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Running head: Dealing with social exclusion in childhood

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Abstract

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

Keywords

Social exclusion
Prosocial behavior
fMRI
Childhood
Meta-analysis

1. Introduction

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

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

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

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

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

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

2. Materials and Methods

2.1 Participants

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

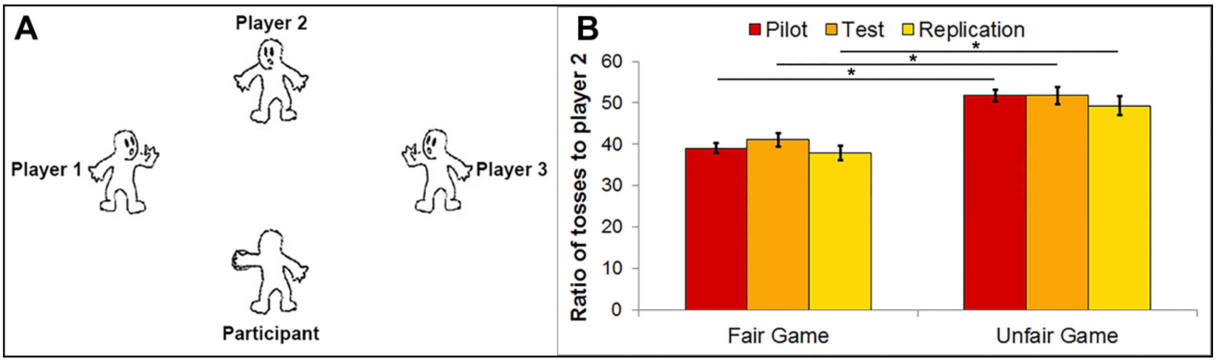
Some participants were excluded from analyses due to excessive head motion during the MRI session or because they did not finish the scanning session (two children from the pilot sample, three children from the test sample, and four from the replication sample). The final pilot sample consisted of 18 children (*M* = 8.15 years, *SD* = 1.06, 55.6% male), the final test sample of 27 children (*M* = 8.23 years, *SD* = 0.67, 40.7% male), and the final replication sample of 26 children (*M* = 8.21 years, *SD* = 0.71, 42.3% male). The three samples did not significantly differ in age ($F(2, 68) = .04$, $p = .96$) or gender ($X^2(2) = 1.08$, $p = .58$). All participants were screened for MRI contra indications, had normal (or corrected to normal) vision, were fluent in Dutch, and had no physical or psychological disorder that disabled their performance on the tasks. Written informed consent was obtained from both parents before the start of the study. Parents received €50 for the participation of their children, and all children received €3.50 and a goodie bag with small presents. The study was approved by the Dutch Central Committee on Research Involving Human Subjects.

2.2 Experimental Design

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

The game consisted of two parts: the Fair Game and the Unfair Game. During the first part (the Fair Game), the game was programmed to ensure that all four players received the ball an equal number of times. During the second part (the Unfair Game), either player 1 or player 3 tossed the ball only once to player 2 (at the top of the screen). After this initial toss, player 1 and player 3 no longer tossed the ball to player 2, thereby creating a situation of *observed* social exclusion for the participant. The participant could therefore choose to compensate for the exclusion by tossing more balls to excluded player 2, or to contribute to the exclusion by tossing more balls to players 1 and 3. The location of the excluded player was always the same for all participants (directly across the participant, at the top of the screen). In both the Fair Game and the Unfair Game, each trial consisted of a ball toss with a duration of 2000 ms. After each ball toss a jitter was added with a duration ranging from 1000-2000 ms in steps of 500 ms. The Fair Game consisted of 120 trials and was played on a laptop outside the MRI scanner. The Unfair Game consisted of 168 trials and was played in the MRI scanner, to enable collection of behavioral and MRI data during the task. During the Unfair Game, participants could indicate their response by pressing a button on a box attached to their right leg. The Unfair Game was presented in two separate parts to provide participants with a small rest period in between. During the entire game, the excluding players were referred to as Players 1 and 3 (on the left and right side of the screen respectively), the excluded player was referred to as Player 2, and the participant was referred to as "Participant" (see Figure 1A).

