other two agency conditions, but only if they were excluded (simple effects analysis: all *ps*$<$0.001).

3.2. Effects of stressor on affective cognitions

For aggression, our repeated measures ANOVA revealed a significant time x inclusion status interaction (F(1.819, 141.820)=3.682, $p = 0.031$, *par.*η^2=0.045), which indicated that included participants reported lower levels of aggression than excluded participants after the Cyberball ($M = 1.02$, SD=0.154 vs. $M = 1.26$, SD=0.587, $p = 0.015$) and after the TSST ($M = 1.07$, SD=0.342 vs. $M = 1.41$, SD=0.735, $p = 0.010$). There was, however, no difference at baseline ($M = 1.07$, SD=0.342 vs. $M = 1.05$, SD=0.309, $p = 0.740$) and no three-way interaction of time x inclusion status x agency was found ($p = 0.690$). Similar results were found for the participants' need to escape from the situation. A significant time x inclusion status interaction (F(2, 156)= 5.276, $p = 0.006$,

Fig. 2. The Virtual Reality (VR) Cyberball paradigm.

par.η^2=0.063) showed that participants in the exclusion condition—when compared to the inclusion condition—reported a higher need to escape after the TSST (M = 3.31, SD= 1.352 vs. M = 2.71, SD=1.154, p = 0.029), but not at baseline (M = 2.07, SD= 1.022 vs. M = 2.14, SD=1.139, p = 0.758) or after Cyberball (M = 2.14, SD= 1.181 vs. M = 2.29, SD=1.216, p = 0.582). In terms of emotional numbness, there were no significant interactions (time x inclusion status: p = 0.355, time x agency: p = 0.850, time x inclusion status x agency: p = 0.252). For perceived social support, a significant three-way interaction (F(4, 156)= 3.302, p = 0.013, *par.*η^2=0.078) revealed that in the ftf condition, inclusion leads to more perceived social support than exclusion after Cyberball (M = 4.43, SD=0.646 vs. M = 1.857, SD=0.770, p<0.001) and after the TSST (M = 3.07, SD=0.997 vs. M = 1.64, SD=0.929, p = 0.001), but not at baseline (M = 2.64, SD=0.745 vs. M = 2.50, SD=1.225, p = 0.725). For the avatar condition, inclusion also leads to more reports of perceived social support than exclusion, but this effect was found only after Cyberball (M = 4.36, SD=0.6333 vs. M = 2.00, SD=0.877, p<0.001) and did not prevail to after the TSST (M = 2.79, SD=1.051 vs. M = 2.21, SD=1.122, p = 0.185)—baseline scores were: M = 2.57, SD=1.223 vs. M = 2.64, SD=1.008 (p = 0.861). Similar results were found for the agent condition, with more perceived social support when participants were included (vs. excluded) after Cyberball (M = 2.86, SD=1.232 vs. M = 2.00, SD=1.038, p = 0.013), but not after the TSST (M = 2.64, SD=1.447 vs. M = 2.14, SD=1.167, p = 0.246) and at baseline (M = 2.71, SD=1.069 vs. M = 2.50, SD=1.092, p = 0.599).

3.3. Salivary cortisol

Salivary cortisol trajectories over the course of the experiment are presented in Fig. 3, and the model summaries of the fixed effects are depicted in Table 2. No significant main effects or interactions were found regarding cortisol reactivity to the Cyberball paradigm (all ps>0.05). Excluded participants showed a higher overall mean cortisol concentration, but this slope did not reach significance (b = 0.20, SE=0.01, p = 0.055). Neither agency nor the interactions agency x inclusion status were associated with changes in cortisol trajectories (all main effects and interactions p>0.05).

