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## **The neural correlates of dealing with social exclusion in childhood**

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1 **The neural correlates of dealing with social exclusion in childhood**

2 Running head: Dealing with social exclusion in childhood

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21 **Abstract**

22 Observing social exclusion can be a distressing experience for children that can be followed  
23 by concerns for self-inclusion (self-concerns), as well as prosocial behavior to help others in  
24 distress (other-concerns). Indeed, behavioral studies have shown that observed social  
25 exclusion elicits prosocial compensating behavior in children, but motivations for the  
26 compensation of social exclusion are not well understood. To distinguish between self-  
27 concerns and other-concerns when observing social exclusion in childhood, participants  
28 (aged 7-10) played a four-player Prosocial Cyberball Game in which they could toss a ball to  
29 three other players. When one player was excluded by the two other players, the participant  
30 could compensate for this exclusion by tossing the ball more often to the excluded player.  
31 Using a three-sample replication (N=18, N=27, and N=26) and meta-analysis design, we  
32 demonstrated consistent prosocial compensating behavior in children in response to  
33 observing social exclusion. On a neural level, we found activity in reward and salience  
34 related areas (striatum and dorsal anterior cingulate cortex (dACC)) when participants  
35 experienced inclusion, and activity in social perception related areas (orbitofrontal cortex)  
36 when participants experienced exclusion. In contrast, no condition specific neural effects  
37 were observed for prosocial compensating behavior. These findings suggest that in  
38 childhood observed social exclusion is associated with stronger neural activity for self-  
39 concern. This study aims to overcome some of the issues of replicability in developmental  
40 psychology and neuroscience by using a replication and meta-analysis design, showing  
41 consistent prosocial compensating behavior to the excluded player, and replicable neural  
42 correlates of experiencing exclusion and inclusion during middle childhood.

43

44 **Keywords**

45 Social exclusion                      Prosocial behavior                      fMRI  
46 Childhood                              Meta-analysis

47

## 48 **1. Introduction**

49 Observing social exclusion occurs often in school-aged children and can be a distressing  
50 experience (Saylor et al., 2013). For example, when children observe that others are  
51 excluded from a game or social event, children may experience distress because they are  
52 concerned about their own inclusion, or they may feel the need to help the other person in  
53 distress, also referred to as prosocial behavior (Padilla-Walker & Carlo, 2014). Children show  
54 basic prosocial behavior from 18 months of age onwards (Warneken & Tomasello, 2006) and  
55 this behavior rapidly develops throughout childhood and adolescence when cognitive  
56 capacity and perspective taking skills continue to grow (Eisenberg, Fabes, & Spinrad, 2006;  
57 Güroğlu, van den Bos, & Crone, 2014). However, the motivations for helping or  
58 compensation behavior remain largely unknown, possibly because these motives are difficult  
59 to unravel on the basis of behavior only. Neuroimaging may prove helpful to examine the  
60 different processes that take place when children observe social exclusion.

61 Social exclusion is often studied by using the Cyberball Game (Williams, Cheung, &  
62 Choi, 2000): a three player ball game where two virtual players no longer toss a ball to an  
63 excluded player, creating a situation of social exclusion. Although Cyberball is a computer  
64 game including virtual players, several studies have shown that both children and  
65 adolescents show more prosocial behavior in subsequent interactions towards individuals  
66 who have been excluded in this game, as indicated by helpful emails and money donations  
67 (Masten, Eisenberger, Pfeifer, & Dapretto, 2010; Masten, Morelli, & Eisenberger, 2011; Will,  
68 van den Bos, Crone, & Güroğlu, 2013). Recently a prosocial version of the paradigm was  
69 developed to examine concurrent compensating behavior when an individual is excluded  
70 (Riem, Bakermans-Kranenburg, Huffmeijer, & van IJzendoorn, 2013). In the Prosocial  
71 Cyberball Game (PCG) participants can compensate for this exclusion by tossing the ball  
72 more often to the excluded player. Studies have shown that compensating behavior followed  
73 observed social exclusion towards the excluded player across childhood, adolescence and  
74 adulthood (Riem et al., 2013; van der Meulen, van IJzendoorn, & Crone, 2016; Vrijhof et al.,

75 2016). Yet, it remains to be determined if children are most concerned about others when  
76 observing exclusion, or about self-inclusion and exclusion.

77         Neuroimaging research in adults revealed that simply observing another person being  
78 excluded is associated with increased activity in areas such as the dorsal anterior cingulate  
79 cortex (dACC) and bilateral insula (Masten, Eisenberger, Pfeifer, Colich, & Dapretto, 2013;  
80 Meyer et al., 2013; Novembre, Zanon, & Silani, 2015). These regions are thought to play a  
81 role in social uncertainty and distress, and may be critically involved in experiencing  
82 concerns about self-exclusion (Cacioppo et al., 2013). Interestingly, previous studies have  
83 shown that the experience of being excluded yourself leads to feelings of decreased self-  
84 worth (Zadro, Williams, & Richardson, 2004), accompanied by an increase in activation of the  
85 dACC and bilateral insula (Cacioppo et al., 2013; Eisenberger, Lieberman, & Williams, 2003;  
86 Rotge et al., 2015). Additionally, a recent study has added to this body of literature by  
87 postulating that co-activation in the dACC and bilateral insula is a measure of social  
88 inclusivity, and that activation in these two areas can therefore be found in both social  
89 exclusion and social inclusion contexts (Dalgleish et al., 2017).

90         In contrast, prosocial compensating behavior (i.e. compensating an excluded player)  
91 in the Prosocial Cyberball Game resulted in increased activation of the temporo-parietal  
92 junction (TPJ), nucleus accumbens (NAcc), and the bilateral insula (van der Meulen et al.,  
93 2016). The TPJ is an area previously associated with perspective taking (Carter & Huettel,  
94 2013) whereas the NAcc is part of the reward network of the brain (Delgado, 2007;  
95 Lieberman & Eisenberger, 2009). Possibly, these regions play an important role in prosocial  
96 compensating behavior. These patterns of neural activity lead to the hypothesis that the  
97 Prosocial Cyberball Game might tap into two different processes: the experience or concern  
98 for possible self-exclusion and the compensation for exclusion of others. Experience of  
99 possible self-exclusion refers to the worry about own participation in the game, whereas  
100 compensation for exclusion is thought to reflect prosocial behavior.

101         The aim of the current study was to investigate the behavioral and neural correlates of  
102 reactions to observed social exclusion in middle childhood. Our target age was children in the

103 age range 7-10 years because this is a critical age for forming intimate friendships and social  
104 connections (Buhrmester, 1990), but the neural reactions to observed social exclusion in this  
105 particular age range have not yet been studied. We used the Prosocial Cyberball Game  
106 (Riem et al., 2013) to study possible reactions to observed social exclusion, namely  
107 experience of possible self-exclusion and prosocial compensating behavior. Previous studies  
108 have called into question whether neuroimaging results survive Type I errors and may lead to  
109 too many false positives (Eklund, Nichols, & Knutsson, 2016). Moreover, recent projects  
110 have raised concerns about whether results from psychological experiments can be  
111 replicated (Open Science, 2015). Therefore, we used a replication approach including a pilot  
112 sample to generate hypotheses, a test sample to test these hypotheses, and a replication  
113 sample to confirm these findings. The test and replication sample consisted of co-twins  
114 because they are similar in many respects: this will optimize the chance for replication, and  
115 lack of replication cannot easily be ascribed to confounding or unmeasured differences  
116 between the two samples.

