## **ORIGINAL RESEARCH**



# Contextual exclusion processing: an fMRI study of rejection in a performance-related context

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**Abstract** Social stress has a major detrimental impact on subjective well-being. Previous research mainly focused on two methods to induce and measure social stress: social exclusion and performance evaluation. For social exclusion researchers frequently focused on the Cyberball task, which in contrast to many psychosocial stress paradigms does not include a performance component. The aim of the current study was to establish an optimized psychosocial stress paradigm by combining both, social exclusion as well as performance evaluation within a single fMRI paradigm. We implemented a modification of the Cyberball task including a performance game (with exclusion and inclusion periods) in addition to the already established exclusion and inclusion periods. This indeed resulted in increased subjective stress in the performance game. Hence, the modified Cyberball version seems to be superior in mapping relevant neural social stress correlates more pronounced and reliably. Exclusion within the

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performance-related context contrasted to the unmodified exclusion was associated with higher activation in the dorsal anterior cingulate cortex and the anterior insula. Moreover, the modified exclusion reflected greater social processing in the precuneus, several temporo-parietal and medial prefrontal areas, as suggested by the additional task aspects of social evaluation and social perspective taking. The findings emphasize that public negative evaluation is effective in substantially enlarging and potentiating the distressing effect of exclusion on a subjective as well as on a neural level. This may have a great potential for further experimental research on social stress.

**Keywords** Cyberball · Social evaluative stress · Salience network · Mentalizing network · Functional magnetic resonance imaging (fMRI)

#### Introduction

Successful social life means to be included in society, in a team, in a working group or in any other social group. Being excluded by others threatens all benefits that result from teamwork and from social interactions in general and might lead to a reduced self-esteem (Zadro et al. 2004). This might be especially pronounced in all situations that put strong emphasis on team performance, because in these circumstances exclusion might be an indication of inadequate performance. Previous studies investigating social exclusion have shown a number of negative effects for the individual (Williams and Nida 2011). For example, exclusion seems to impair self-regulation (Baumeister et al. 2005), increase the feeling of distress and negative affect (Chow et al. 2008; Zadro et al. 2004), and might even change our perspective on ourselves and what we expect others to think about us (Bastian and Haslam



2010). All things considered, social exclusion threatens our fundamental needs such as the need to belong, the need for control and the need for a meaningful existence (Smith and Williams 2004; Zadro et al. 2004).

Physiological responses to rejection or exclusion confirm their power as a stressor for the individual. Increased cortisol levels have been observed in several laboratory settings in which participants were excluded of a real life conversation (Blackhart et al. 2007; Stroud et al. 2002). In experimental investigations, however the simulated exclusion from an online ball-tossing game (Cyberball) has not consistently been associated with increased cortisol levels (Bass et al. 2014; Seidel et al. 2013; Zöller et al. 2010) - possibly due to the mild nature of exclusion as social stressor and inter-individual variability in appraisal of this experimental stressor. In the Cyberball task, the participant is told that he or she will play a virtual ball-tossing game with two or more participants. Unknown to the participant, the game is rigged and consists of computer-regulated inclusion and exclusion periods. During the latter, the participant does not receive the ball anymore. This exclusion period is the key component for the induction of social stress as rejection elicits social threat (Williams 2007a). Independent of the inconsistent hormonal stress response, the Cyberball task still has been characterized by high effect sizes with regard to negative affect (Blackhart et al. 2009) and elicits well-studied neural responses (Cacioppo et al. 2013; Rotge et al. 2014).

The core component in the Cyberball task is the feeling of being rejected by a social group, but there is some evidence for additional factors influencing individual stress reactions to social rejection in this paradigm (Williams 2007b). On the one hand, individual differences regarding the need to belong or self-esteem seem to be important for emotional, hormonal and neural responses (Beekman et al. 2015; Ford and Collins 2010; Onoda et al. 2009). On the other hand, there are external factors which seem to influence the perception of exclusion in the Cyberball task. For example, several studies manipulated information about the team members to imply different reasons for exclusion. They found that permanently discriminating reasons, like gender or ethnicity increase distress or prolong negative emotional states (Goodwin et al. 2010; Masten et al. 2011b; Wirth and Williams 2009). Some contexts that enhance a positive self-identification after exclusion might have a buffering function as well (Wirth and Williams 2009). In summary, these studies indicate that emotions and even neural responses to exclusion are modulated by an individual's appraisal of the situation (Masten et al. 2011b).

The current study aimed to modify the experience and appraisal of exclusion by increasing the stress level within the Cyberball game. As the aforementioned findings show, the exclusion period in the Cyberball task itself is distressing. However, the task lacks one component which is essential to many other psychosocial stress tasks like the Montreal

Imaging Stress Task (Dedovic et al. 2005) and the Trier Social Stress Test (Kirschbaum et al. 1993): a public performance monitoring in combination with (fake) negative feedback. Combining both the public performance evaluation and the subsequent exclusion in one task might be a more powerful psychosocial stressor compared to the mere exclusion in previous Cyberball studies. We therefore modified the Cyberball task such that in addition to the established inclusion and exclusion conditions (here free game, FG), a performance related condition (performance game, PG) was incorporated. In this condition individuals are not only excluded from a ball tossing game but they are also pressured by time constraints and public negative feedback about their performance. Following the assumption that the public evaluation of performance contributes to one's stress response, we expect an increased stress level during the PG, even while subjects are included. Moreover, we expect that this additional stress component reinforces the negative experience of exclusion. When provided with negative feedback on performance, the attribution of exclusion will most likely be performance-related and the reason for exclusion might be more obvious than in the free game. Thus, performance feedback in the Cyberball task would prompt a new appraisal of the exclusion situation increasing self-evaluative cognition, a core component for social stress (Gruenewald et al. 2007). Being socially excluded after negative performance feedback would encompass a negative outcome, which attributed to the self would result in what has been conceptualized as mental pain or social stress (Tossani 2013).

