Neural and Cortisol Responses during Play with Human and Computer Partners in Children with Autism

E. Kale Edmiston¹, Kristen Merkle², Blythe A. Corbett*,¹,³,⁴

Vanderbilt University, Nashville, TN, USA

¹Vanderbilt Brain Institute, ²Vanderbilt University Institute of Imaging Science, ³Department of Psychiatry, ⁴Department of Psychology

CORRESPONDENCE

Blythe A. Corbett, PhD.

Associate Professor Department of Psychiatry & Psychology

Vanderbilt University

PMB 40, 230 Appleton Place

Nashville, TN 37203

Phone: (615) 936-0280

FAX: (615) 322-8236

Email: blythe.corbett@vanderbilt.edu

Title: 100 words

Running Head: 40 words

Abstract: 200 words

Text: 5,549 words main text

Key Words: autism, insula, social exchange, Prisoner’s dilemma, fMRI, temporal parietal junction
ABSTRACT

Background: Children with autism spectrum disorder (ASD) exhibit impairment in reciprocal social interactions, including play, which can manifest as failure to show social preference or discrimination between social and nonsocial stimuli.

Methods: To explore mechanisms underlying these deficits, we collected salivary cortisol from forty-two children 8-to-12 years with ASD or typical development during a playground interaction with a confederate child. Participants underwent functional MRI during a Prisoner’s Dilemma game requiring cooperation or defection with a human (confederate) or computer partner. Search region of interest analyses were based on previous research (e.g., insula, amygdala, temporal parietal junction).

Results: There were significant group differences in neural activation based on partner and response pattern. When playing with a human partner, children with ASD showed limited engagement of a social salience brain circuit during defection. Reduced insula activation during defection in the ASD children relative to TD children, regardless of partner type, was also a prominent finding. Insula and temporal parietal junction BOLD during defection was also associated with stress responsivity and behavior in the ASD group under playground conditions.

Discussion: Children with ASD engage social salience networks less than TD children during conditions of social salience, supporting a fundamental disturbance of social engagement.
INTRODUCTION

Social cognition requires the ability to interpret another person’s behavior, interact in complex social groups, empathize, and predict how others will feel, think, and act (Baron-Cohen et al. 1985). Impairment in social cognition is a distinguishing feature of autism spectrum disorders (ASD) (APA 2000). Children with ASD have reduced social engagement with peers during play (Corbett et al. 2010; Hauck et al. 1995) especially under conditions of solicited cooperative play by a peer (Corbett et al. 2013). In addition to reduced reciprocal social interaction during play, engaging with typically developing peers is often accompanied by increased physiological arousal as indexed by elevated cortisol responses (Corbett et al. 2010; Schupp et al. 2013). While play is a fundamental milestone in childhood, facilitating the development of important cognitive and social skills (Boucher 1999), it is often an area of significant delay and impairment in children with ASD (e.g., Farmer-Dougan and Kaszuba 1999; Hauck et al. 1995; Ingram et al. 2007; Macintosh and Dissanayake 2006; Schupp et al. 2013).

The psychobiological investigation of play allows for study of brain networks involved in social behaviors (Panksepp et al. 1984). Various disciplines have used social exchange games to explore aspects of human social behavior, such as motivation, reward, and cooperation (King-Casas et al. 2005; Panksepp et al. 1984; Rilling et al. 2002; Rilling et al. 2004; Rilling et al. 2007; Sally 2003). The Prisoner’s Dilemma (PD) provides a well-established paradigm for reciprocal interactions (Axelrod and Hamilton 1981; Deykin et al. 2007; Patel et al. 2006; Rilling et al. 2002). The PD relies on either cooperation or defection with partners, rendering four possible outcomes. Cooperation is associated with positive feelings of trust and friendship, while defection is associated with negative feelings including anger or contempt.
where the responses of the players are conflicting, the cooperator often feels anger or frustration, while the defector may experience feelings of guilt, anxiety or alternatively, happiness.

Exchange games such as the PD have been used with fMRI to explore the correlates of social cognition (Patel et al. 2006; Rilling et al. 2002). In typical populations, these paradigms frequently recruit limbic and striatal brain regions involved in reward (e.g., anterior cingulate (ACC)), learning (e.g., caudate), and affective processing (e.g., amygdala, insula) (Delgado et al. 2005; King-Casas et al. 2005; Tomlin et al. 2006). Recruitment of brain regions varies by player choice; cooperative play has been associated with activity in the nucleus accumbens, caudate, orbital frontal cortex, dorsolateral prefrontal cortex and rostral anterior cingulate cortex (Rilling et al. 2002; Rilling et al. 2007), whereas defection tends to recruit the amygdala and anterior insula (Rilling et al. 2002; Sanfey et al. 2003). However, activation patterns are highly dependent on the opponent player (e.g., human vs. computer partner) (Kircher et al. 2009; McClure-Tone et al. 2011; Suzuki et al. 2011). Game playing can also be enhanced through the use of putative human partners that are presumed to be playing outside the scanner (e.g., (Rilling et al. 2008a).