117 On a behavioral level we hypothesized that observing social exclusion would lead to  
118 prosocial compensating behavior (Riem et al., 2013; van der Meulen et al., 2016; Vrijhof et  
119 al., 2016). On a neural level we expected that both experiencing self-exclusion and self-  
120 inclusion would result in activity in dACC and bilateral insula (Cacioppo et al., 2013; Dalgleish  
121 et al., 2017; Eisenberger et al., 2003; Rotge et al., 2015). Furthermore, we expected that  
122 engaging in prosocial compensating behavior would lead to activity in dACC and bilateral  
123 insula (Masten et al., 2013; Masten et al., 2010) and TPJ, and NAcc, similar to what has  
124 been found in adults (van der Meulen et al., 2016). Although TPJ, dACC and bilateral insula  
125 show a sharp increase in cortical thickness during middle childhood (Mills, Lalonde, Clasen,  
126 Giedd, & Blakemore, 2014; Pfeifer & Peake, 2012), not much is known about the functional  
127 role of these regions in observing social exclusion in middle childhood. The power of our  
128 experimental design suggests that the present set of studies is particularly sensitive to  
129 detecting brain-behavior relationships of higher socio-affective functions and their  
130 development in a developmental sample.

131

132

## 133 **2. Materials and Methods**

### 134 **2.1 Participants**

135           Three samples were recruited for this study: a pilot sample, a test sample and a  
136 replication sample. The pilot sample consisted of 20 children aged 7-10 years ( $M = 8.13$   
137 years,  $SD = .97$ , 50% male). This sample was composed of 9 opposite sex twin pairs and 2  
138 singletons, recruited from an existing database at Leiden University. The test and replication  
139 sample consisted of 30 same sex twin pairs ( $M = 8.19$  years,  $SD = .68$ , 46.7% male). Co-  
140 twins in the twin pairs were randomly divided over the test and replication sample upon  
141 inclusion, such that one child from each pair was placed in the test sample and one child was  
142 placed in the replication sample. These participants were recruited for the longitudinal twin  
143 study of the Leiden Consortium on Individual Development (L-CID). Families with twin  
144 children aged 7-8 years at the moment of inclusion were recruited from municipalities in the  
145 western region of the Netherlands, by sending invitations to participate to their home  
146 addresses (obtained through the municipal registries).

147           Some participants were excluded from analyses due to excessive head motion during  
148 the MRI session or because they did not finish the scanning session (two children from the  
149 pilot sample, three children from the test sample, and four from the replication sample). The  
150 final pilot sample consisted of 18 children ( $M = 8.15$  years,  $SD = 1.06$ , 55.6% male), the final  
151 test sample of 27 children ( $M = 8.23$  years,  $SD = 0.67$ , 40.7% male), and the final replication  
152 sample of 26 children ( $M = 8.21$  years,  $SD = 0.71$ , 42.3% male). The three samples did not  
153 significantly differ in age ( $F(2, 68) = .04$ ,  $p = .96$ ) or gender ( $X^2(2) = 1.08$ ,  $p = .58$ ). All  
154 participants were screened for MRI contra indications, had normal (or corrected to normal)  
155 vision, were fluent in Dutch, and had no physical or psychological disorder that disabled their  
156 performance on the tasks. Written informed consent was obtained from both parents before  
157 the start of the study. Parents received €50 for the participation of their children, and all

158 children received €3.50 and a goodie bag with small presents. The study was approved by  
159 the Dutch Central Committee on Research Involving Human Subjects.

160

## 161 **2.2 Experimental Design**

162 To measure reactions to observed social exclusion we used an experimental fMRI adapted  
163 version of the Prosocial Cyberball Game (PCG) (Riem et al., 2013; van der Meulen et al.,  
164 2016; Vrijhof et al., 2016). In this game, participants see four classical Cyberball figures on  
165 the screen (Williams et al., 2000). The participant is represented by the figure at the bottom  
166 of the screen, and the three other figures are placed at the left, the right, and the top of the  
167 screen (see Figure 1A). Participants were told that they were going to play a computerized  
168 ball tossing game with three other players. No mention was made of exclusion, in order to  
169 avoid influencing their behavior. Thus, prosocial compensating is not confounded with  
170 varying biases between participants to follow the explicit or implicit experimenter suggestions  
171 for desirable behavior. Participants were asked to imagine that they were actually playing the  
172 game by thinking about the setting and the other players of the game. Previous studies have  
173 shown that there were no differences in reduced feelings of belonging and self-esteem  
174 between conditions where participants believed that other players were present, or merely  
175 imagined that other players were present (Zadro et al., 2004). Since imagining playing with  
176 others is a strong manipulation in research on gaming (Konijn, Bijvank, & Bushman, 2007)  
177 and does not rely on deception, we also used this manipulation for the PCG.

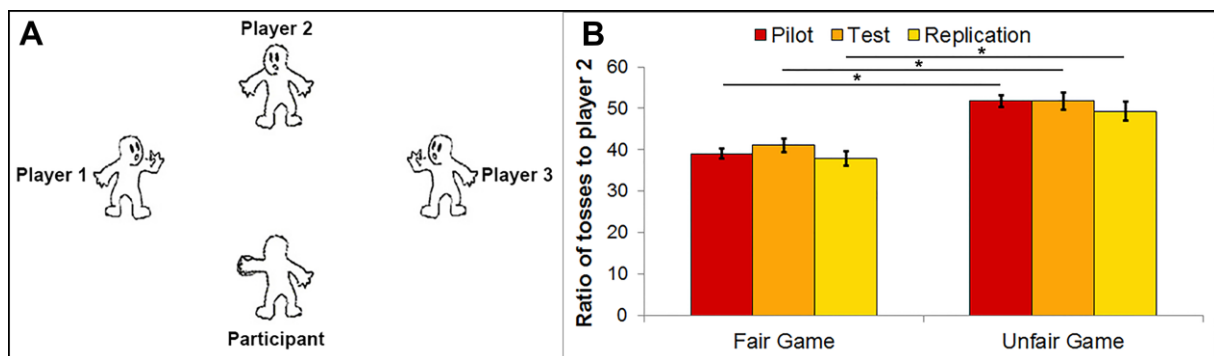
178 The game consisted of two parts: the Fair Game and the Unfair Game. During the first  
179 part (the Fair Game), the game was programmed to ensure that all four players received the  
180 ball an equal number of times. During the second part (the Unfair Game), either player 1 or  
181 player 3 tossed the ball only once to player 2 (at the top of the screen). After this initial toss,  
182 player 1 and player 3 no longer tossed the ball to player 2, thereby creating a situation of  
183 *observed* social exclusion for the participant. The participant could therefore choose to  
184 compensate for the exclusion by tossing more balls to excluded player 2, or to contribute to  
185 the exclusion by tossing more balls to players 1 and 3. The location of the excluded player