A significant interaction of inclusion status x TSST reactivity (p = 0.005) with an estimate of b = 0.18 (SE=0.06) indicated that, in response to the TSST, excluded participants had higher cortisol concentrations (i.e., positive reactivity slope) than included participants. Furthermore, we found a significant interaction of agency x TSST recovery (p = 0.039). Simple effects analysis revealed that after the TSST, only participants in the ftf (b = 0.16, SE=0.05, p = 0.005) and avatar (b = 0.11, SE=0.05, p = 0.046) conditions—not in the agent condition (b=−0.004, SE=0.06, p = 0.946)—showed a cortisol recovery. We also found an inclusion status x agency x TSST reactivity interaction (p = 0.047). Simple effects indicated that a positive reactivity slope was only prevalent in individuals who were excluded ftf (b = 0.25, SE=008, p = 0.002) or by an avatar (b = 0.30, SE=0.008, p<0.001), but not for those who were excluded by an agent (b=−0.008, SE=0.08, p = 0.917). No TSST reactivity for cortisol was found in any of the inclusion conditions (all ps>0.320).

3.4. Heart rate

HR trajectories over time are depicted in Fig. 4. We found a significant effect of inclusion status (F = 9.299, p = 0.003), time (F = 85.015, p<0.001), and a significant interaction of inclusion status x time (F = 3.553, p = 0.031) on HR reactivity over the course of the Cyberball paradigm. Simple effects revealed that excluded participants (as compared to included participants) had a higher HR during the Cyberball (b = 5.95, SE=2.48, p = 0.027), but not before or after (ps=0.155 and 0.123, subsequently). No other effects or interactions were significant (all ps>0.071). See Table 3 for estimates of fixed effects.

Concerning the TSST, a significant effect of time (F = 89.449, p<0.001) and interaction of inclusion status x time (F = 3.990, p<0.001) was found, indicating that the TSST induced a stress reaction in all conditions and that the response was higher in excluded participants. Furthermore, a significant three-way interaction of inclusion status x agency x time was found (F = 1.777, p = 0.042). In the ftf condition, excluded participants (compared to included participants) had higher HR in the preparation phase (b = 9.24, p = 0.029) and during the TSST (bs=13.55 and 13.62, ps=0.002 and 0.001). Similarly, participants excluded by an avatar as compared to avatar-inclusion condition had a higher HR during the TSST (bs=13.31 and 16.42; ps=0.002 and <0.001) but not in the preparation phase (b = 6.87, p = 0.104). There was no change in HR if participants were included or excluded by an agent in the preparation phase (b=−0.25, p = 0.952) or during the TSST (bs= 2.87 and 2.76; ps =0.495 and 0.512). No difference between conditions was found in the recovery time points after the TSST (ps>0.092).

3.5. Heart rate variability

HRV trajectories over time are depicted in Fig. 5. In response to the Cyberball, there was a significant effect of time but no other significant effects or interactions (ps>0.071). All conditions showed a lower HRV during Cyberball (b=−979, p<0.001). See Table 3 for estimates of fixed effects.

In response to the TSST, there was a significant effect of inclusion status (F = 6.482, p = 0.013), time (F = 37.165, p<0.001), and inclusion status x time (F = 2.995, p = 0.007), and inclusion status x agency x time

(F = 2.294, p = 0.006). This indicated that the TSST led to a reduced HRV in previously excluded individuals. Furthermore, excluded participants (compared to included participants) had a lower HRV in the ftf condition in the TSST preparation phase (b=−12.76, p = 0.034), and during the TSST (bs=−13.27 and −12.52, ps=0.027 and 0.037). In the ftf condition, excluded participants (compared to included participants) had a lower HRV during the TSST during the second phase of the TSST (arithmetic task; b=−13.96, p = 0.029) but not during the preparation phase (b=−4.04, p = 0.498) or the first TSST phase (speech task, b=−10.64, p = 0.076). There was no change in HRV if participants had previously been included or excluded by an avatar in the preparation to the TSST (b=−0.25, p = 0.952) or during the TSST (bs=2.87 and 2.76; ps=0.495 and 0.512). There was no change in HRV if participants were included or excluded by an agent in the preparation to the TSST (b=−1.61, p = 0.788) or during the TSST (bs=1.63 and −1.52; ps=0.784 and 0.798). No difference between conditions was found in the recovery time points after the TSST (ps>0.120).