These paradigms have been employed in studies of children, adolescents and adults with ASD to elucidate behavioral patterns and neural networks implicated in the neuropathology of the disorder. For example, Sally and Hill (2006) demonstrated that children with autism had difficulty shifting strategy compared to the control group during the PD (Sally and Hill 2006). In a study of monetary reward processing, adult males with ASD showed enhanced activation of left rostral ACC during monetary vs. social reward, which may reflect enhanced performance monitoring to achieve a goal-directed behavior (Schmitz et al. 2008).
The aim of the investigation was to examine behavioral, physiological, and neural patterns of play to elucidate elements of social cognition in autism. We employed a playground peer interaction paradigm to allow for examination of physiological response and behavioral variables during natural play with a peer (Corbett et al. 2013). Subsequently, we utilized the PD game with human and computer partners during fMRI to examine whether deficits in peer interactions were related to alterations in the neural circuitry involved in social decision making. To ascertain the response to social and nonsocial partners, we explicitly modeled the outcome phase of the task. Based on previous child studies (McClure-Tone et al. 2011), relevant brain search regions of interest (ROIs) were identified and included the ACC, insula, medial prefrontal cortex, TPJ, caudate, and precuneus.

Based on failure to show preference for social stimuli (Dawson et al. 2002; Klin, 1991; Riby and Hancock 2008; Wilson et al. 2010) and greater self-play (Corbett et al. 2013), it was hypothesized that children with ASD would show a similar behavioral response regardless of whether they were playing with a human or computer partner. In line with previous findings of atypical response to social stimuli in ASD (Chiu et al. 2008; Corbett et al. 2009), we hypothesized that children with ASD compared to peers would show differences in social salience circuitry, including the amygdala, insula, temporal cortex, precuneus, and anterior cingulate cortex, reflecting reduced recruitment for social stimuli during both cooperative and defection trials. Conversely, it was hypothesized that neurotypical children would show a pattern of activations similar to healthy adult participants, such as recruitment of the insula during defection, and greater activity in the anterior cingulate, amygdala, caudate, and temporal cortex during play with a human partner (Rilling et al. 2004; Rilling et al. 2008b). Finally, based on our previous studies (Corbett et al. 2013; Kidd et al. 2012), exploratory analyses were conducted to
determine if elevated cortisol levels in children with ASD would be associated with activation in
social salience brain regions, such as the insula, TPJ and amygdala.

METHODS

Participants

Participants were recruited from study registries, clinics, and schools. Inclusion criteria
for all participants were: IQ $\geq 80$ (Wechsler 1999), prepubertal development (Petersen et al.
1988), and an absence of known neurological, psychiatric, or medical conditions based on semi-
structured parental interview. Scanning required having no major contraindication for MRI (e.g.,
metal implants, seizures, claustrophobia). Only participants meeting all of the following three
criteria were included in the ASD group: 1) confirmed diagnosis of an ASD based on diagnostic
criteria (APA 2000), 2) clinical judgment (B.A.C) and 3) current assessment with *Autism
Diagnostic Observation Schedule* (Lord et al. 1999). In addition to the ADOS, the Social
Communication Questionnaire (SCQ; (Rutter et al. 2003)) was used to rule out autism for the
typical children (score $>10$ excluded).

Our initial sample consisted of 42 (21 children with ASD, 21 TD) medication-free,
prepubescent, right-handed children (5 female) between 8-to-12 years. Fourteen children (9
ASD, 5 TD) were excluded from the fMRI analyses due to excessive motion and failure to
complete the task resulting in an attrition rate of 30%, which is comparable to pediatric fMRI
studies (Yerys et al. 2009). There were no differences in symptom severity between those
children who were excluded due to motion and those who were not. The final fMRI analyses
were conducted on 28 children (12 ASD and 16 TD). Demographic and psychological
characteristics of the sample are presented in Table 1.
The Institutional Review Board of Vanderbilt University approved the study and procedures were followed consistent with the Declaration of Helsinki (BMJ 1991; 302: 1194). Parents completed written informed consent and children assented to participate. Participants received compensation for participating in the playground study visit as well as gift cards, the value of which was based on dollars earned during the fMRI task.

Procedures

The study required three visits to the University over one month: 1) diagnostic and psychological assessment, 2) the peer interaction playground with confederate child, and 3) fMRI prisoner’s dilemma paradigm.

Visit 1.

Diagnostic and Neuropsychological Methods

*Autism Diagnostic Observation Schedule* (ADOS; (Lord et al. 1999)) is a semi-structured interview designed to assess behaviors indicative of autism, which was administered by an ADOS trained, research-reliable psychologist.