186 was always the same for all participants (directly across the participant, at the top of the  
 187 screen). In both the Fair Game and the Unfair Game, each trial consisted of a ball toss with a  
 188 duration of 2000 ms. After each ball toss a jitter was added with a duration ranging from  
 189 1000-2000 ms in steps of 500 ms. The Fair Game consisted of 120 trials and was played on  
 190 a laptop outside the MRI scanner. The Unfair Game consisted of 168 trials and was played in  
 191 the MRI scanner, to enable collection of behavioral and MRI data during the task. During the  
 192 Unfair Game, participants could indicate their response by pressing a button on a box  
 193 attached to their right leg. The Unfair Game was presented in two separate parts to provide  
 194 participants with a small rest period in between. During the entire game, the excluding  
 195 players were referred to as Players 1 and 3 (on the left and right side of the screen  
 196 respectively), the excluded player was referred to as Player 2, and the participant was  
 197 referred to as "Participant" (see Figure 1A).

198

199 **Figure 1. (A)** Screenshot of Prosocial Cyberball Game. **(B)** Ratio of tosses of the participant  
 200 to Player 2 in the PCG across the three samples.



201

202

### 203 2.3 Procedure

204 Participants were given an extensive explanation and practice session in a mock scanner to  
 205 familiarize them with the procedure of an MRI scan. All participants played the Fair Game of  
 206 the PCG before the scanning session. Co-twins were then randomly assigned to either start  
 207 with the scan session (and thus perform the Unfair Game of the PCG) or to start with other

208 behavioral tasks that were part of the larger L-CID study. All twin pairs (from the pilot sample  
209 or from the test/replication sample) were randomly assigned to one of two procedures on the  
210 day of data collection.

211

## 212 **2.4 MRI data acquisition**

213 MRI scans were made with a Philips 3.0 Tesla scanner, using a standard whole-head coil.  
214 Data for the pilot sample were collected on a Philips Achieva TX MR, whereas data for the  
215 test and replication sample were collected on a Philips Ingenia MR. The functional scans  
216 were acquired using a T2\*-weighted echo-planar imaging (EPI). The first two volumes were  
217 discarded to allow for equilibration of T1 saturation effects (TR = 2.2 s; TE = 30 ms;  
218 sequential acquisition, 37 slices; voxel size = 2.75 x 2.75 x 2.75 mm; Field of View = 220 x  
219 220 x 112 mm). For the pilot sample the Field of View was 220 x 220 x 114.68 mm, with a  
220 sequential acquisition of 38 slices, and all other parameters were equal. After the functional  
221 runs, a high resolution 3D T1-weighted anatomical image was collected (TR = 9.8 ms, TE =  
222 4.6 ms, 140 slices; voxel size = 1.17 x 1.17 x 1.2 mm, and FOV = 224 x 177 x 168 mm). For  
223 the pilot sample the TR was 9.76, the TE was 4.59, the voxel size was 0.875, and all other  
224 parameters were equal. Participants could see the stimuli projected on a screen via a mirror  
225 attached to the head coil. Foam inserts were used within the head coil to restrict head  
226 movement.

227

## 228 **2.5 MRI data analyses**

229 All data were analyzed with SPM8 (Wellcome Department of Cognitive Neurology, London).  
230 Images were corrected for slice timing acquisition and differences in rigid body motion.  
231 Functional volumes were spatially normalized to T1 templates. The normalization algorithm  
232 used a 12-parameter affine transform together with a nonlinear transformation involving  
233 cosine basis functions and resampled the volumes to 3 mm cubic voxels. Templates were  
234 based on the MNI305 stereotaxic space (Cocosco, Kollokian, Kwan, & Evans, 1997).  
235 Functional volumes were spatially smoothed with a 6 mm full width at half maximum (FWHM)

236 isotropic Gaussian kernel. As a final step, the ArtRepair module (Mazaika, Hoefft, Glover, &  
237 Reiss, 2009) was used to address any head motions in the data. The threshold was set at 2  
238 mm, and participants were excluded if more than 20% of the dynamics of the two functional  
239 runs were affected.

240         The start of each ball toss was modeled separately with a zero duration event. Since  
241 imaging data were collected during the Unfair Game but not during the Fair game, only the  
242 Unfair game was taken into account for these analyses. To study participant's experience of  
243 possible self-exclusion we differentiated between the participant receiving tosses from  
244 excluding Players 1 and 3 ("Experienced Inclusion") versus the participant not receiving the  
245 ball from these players ("Experienced Exclusion"). To study participant's compensation for  
246 observed exclusion of Player 2, we differentiated between the participant's tossing to this  
247 excluded Player 2 ("Compensating") versus his or her tosses to the excluding Players 1 and  
248 3 ("Tossing to excluders").

249         The trial functions were used as covariates in a general linear model; along with a  
250 basic set of cosine functions that high-pass filtered the data. The least-squares parameter  
251 estimates of height of the best-fitting canonical HRF for each condition were used in pair-  
252 wise contrasts. Motion regressors were included in the first level analysis. The resulting  
253 contrast images were computed on a subject-by-subject basis and then submitted to group  
254 analyses.

255

### 256 2.5.1 Whole brain analyses

257 We computed two different contrasts to study the various reactions to observed social  
258 exclusion. First, to investigate the neural response to being potentially excluded from the  
259 game by the other two players, we tested the contrast: *Experienced Inclusion* > *Experienced*  
260 *Exclusion* (and the reversed contrast). In accordance with the programming of the game,  
261 over the three samples the percentage of tosses from excluding Players 1 and 3 to the  
262 participant (M = 50.08, SD = .74) was comparable to the number of tosses from Players 1  
263 and 3 to each other (M = 49.92, SD = .74). Over the three samples the percentage of tosses

264 to the excluded player ( $M = 50.86$ ,  $SD = 10.20$ ) was comparable to the number of tosses to  
265 the two excluding players combined ( $M = 49.14$ ,  $SD = 10.20$ ). Second, to investigate the  
266 neural response to prosocial compensating behavior, we tested the contrast: *Compensating*  
267 *> Tossing to excluders* (and the reversed contrast). Significant task-related responses  
268 exceeded a cluster-corrected threshold of  $p < .05$  FDR-corrected, with a primary threshold of  
269  $p < .005$  (Woo, Krishnan, & Wager, 2014).

270

### 271 *2.5.2 Region of interest analyses to test for replication effects*

272 To further specify the effects of the whole brain analyses and to test for replication  
273 effects, functional ROIs were defined. We extracted functional clusters of activation from the  
274 whole brain contrasts in the pilot sample with the use of the MarsBar toolbox (Brett, Anton,  
275 Valabregue, & Poline, 2002). Functional clusters that encompassed multiple anatomical  
276 regions were masked with anatomical templates from the MarsBar-AAL (Tzourio-Mazoyer et  
277 al., 2002) to separate the different anatomical regions. We then used the ROIs from the pilot  
278 sample to extract parameter estimates from the test sample. The same approach was used  
279 for the analysis of the results from the test sample to the replication sample.