In addition to the study of behavioral stress effects of Cyberball exclusion, the task has been applied frequently to investigate neural correlates of rejection. Eisenberger et al. (2003) were the first to investigate the neural response to exclusion in the Cyberball task; and they proclaimed the dorsal anterior cingulate cortex (dACC) as a target region for the processing of exclusion related distress. The dACC is also referred to as anterior middle cingulate cortex (aMCC), as defined by Vogt (2005) based on cytoarchitectonic information and structural and functional connectivity. To prevent confusion, we will refer to this region as dACC/aMCC throughout this manuscript. Some subsequent studies suggested that the aMCC/dACC in combination with the anterior insula is a target region for the processing of social pain as these regions overlap with what has been described as the physical pain network (DeWall et al. 2012; Kross et al. 2011). Later models for exclusion processing refer to the aMCC/ dACC as a neural alarm system (Eisenberger and Lieberman 2004; Kawamoto et al. 2015). Yet, besides the anterior insula and aMCC/dACC, other regions have repeatedly been related to exclusion, such as the inferior orbitofrontal cortex (OFC) (Cacioppo et al. 2013) or other regions involved in emotion regulation like the ventrolateral prefrontal cortex (vIPFC) (Goldin et al. 2008; Sebastian et al.



2011), or in mentalizing like the temporo-parietal junction (TPJ), medial prefrontal cortex (mPFC) and precuneus (DeWall et al. 2012; Schurz et al. 2014). Common regions underlying social stressors such as rejection and public evaluative stress have been suggested in a recent meta-analysis (Kogler et al. 2015) emphasizing the role of the anterior insula and inferior frontal gyrus.

Our hypotheses are based on the assumption that the additional stress component in PG, implemented by the public performance evaluation, adds to the feeling of distress. We therefore predict increased subjective stress and reduced positive affective in the PG inclusion and exclusion conditions, and in exclusion compared to inclusion conditions (1). Moreover, we hypothesize reduced positive affect for exclusion situations compared to inclusion periods in both game contexts (2). In PG, we predict exclusion to result in less positive affect and increased stress than in FG (3). On the neural level, we hypothesize the contrast of exclusion versus inclusion to demonstrate increased activity in exclusion related regions encompassing structures in the vIPFC (inferior frontal gyrus, IFG), dorsomedial prefrontal cortex (dmPFC), ventromedial prefrontal cortex (vmPFC) including OFC and ventral anterior cingulate cortex (vACC), and insula (4), regardless of context (PG, FG). In addition, we predict that the increase of social evaluative threat - parallel to the expected behavioral effects (stress and positive affect) -intensifies the neural response to exclusion, thereby eliciting increased activity in the anterior insula and aMCC/dACC for exclusion in PG compared to FG (5).

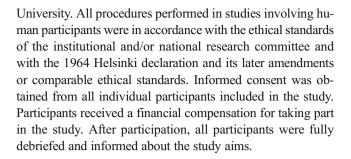
## Materials and methods

# **Participants**

Twenty men and 20 women (mean age: 27.80 years; SD: 7.86) took part in the experiment. All participants had normal or corrected vision, no MRI contraindications and no history of traumatic brain injury, psychiatric or neurological illness. According to the Edinburgh Handedness Inventory (Oldfield 1971), all subjects were fully right handed. Participants completed tests of crystallized verbal intelligence (Lehrl et al. 1995), executive functions (Reitan 1956) and working memory (Von Aster et al. 2006) (see Online Resource 1). Participants were recruited through public postings in university buildings at RWTH Aachen University. None of the participants had ever taken part in a Cyberball task or any other experiment on social exclusion.

#### Compliance with ethical standards

The authors declare that there are no competing interests. Experimental procedures were approved by the Ethics Committee of the Medical Faculty of the RWTH Aachen



#### **Procedure**

Upon arrival, participants were told that they would be taking part in a group study designed to test their mental visualisation abilities while playing a ball-tossing game on the computer with two unknown teammates. Two virtual players located on the right and left side of the screen represented the teammates. The virtual players were named "Dieter" and "Nora," with their respective names written above virtual representations of the figures. Dieter always started the game by throwing the ball to the participant, who was represented by a hand in the lower center of the screen. To throw the ball, participants could press one of two buttons of a keyboard (LumiTouch) with the first or second finger of the right hand, directing the ball to either the left or right teammate, respectively. Before the experimental session began, participants were also informed that there would be two conditions, the "Free Game" (FG) and the "Performance Game" (PG). The virtual game was implemented using Presentation® software (Version 16, www.neurobs.com) and presented by MRI compatible video glasses (VisuaStimDigital; Resonance Technology, resolution: 800x600).

The fMRI task consisted of 20 blocks (10 FG blocks and 10 PG blocks) with each block lasting 50s. Additionally, both FG and PG were conducted in an inclusion and an exclusion condition (5 blocks each). We pseudo-randomized the order of the context in which the game started, such that half of the participants started with 10 FG blocks and the other half started with 10 PG blocks. Independent of the order, the experiment always began with an inclusion block in order to increase the credibility of the paradigm. The following blocks of inclusion and exclusion within a certain context were completely randomized.