*Social Communication Questionnaire (SCQ; (Rutter et al. 2003)) was used as a screening tool for ASD (scores of ≥15 is suggestive of ASD). The exclusion criteria for a typically developing child was a score ≥10; however, no TD participants exceeded this score.

*Wechsler Abbreviated Scale of Intelligence (WASI, (Wechsler 1999)) was used to obtain an estimate of each child’s intellectual functioning. An estimated IQ ≥ 80 was required for participation in the study.*
Visit 2.

Peer Interaction Playground Paradigm: The naturalistic playground paradigm includes two research participants (a child with ASD, and a TD child) and a confederate child of the same age and gender who provided structure to the otherwise natural interaction. The paradigm is fully described elsewhere (Corbett et al. 2010; Schupp et al. 2013) and in supplementary materials. Briefly, the 20-minute play is subdivided into periods of prescribed free and cooperative play facilitated by a confederate child on the playground communicating with the research staff via concealed audio technology. Interactions were video recorded using state-of-the-art equipment. Observer XT Version 8.0 software was used for the collection and analysis of the interaction observational data (Noldus 2008). Data were analyzed based on a predefined list of operationalized behaviors (Corbett et al. 2010; Schupp et al. 2013).

Physiological Arousal During Play: Salivary samples were obtained to measure cortisol, a primary stress hormone, in order to assess physiological arousal in response to the social interaction at the beginning (20-min) and end (40-min) of play (there is a 20-min lag in the detection of cortisol in saliva which guides the collection of saliva in 20 minute intervals). Our established methods for home and protocol collection and analysis are fully described elsewhere and the complete data and analyses are published elsewhere (Corbett et al. 2013).

Visit 3.

Participants engaged in the fMRI study described below. Importantly, the confederate from the playground was the same child that participants were told they would play with during a game in the scanner.
Subject Preparation for MRI scanning: Subjects underwent preparatory procedures designed for children. As part of the training on the PD paradigm immediately prior to the actual scan, children were tested for their understanding of the task and appreciation for the concept of money. Scans were conducted following same-day mock scanner exposure to minimize anticipatory stress (Corbett et al. 2008). The mock scanner exposure occurs approximately thirty minutes prior to starting the actual scan. The actual scan lasted approximately 45-60 minutes, including prep time.

We followed the general fMRI design used by Rilling (Patel et al. 2006; Rilling et al. 2002). The paradigm was simplified, colored and shortened to be more appropriate for children. Each run consisted of 20 rounds lasting approximately 8 minutes and there were 2 runs. The order of the runs was counterbalanced across subjects.

At the start of each round, the Payoff Matrix (Figure 1A) was back-projected onto a translucent screen placed near the end of the MRI gantry and viewed through a periscopic prism system attached to the head coil.

The payoff matrix was displayed for 4 s during the decision portion of the task, followed by a 4 s fixation, a white cross hair on a black background. The outcome matrix was presented at 8 s and remained on the screen for 4 s followed by a 4 s fixation (see Figure 1B). The outcome was revealed by highlighting each player’s choice and the resulting payoff for that round. Four possible outcomes per round exist: Player A and B cooperate (CC), Player A cooperates and Player B defects (CD), Player A defects and Player B cooperates (DC) or Player A and B defects (DD). Each round lasted 16 s. Scan acquisition parameters are detailed in the supplemental materials.
**Data Analysis**

*Behavioral Data:*

Between group difference of the behavioral data for response choices and reaction time were calculated using independent sample t-tests using SPSS 18.0 (Chicago, IL).

*fMRI Data:*

*fMRI Data Preprocessing*

Data were preprocessed using SPM8 (Wellcome Department of Neurology, http://www.fil.ion.ucl.ac.uk/spm8) and Matlab (Version 7.1, The Mathworks, Inc., Natick, MA). Data were slice-time corrected, motion-corrected by alignment to the mean image across runs, and coregistered with the structural scan. Images were normalized to the MNI T1 template and resampled to 1 mm³ voxels. Data was smoothed using a 6 mm FWHM Gaussian kernel. All participants included for analysis had translation less than 3 mm and rotation less than 3 degrees. Functional images were analyzed for BOLD response to both outcome and decision (see Figure 1B) such that each epoch of the decision (0-8 s) and outcome (9-16 s) were analyzed separately. Reaction time, choices and outcome variables were convolved with the hemodynamic response function. The aim of the study was to ascertain responses to partner actions both on the playground and in the scanner; therefore, we focused on differences during the outcome phase of the paradigm.