280 Next, one-sided paired sample t-tests were used to test whether the activation in the  
281 first sample was significantly different between the conditions in the second sample. We  
282 corrected for multiple testing with a Bonferroni correction of  $\alpha = .10$ , dependent on the  
283 number of extracted ROIs, because we were looking for replication of previously found  
284 results. Outlier scores ( $z$ -value  $< -3.29$  or  $> 3.29$ ) were winsorized (Tabachnick & Fidell,  
285 2013).

286 To specifically explore the neural response during prosocial behavior across all three  
287 samples and to align our activation patterns with those found in adults, we used additional  
288 independent ROIs that were used in a study on prosocial neural responses in adults (see van  
289 der Meulen et al. (2016)). In the adult study, Neurosynth templates were used to create  
290 masks of the dorsal anterior cingulate cortex (dACC), bilateral insula, medial prefrontal cortex  
291 (mPFC), temporo-parietal junction (TPJ), and nucleus accumbens (NAcc). We used these

292 masks to extract parameter estimates for the conditions “Compensating” and “Tossing” in all  
293 three samples. Combined effect sizes were computed with the Comprehensive Meta-  
294 Analysis (CMA) program (Borenstein, Rothstein, & Cohen, 2005).

295

### 296 **2.5.3 Meta-analysis**

297 We used an activation likelihood estimate (ALE) meta-analysis of whole brain results to test  
298 for commonalities across the three samples, for those contrasts that resulted in replicable  
299 effects. Given that the purpose of this meta-analysis was to test for commonalities among  
300 three samples that may not be observed in single studies, we used a less conservative  
301 threshold, which was then analyzed with a more stringent threshold at a meta-analytic level  
302 Coordinates from whole brain analyses conducted at a threshold of  $p < .001$  uncorrected, 10  
303 contiguous voxels, were entered in the Gingerale program (version 2.3.6,  
304 <http://www.brainmap.org/ale/>). We used a cluster correction of  $p < .05$ , with 1000  
305 permutations and an initial primary voxel-wise threshold of  $p < .001$ .

306

307

## 308 **3. Results**

### 309 **3.1 Behavioral results**

310 The main behavioral outcome from the PCG is prosocial compensating behavior to Player 2,  
311 defined as an increase in ratio of tosses to Player 2 from the Fair game to the Unfair game.  
312 We calculated this ratio by dividing the number of tosses to Player 2 by the total number of  
313 tosses to all players (van der Meulen et al., 2016; Vrijhof et al., 2016). Paired t-tests were  
314 performed to study prosocial compensating behavior. Analyses that compare the first and  
315 second part of the Unfair Game (as these were presented as separate runs during the scan  
316 session) can be found in Supplement A.

317 First, in the pilot sample we found a significant difference in ratio of tosses to Player 2  
318 in the Fair Game compared to the Unfair Game ( $t(17) = -5.68, p = < .001, d = 2.20$ ). This

319 finding was replicated in the test sample ( $t(26) = -5.27, p < .001, d = 1.11$ ), and in the  
 320 replication sample ( $t(25) = -4.04, p < .001, d = 1.10$ ; see Table 1 for descriptives). Second,  
 321 because children differed in their percentage of tosses to Player 2 in the Fair Game (see  
 322 Figure 1B), we took these base-line differences into account by calculating a difference score  
 323 between percentage of tosses to Player 2 in the Unfair Game minus the percentage of tosses  
 324 to Player 2 in the Fair Game. Thus, for each participant a compensating score was  
 325 calculated. We used an ANOVA to test whether there was a difference in compensating  
 326 scores for the three samples, and found no significant difference ( $F(2, 68) = .15, p = .86$ ).  
 327 This shows that levels of prosocial compensating behavior were the same across the three  
 328 samples during middle childhood.

329

330 **Table 1.** Descriptives of percentage of tosses of participant in Prosocial Cyberball Game.

331 Data represents means (with standard deviations in parentheses).

		PILOT	TEST	REPLICATION
Fair Game	To player 1	30.47 (5.84)	30.52 (7.08)	31.21 (6.15)
	To player 2	39.03 (5.34)	41.05 (8.14)	37.84 (9.03)
	To player 3	30.49 (5.51)	28.43 (6.37)	30.95 (6.57)
Unfair Game	To player 1	36.64 (6.22)	25.12 (7.66)	26.40 (7.10)
	To player 2	51.74 (6.19)	51.76 (10.75)	49.31 (11.87)
	To player 3	24.62 (6.92)	23.12 (6.58)	24.29 (8.54)

332

### 333 3.2 Neural reactions to Playing with Others

#### 334 3.2.1 Experienced Inclusion > Experienced Exclusion

335 First, we tested the contrast Experienced Inclusion > Experienced Exclusion in the pilot  
 336 sample with a whole brain analysis. The contrast was defined as receiving the ball from  
 337 excluding Players 1 and 3 (“Experienced Inclusion”) versus not receiving the ball from  
 338 excluding Players 1 and 3 (“Experienced Exclusion”). The Experienced Inclusion >  
 339 Experienced Exclusion analysis resulted in significant activation in several clusters that  
 340 spanned medial prefrontal cortex (PFC; including pre-supplementary motor area (SMA),

341 ACC), bilateral insula, bilateral striatum (including caudate, pallidum, putamen) and left pre-  
 342 and postcentral gyrus (See Table 2 and Figure 2A). These were separated in 18 anatomically  
 343 defined subclusters from which parameter estimates were extracted. When no significant  
 344 differences were found between hemispheres, results were collapsed across left and right  
 345 hemispheres. This resulted in a total of 12 regions that were analyzed in the test sample (see  
 346 Figure 2B). Out of these 12 regions, bilateral caudate, insula, pallidum, and putamen,  
 347 anterior and mid cingulum, left pre- and postcentral gyrus, and SMA, had significantly more  
 348 activation for Experienced Inclusion than for Experienced Exclusion (all  $p < .008$ ) in the test  
 349 sample (see Figure 2C).

350 Next, we examined the contrast Experienced Inclusion > Experienced Exclusion in the  
 351 test sample. This analysis resulted again in activation in several clusters that spanned medial  
 352 PFC (including pre-SMA, ACC), bilateral insula, bilateral striatum (including caudate,  
 353 pallidum, putamen) and left pre- and postcentral gyrus (See Table 2 and Figure 2D). These  
 354 were separated in 14 anatomically defined subclusters from which parameter estimates were  
 355 extracted. After collapsing results over hemispheres there were 10 regions included in the  
 356 analysis for replication in the replication sample (see Figure 2E). Out of these 10 regions,  
 357 bilateral insula and putamen, mid cingulum, left pre- and postcentral gyrus, and SMA had  
 358 significantly more activation for Experienced Inclusion than for Experienced Exclusion (all  $p <$   
 359  $.01$ ) in the replication sample (see Figure 2F). For completeness the results of the contrast  
 360 Experienced Inclusion > Experienced Exclusion in the replication sample are also reported in  
 361 Table 2.