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



2.3 Procedure

Participants were given an extensive explanation and practice session in a mock scanner to familiarize them with the procedure of an MRI scan. All participants played the Fair Game of the PCG before the scanning session. Co-twins were then randomly assigned to either start with the scan session (and thus perform the Unfair Game of the PCG) or to start with other behavioral tasks that were part of the larger L-CID study. All twin pairs (from the pilot sample or from the test/replication sample) were randomly assigned to one of two procedures on the day of data collection.

2.4 MRI data acquisition

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

2.5 MRI data analyses

All data were analyzed with SPM8 (Wellcome Department of Cognitive Neurology, London). Images were corrected for slice timing acquisition and differences in rigid body motion. Functional volumes were spatially normalized to T1 templates. The normalization algorithm used a 12-parameter affine transform together with a nonlinear transformation involving cosine basis functions and resampled the volumes to 3 mm cubic voxels. Templates were based on the MNI305 stereotaxic space (Cocosco, Kollokian, Kwan, & Evans, 1997). Functional volumes were spatially smoothed with a 6 mm full width at half maximum (FWHM) isotropic Gaussian kernel. As a final step, the ArtRepair module (Mazaika, Hoeft, Glover, & Reiss, 2009) was used to address any head motions in the data. The threshold was set at 2 mm, and participants were excluded if more than 20% of the dynamics of the two functional runs were affected.

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

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

2.5.1 Whole brain analyses

We computed two different contrasts to study the various reactions to observed social exclusion. First, to investigate the neural response to being potentially excluded from the game by the other two players, we tested the contrast: *Experienced Inclusion > Experienced Exclusion* (and the reversed contrast). In accordance with the programming of the game, over the three samples the percentage of tosses from excluding Players 1 and 3 to the participant (M = 50.08, SD = .74) was comparable to the number of tosses from Players 1 and 3 to each other (M = 49.92, SD = .74). Over the three samples the percentage of tosses to the excluded player (M = 50.86, SD = 10.20) was comparable to the number of tosses to the two excluding players combined (M = 49.14, SD = 10.20). Second, to investigate the neural response to prosocial compensating behavior, we tested the contrast: *Compensating > Tossing to excluders* (and the reversed contrast). Significant task-related responses exceeded a cluster-corrected threshold of $p < .05$ FDR-corrected, with a primary threshold of $p < .005$ (Woo, Krishnan, & Wager, 2014).

2.5.2 Region of interest analyses to test for replication effects

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

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

To specifically explore the neural response during prosocial behavior across all three samples and to align our activation patterns with those found in adults, we used additional independent ROIs that were used in a study on prosocial neural responses in adults (see van der Meulen et al. (2016)). In the adult study, Neurosynth templates were used to create masks of the dorsal anterior cingulate cortex (dACC), bilateral insula, medial prefrontal cortex (mPFC), temporo-parietal junction (TPJ), and nucleus accumbens (NAcc). We used these masks to extract parameter estimates for the conditions “Compensating” and “Tossing” in all three samples. Combined effect sizes were computed with the Comprehensive Meta-Analysis (CMA) program (Borenstein, Rothstein, & Cohen, 2005).