4. Discussion

This study investigated—for the first time—HPA and SNS responses to a novel ecologically valid paradigm (VR) inducing ostracism or social inclusion via VR or ftf and evaluated whether this experience had prolonged effects on stress reactivity to a subsequent social-evaluative stressor. In sum, this research showed that real-life and virtual ostracism via avatars and agents significantly affected fundamental social needs (belonging, control, meaningful existence, self-esteem). Additionally, HPA and SNS reactivity to real-life and virtual ostracism was comparable, indicating that excluded participants showed an increased SNS reactivity, but no significant increase in HPA reactivity during Cyberball. In contrast to excluded participants who showed elevated HPA and SNS reactivity, included participant revealed a blunted HPA response in the subsequent TSST. Similarly, HPA reactivity was blunted during TSST if participants interacted with an agent prior to the acute stressor.

4.1. Differences between ostracized and included participants

On the one hand, our findings contradict prior research by Weik et al. [24,25] who found excluded individuals to show suppressed cortisol reactivity during a subsequent stressor, on the other hand, our result is partly in line with Williamson et al., [27] who reported SNS increases in excluded but not included individual during a subsequent stressor. However, it is difficult to fully interpret the current findings on the basis of past research as the existing studies differ from our setup in several aspects.

Both research groups [24,25,27] used the traditional Cyberball paradigm which consists of 2D stick figure players and which has been criticized for its lack of mundane realism and its limited ecological validity (e.g., [31]). Research on VR-based adaptations of the Cyberball game (see [33]) suggests that there may be differences in effectiveness between the low immersion original and the fully immersive extension. Furthermore, Weik et al. [24,25] used a public speaking task instead of the TSST. Arguably, the TSST is expected to induce a larger cortisol secretion than a speech conducted merely in front of a camera, because TSST specifically manipulates the aspect uncontrollability via an expressionless jury of two to three confederates [58]. In contrast to Weik et al. [24,25], and in line with our setup, Williamson et al. [27], introduced the TSST, yet only assessed SNS related stress reactivity. Also, the fact, that this research group tested a mixed gender sample, makes direct comparisons with our female sample difficult given that males and females differ in their psychophysiological stress response [45,46].

In sum, at this point it is impossible to draw firm conclusions and to generalize the current findings to different settings, as research on the long-term effects of ostracism experiences on subsequent stress reactivity may still be regarded in its infancy. Further studies are needed

Table 1
Descriptive statistics and results of the 2 × 3 ANOVAs regarding the manipulation check variables.

	Inclusion Status												2 × 3 ANOVAs					
	Included						Excluded						Main Effect Inclusion Status		Main Effect Agency		Interaction	
	Face-to-face		Avatar		Agent		Face-to-face		Avatar		Agent							
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F*	*p*	*F*	*p*	*F*	*p*
Manipulation Check Cyberball																		
Rejection Sensitivity	1.21	0.426	1.50	0.855	1.64	0.842	1.71	0.825	1.43	0.514	1.36	0.633	0.096	.757	0.024	.976	2.335	.104
Confidence that Interaction was with Computer Algorithm	1.29	0.825	7.14	2.507	6.86	2.179	3.79	3.355	6.14	2.143	7.64	1.737	2.392	.126	**36.130**	**<0.001**	**4.206**	**.018**
Ball tosses (reported. in%)	49.86	22.260	55.71	10.894	55.00	13.693	27.86	8.484	32.86	14.507	39.08	15.381	**38.145**	**<0.001**	2.141	.125	0.432	.651
Basic Needs Scale	6.88	1.015	6.48	1.004	5.87	1.063	4.34	1.194	4.66	0.988	4.20	1.041	**75.753**	**<0.001**	2.553	.084	1.329	.271
Manipulation Check TSST																		
Social Evaluation	3.50	0.650	3.36	0.633	3.57	0.514	3.29	0.994	3.14	0.770	3.36	0.633	1.884	.174	0.651	.524	<0.001	.999
Responded Like in Real Interview	1.86	0.864	2.07	0.730	1.77	0.927	1.93	0.730	1.79	0.699	2.29	1.069	0.294	.589	0.186	.831	1.549	.219
Perceived Friendliness of Interviewers	2.14	0.949	2.00	0.961	2.93	0.917	1.79	0.802	1.57	0.646	2.86	1.100	2.085	.153	**12.030**	**<0.001**	0.304	.739
Perceived Artificiality of Situation	2.43	1.22	1.93	1.072	2.29	.995	3.93	.267	3.57	.646	1.86	.864	**21.187**	**<0.001**	**10.755**	**<0.001**	**11.547**	**<0.001**