Regardless of exclusion or inclusion in FG or PG, within each block there was a short inclusion phase lasting 14.7 s. In the following 35.3 s, participants were either included or excluded. The participant received the ball in 60 % of all cases in inclusion phases. While some studies excluded the participant completely during exclusion phases, in our study, the subject received the ball in 3 % of all throws similar to other studies (Gradin et al. 2012; Kawamoto et al. 2012).

In FG, participants were told to just visualize and throw the ball to the player they preferred. The context was changed in PG by instructing subjects that fast reactions to the virtual ball would be essential for good group performance- the group as a



whole would earn more points for faster reaction times. It was further explained that the faster the participants threw the ball back into play, the more points they would contribute to the overall group performance. To indicate reaction times, participants were told that whenever one of the players reacted too slowly, he or she would be warned by a red frame that would pop up and encircle his or her virtual figure (Fig. 1). During the PG inclusion phase and also in the preliminary phase before the PG exclusion phase, the red frame was presented in 20 % of the throws of the teammates, whereas the participant was presented with a red frame in 50 % of all throws. During actual exclusion periods, the virtual excluders still got negative feedback in 20 % of all throws.

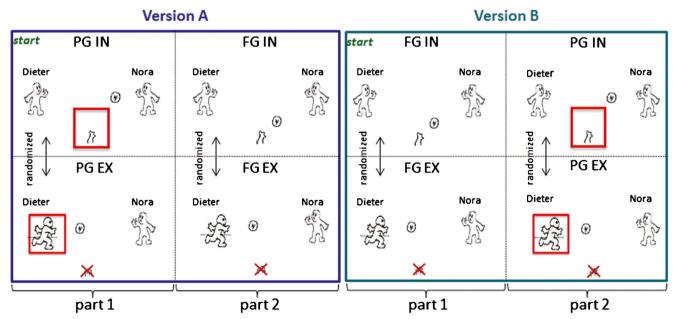
After each block, participants were asked to answer two questions. The first assessed their subjective stress level (*Do you feel stressed*?). The second question assessed positive affect (*Do you feel well*?). Subjects answered these questions with their first or second finger on the fibre optic response pad (LumiTouch). Pressing the keys moved a bar on a 9-point Likert-like scale from 1 (*not at all*) to 9 (*extremely*). Participants were presented a visual feedback in response to their choice. After five seconds, the next question appeared. The two questions were followed by a 16 s baseline phase in which a fixation cross was presented. (Fig. 2).

# Behavioral data analysis

As eleven participants expressed doubts about the cover story when asked directly, stress and positive affect scores of believers versus non-believers were compared by independent sample t-tests. For the analysis of stress and positive affect, scores were generated by averaging the mean rating after each block separated into condition per context. Self-perceived stress was analysed by a 2x2 repeated measures ANCOVA with context (PG versus FG) and condition (IN versus EX) as within-subject factors. Order of context (start PG versus start FG) was included as covariate of no interest. The same analysis was conducted for positive affect. As one subject was identified as an outlier because of a wrong interpretation of the scale for positive affect in the first block, he was excluded from this analysis.

#### fMRI data acquisition and analyses

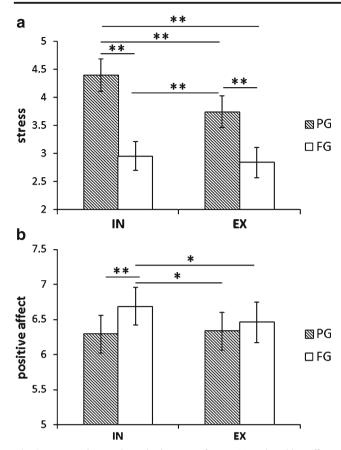
Imaging data were acquired using a Siemens 3 T Trio scanner (Siemens AG; Erlangen, Germany) equipped with a 12-channel head matrix coil located in the Department of Psychiatry, Psychotherapy and Psychosomatics, RWTH Aachen University. Foam pads were used to properly stabilize the head. Each fMRI session consisted of one functional run and one anatomical run, which was always performed at the end of the session. A time series of 750 functional images per participant was acquired, using a spin-echo EPI sequence with the following acquisition parameters: TR = 2000 ms; TE = 28 ms, flip angle =77°, FOV = 192 \* 192 mm, matrix size =64 x 64 mm, 34 slices, voxel size =3x3x3.75 mm³, interleaved, slice gap 0.8 mm. Functional scans lasted 25 min, including a pre-baseline (28 s). Structural scans were acquired using a T1-weighted MPRAGE sequence with the



**Fig. 1** The figure presents the order of the modified Cyberball: In version A, Cyberball started with an inclusion block (PG IN) in the performance-related context, followed by randomized blocks of inclusion and exclusion (PG EX); part 2 was applied in a neutral context starting with

inclusion (FG IN), followed by inclusion and exclusion (FG EX); in version B (reversed), Cyberball started with the neutral context in part 1, followed by the performance-related context in part 2



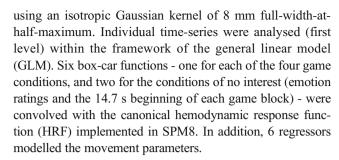


**Fig. 2** Mean ratings and standards errors of stress (a) and positive affect (b) are indicated. PG = performance game; FG = free game; EX = exclusion, IN = inclusion. \*significant at p < .05. \*\*significant after Bonferroni correction

following acquisition parameters: TR = 2300, TE = 3.03 ms, flip angle =9°, FOV = 256 \* 256 mm, matrix size =64 x 64; 176 slices, voxel size = $1x1x1mm^3$ .