2.5.3 Meta-analysis

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

3. Results

3.1 Behavioral results

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

First, in the pilot sample we found a significant difference in ratio of tosses to Player 2 in the Fair Game compared to the Unfair Game ($t(17) = -5.68$, $p = < .001$, $d = 2.20$). This finding was replicated in the test sample ($t(26) = -5.27$, $p < .001$, $d = 1.11$), and in the replication sample ($t(25) = -4.04$, $p < .001$, $d = 1.10$; see Table 1 for descriptives). Second, because children differed in their percentage of tosses to Player 2 in the Fair Game (see Figure 1B), we took these base-line differences into account by calculating a difference score between percentage of tosses to Player 2 in the Unfair Game minus the percentage of tosses to Player 2 in the Fair Game. Thus, for each participant a compensating score was calculated. We used an ANOVA to test whether there was a difference in compensating scores for the three samples, and found no significant difference ($F(2, 68) = .15$, $p = .86$). This shows that levels of prosocial compensating behavior were the same across the three samples during middle childhood.

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

3.2 Neural reactions to Playing with Others

3.2.1 Experienced Inclusion > Experienced Exclusion

First, we tested the contrast Experienced Inclusion > Experienced Exclusion in the pilot sample with a whole brain analysis. The contrast was defined as receiving the ball from excluding Players 1 and 3 (“Experienced Inclusion”) versus not receiving the ball from excluding Players 1 and 3 (“Experienced Exclusion”). The Experienced Inclusion > Experienced Exclusion analysis resulted in significant activation in several clusters that spanned medial prefrontal cortex (PFC; including pre-supplementary motor area (SMA), ACC), bilateral insula, bilateral striatum (including caudate, pallidum, putamen) and left pre- and postcentral gyrus (See Table 2 and Figure 2A). These were separated in 18 anatomically defined subclusters from which parameter estimates were extracted. When no significant differences were found between hemispheres, results were collapsed across left and right hemispheres. This resulted in a total of 12 regions that were analyzed in the test sample (see Figure 2B). Out of these 12 regions, bilateral caudate, insula, pallidum, and putamen, anterior and mid cingulum, left pre- and postcentral gyrus, and SMA, had significantly more activation for Experienced Inclusion than for Experienced Exclusion (all $p < .008$) in the test sample (see Figure 2C).

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

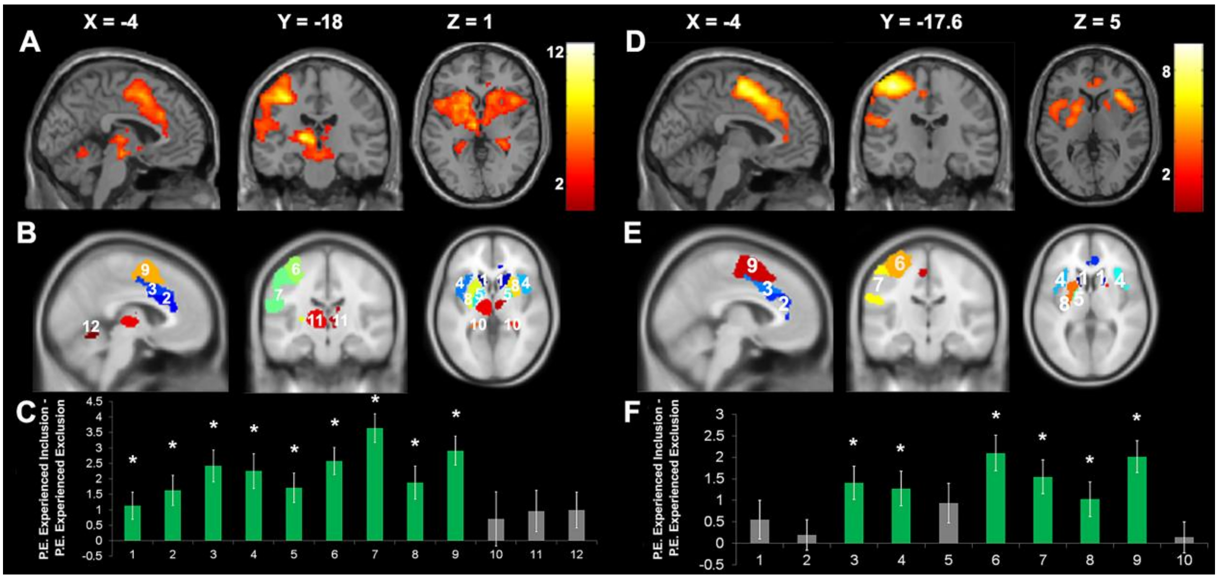
Table 2. Whole brain table for neural activation in the contrast “Experienced Inclusion > Experienced Exclusion” for the pilot and test sample, with a cluster corrected threshold of $p < .05$ FDR-corrected, at an initial threshold of $p < .005$