Note. Significant main effects/interactions (p <0.05) are denoted in bold.

Table 2
Model summary of fixed effects of the salivary cortisol model for reactivity to the Cyberball paradigm as well as reactivity and recover to the TSST.

	NumDF	*DenDF*	*F*	*p*
Cyberball				
Inclusion Status	1	134.15	0.942	.334
Agency	2	134.18	0.256	.774
Reactivity Cyberball	1	92.38	0.006	.938
Inclusion Status x Reactivity Cyberball	1	126.30	0.469	.495
Agency x Reactivity Cyberball	2	104.38	2.491	.088
Inclusion Status x Agency x Reactivity Cyberball	2	135.41	2.618	.077
TSST				
Inclusion Status	1	83.13	0.245	.622
Agency	2	83.13	0.182	.834
Reactivity TSST	1	122.60	7.861	**.006**
Recovery TSST	1	156.78	0.040	.842
Inclusion Status x Agency	2	83.13	0.708	.450
Inclusion Status x Reactivity TSST	1	122.60	8.364	**.005**
Inclusion Status x Recovery TSST	1	156.78	2.091	.150
Agency x Reactivity TSST	2	122.60	2.299	.105
Agency x Recovery TSST	2	156.77	3.324	**.039**
Inclusion Status x Agency x Reactivity TSST	2	122.60	3.127	**.047**
Inclusion Status x Agency x Recovery TSST	2	156.77	0.496	.610

Note: Statistically significant results with $p<0.05$ are denoted in bold.

which make use of fully immersive, sensorially enriched and ecologically valid virtual environments to approximate real-life social exclusion experiences. Additionally, both stress axes, HPA and SNS, should be considered, as there is evidence which suggests a different mode of action depending on the type of stressor [9].

Further, our results are partly in line with Williamson et al. [27], who report a decreased SNS activity in response to a subsequent acute stressor after an inclusion paradigm. As noted by former studies [9,47] this SNS pattern reflect a neural networking associated with distress including the dorsal anterior cingulate cortex (dACC), which is an area directly associated with processes involved in detecting acute threat. Thus, our finding may be interpreted as a stress-protective mechanism [27]. As indicated, our results show the same patterns between HPA axis and SNS reactivity. Consistent with this, past research has revealed that social support leads to reduced dACC activation which, in turn, is associated with a lower HPA and SNS reactivity [28]. Following this, it can be assumed that the social connection to others seems to produce a short-time resilience against acute socio-evaluative stressors, which in our case led participants to perceive the TSST not as an acute threat. This is supported by the fact that our included participants reported higher levels of perceived social support than excluded participants. Similar conclusions may be drawn from the study of Häusser et al. [29] showing that social identity, in terms of experiencing oneself as being part of a group, buffers neuroendocrine stress reactions when exposed to an acute stressor.

4.2. Comparability of virtual and real-life stressors

Following our results, there was no effect of agency regarding acute stress reactivity during Cyberball of the HPA axis and the SNS. Surprisingly, there was only a minor increase in salivary cortisol and HR during the subsequent TSST stressor task for participants who had been included or excluded by an agent, but this was significantly lower than in excluded participants in the ftf or avatar conditions. Hence, in our study, only participants, who had been included by another human via an avatar or ftf, reported increased levels of perceived social support, not those who were included by a computer agent. This is in line with a meta-analysis [48] on the effects of agency on social influence which found avatars to be more influential than agents, particularly in competitive and collaborative—rather than neutral—tasks. Also, the current results parallel more recent research which—similar to the present study—used virtual reality to test the buffering hypothesis of social stress [30] and succeeded in showing that SNS reactivity to a stressor can be suppressed if the preceding social support is provided by a person ftf or via an avatar, but not if the support is attributed to a computer algorithm.