Imaging data were analysed using SPM8 software (http:// www.fil.ion.ucl.ac.uk/spm/). The first six volumes of each fMRI run were discarded so that the scanner could reach a stable magnetized state, preventing artefacts from transient signal changes at the beginning of the functional run. The images of the time-series were realigned with a two-pass procedure, with the first image (first pass) and the mean image (second pass) used as references. None of the subjects exceeded the predefined movement limits of 3 mm, or 3°. Data were high-pass filtered at 156 s to remove lowfrequency drifts. Each anatomical scan was co-registered according to its mean EPI scan and subsequently used to determine spatial normalization parameters by means of the unified segmentation approach. These normalization parameters were applied to the functional scans, thus transforming the timeseries into the standard space defined by the Montreal Neurological Institute (MNI).

During normalization, all images were resampled to a voxel size of 2x2x2 mm<sup>3</sup>. Afterwards, images were smoothed



# Group level, whole brain analysis

For the whole brain analysis a general linear model (GLM) with random effects was conducted with the four conditions (PG EX, PG IN, FG EX, FG IN) entered as independent regressors. A voxel-level threshold of p = .05 corrected for multiple comparisons (FWE correction) was used for all contrasts. In order to test the main effect of exclusion, both exclusion conditions were contrasted against the inclusion conditions. Subsequently, in both the performance related context (PG) and the unmodified context (FG) we conducted t-contrasts of exclusion conditions compared to the unmodified inclusion condition (FG IN). We contrasted both exclusion conditions to the FG IN condition as this condition equals the original inclusion condition in other Cyberball experiments and does not contain additional stress components (public performance evaluation), which would influence the contrast. Subsequently, we applied a logical AND conjunction analvsis of both contrasts (PG EX > FG IN  $\cap$  FG EX > FG IN), which would contain regions that consistently were activated in both the PG exclusion period and the FG exclusion period compared to control. As opposed to the main effect, this conjunction analysis specifically focuses on the common effect of both exclusion conditions compared to a "clean" control condition provided by the FG IN condition. Furthermore, both exclusion conditions were compared applying a t-contrast of PG EX > FG EX.

# **Results**

#### Behavioral data

Age and neuropsychological test scores of men and women did not differ significantly (see Online Resource 1). For a manipulation check we tested credibility as a potentially influencing factor on our behavioral data. Participants who indicated some doubt about our paradigm did not show different scores for stress (t(38) = -.800, p = .429) or positive affect (t(38) = -.302, p = .764) compared with participants that completely believed in our paradigm. For validation purposes regarding a successful manipulation of the game, the average tosses within each condition (for the 35.3 s which were analyzed in the fMRI block) were compared. Ball tosses significantly differed (all p < .001), with the largest number of tosses



in PG IN and the lowest number in FG IN (Online Resource 2).

Behaviorally, subjectively perceived stress was analyzed depending on context and condition, with order as a covariate. There was a main effect of context (F(1,38) = 10.30, p = .003,  $\eta_p^2 = .213$ ) but no effect of condition (F(1,38) = 1.09, p = .303,  $\eta_p^2 = .028$ ). However, the interaction of context and condition was significant (F(1,38) = 4.08, p = .050,  $\eta_p^2 = .097$ ). Post-hoc tests showed that inclusion in PG was more stressful than inclusion in FG (t(39) = 6.71, p < .001), and more stressful than exclusion in PG (t(39) = 5.31, p < .001) and FG (t(39) = 6.31, p < .001). Interestingly, exclusion in PG was perceived as more stressful than inclusion in FG (t(39) = 4.82, p < .001) and exclusion in FG (t(39) = 5.11, p < .001). In FG, however, stress ratings for inclusion and exclusion did not differ significantly (t(39) = -.907, p < .370).

For positive affect, there was a main effect of condition  $(F(1,37) = 5.42, p = .025, \eta_p^2 = .128)$  showing higher positive affect in the inclusion conditions. Specifically, post-hoc tests revealed that positive affect in FG IN was significantly higher than FG EX (t(38) = 2.18, p = .036). Similarly, positive affect was higher in the FG IN than in PG EX (t(38) = 2.53, p = .016)and PG IN (t(38) = 2.80, p = .008). Positive affect in the two exclusion conditions and the inclusion condition in PG did not differ (all p > .33). Furthermore, there was an interaction of order and condition  $(F(1,37) = 4.25, p = .046, \eta_p^2 = .103)$ . For all individuals that started with PG, results demonstrated a more positive affect of inclusion compared to exclusion (t(19) = 2.45, p = .024). However, when individuals started with FG their affective ratings did not differ significantly (t(18) = .61, p = .547). There was no main effect of context or the covariate order concerning positive affect.

# **Brain activity**

The main effect of EX > IN (Online Resource 3, Table 1; Online Resource 4) showed increased activation in several prefrontal regions including a large cluster in the vmPFC, the left vlPFC (IFG) and the dmPFC (middle frontal gyrus) and in the occipital cortex as well. Further, higher activation for exclusion was observed in limbic areas including bilateral hippocampus and amygdala. There was no increased activation for the anterior insula or the aMCC/ dACC comparing exclusion to inclusion. In addition, results for the reverse contrast (IN > EX) are presented in Table 1 and the contrasts for PG > FG and FG > PG are represented in Table 2 (all Online Resource 3), mainly demonstrating higher activation in temporal, parietal and occipital brain regions.