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
PILOT					

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

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

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



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

3.2.2 Experienced Exclusion > Experienced Inclusion

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

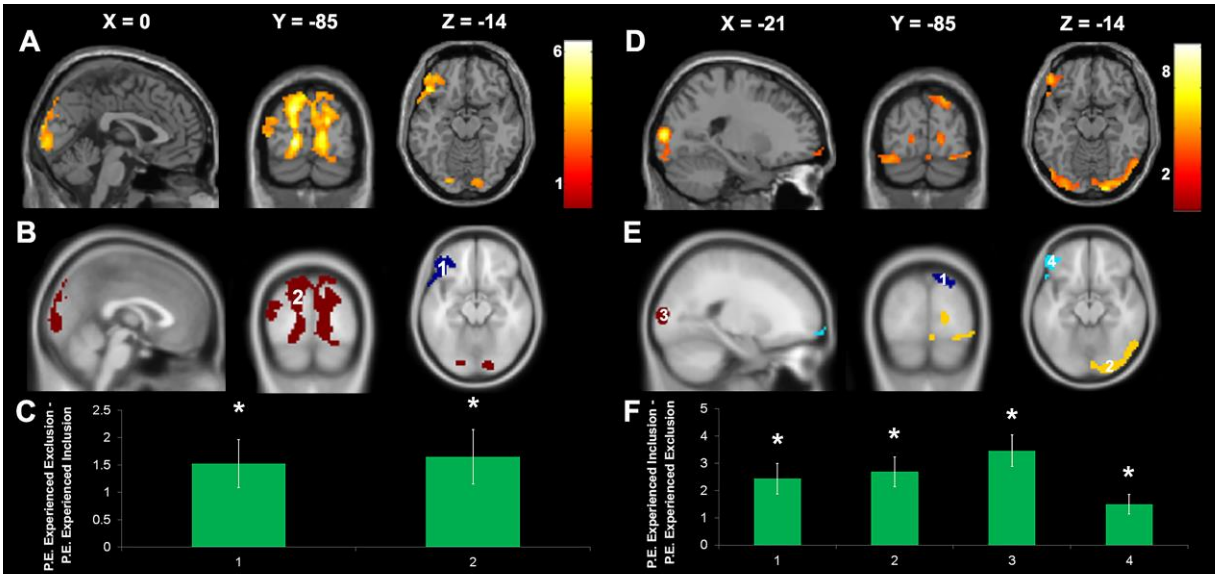
The analysis of parameter estimates extracted from the ROIs from this contrast and tested in the test sample showed that both regions were replicated in the test sample as showing greater activation for Experienced Exclusion than Experienced Inclusion (all *p* < .005; see Table 3 and Figure 3D). As a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

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

			MNI Coordinates		
Name	Voxels	T-Value	X	Y	Z
PILOT					
Experienced Exclusion > Experienced Inclusion					
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
TEST					
Experienced Exclusion > Experienced Inclusion					
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