We used the general linear model in SPM8 for first level statistical analysis. Separate regressors were constructed modeling each of the four task outcomes. Because of the low number of CC trials in both groups, CC and DC trials were combined to create a “Co-Player Cooperation” contrast, and CD and DD trials were combined to create a “Co-Player Defection” contrast (McClure-Tone et al. 2011). The subsequent responses were examined for both the
computer co-player and the human co-player runs. These first (participant)-level contrast images were then used for second (group)-level analyses.

Within-group activation was examined by separate analyses of each contrast (Co-Player Defected, Co-Player Cooperated) for both the computer and human runs, in each group (ASD and TD). We then compared activation between the groups for both co-player conditions, for a total of four comparisons, in a set of a priori search ROIs thought to represent a social salience network, including the precuneus (Assaf et al. 2010), anterior cingulate cortex (Watanabe, 2012), insula (Pfeifer et al. 2013), amygdala, and temporal pole (Carter et al. 2012a), and caudate as defined by the aal atlas. We also performed spherical ROI analyses in SPM8 for the mPFC and temporal parietal junction (TPJ), as defined by McClure-Tone; the TPJ ROI consisted of a sphere with a radius of 15 mm, centered at coordinates 48, −54, 27, the peak that corresponded best to those used in prior PD game studies (McClure-Tone et al. 2011). These regions have all shown enhanced activation or activation differences during performance of the prisoner’s dilemma task in previous studies of various populations, including healthy adults (Emonds et al. 2012; Patel et al. 2006; Singer et al. 2004; Suzuki et al. 2011), adults with varying degrees of pro-social personality traits (Emonds et al. 2014), adolescents with without anxiety disorders (McClure-Tone et al. 2011), and adults with and without psychopathic traits (Rilling et al. 2007), for review see (Stallen and Sanfey 2013) and are thought to represent a network for detection of socially salient stimuli. In order to be comprehensive, and in line with previous fMRI studies in pediatric psychiatric samples (Cascio et al. 2013; McClure-Tone et al. 2011), we report results from all search ROI analyses at uncorrected p<0.05. Subsequently, to obtain an extent-based cluster threshold for each ROI, the statistical significance of these clusters was determined based on simulations performed with AlphaSim.
which indicated that a family-wise error rate of $\alpha = 0.05$ was achieved with the following cluster sizes for our search ROIs: 132 voxels for the precuneus, 90 voxels for the insula, 127 voxels for the temporal pole, 34 voxels for the amygdala, 118 voxels for the TPJ, and 105 voxels for the caudate. In order to correct for multiple comparisons in our ROI analyses, we have instead used the Bonferroni-Holm method (Holm, 1979). Bonferroni-Holm is similar to a traditional Bonferroni correction procedure, in that it provides sufficient control for avoidance of Type I Error without the great sacrifice of power that occurs with traditional Bonferroni correction. In Bonferroni-Holm, tests are conducted in a sequential, step-down manner such that the tests are ordered by significance and tested with a step-wise adjustment of the alpha levels on the basis of the number of remaining tests.

We conducted between-group whole brain analyses to identify other regions that may be involved in social salience processing during the PD (topologically FDR corrected $q < 0.01$) (Chumbley and Friston 2009).

RESULTS

Behavioral Performance:

Using independent sample t-tests behavioral response patterns between children with ASD and TD children were examined based on whether the participant chose to Cooperate (C), Defect (D) or not respond (NR). As shown in Table 2, there were no significant between group differences for behavioral response patterns or reaction time for Cooperate (CC and CD) or Defect (DC and DD) conditions when playing with either Human or Computer partners (all $p > 0.05$). There were no significant differences between the groups based on NR trials when playing with either
partner type (all $p > 0.05$). On average the children with ASD cooperated roughly a third of the time for the Computer (30.5%) and Human (29.7%) conditions; whereas they defected two-thirds of the time for both the Computer (66.4%) and Human (66.6%) conditions, respectively. The typically developing children had a similar profile for cooperation with the Computer (28%) and Human (37.6%) and defected twice as much for computer (69%) and Human (64.8%) partners. Because so few participants chose to cooperate, we were underpowered to detect between or within group differences for cooperation trials and therefore only discuss the results of defection trials.

**Money Earned**

Amounts earned across the runs were computed and children received half of the money earned across 40 trials. Earnings were rounded to the nearest $5 denomination and given in the form of gift cards. The average amount earned was $40.00 and there were no significant differences between children with ASD or TD ($p > 0.05$) suggesting that both groups perceived money to be a salient reward.

**Social and Reward Circuitry Activation:**

**Computer Partner**

**Defection (DD and CD):**

Within-Group: There were no significant findings for the ASD search ROI within-group analyses. Whole brain analysis showed significant activation of the R cerebellar lobe, L sensorimotor cortex, and R globus pallidus/putamen (see Table 3 & Supplemental Table 1). The TD group showed activation in the R TPJ and bilateral precuneus. TD whole brain analyses identified additional significant regions including L putamen with submaxima extending to
somatosensory and motor cortices, R insula with submaxima extending to the globus pallidus and putamen, and R motor cortex.