362

363 **Table 2.** Whole brain table for neural activation in the contrast “Experienced Inclusion >  
 364 Experienced Exclusion” for the pilot and test sample, with a cluster corrected threshold of  $p <$   
 365  $.05$  FDR-corrected, at an initial threshold of  $p < .005$

Name	Voxels	T-Value	MNI Coordinates		
			X	Y	Z
<b>PILOT</b>					

*Experienced Inclusion > Experienced Exclusion*

R Cerebellum	495	12.78	27	-55	-26
R Precuneus		9.75	15	-52	20
Cerebellar Vermis		7.54	5	-55	-11
L Thalamus	2740	11.94	-12	-16	7
		8.12	-12	-7	-2
L IFG		7.77	-51	8	7
L Postcentral Gyrus	2006	10.26	-36	-22	49
		8.22	-48	-22	49
L Anterior Cingulate Cortex		9.19	-12	23	31

**TEST**

*Experienced Inclusion > Experienced Exclusion*

L Postcentral Gyrus	2714	9.54	-45	-37	58
		8.58	-51	-25	58
L Precentral Gyrus		9.51	-39	-25	58
R Insula	393	5.97	33	23	7
		4.18	35	17	-8
R Putamen		3.53	21	8	-5
L Insula	877	5.56	-30	14	13
		4.52	-39	-7	22
L Pallidum		5.21	-21	2	-2
L Middle Frontal Gyrus	223	4.12	-33	47	28
		3.95	-35	47	37
		3.79	-45	41	31

**REPLICATION**

*Experienced Inclusion > Experienced Exclusion*

R SMA	1456	8.46	6	2	55
L Precentral Gyrus		7.46	-36	-28	61
L SMA		6.69	-6	2	49

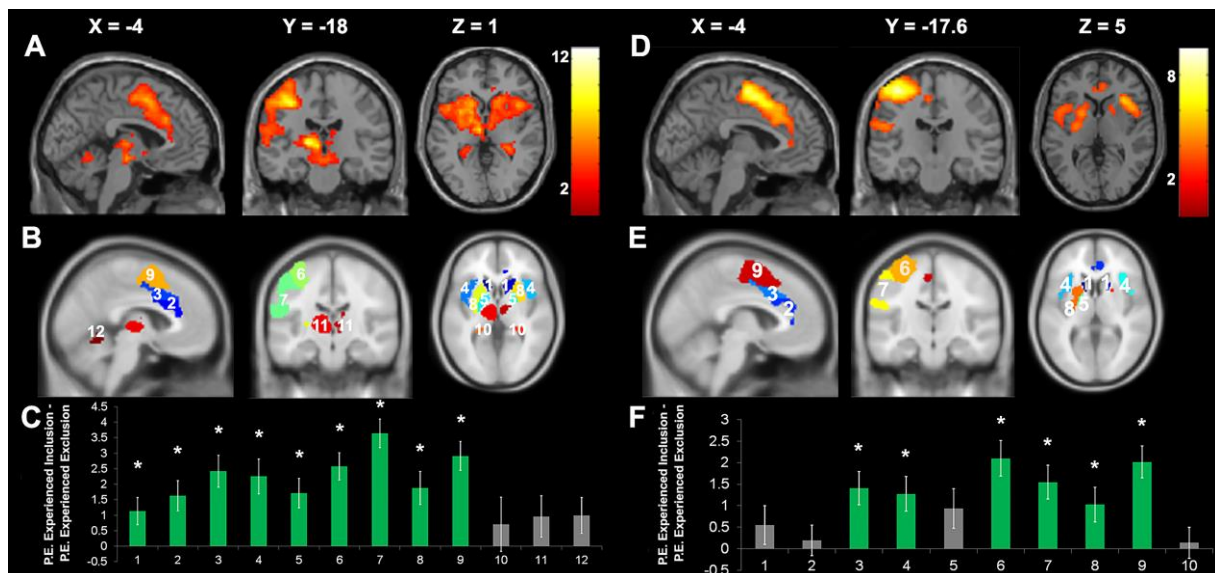
366

367

368 **Figure 2. (A)** Whole brain results for the contrast “Experienced Inclusion > Experienced  
 369 Exclusion” in the pilot sample. **(B)** Representation of anatomically separated ROI subclusters  
 370 based on whole brain results: bilateral caudate (1), anterior cingulum (2), mid cingulum (3),



371 bilateral insula (4), bilateral pallidum (5), left postcentral gyrus (6), left precentral gyrus (7),  
 372 bilateral putamen (8), SMA (9), bilateral hippocampus (10), bilateral thalamus (11) and  
 373 cerebellum (12). **(C)** Difference scores of activity in ROI subclusters in test sample. **(D)**  
 374 Whole brain results for the contrast “Experienced Inclusion > Experienced Exclusion” in the  
 375 test sample. **(E)** Representation of anatomically separated ROI subclusters based on whole  
 376 brain results: bilateral caudate (1), anterior cingulum (2), mid cingulum (3), bilateral insula (4),  
 377 bilateral pallidum (5), left postcentral gyrus (6), left precentral gyrus (7), bilateral putamen (8),  
 378 SMA (9), and left middle frontal gyrus (10). **(F)** Difference scores of activity in ROI  
 379 subclusters in replication sample.



380  
 381 P.E. = parameter estimates. Error bars represent standard errors of the mean. Green bars and asterisks (\*)  
 382 indicate replicated results.

### 384 3.2.2 Experienced Exclusion > Experienced Inclusion

385 Next, we tested the reversed contrast: Experienced Exclusion > Experienced Inclusion. In the  
 386 pilot sample, this analysis resulted in two regions, a cluster in the left orbitofrontal lobe and a  
 387 cluster in the occipital lobe (see Table 3 and Figure 3A). Two participants in the test sample  
 388 had neural masks that did not completely cover these specific regions. Therefore one  
 389 participant was excluded from analysis of activity in the left orbitofrontal lobe and one  
 390 participant was excluded from analysis of activity in the left calcarine gyrus.

391 The analysis of parameter estimates extracted from the ROIs from this contrast and  
 392 tested in the test sample showed that both regions were replicated in the test sample as  
 393 showing greater activation for Experienced Exclusion than Experienced Inclusion (all  $p <$   
 394  $.005$ ; see Table 3 and Figure 3D). As a next step, the same whole brain analysis was  
 395 performed in the test sample, which resulted in four regions: a cluster in the right paracentral  
 396 lobe, two clusters in the occipital lobe, and a cluster in the left middle orbital gyrus. ROI  
 397 values were extracted to test for replication in the replication sample. All four regions were  
 398 replicated in the replication sample as showing greater activation for Experienced Exclusion  
 399 than Experienced Inclusion (all  $p < .001$ ). For completeness the results of the contrast  
 400 Experienced Inclusion > Experienced Exclusion in the replication sample are also reported in  
 401 Table 3.