Nevertheless, in our study, participants in the agent condition did not respond to the subsequent TSST as intended. This might be associated with the fact that both included and excluded participants in the agent

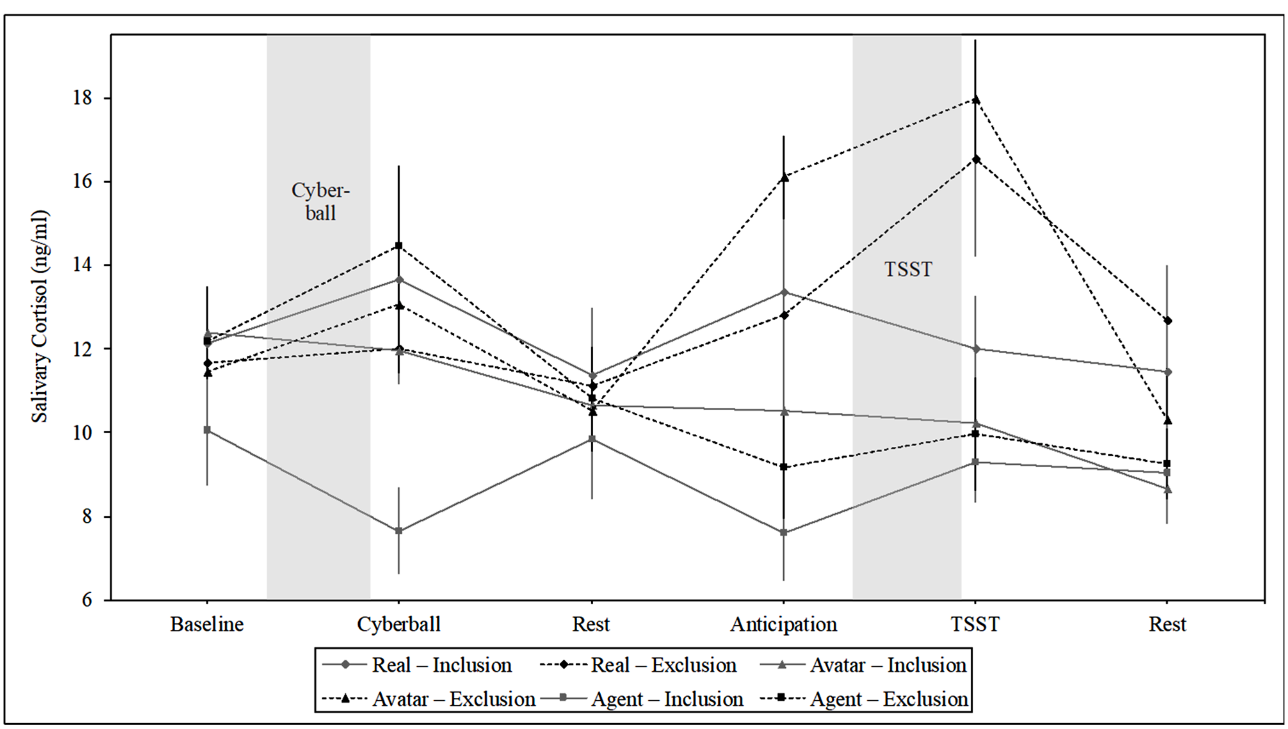

Fig. 3. Salivary cortisol ($M \pm$ SEM) trajectories of the two inclusion status conditions at predefined time points.
Note: TSST = Trier Social Stress Test.

Table 3
Model summary of fixed effects of heart rate and heart rate variability models for the reactivity to the Cyberball paragidm as well as the TSST.