Between-Group: Search ROI comparisons revealed that, when playing with a Computer partner during Defection trials, ASD participants showed significantly less activation than the TD children in the R insula (Figure 2, Table 4). The whole brain analyses revealed statistically significant R insula activation (ASD<TD) (cl=77, t=5.16; 51,11,1, Supplemental Table 2).

**Human Partner**

**Defection (DD and CD):**

Within-Group: Strikingly, the ASD group did not show any significant search ROI or whole brain BOLD signal activity during defection with a human partner. The TD group displayed significant activations in the R temporal pole, R TPJ, L insula, and bilateral caudate. TD whole brain analyses identified significant areas of activation in bilateral DLPFC, bilateral putamen, bilateral parietal cortex, L motor cortex, L dorsal medial cingulate cortex, R fusiform gyrus, and R cerebellar lobe.

Between-Group: Between-group comparisons showed that during defection trials with a putative human partner, the ASD group demonstrated significantly less activation than the TD children in left insula and TPJ, as well as the bilateral caudate (Figure 3). The amygdala was also less active in ASD participants compared to TD, although this finding was just under cluster extent thresholding (cl=30). There were no findings in which ASD children showed more activation (Table 4). Whole brain analyses (ASD<TD) revealed significant differences in the R caudate, L
angular gyrus, L insula/putamen, L supplementary motor area, and R motor cortex (Supplemental Table 2).

We also performed analyses comparing human to computer co-player trials. When collapsing across co-player conditions, there were no significant differences between activation in the human vs. computer conditions, either within or between groups. Taken together, the results show that when children with ASD played the PD game with the Human partner, they showed a lack of engagement of prototypical social salience regions during defection, including the insula and TPJ.

**Summary of BOLD Findings that Meet Cluster Extent Thresholding Criteria**

In this study, TD participants recruited the bilateral precuneus and caudate, as well as the right TPJ during defection with a computer co-player. During defection with a human co-player TD participants recruited the left insula, and right temporal pole, in addition to the bilateral caudate and right TPJ. Between-group contrasts show significantly less activation of the right insula during computer defection trials in the ASD group compared to the TD group. During human defection trials, there was significantly less activation of the left insula and bilateral TPJ in the ASD group compared to the TD group.

**Neural-Behavioral correlational analyses**

Significant behavioral differences between the ASD and TD groups have been consistently reported during the peer interaction paradigm in cooperative play, verbal interaction and self-play (all $p < 0.05$) (Corbett et al., 2014; Corbett et al., 2010). Associations between these play behavior variables and the ROI activations for the total group (ASD and TD) were investigated
using Pearson product correlations (Table 5). For Human Defection, significant correlations were observed for the R insula and bilateral TPJ and self-play.

**Neural-Physiological Correlational Analyses**

Significant differences between the groups were found on salivary cortisol during play with the confederate child ($p<0.05$) showing higher arousal for the ASD group (Corbett et al., 2014). Using exploratory Pearson product partial correlational analyses controlling for baseline cortisol levels, we examined whether activation magnitudes for significant ROI regions during social processing were related to physiological arousal during the playground interaction with peer confederates (Figure 4). Total-group comparisons are presented in Table 5.

**DISCUSSION**

We examined the neural, behavioral, and physiological patterns of play during defection with human and computer partners in an effort to elucidate aspects of social salience in autism. Regarding behavioral responses, both groups showed comparable reaction time and earned essentially the same amount of dollar rewards. ASD and TD children showed similar response patterns during the PD paradigm, with both groups engaging in competitive play twice as much as cooperative play. Preadolescents tend to be more competitive than younger children in the PD game (Tedeschi et al. 1969). This bias for defection has also been reported for adolescents with and without anxiety during the PD paradigm (McClure-Tone et al. 2011). Due to insufficient power to interpret cooperative trial findings, we only discuss defection trials below.

Regarding neural activation, there were significant group differences based on partner type (Human versus Computer). Children with ASD are characterized by impairment in
reciprocal social interaction, which may be attributed to limited social motivation or salience (Chevallier et al. 2012). Thus, it was hypothesized that children with ASD would show differential recruitment of social salience circuitry compared to typically developing children during defection conditions, which is supported in part by the findings detailed below. The results are explicated based on brain structure and their relationship to physiological arousal and playground behavior when playing with the confederate outside of the scanner.