Clusters showing increased activation in FG EX > FG IN and PG EX > FG IN are presented in Fig. 3, the corresponding peak voxels are reported in Table 1. The visual overlay presented in Fig. 3 does not depict a statistical comparison or

contrast and is only included for display purposes. It should not be interpreted independently from the results of the formal statistical tests depicted in the left and right panels of Fig. 3. The overlapping activation of both exclusion contrasts compared to the FG IN condition resulted in higher activation of the IFG, vmPFC (vACC, inferior OFC), the posterior insula, the middle and posterior cingulate cortex, angular gyrus, middle temporal and post central gyrus and paracentral lobule.

Contrasting exclusion conditions directly showed increased activation in several regions for PG EX > FG EX (Table 1). Especially the dmPFC, several temporo-parietal regions, precuneus and the striatum were activated in PG EX more than in FG EX. In line with our hypothesis, contrasting PG EX > FG EX showed increased activation of the insula and aMCC/dACC. In FG EX, only visual areas were activated more than in PG EX (Table 2).

#### **Discussion**

This study investigated the neural correlates of exclusion after adding a negative performance evaluation in direct comparison with naïve exclusion in the Cyberball paradigm for the first time. The aim of placing public performance evaluation before excluding a participant was to increase social evaluative threat, which in turn was assumed to increase brain activity within a network related to social rejection and stress. Applying a modified version of an established exclusion paradigm, we found that negative performance evaluation within the Cyberball task served as an improved psychosocial stressor increasing subjectively perceived stress in inclusion and exclusion periods compared to naive exclusion. Our results thus clearly indicate that social evaluation contributes to the stressfulness of the paradigm. Exclusion periods, which were the focus of measuring stressful experience within the Cyberball paradigm, seemed to be more stressful after negative performance feedback than after naïve exclusion. Inclusion in the performance condition was the most effective stressor on a behavioral level, which may explain the higher stress level in exclusion as the effect of a preceding (and persisting) stressful experience. Taken together, the modified version may be superior in reliably producing correlates related to a stress response in a more classical sense (Seidel et al. 2013; Zöller et al. 2010) as implied by behavioral ratings.

# Neural responses to performance-related exclusion versus naïve exclusion

Both naïve and performance-related exclusion were associated with increased activation in the vmPFC, in the operculum, and posterior insula, which is in line with previous studies investigating neural responses to exclusion and empathy for social exclusion (Bolling et al. 2011; Cacioppo et al. 2013;



Table 1 MNI coordinates (x,y,z) for all peak voxels of significant clusters, T-value and cluster size k in voxel for exclusion versus control (FG EX > FG IN, and PG EX > FG IN) and the statistical overlap of both contrasts (PG EX > FG IN)  $\cap$  FG EX > FG IN)

Region	x	у	z	t	k
FG EX > FG IN	,		,		'
R Postcentral Gyrus	44	-32	64	8.62	996
vmPFC (vACC, OFC)	0	40	-8	6.05	396
Rolandic Operculum	38	-16	18	9.12	337
L ParaHippocampal Gyrus	-22	-32	-14	6.38	170
L Angular Gyrus	-44	-74	44	6.22	136
L Superior Occipital Gyrus	-12	-102	18	5.93	104
L Middle Temporal Gyrus	-60	-4	-22	5.98	70
L IFG (pars Triangularis)	-58	30	6	5.24	43
L Posterior Cingulate Cortex	-8	-48	20	5.68	40
R Paracentral Lobule	10	-34	54	5.84	35
Cuneus	2	-90	26	5.26	19
L Hippocampus	-42	-34	-10	5.33	2
L MCC	-4	-38	42	5.31	17
L IFG (pars Orbitalis)	-38	34	-18	5.37	17
L Superior Frontal Gyrus	-12	58	28	5.27	16
R Hippocampus	28	-18	-20	5.12	15
R Middle Temporal Gyrus	52	-4	-28	5.21	13
L Posterior Insula	-38	-12	20	5.52	10
PGEX > FGIN					
R Precentral Gyrus	34	-26	52	9.60	5134
R Paracentral Lobule	6	-34	54	8.64	
R Postcentral Gyrus	18	-36	68	8.29	
R Precuneus	6	-42	40	7.73	
L MCC	0	-24	40	6.58	
L Angular Gyrus	-44	-72	44	8.51	149
L IFG (pars Orbitalis)	-46	32	-18	6.70	585
L IFG (pars Triangularis)	-56	30	6	6.41	
Superior Medial Gyrus	0	62	24	6.27	557
Lingual Gyrus	-12	-82	-10	7.50	489
L Middle Temporal Gyrus	-68	-46	-12	7.57	355
L Cuneus / Precuneus	-6	-76	34	5.60	229
R Rolandic Operculum/Posterior Insula	40	-16	18	7.40	222
R Middle Temporal Gyrus	62	-14	-14	6.31	211
L Middle Temporal Gyrus	-62	-6	-14	6.25	181
L Inferior Temporal Gyrus	-50	-10	-30	5.34	10.
L Middle Frontal Gyrus	-22	22	44	5.95	150
L Superior Temporal Gyrus	-46	-16	-4	5.50	136
L Cerebellum	-12	-52	-18	5.80	9:
R IFG (pars Orbitalis)	34	36	-12	6.20	90
L Mid Orbital Gyrus	-2	34	-14	5.31	5
R IFG (pars Triangularis)	53	32	10	5.87	48
R Middle Frontal Gyrus	32	56	6	5.67	30
R Cerebellum	20	-80	-30	7.24	31
R Fusiform Gyrus	28	-54	-8	5.61	27
L ACC (pregenual)	-4	46	8	5.27	27
$PGEX > FGIN \cap FGEX > FGIN (Conjustive Conjustive Conj$		10	Ü	2.27	2.
R Postcentral Gyrus	36	-26	52	8.50	944
R Rolandic Operculum	40	-16	18	7.39	17
K Kolalidic Opercuidili	40	-10	10	1.39	1