	Heart Rate				Heart Rate Variability			
	Num Df	*Den DF*	*F*	*p*	*Num Df*	*Den DF*	*F*	*p*
Cyberball								
Inclusion Status	1	78.00	3.356	.071	1	78.00	1.929	.169
Agency	2	78.00	1.972	.146	2	78.00	1.484	.233
Time	2	156.00	85.015	**<0.001**	2	156.00	70.126	**<0.001**
Inclusion Status x Time	2	156.00	3.553	**.031**	2	156.00	0.221	.802
Agency x Time	4	156.00	1.781	.135	4	156.00	2.203	.071
Inclusion Status x Agency x Time	6	116.75	1.118	.356	6	116.75	0.467	.832
TSST								
Inclusion Status	1	78.00	9.299	**.003**	1	78.00	6.482	**.013**
Agency	2	78.00	2.122	.127	2	78.00	0.296	.745
Time	6	468.00	89.449	**<0.001**	6	468.00	37.165	**<0.001**
Inclusion Status x Time	6	468.00	3.990	**<0.001**	6	468.00	2.995	**.007**
Agency x Time	12	468.00	0.738	.714	12	468.00	1.570	.097
Inclusion Status x Agency x Time	14	270.86	1.777	**.042**	14	270.86	2.294	**.006**

Note: Statistically significant results with $p<0.05$ are denoted in bold.

condition perceived the TSST interviewers as more artificial and friendlier. Following this assumption, a study by Wiemers et al. [49] showed that participants who were exposed to a TSST and perceived the interviewers as friendly did not respond with HPA axis activation. Additionally, participants in our agent condition reported that they perceived the TSST more as an artificial situation than participants of the other groups, independent from inclusion status. As hypothesized by prior research (e.g., [35,37]), this may be due to interfering cognitive processes (i.e., attributions) which take place following the social interaction. Hence, it is possible that participants may have attributed the interaction with the computer agent to a computer algorithm and may have, thus, devaluated the whole experience.

4.3. Implications for future research

Research on factors unfolding a potentially protective effect in the face of ostracism experiences both online and offline (e.g., ftf bullying vs. cyberbullying, [50]) is warranted. In this context, the manipulation of agency in such experiments is notably important in order to be able to draw conclusions about virtual social interactions and their intricate effects on human behavior. In our study, however, the agent condition may not have been entirely successful and it remains unclear whether the mere knowledge that one had interacted with a computer may have corrupted the socio-evaluative task or whether other factors (e.g., the uncanny valley effect, see [51]) may be held responsible. The participants' attribution post Cyberball about the agency might affect the following stress responses and behavior, which is a crucial factor for experimental research. As hypothesized by the Temporal Need Threat Model [59], the reactions to the ostracism experience seem to follow distinct phases, first including an acute stress response which is followed by a reflection of the experience and activation of according coping mechanisms. Correspondingly, the consideration of additional psychophysiological markers such as oxytocin which is known to dampen cortisol release under stress [18,25] may contribute to a better understanding of the complex mechanisms shaping stress reactivity.

4.4. Strengths and limitations

A definite strength of the current study is that we used a multimodal assessment approach including HPA and SNS reactivity and controlled for some attributional and affective influences. Moreover, as indicated by Weik et al. [24], we chose HR and HRV as a more sensitive measure of SNS compared to catecholamines in plasma. Apart from simultaneously considering both stress systems, transferring the Cyberball game into VR has several advantages. By doing so, the present research, on the one hand, met the often voiced critique about the traditional paradigm's lack