**Insula**

During both the Computer and Human co-player defection contrasts, the ASD group showed less activation in the insula than TD children. The insula is involved in aspects of self-awareness (Craig 2004), as well as interoceptive awareness enhanced by interpersonal and negative emotional experiences (Critchley et al. 2004). In the PD task, the TD children recruited the insula during defection in response to social partners, suggesting self-reflection amidst a mildly negative or frustrating outcome. Engagement of the insula under co-player defection conditions for the TD children replicates previous findings in adults (Rilling et al. 2008b) and supports our hypothesis predicting similar activation in salience regions between TD children and adults.

The contrast between the groups based on partner demonstrates atypical and opposing responsiveness of networks underlying reciprocal social exchange. This notion is supported by a recent investigation in which youth with ASD exhibited hypoactivation of the anterior insula during self-appraisal and greater activation of the insula during “other” appraisal (Pfeifer et al. 2013). Other studies show that insula activity in ASD is heightened in response to rewarding stimuli, such as food and objects, related to restricted interests suggesting altered salience or
reward processing specific to nonsocial stimuli (Cascio et al. 2012; Cascio et al. 2013). There is evidence that activation of anterior insula is positively associated with social anxiety and self-awareness (Terasawa et al. 2013). Individuals with ASD often exhibit heightened autonomic arousal and increased stress during face-to-face encounters and reduced stress during self-play (Corbett et al. 2010; Hirstein et al. 2001; Schupp et al. 2013). In the current study, insula activation was strongly correlated with self-play, suggesting that children who engaged in more self-play demonstrated enhanced recruitment of the insula when defecting with human partners. These relationships contributed to the Neural-Behavioral-Physiological Model presented in Figure 5. Taken together, the findings suggest aberrant functioning of prototypical networks underlying social exchange, salience and self-perception.

**Temporal Parietal Junction (TPJ)**

The TPJ is part of a social cognition network (Assaf et al. 2009), and may have a specialized role in predicting behavior in social situations (Carter et al. 2012b; Frith and Frith 2003). In the present study, TD children showed greater activation of the TPJ than ASD children during defection with the human partner. TPJ recruitment seems to be especially sensitive in individuals showing a strong preference for human over computer partners (Carter et al. 2012b); enhanced activation of TPJ in the TD group during defection trials may be related to greater self-reflection in the TD group relative to the ASD participants. Adults with ASD do not recruit the TPJ; similar to ASD children in the present study (Lombardo et al. 2011). In our study, bilateral TPJ BOLD was positively associated with enhanced self-play, which corroborates the ASD profile of reduced mentalizing ability, increased independent play and less social interaction with others. Taken together, our findings of reduced activity in the TPJ in our ASD participants
relative to TD children support the idea that the TPJ may be a critical region of interest in elucidating social salience deficits in autism.

**Caudate**

In this study, the caudate was less active in the ASD group compared to the TD group during defection with a human, but not a computer, partner. Previous findings have indicated decreased activity of the dorsal striatum in ASD compared to TD during processing of social reward (Delmonte et al., 2012) although other studies found no differences between participants with ASD and TD groups (Cascio et al., 2012; Dichter et al, 2012b). Although the caudate is commonly associated with reward processing, it has a more generalized function of stimulus valuation and motivated behavior via projections from the amygdala (Zorrilla and Koob, 2013) which may help to explain our findings during defection trials.

The amygdala is another brain structure that subserves social and emotional salience and processing, including the “motivational value of stimuli” (Zald 2003). Engagement of the amygdala during defection is a common finding in PD paradigms when playing with human partners (e.g., (Rilling et al. 2007). During the human co-player defection, children with ASD recruited the right amygdala less than the TD children suggesting that they processed the defection outcome with a human partner to be less salient or less negatively arousing than TD children. However, this finding was just under cluster extend threshholding.

Our findings illustrate a disturbed social salience network in children with ASD, demonstrated by diminished and atypical recruitment of regions implicated in a social salience network in interpersonal exchanges. The most prominent finding in this study is reduced insula activity during defection trials in ASD relative to TD, in both social and nonsocial conditions,
which is consistent with previous reports (for review see Dichter 2012a). Also in line with our exploratory cortisol regression analyses, there is evidence that individuals with ASD exhibit heightened autonomic arousal during face-to-face encounters (Corbett et al. 2013), which has been interpreted to reflect a strong negative response to social stimuli (Hirstein et al. 2001). In this investigation, associations were observed between heightened cortisol levels and decreased activation of the left insula and bilateral TPJ. Thus, it may be that social interaction with others is not simply less salient to children with ASD e.g., (Dawson et al. 1998; Weigelt et al. 2012), but that it may be aversive. It is also likely that a history of difficulty engaging with peers and experiencing social rejection, may contribute to heightened negative reactivity during social interaction (Church et al. 2000). These negative social experiences can exacerbate social anxiety and stress especially with peers (Bellini 2006; Corbett et al. 2010; Schupp et al. 2013; White et al. 2009; White and Roberson-Nay 2009).