402

403 **Table 3.** Whole brain table for neural activation in the contrasts “Experienced Exclusion >  
 404 Experienced Inclusion” for the pilot and test sample, with a cluster corrected threshold of  $p <$   
 405  $.05$  FDR-corrected, at an initial threshold of  $p < .005$

Name	Voxels	T-Value	MNI Coordinates		
			X	Y	Z
<b>PILOT</b>					
<i>Experienced Exclusion &gt; Experienced Inclusion</i>					
L Calcarine Gyrus	1422	6.79	-9	-91	-5
L Superior Occipital Gyrus		5.42	-18	-85	34
R Cuneus		5.41	9	-91	25
L Inferior Frontal Gyrus	264	6.75	-39	26	-17
		5.05	-18	17	-23
		4.77	-51	38	-8
<b>TEST</b>					
<i>Experienced Exclusion &gt; Experienced Inclusion</i>					
R Cuneus	467	8.12	21	-91	10
R Lingual Gyrus		5.14	15	-97	-11
R Calcarine Gyrus		5.10	18	-97	-2

L Middle Occipital Gyrus	373	7.58	-18	-94	7
		4.80	-48	-79	-17
L Inferior Occipital Gyrus		4.47	-33	-94	-11
L Inferior Frontal Gyrus	326	5.96	-57	41	1
		5.21	-57	23	-11
		4.73	-51	41	-14
R Paracentral Lobe	543	4.85	-3	-58	76
		4.59	0	-25	73
R Precuneus		4.58	3	-73	54

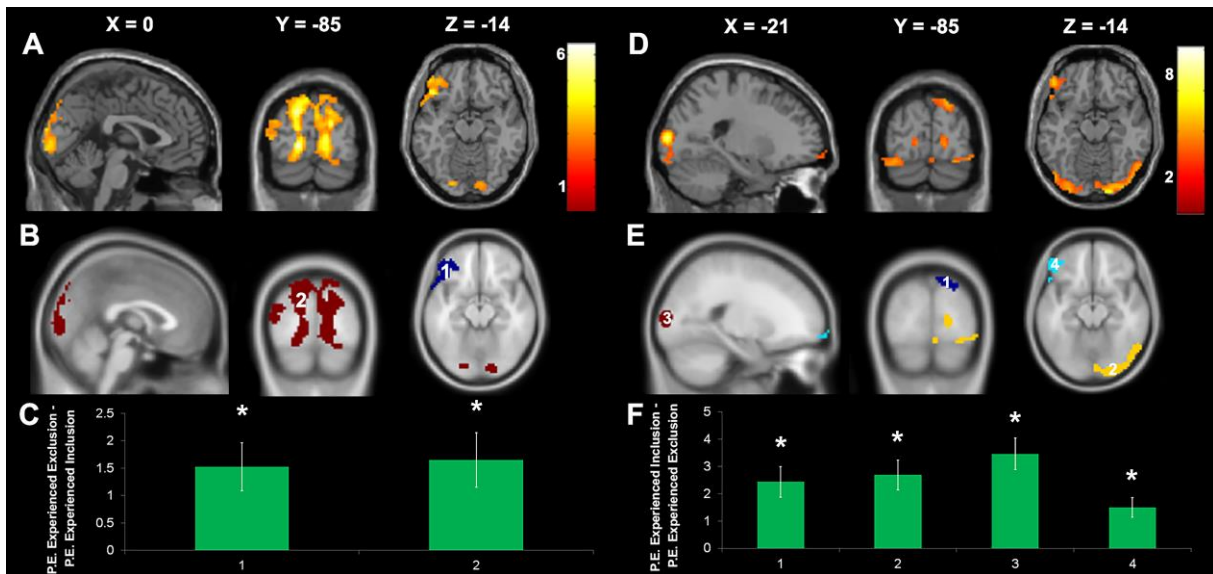
## REPLICATION

### *Experienced Exclusion > Experienced Inclusion*

R Superior Occipital Gyrus	2758	7.34	24	-91	10
L Superior Occipital Gyrus		6.62	-15	-91	4
L Middle Occipital Gyrus		6.44	-27	-91	13
R Superior Frontal Gyrus	1721	7.23	21	32	64
L Superior Frontal Gyrus		6.87	-12	38	61
R Superior Frontal Gyrus		6.82	15	44	58
L Temporal Pole	1052	7.09	-57	17	-23
L Inferior Frontal Gyrus		6.63	-54	35	-17
		6.46	-57	26	-11
R Inferior Frontal Gyrus	387	5.06	33	29	-23
		5.03	30	38	-17
		4.70	42	29	-23

406

407 **Figure 3. (A)** Whole brain results for the contrast “Experienced Exclusion > Experienced  
408 Inclusion” in the pilot sample. **(B)** Representation of anatomically separated ROI subclusters  
409 based on whole brain results: left IFG (1), and calcarine gyrus (2). **(C)** Difference scores of  
410 activity in ROI subclusters in the test sample. **(D)** Whole brain results for the contrast  
411 “Experienced Exclusion > Experienced Inclusion” in the test sample. **(E)** Representation of  
412 anatomically separated ROI subclusters based on whole brain results: right paracentral  
413 lobule (1), right cuneus (2), left middle occipital gyrus (3), and left middle orbital gyrus (4). **(F)**  
414 Difference scores of activity in ROI subclusters in the replication sample.



415  
 416 P.E. = parameter estimates. Error bars represent standard errors of the mean. Green bars and asterisks (\*)  
 417 indicate replicated results.

418

### 419 3.3 Whole brain ALE meta-analysis

420 To investigate common activation in the contrast Experienced Inclusion > Experienced  
 421 Exclusion and its reversal, we performed a meta-analysis across the three samples. We  
 422 found common activation in the contrast Experienced Inclusion > Experienced Exclusion in  
 423 three clusters, namely the SMA/anterior cingulate, putamen/pallidum, and pre/postcentral  
 424 gyrus (see Figure 4A, for coordinates see Table 3). For the reversed contrast, Experienced  
 425 Exclusion > Experienced Inclusion, we found common activation in three clusters, including  
 426 clusters in the occipital lobe and left orbitofrontal cortex (OFC; see Figure 4B, for coordinates  
 427 see Table 4).

428

429 **Table 4.** Whole brain table for common activation across the three samples for the contrasts  
 430 “Experienced Inclusion > Experienced Exclusion” and “Experienced Exclusion > Experienced  
 431 Inclusion”.