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



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

3.3 Whole brain ALE meta-analysis

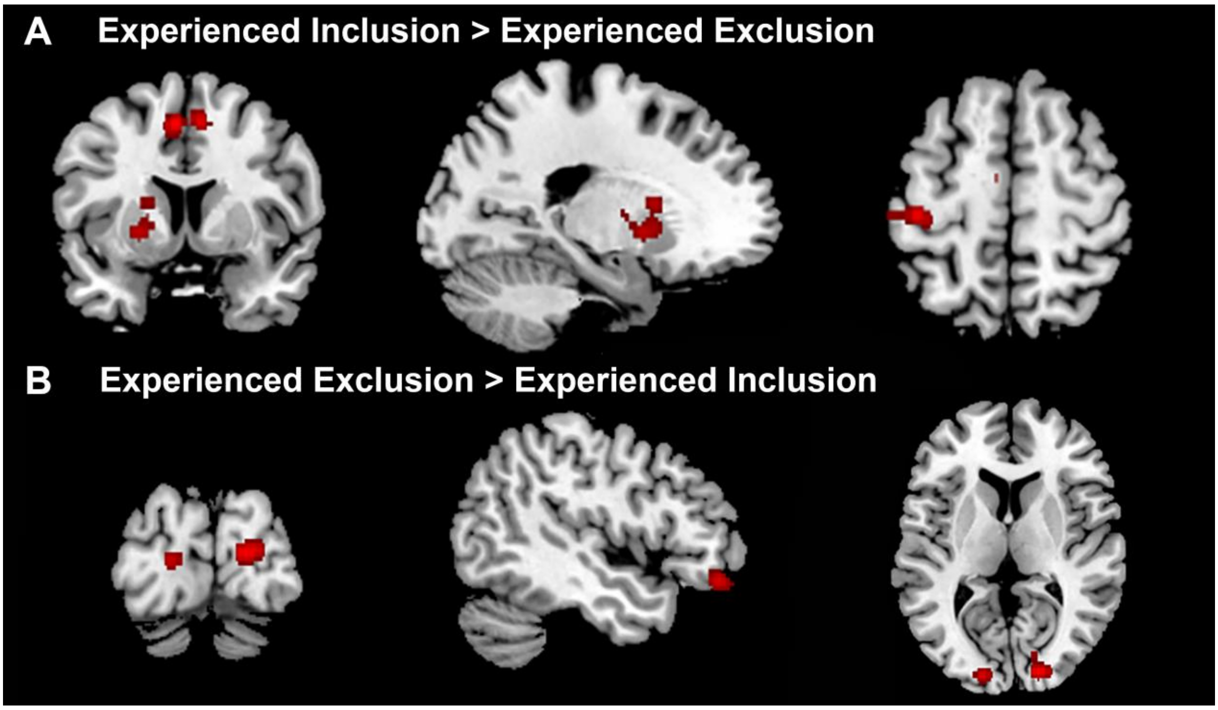
To investigate common activation in the contrast Experienced Inclusion > Experienced Exclusion and its reversal, we performed a meta-analysis across the three samples. We found common activation in the contrast Experienced Inclusion > Experienced Exclusion in three clusters, namely the SMA/anterior cingulate,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

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

		MNI Coordinates		
Name	Voxels	X	Y	Z
Experienced Inclusion > Experienced Exclusion				
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
Experienced Exclusion > Experienced Inclusion				
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

Figure 4. Results from the whole brain ALE meta-analysis for the contrasts **(A)** Experienced Inclusion > Experienced Exclusion and **(B)** Experienced Exclusion > Experienced Inclusion



3.4 Neural reactions to Prosocial Compensating Behavior

3.4.1 Compensating versus Tossing to excluders

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

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

			MNI Coordinates		
Name	Voxels	T-Value	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
Tossing to excluders > Compensating					
R Calcarine Gyrus	195	6.22	12	-76	7
R Lingual Gyrus		3.28	9	-58	1
		3.89	15	-54	-5