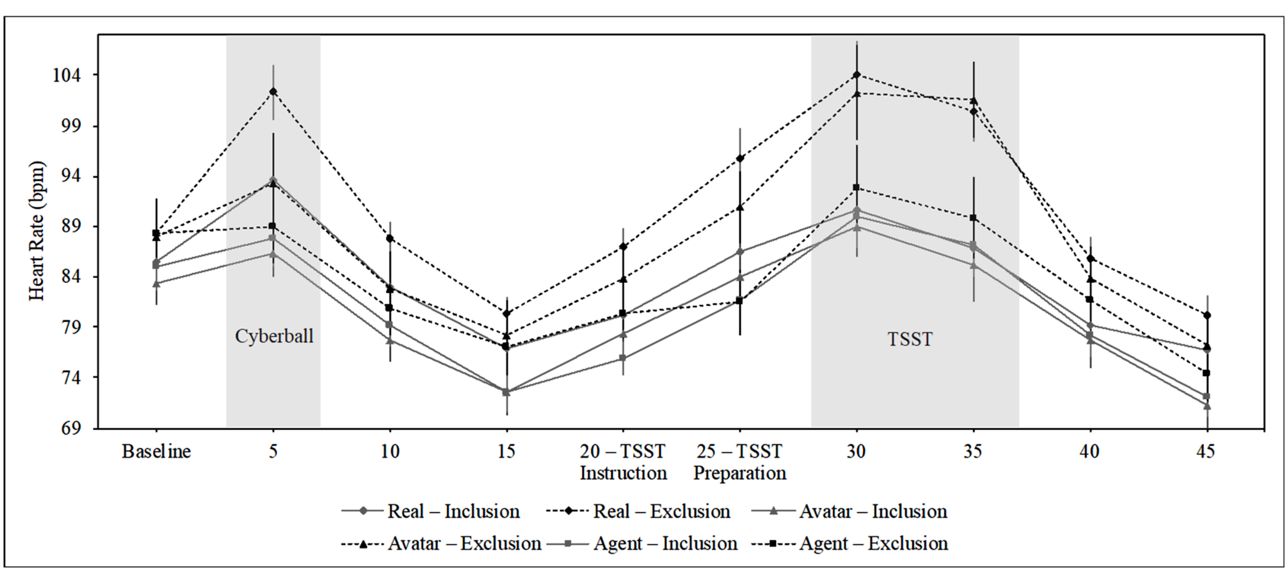

Fig. 4. Heart rate (beats per minute, bpm, $M \pm$ SEM) of the two inclusion status conditions at predefined time points.
Note: TSST = Trier Social Stress Test.