Name	Voxels	MNI Coordinates		
		X	Y	Z
<i>Experienced Inclusion &gt; Experienced Exclusion</i>				
L SMA	3736	-6	6	50

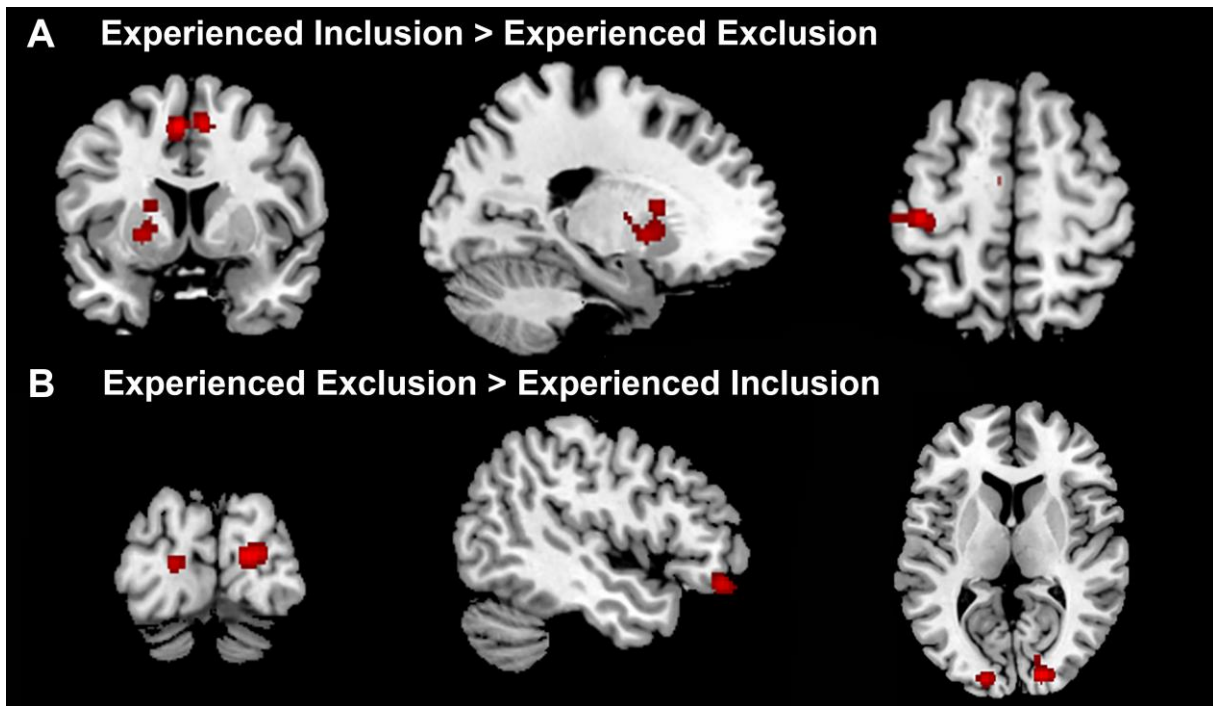
		-8	10	44
		-6	-10	60
		-12	-10	60
R SMA		8	8	50
L Anterior Cingulate Cortex		-10	24	31
R Middle Cingulate Cortex		8	16	44
L Middle Cingulate Cortex		-8	16	38
L Putamen	1680	-22	4	-2
		-18	10	12
		-18	10	0
		-24	-6	10
L Pallidum		-18	-4	4
L Caudate		-16	16	4
L Precentral Gyrus	1064	-40	-24	58
L Postcentral Gyrus		-50	-24	58
		-48	-22	50
<i>Experienced Exclusion &gt; Experienced Inclusion</i>				
R Cuneus	1176	18	-91	8
R Calcarine Gyrus		16	-80	10
L Orbitofrontal Cortex	1136	-50	42	-14
L Superior Occipital Gyrus	880	-16	-92	6

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432

433 **Figure 4.** Results from the whole brain ALE meta-analysis for the contrasts **(A)** Experienced

434 Inclusion > Experienced Exclusion and **(B)** Experienced Exclusion > Experienced Inclusion



435

436

437 **3.4 Neural reactions to Prosocial Compensating Behavior**

438 *3.4.1 Compensating versus Tossing to excluders*

439 In the pilot sample, the contrast Compensating > Tossing to excluders resulted in one cluster  
 440 in the occipital lobe (see Table 5). The reversed contrast resulted in another single cluster in  
 441 the occipital lobe. ROIs were extracted for replication, but these regions were not replicated  
 442 in the test sample. In the test sample, the contrast Compensating > Tossing to excluders and  
 443 the reversed contrast did not result in significant activations. Because we found no significant  
 444 activations in the test sample, we did not test this contrast in the replication sample.

445

446 **Table 5.** Whole brain table for neural activation in the contrast Compensating > Tossing to  
 447 excluders (and reversed), with a cluster corrected threshold of  $p < .05$  FDR-corrected, at an  
 448 initial threshold of  $p < .005$

Name	Voxels	T-Value	MNI Coordinates		
			X	Y	Z

**PILOT**

*Compensating > Tossing to excluders*

L Cuneus	149	5.42	-6	-94	16
		4.35	-5	-91	25
L Calcarine Gyrus		5.08	3	-94	13
<i>Tossing to excluders &gt; Compensating</i>					
R Calcarine Gyrus	195	6.22	12	-76	7
R Lingual Gyrus		3.28	9	-58	1
		3.89	15	-54	-5

---

449

#### 450 3.4.2 Meta-analytic results for independent ROIs

451 The absence of neural effects for prosocial compensating behavior was unexpected  
452 considering the behavioral results and the results of previous studies on neural correlates of  
453 Cyberball (van der Meulen et al., 2016). Therefore, we performed a meta-analysis on pre-  
454 defined ROIs from an adult study (van der Meulen et al., 2016): the bilateral insula, left and  
455 right TPJ, and bilateral NAcc. Parameter estimates from these ROIs were extracted and  
456 combined in a meta-analysis. However, we found no significant pattern of activation during  
457 prosocial behavior across the three samples (see supplementary table S1).

458

#### 459 3.5 Relation with prosocial compensating behavior

460 Lastly, we were interested in whether activity in areas that were observed in the meta-  
461 analyses was related to prosocial compensating behavior. Therefore, we created spheres  
462 based on the coordinates of the clusters found in the meta-analyses. We chose coordinates  
463 for the ACC, putamen, pre-/postcentral gyrus, SMA in the “Experienced Inclusion >  
464 Experienced Exclusion” contrast, and coordinates for the OFC in the “Experienced Exclusion  
465 > Experienced Inclusion” contrast (see Table 3). Spheres were created with a diameter of 5  
466 mm. The resulting spheres were then submitted to ROI analyses for each of the three  
467 samples, and resulting parameter estimates were correlated with prosocial compensating  
468 behavior (defined as the compensating score obtained in the PCG). In all three samples no  
469 significant associations were found between prosocial compensating behavior and parameter  
470 estimates from any of the ROIs.

471

472

#### 473 **4. Discussion**

474 This study examined the neural correlates of observing social exclusion in a four-player  
475 Prosocial Cyberball Game during middle childhood. As expected, the exclusion of a fourth  
476 player by two others resulted in increased ball tossing by the participant to the excluded  
477 player. This is consistent with earlier findings of helping or compensating behavior in children  
478 who observed social exclusion of others (Vrijhof et al., 2016; Will et al., 2013). The behavior  
479 was robust across three samples. Furthermore, in a meta-analysis across the three samples  
480 there was increased activity in striatum and dACC when participants experienced inclusion  
481 themselves, and increased activity in orbitofrontal cortex when participants experienced  
482 exclusion, consistent with prior studies showing that these are important areas for the  
483 feelings of inclusion and exclusion in traditional Cyberball games (Lieberman & Eisenberger,  
484 2009). However, contrary to our expectations, there were no neural regions that  
485 distinguished between compensating an excluded player and tossing the ball to the non-  
486 excluded players. The pattern of increased activity in social-affective brain regions as  
487 previously found in adults (van der Meulen et al., 2016) could not be confirmed in 7-10-year-  
488 old children, even when we used specific regions of interest in the social brain network or in a  
489 meta-analysis.