Our findings contribute to the expanding literature showing dysfunction of social salience and motivational valuation systems in both children (Kohls et al. 2013; Scott-Van Zeeland et al. 2010) and adults with ASD (Dichter et al. 2012b). The fact that there is relative similarity across studies despite differences in design and subject characteristics, lends credence for the replicated findings and interpretation. Whereas some find disruption primarily for social rewards (Scott-Van Zeeland et al. 2010), others have reported disruption for nonsocial (Dichter et al. 2012b; Schmitz et al. 2008) or both reward types (Kohls et al. 2013). There is a convergence of data supporting disturbed salience networks in children with ASD. The findings support the idea that engaging with social agents is less intrinsically salient for individuals with ASD. However, the data are unable to answer whether this may be due to limited social motivation (Chevallier et al. 2012), aberrant salience processing (Scott-Van Zeeland et al. 2010), or to a history of negative
social exchanges with peers (e.g., Humphrey and Symes 2011; White et al. 2009)). Nevertheless, the results add to the growing literature showing diminished responsivity to social stimuli in children with ASD relative to typically developing children.

As part of the PD paradigm, the current study incorporated money earned during the game in the form of gift cards. While some studies have shown disruption in BOLD signal in response to monetary stimuli in ASD (Kohls et al. 2013), there were no significant differences between the groups based on money earned suggesting that both were incentivized by financial reward. A recent investigation of adults with low empathy suggests heightened activation of putative reward regions in response to monetary incentives versus social stimuli (Gossen et al. 2013). To date, only one study of children with ASD has compared brain reward system activity to primary (i.e., food) versus secondary (i.e., monetary) rewards, as well as to social and nonsocial rewards, finding similar activation patterns between ASD and TD groups for monetary rewards but increased activity to primary rewards compared to TD controls (Cascio et al. 2012). Given these mixed findings, the relative salience of secondary rewards such as monetary rewards in ASD warrants further investigation. In the present study, qualitative observations before and after the scan related to the promise of earning money suggest participants were equally enthusiastic across the groups. However, the design of this study does not allow for the interpretation of the relationship of the reported findings to monetary reward processing. Future fMRI studies should investigate monetary versus social salience processing in people with ASD. Unique features of the study pertain to the inclusion of a confederate child, which the participants actually engaged with on a playground paradigm and thereby served as a known social partner during the PD. While enhancing ecological validity, it likely contributed to the believability that participants were actually playing with a human partner. Moreover, the
children were all medication-free; therefore, confounding influences that disturb the regulation of corticolimbic activity were removed (e.g., (Rubia et al. 2009).

Limitations

Despite these strengths there are several limitations to acknowledge. There were significantly fewer cooperative than competitive trials, making negative findings in cooperative tasks less interpretable. However, this preponderance for defection has also been observed in other studies (McClure-Tone et al. 2011). Importantly, interpretations regarding social cooperation were less tenable than more general interpretation about salience processing differences (both social and nonsocial) between our ASD and TD groups. The study was limited by a small sample size, especially in the ASD group. While the attrition rate is comparable to other pediatric studies (Yerys et al. 2009), it may have contributed to Type II error. Salivary cortisol was measured during a previous peer interaction exchange with the human confederate rather than on the scan day. Our research measuring biobehavioral profiles provides some assurance that these values reflect a strong measure of physiological arousal status during natural social play conditions (Corbett et al. 2010; Corbett et al. 2013; Schupp et al. 2013). Even so, it is unclear if elevated BOLD activity can be reliably compared with states of physiological arousal in a trait-like manner. Finally, the study involved multiple comparison analyses, which were not corrected because the groups were not identical; we were interested in the results of individual hypotheses, and the correlational analyses were deemed exploratory.

Summary

Children with ASD demonstrate a lack of engagement of social salience networks during play with social agents, yet engage such structures with nonhuman partners. It remains unclear
the degree to which the behavioral and neural response patterns of social salience are modifiable and if so, under what conditions. Future studies are needed to explore the saliency of social and nonsocial stimuli, the distinction between perceptual and motivational factors in social exchange, and the plasticity of social salience circuitry in response to treatment for persons with ASD.
ACKNOWLEDGEMENTS

This work was supported by National Institute of Health (NIH) R01MH085717 awarded to Blythe A. Corbett, Ph.D., CTSA award No. UL1TR000445 from the National Center for Advancing Translational Sciences to Vanderbilt University, and NICHD Grant P30HD15052 to the Vanderbilt Kennedy Center for Research on Human Development, as well as The Dan Marino Foundation and Vanderbilt Brain Institute Clinical Neuroscience Scholars Program (E.K.E). We are grateful to Cameron Carter, M.D. for his mentorship on the previous unpublished pilot project, David H. Zald, Ph.D. for his comments on the manuscript, and to Jim Rilling, Ph.D. for initially sharing the paradigm.