Table 1 (continued)

Region	X	у	z	t	k
L Angular Gyrus	-48	-72	40	6.38	134
L Middle Temporal Gyrus	-60	-4	-22	5.85	43
L IFG (P. triangularis)	-58	30	6	5.24	38
R Paracentral Lobule	10	-34	54	5.80	34
L Mid Orbital Gyrus /vACC	-2	34	-14	5.27	33
L Posterior Cingulate Cortex	-8	-48	22	5.57	32
L MCC	-4	-38	42	5.29	15
L IFG (P. orbitalis)	-38	34	-18	5.33	15
L Cerebelum (IV-V)	-22	-44	-22	5.25	8

Kross et al. 2011; Masten et al. 2011a; Wudarczyk et al. 2015). However and most interestingly, as opposed to naïve exclusion we demonstrated enhanced activity in the aMCC/dACC and anterior insula in performance-related exclusion. The direct comparison of different exclusion situations may contribute to the clarification of the roles for the aMCC/dACC and anterior insula within social exclusion as we observed one highly important difference between both

**Table 2** MNI coordinates (x,y,z) for all peak voxels of significant clusters, T-value and cluster size (k) in voxels for PG EX > FG EX and FG EX > PG EX

Region	X	у	Z	t	k
PG EX > FG EX					
R Inferior Parietal Lobule	42	-48	38	7.470	3757
R Angular Gyrus	34	-62	40	7.360	
R Superior Occipital Gyrus	24	-82	20	7.310	
L Lingual Gyrus (V1-V4)	-10	-82	-10	11.630	1797
R Middle Frontal Gyrus	24	18	52	6.850	1124
L Superior Medial Gyrus	2	24	42	6.320	
R aMCC	10	16	30	5.490	
R Superior Frontal Gyrus	18	46	28	6.710	1018
L Inferior Parietal Lobule	-38	-50	38	6.700	908
R Precuneus	4	-48	76	7.060	471
L Paracentral Lobule					
R Inferior Temporal Gyrus	62	-50	-18	6.370	271
R Middle Temporal Gyrus					
L Middle Occipital Gyrus	-28	-82	20	7.640	248
R Putamen/ Insula	30	14	-2	6.420	108
R Caudate Nucleus	18	0	16	5.820	90
L Middle Frontal Gyrus	-34	14	38	5.880	88
L Middle Temporal Gyrus	-64	-52	-12	5.880	47
L Cerebellum	-34	-70	-32	6.140	25
L Anterior Insula	-31	16	-2	5.080	8
FGEX > PGEX					
R V1/ V2	12	-104	2	7.99	566
L Middle Occipital Gyrus	-22	-98	6	7.48	468

exclusion conditions. Subjective stress in the performancerelated exclusion increased but there was no difference for exclusion conditions in affective ratings. This facilitates a more specific interpretation for the role of aMCC/ dACC and anterior insula. The findings oppose the assumption of a processing of affective pain components. Instead they underline the association of stress and activity in the anterior insula as suggested by a recent meta-analysis (Kogler et al. 2015) and aMCC/dACC. Moreover, increased dACC activation in the performance game could be interpreted as the response to increased social conflict in the exclusion period in the performance game as suggested by investigations of expectancy violation contrasted to social exclusion (Bolling et al. 2011; Somerville et al. 2006). In agreement with both of these studies, the performance situation may indeed have elicited neural activation in response to expectancy violation because the aim of the game situation was more precisely defined. Hence, exclusion would have violated the participants' expectations more strongly. In contrast to this rather cognitive component of exclusion processing, the emotional aspect of exclusion independent of the context might be associated with vmPFC activation more than with aMCC/dACC activation.

Implications of previous neuroimaging studies would ascribe emotional salience as a main function of the insula and the aMCC/dACC (Taylor et al. 2009). As parts of the salience network (Seeley et al. 2007), both regions may work as hubs in the neural alarm system (Eisenberger and Lieberman 2004; Kawamoto et al. 2015) detecting salient changes in the social context when being excluded. A possible and convincing explanation for the higher activation in the performance-related exclusion seems to be that both exclusion situations have a different social relevance here. Especially for the right frontoinsular cortex, a causal role in activating regions involved in moral reasoning has been suggested (Chiong et al. 2013). For an increase in social stress, public negative feedback evaluating both the performance of the participant as well as the performance of the teammates is implemented in the performance game. Consequently, the performance situation provides a context for increased self-evaluative and other



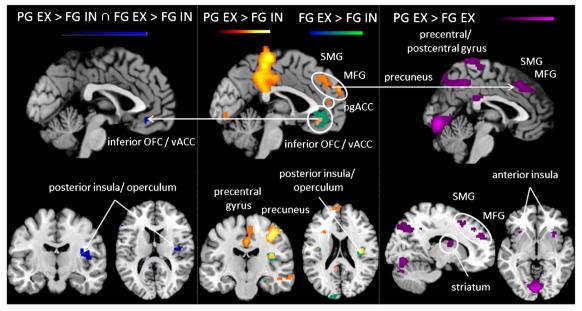


Fig. 3 Significant clusters for the conjunction (left part) of performance exclusion versus free game inclusion and free game exclusion versus free game inclusion; significant clusters of the visual overlap of performance game exclusion versus free game inclusion and free game exclusion versus free game inclusion (middle part)\*; significant clusters of the comparison of performance exclusion and free game exclusion (right

part); FWE voxel level threshold of p < .05 was used for all clusters. \*the visual overlap in Fig. 3 (middle part) does not depict a statistical comparison or contrast, and should not be interpreted independently from the results of the formal statistical tests depicted in the left and right panels of Fig. 3

related thoughts since reactions were important for the group aim.