490 The strongest and most consistent findings were observed for the contrast  
491 experienced self-inclusion versus experienced self-exclusion. That is to say, experienced  
492 self-inclusion (receiving the ball from the two excluding players) was associated with  
493 increased activity in the striatum and the dACC in each of the three samples, and this was  
494 confirmed in a meta-analysis. These neural regions have also been consistently implicated in  
495 reward processing (Bhanji & Delgado, 2014; Delgado, 2007), and dACC activity specifically  
496 has been argued to signal evaluation and appraisal of an upcoming event (Shenhav, Cohen,  
497 & Botvinick, 2016). These findings may indicate that self-inclusion is important for children in



498 ball tossing games. Indeed, prior studies showed that children who were not included by their  
499 peers reported feeling less happy and more angry (Saylor et al., 2013), and showed higher  
500 levels of cortisol, an indication of increased levels of stress (Gunnar, Sebanc, Tout, Donzella,  
501 & van Dulmen, 2003).

502         The reversed contrast, experienced self-exclusion (not receiving the ball from the two  
503 excluding players) was associated with activation in the orbitofrontal cortex. This region was  
504 previously observed in adults in a meta-analysis on social exclusion (Cacioppo et al., 2013),  
505 possibly indicating that this region is generally observed across children and adults when not  
506 being included. The orbitofrontal cortex is thought to play a role in managing social  
507 perceptions (Hughes & Beer, 2012). It should be noted that prior studies, including meta-  
508 analyses (Cacioppo et al., 2013), also pointed to the dACC and bilateral insula as important  
509 regions for exclusion, whereas in the current study the dACC was observed for inclusion.  
510 However, the role of the dACC and insula in exclusion has been debated, and possibly it is  
511 signaling the salience of an event (Menon & Uddin, 2010; Seeley et al., 2007) rather than  
512 specific activation for social events. Taken together, across three samples and confirmed by  
513 a meta-analysis, we observed consistent neural activation patterns for experienced self-  
514 inclusion and self-exclusion in 7-10-year-old children, validating this as a paradigm to  
515 investigate responses to a situation of social exclusion.

516         We found no evidence in the current study for neural regions that correlate with  
517 prosocial compensating behavior, that is to say, ball tossing to the excluded player versus  
518 ball tossing to the other players. This is surprising, because behaviorally there was a strong  
519 and consistent compensating pattern in all three samples. We previously observed in adults  
520 that bilateral insula, TPJ and NAcc were activated when tossing to an excluded player versus  
521 tossing to the other players (van der Meulen et al., 2016). However, previous studies that  
522 examined giving behavior in children and adolescents observed that children do not yet  
523 differentiate between intentions for giving (Güroğlu, van den Bos, & Crone, 2009) and that  
524 activity in TPJ associated with intention understanding develops during adolescence  
525 (Güroğlu, van den Bos, van Dijk, Rombouts, & Crone, 2011). Even though children as young

526 as four years old understand the norms for fair distributions of goods, they only behave in  
527 accordance with those norms when they reach the age of eight (Smith, Blake, & Harris,  
528 2013). Furthermore, it is unclear when children's motivations for fair behavior shift from a  
529 desire to follow the norms to the understanding of someone else's needs. The current study  
530 cannot give a conclusive answer to this question because there was no comparison group  
531 with older participants. However, earlier research has indicated that activity in TPJ increases  
532 with age, especially for situations where perspective taking is required (Crone, 2013).  
533 Therefore, it would be interesting for future studies to test whether this developmental  
534 increase extends to other social brain regions, and whether this increase in activity can be  
535 related to changing motives for prosocial compensating behavior.

536         This study has significant strengths, such as the replication design that was used to  
537 test and replicate results from one sample to two other samples. The addition of a meta-  
538 analytic approach further confirmed our results. Furthermore, the current study is one of the  
539 first to investigate behavioral and neural correlates of prosocial compensating behavior in  
540 middle childhood. Nevertheless, there also were some limitations that should be addressed  
541 in future studies. First, the two processes studied (prosocial compensating behavior and  
542 experience of possible self-exclusion) are dependent on each other, as the participant first  
543 has to receive the ball from the excluders before they are able to engage in prosocial  
544 compensating behavior. This might provide a bias for the analysis used in this study although  
545 the number of tosses in each contrast was comparable. Second, the contrast used to study  
546 neural findings for prosocial compensating behavior (tossing to excluded player vs tossing to  
547 other players in the unfair situation) might not be the optimal situation to study these  
548 reactions. Ideally, a comparison similar to the difference score in the behavioral results would  
549 be made: a comparison in tossing to player 2 during the unfair situations versus tossing to  
550 player 2 during the fair situation. However, given that imaging data was not collected during  
551 the fair situation, we believe that we have chosen the best possible contrast to measure  
552 prosocial behavior, as it only includes behavior from the participant (tossing to excluded or to  
553 other players) and is therefore comparable in for example motion and time-one-task

554 confounds. Third, the test and replication sample were not completely independent from each  
555 other. For these two samples same-sex co-twins were randomly assigned to the test or  
556 replication sample. Therefore, the results could be more similar for the test and replication  
557 sample than for the pilot sample. In fact, the replication step from test to replication sample  
558 was optimized in that the two samples were perfectly matched on age, gender, family  
559 background, and in about half of the cases even on genetic make-up. A randomized co-twin  
560 design leaves much less room for alternative interpretations in case of non-replication.  
561 Finally, the sample size of our three samples was too small to examine individual differences  
562 in motives for prosocial compensating behavior. This would be an important step in  
563 investigating the underlying reasons for children to engage in prosocial behavior in the  
564 Prosocial Cyberball Game, and therefore this question should be addressed in a larger  
565 sample.

566           In conclusion, the current study confirmed the hypothesis that children ages 7-10-  
567 years show prosocial compensating behavior in a relatively new paradigm in children: the  
568 Prosocial Cyberball Game. Interestingly, we found no strong evidence for specific neural  
569 activity related to prosocial compensating behavior towards the excluded player, but robust  
570 evidence was found for neural contributions to feelings of self-inclusion and –exclusion. The  
571 relation between prosocial compensating behavior and neural activity during self-inclusion  
572 and –exclusion is not yet clear, but possibly these findings highlight the switch from self to  
573 other motivations to engage in prosocial compensating behavior in late childhood and  
574 emerging adolescence. Alternatively, there may be important individual differences between  
575 children that emerge in larger samples. These hypotheses will be tested in a future  
576 longitudinal design, as these children will be followed over several years. Here, we presented  
577 a new approach to the hotly debated issue of replicability in behavioral and neuroscience  
578 showing that answers might be dependent on specific contrasts and underlying neural  
579 mechanisms even within the same paradigm.

580

581

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586

587

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