3.4.2 Meta-analytic results for independent ROIs

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

3.5 Relation with prosocial compensating behavior

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

4. Discussion

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

The strongest and most consistent findings were observed for the contrast experienced self-inclusion versus experienced self-exclusion. That is to say, experienced self-inclusion (receiving the ball from the two excluding players) was associated with increased activity in the striatum and the dACC in each of the three samples, and this was confirmed in a meta-analysis. These neural regions have also been consistently implicated in reward processing (Bhanji & Delgado, 2014; Delgado, 2007), and dACC activity specifically has been argued to signal evaluation and appraisal of an upcoming event (Shenhav, Cohen, & Botvinick, 2016). These findings may indicate that self-inclusion is important for children in ball tossing games. Indeed, prior studies showed that children who were not included by their peers reported feeling less happy and more angry (Saylor et al., 2013), and showed higher levels of cortisol, an indication of increased levels of stress (Gunnar, Sebanc, Tout, Donzella, & van Dulmen, 2003).

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

We found no evidence in the current study for neural regions that correlate with prosocial compensating behavior, that is to say, ball tossing to the excluded player versus ball tossing to the other players. This is surprising, because behaviorally there was a strong and consistent compensating pattern in all three samples. We previously observed in adults that bilateral insula, TPJ and NAcc were activated when tossing to an excluded player versus tossing to the other players (van der Meulen et al., 2016). However, previous studies that examined giving behavior in children and adolescents observed that children do not yet differentiate between intentions for giving (Güroğlu, van den Bos, & Crone, 2009) and that activity in TPJ associated with intention understanding develops during adolescence (Güroğlu, van den Bos, van Dijk, Rombouts, & Crone, 2011). Even though children as young as four years old understand the norms for fair distributions of goods, they only behave in accordance with those norms when they reach the age of eight (Smith, Blake, & Harris, 2013). Furthermore, it is unclear when children's motivations for fair behavior shift from a desire to follow the norms to the understanding of someone else's needs. The current study cannot give a conclusive answer to this question because there was no comparison group with older participants. However, earlier research has indicated that activity in TPJ increases with age, especially for situations where perspective taking is required (Crone, 2013). Therefore, it would be interesting for future studies to test whether this developmental increase extends to other social brain regions, and whether this increase in activity can be related to changing motives for prosocial compensating behavior.

This study has significant strengths, such as the replication design that was used to test and replicate results from one sample to two other samples. The addition of a meta-analytic approach further confirmed our results. Furthermore, the current study is one of the first to investigate behavioral and neural correlates of prosocial compensating behavior in middle childhood. Nevertheless, there also were some limitations that should be addressed in future studies. First, the two processes studied (prosocial compensating behavior and experience of possible self-exclusion) are dependent on each other, as the participant first has to receive the ball from the excluders before they are able to engage in prosocial compensating behavior. This might provide a bias for the analysis used in this study although the number of tosses in each contrast was comparable. Second, the contrast used to study neural findings for prosocial compensating behavior (tossing to excluded player vs tossing to other players in the unfair situation) might not be the optimal situation to study these reactions. Ideally, a comparison similar to the difference score in the behavioral results would be made: a comparison in tossing to player 2 during the unfair situations versus tossing to player 2 during the fair situation. However, given that imaging data was not collected during the fair situation, we believe that we have chosen the best possible contrast to measure prosocial behavior, as it only includes behavior from the participant (tossing to excluded or to other players) and is therefore comparable in for example motion and time-one-task confounds. Third, the test and replication sample were not completely independent from each other. For these two samples same-sex co-twins were randomly assigned to the test or replication sample. Therefore, the results could be more similar for the test and replication sample than for the pilot sample. In fact, the replication step from test to replication sample was optimized in that the two samples were perfectly matched on age, gender, family background, and in about half of the cases even on genetic make-up. A randomized co-twin design leaves much less room for alternative interpretations in case of non-replication. Finally, the sample size of our three samples was too small to examine individual differences in motives for prosocial compensating behavior. This would be an important step in investigating the underlying reasons for children to engage in prosocial behavior in the Prosocial Cyberball Game, and therefore this question should be addressed in a larger sample.

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

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