The additional pronounced activation in mentalizing regions like the mPFC (superior medial gyrus, medial frontal gyrus) and precuneus during performance related exclusion (Schurz et al. 2014) may be a specific aspect of our paradigm. Our results suggest that social evaluative threat contributes to the negative experience of exclusion and seems to be particularly linked to an enhanced recruitment of a social monitoring and perspective taking system (Kawamoto et al. 2015), reflecting an even stronger social orientation and evaluation of the stressed individual.

Mentalizing regions have been suggested before as important structures within the dynamic process of exclusion as part of the social monitoring system (Kawamoto et al. 2015). This system is thought to adaptively regulate social behavior by processing social cues with new information regarding the current situation. Evaluation of the behavior of the excluders might determine if a situation is processed as intentional or unintentional exclusion (Chow et al. 2008). Although former studies already reported recruitment of mentalizing regions using the Cyberball task (Karremans et al. 2011; Moor et al. 2012), we demonstrated that performance-related exclusion seemed to engage mentalizing regions to a larger extent than naïve exclusion. Other studies showed that social evaluative threat related to performance results in lowered self-esteem (Dedovic et al. 2014) and rumination (Nepon et al. 2011).

These self- and other-related thoughts might be reflected in the increased activation of the mentalizing network.

Together with reports of high subjective stress levels, our findings indicate that using the Cyberball task as a psychosocial stress paradigm might profit from adding performance evaluation precedent to exclusion. Exclusion seems to be more alarming and demands a stronger contention with the social environment when performance is evaluated. Future investigation of hormonal reactions, such as cortisol levels, would contribute to the understanding of exclusion related stress and its modification by increased social evaluative threat.

# **Emotional responses to naive and performance related exclusion**

The present results confirm previous observations that the experience of exclusion is highly undesirable, leading to more negative mood and distress (Bernstein and Claypool 2012; Williams et al. 2000). In the current study, affective ratings were assessed online throughout the task, facilitating a direct report of the affective state of the participants. In contrast to previous studies, which assessed distress exclusively related to rejection, we assessed a general subjective stress level. Although our results do not support the hypothesis of a negative influence on the general stress level by the experience of exclusion compared to inclusion, the second part of our



hypothesis was supported: public performance evaluation increased general stress levels both during inclusion and exclusion periods compared to the established inclusion condition (FG IN), confirming that social evaluative threat increases social stress (Dickerson and Kemeny 2004). Moreover, the relatively lower general stress level in the exclusion period in the free game Cyberball period suggests that negative public evaluation might affect subjective stress levels even more than naïve exclusion. Hence, being excluded because of a bad performance seems to be worse than being excluded without a rationale. On the other hand, in contrast to inclusion in the performance situation, the exclusion was perceived as less stressful. One might speculate that a bad performance as reason for the exclusion might have a protective effect because it offers the possibility to an external attribution for the exclusion. Wirth and Williams (2009) found a similar effect when subjects were excluded because they belonged to a random and temporary defined group and their group membership served as external attribution for the exclusion. However, in the current context it might be debatable if the attribution to a bad performance can actually have a similar protective effect. In contrast to a neutral group membership, being evaluated as worse than the teammates is unlikely to improve the evaluation of the situation. Instead, the observation of more positive feelings within the performance exclusion, compared to the performance inclusion, could reflect the relief of not receiving any negative feedback as long as one does not take part in the game anymore. Actually, receiving negative feedback – which may imply a very negative future consequence - might be worse than experiencing the negative consequence by being excluded. A confirmation of this assumption would be an interesting research aim in future studies.

As a replication of previous findings on negative effects of exclusion, we aimed to show a worsening of mood in response to exclusion. Online ratings of affective state confirmed the negative effect of exclusion. In contrast to our control condition, the unmodified inclusion, exclusion was associated with a less positive mood. Since the negative performance feedback in PG might have influenced mood ratings independently, our results are still similar to previous findings (Sebastian et al. 2011) demonstrating more negative affect in exclusion compared to an acceptance condition (unmodified inclusion, here FG IN). Importantly, the negative effect of exclusion demonstrated by subjective ratings emerged in both contexts, which validates our modification and the fMRI analyses. At the same time, in contrast to a more pronounced stress response in the performance-related exclusion compared to naïve exclusion, affective ratings were comparable for both conditions. Our interpretation of neural differences between exclusion conditions according to behavioral differences therefore is mainly related to the diverse perception of stress. In contrast, exclusion conditions compared to the control condition (FG IN) consistently differed with regard to affective ratings, which hence constitutes the basis for an interpretation of neural responses in these comparisons.