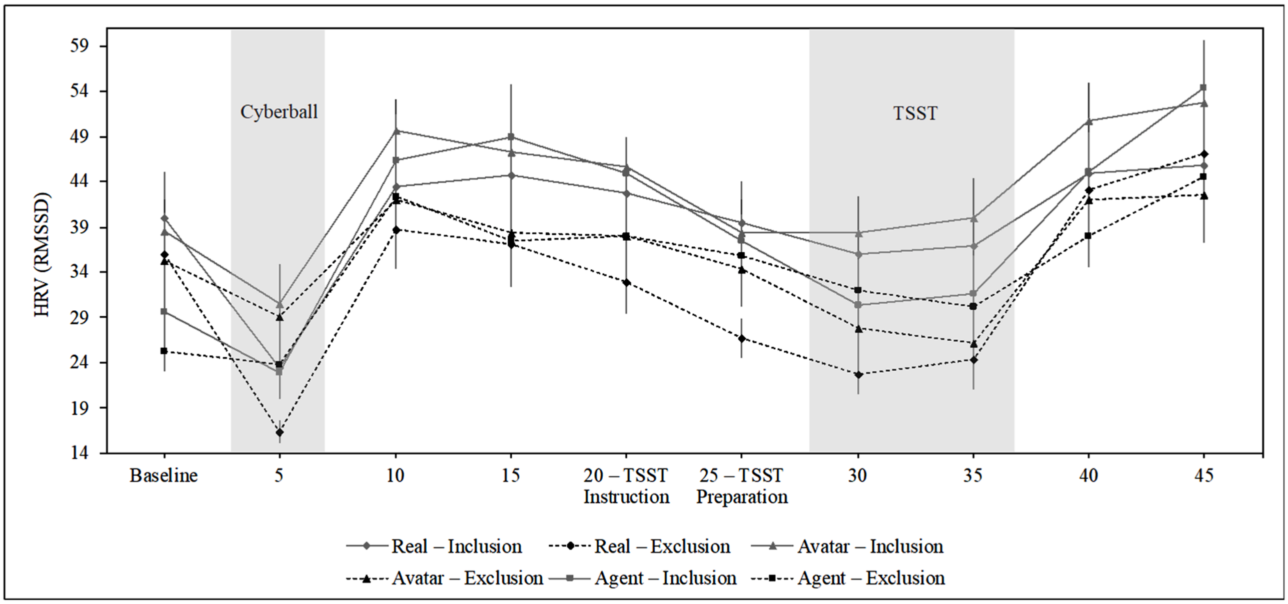

Fig. 5. Heart rate variability, HRV (RMSSD; $M \pm$ SEM) of the two inclusion status conditions at predefined time points.
Note: TSST = Trier Social Stress Test.

of mundane realism (see: [31,32,35]). On the other hand, it payed tribute to the fact that, nowadays, many social interactions—and according experiences of social exclusion or social support—take place in virtual spaces (e.g., on social networking). A possible limitation, however, lies in the fact that—according to current technological standards—the used equipment (HMZ-T1) may be criticized for not providing experiences comparable to those of recent developments (e.g., Oculus rift, HTC vive). However, past research has repeatedly suggested that the sense of presence—a precondition for ecologically valid reactions to the virtual environment—is not so much contingent on image fidelity, but rather depends on the possibility of action and interaction with the virtual content (see [52]). Future studies may put more emphasis on accounting for the sense of presence and the quality of presented images as a control variable.

The lack of neutral control conditions for both the TSST and the Cyberball paradigm may be considered another limitation. Introducing a naïve Cyberball condition (no support and ostracism) or a TSST control group (no social stressor) may additionally strengthen the experiment. However, the TSST has repeatedly been shown to be a reliable tool for eliciting social stress responses in laboratory settings [53], and, hence, comparing stress responses during and post TSST to a baseline may be regarded a valid method of control. With regards to the Cyberball Paradigm, the inclusion paradigm typically represents the control condition, and all detected effects are attributed to the exclusion manipulation. However, some authors (e.g., [27,54]) have suggested that the inclusion condition may not only be regarded a control but may be seen as "an active manipulation of belonging and connectedness" ([27], p. 417). Hence, these authors interpreted the found effects to the inclusion manipulation. This was the starting point for the current study, and could be used to guide future research on exploring the value of the inclusion condition itself. However, our results are limited because no neutral control was used and we were not able to determine the direction of the effects whether inclusion blunted the stress response or exclusion would have magnified the response.

Finally, one may argue that placing the Cyberball paradigm in a park environment may have exerted a confounding influence on stress reactivity as past virtual nature scenarios have been found to have relaxing effects (e.g., [55,56]). Also, alongside physiological measurements, we only used brief self-report questionnaires. However, we screened for possible factors that possibly make participants more vulnerable to ostracism (e.g., mobbing experiences, adverse events). More differentiated assessments as well as ancillary behavioral measures (e.g., eye tracking) would enrich future research design.

5. Conclusion

In sum, our results are consistent with the bigger picture of the psychobiological underpinnings of social exclusion and inclusion phenomena. Moreover, virtual social interactions turned out to be effective and comparable to real-life ftf interactions, encouraging— on the one hand—future experimental research to make use of VR as an ecologically valid tool, and emphasizing— on the other hand—the fact that virtual experiences have 'real' effects. The latter is even more important in the context of cyberbullying, where virtually inflicted pain may have detrimental consequences on overall health and wellbeing. On a positive note, however, virtually provided support may be, in turn, successfully applicable in therapeutic contexts.

Author contributions

ODK designed the study, ODK, JXK and AF were involved in the recruitment of participants and acquisition of data, LB developed the virtual reality system, LMG and RP performed analysis of saliva cortisol and provided lab resources, ODK and AG analyzed the data, ODK, AG and AF wrote the first draft of the manuscript, LMG, JXK, HH, IKE and RP contributed extensively to the first draft, AG prepared the figures and tables, IKE and HH provided lab resources for assessments and supervised the study. All authors have approved the final manuscript.

Declaration of Competing Interest

none

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