# Neural responses of exclusion versus inclusion

Replicating previous findings on exclusion, in our modified paradigm the main effect of exclusion contrasted to inclusion confirmed increased activity in regions which have been described in several studies for healthy adult samples (Karremans et al. 2011; Kawamoto et al. 2012; Onoda et al. 2009; Sebastian et al. 2011). Most consistently, higher activity in exclusion has been reported for regions which are associated with emotion processing and regulation, like the ACC, the medial and lateral PFC and limbic regions (Kohn et al. 2014; Moor et al. 2012; Sebastian et al. 2011; Will et al. 2016). In agreement with these studies, exclusion compared to inclusion was associated with increased activity in the ACC, vmPFC, vIPFC and parahippocampal gyrus in the current study, although the contrast included the performance game inclusion as counterpart for the comparison. As the conjunction analysis demonstrated, the vACC, inferior OFC and IFG showed increased activity both in the performance- related exclusion as well as in the naïve exclusion, confirming that the modification of the Cyberball task is associated with a comparable network as reported by previous studies. Moreover, as behavioral results have already indicated, a reduced positive affect in exclusion conditions and the resulting need to regulate emotions may explain the increased involvement of emotion related brain regions.

The main contrast of exclusion versus inclusion did not show increased activation of the aMCC/ dACC, which has been suggested as target region for the processing of exclusion or social pain (Eisenberger et al. 2003). The "cleaner" comparison of exclusion independent of the context against a control condition (FG IN) as provided by the conjunction analysis confirmed this as there was only increased activation in the ventral portion of the ACC for both exclusion conditions, not in the dorsal portion. Methodological explanations for a missing activation of the aMCC/dACC have been suggested by a recent meta-analysis (Rotge et al. 2014) featuring the length of exclusion periods and sample characteristics and also the construct of self-reported distress in relation to brain activity in this region. Our results concerning the contrast of exclusion and inclusion are in line with previous studies which likewise failed to replicate increased activity of the aMCC/dACC but instead found the vACC responding to exclusion and related to self-reported distress (Masten et al. 2009; Onoda et al. 2009; Sebastian et al. 2011). Likewise, in our study, the vACC was consistently found for exclusion compared to inclusion in both the classic Cyberball conditions as well as in the modified exclusion conditions. The negative effect on mood as a result of exclusion as demonstrated here has been shown reliably by numerous studies (for an overview see



Hartgerink et al. 2015). Once again it supports the assumption of emotion related regions being involved in the processing of exclusion as suggested within the model of dynamic processing of exclusion (Kawamoto et al. 2015). An interesting observation in this context may be added: Note that exclusion conditions did not differ with regard to mood ratings. Likewise, activity in the vmPFC, which has previously been related to emotion processing in exclusion (Sebastian et al. 2011), did not differ between exclusion conditions. This observation could be valuable for the design of future studies, as online ratings of various affective states may contribute to the clarification of the roles for several brain regions in the processing of exclusion.

#### Limitations

We were able to show increased stress levels for exclusion in PG compared to a control inclusion condition, but not in the usual context (FG). In the latter, stress effects became visible in a reduced positive affect. Since we did not apply a specific exclusion related assessment of distress as in former studies (Williams and Nida 2011), this could explain the missing stress effect for the usual Cyberball condition. However, inconsistent evidence for a hormonal stress reaction (Seidel et al. 2013; Zöller et al. 2010) in the Cyberball task would support the critical notion that social exclusion does not necessarily elicit a strong stress response. Hence, the modification we propose could be an effective way to increase stress responses. Increased stress in the performance game inclusion period compared to the exclusion period of the performance game was most likely a result of not receiving any negative feedback at the time of exclusion. This may have reduced the stress, but nevertheless, the stress level was still increased compared to the free game exclusion. Furthermore, this made the exclusion conditions highly comparable, only distinct in the relevant aspect and hence increases the validity of the neural results and their interpretation. The only difference between both exclusion conditions was the threat of receiving negative feedback (which was the contextual factor of interest) and observing the teammates receiving negative feedback. Another limiting point of our study is that both the naïve exclusion and the performance-related exclusion were implemented in one paradigm, which might have influenced emotional and neural responses to the respective other game. However, a pseudo randomization ensured that half of the participants started with the PG and the other half with the FG, which controlled for this influence. Note that fundamental needs as well as specific negative emotions such as anger or disappointment were not assessed in the present study, limiting comparability with previous findings. Further, the inclusion condition during PG cannot be used as a control condition, due to differential behavioral responses and a lack of comparability. However, it served perfectly as an initiator for a more stressful preceding context for the exclusion in the performance game.

#### Conclusion

The applied modification of the Cyberball paradigm constitutes an improved psychosocial stressor and therefore may be more powerful for the induction of social stress and the examination of related neural responses. Behavioral findings demonstrate that subjectively perceived stress levels can be enhanced by applying social evaluative threat to the established Cyberball paradigm. Stress increased compared to naïve exclusion. Inclusion in the performance condition represents a powerful stressful situation and can hence not serve as an adequate control condition. For a future application of the Cyberball task as stress paradigm, the apposition of public performance evaluation seems to be encouraging and might thwart against the missing hormonal stress reaction on the Cyberball task reported in some studies (Seidel et al. 2013; Zwolinski 2012).

Affective responses to exclusion on a behavioral level as well as corresponding neural responses related to a less positive affect are not influenced by enhancing the stressful experience of exclusion. Underlying neural processes associated to a performance-related exclusion in contrast to naïve exclusion highlight the important role of the salience network as potential initiator for social monitoring, and the mentalizing network in exclusion processing after preceding and persisting stress. The modified Cyberball version therefore is an exemplary model showing that specifically public performance evaluation triggers a high stress response, which has behavioral and neural repercussions on exclusion processing.

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