Assaf, M., et al. (2009), 'Brain Activity Dissociates Mentalization from Motivation During an Interpersonal Competitive Game', *Brain Imaging Behav*, 3 (1), 24-37.


Chiu, P. H., et al. (2008), 'Self responses along cingulate cortex reveal quantitative neural phenotype for high-functioning autism', *Neuron*, 57 (3), 463-73.


Gossen, A., et al. (2013), 'Neural evidence for an association between social proficiency and sensitivity to social reward', *Soc Cogn Affect Neurosci*.


King-Casas, B., et al. (2005), 'Getting to know you: reputation and trust in a two-person economic exchange', *Science*, 308 (5718), 78-83.


Riby, D. M. and Hancock, P. J. (2008), 'Viewing it differently: social scene perception in Williams syndrome and autism', *Neuropsychologia*, 46 (11), 2855-60.


Rubia, K., et al. (2009), 'Methylphenidate normalises activation and functional connectivity deficits in attention and motivation networks in medication-naive children with ADHD during a rewarded continuous performance task', *Neuropharmacology*, 57 (7-8), 640-52.


Table 1. Demographic Between Group Data

<table>
<thead>
<tr>
<th>Demographic</th>
<th>ASD Mean (SD)</th>
<th>TD Mean (SD)</th>
<th>F</th>
<th>P value</th>
<th>Eta square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>10.14 (0.79)</td>
<td>10.13 (1.52)</td>
<td>0.001</td>
<td>0.98</td>
<td>0.00</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>105.2 (22.65)</td>
<td>121.47 (16.14)</td>
<td>4.74</td>
<td>0.04</td>
<td>0.16</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>107.20 (21.93)</td>
<td>114.41 (14.32)</td>
<td>1.08</td>
<td>0.31</td>
<td>0.04</td>
</tr>
<tr>
<td>Estimated Full IQ</td>
<td>107.00 (20.55)</td>
<td>119.94 (15.53)</td>
<td>3.44</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>SCQ</td>
<td>21.30 (7.95)</td>
<td>2.29 (2.11)</td>
<td>88.89</td>
<td>0.001</td>
<td>0.78</td>
</tr>
<tr>
<td>SRS</td>
<td>87.00 (21.03)</td>
<td>20.53 (15.17)</td>
<td>90.77</td>
<td>0.001</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Note: SD = Standard Deviation, IQ = Intellectual Quotient, SCQ = Social Communication Questionnaire, SRS = Social Responsiveness Scale. There were 3 TD females in the final analysis.
Table 2. Behavioral Response Choices and Reaction Time between the Groups.

<table>
<thead>
<tr>
<th>Mean Number of Trials Per Run</th>
<th>ASD Mean (SD)</th>
<th>TD Mean (SD)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooperate (C) Computer</td>
<td>6.11 (4.11)</td>
<td>5.6 (3.71)</td>
<td>0.181</td>
<td>0.67</td>
</tr>
<tr>
<td>Defect (D) Computer</td>
<td>13.28 (4.07)</td>
<td>13.8 (3.58)</td>
<td>0.198</td>
<td>0.66</td>
</tr>
<tr>
<td>Cooperate (C) Human</td>
<td>5.94 (3.91)</td>
<td>6.52 (4.21)</td>
<td>0.207</td>
<td>0.65</td>
</tr>
<tr>
<td>Defect (D) Human</td>
<td>13.33 (3.91)</td>
<td>12.96 (4.25)</td>
<td>0.086</td>
<td>0.77</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean Reaction Time Per Run (ms)</th>
<th>ASD Mean (SD)</th>
<th>TD Mean (SD)</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooperate (C) Computer</td>
<td>1110.37 (420.70)</td>
<td>1016.29 (407.32)</td>
<td>0.437</td>
<td>0.51</td>
</tr>
<tr>
<td>Defect (D) Computer</td>
<td>1088.07 (374.97)</td>
<td>1005.89 (348.79)</td>
<td>0.439</td>
<td>0.51</td>
</tr>
<tr>
<td>Cooperate (C) Human</td>
<td>1013.35 (261.39)</td>
<td>1067.72 (567.06)</td>
<td>0.112</td>
<td>0.74</td>
</tr>
<tr>
<td>Defect (D) Human</td>
<td>1026.01 (243.43)</td>
<td>983.52 (332.28)</td>
<td>0.168</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Note: ASD = autism spectrum disorder, TD = typically developing, SD = standard deviation, ms = milliseconds.
Table 3: Within Group Search ROI Brain Findings

<table>
<thead>
<tr>
<th>Group</th>
<th>Computer Co-Player Defection</th>
<th>Peak Voxel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Brain Regions (hemisphere)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cluster Size</td>
<td>t-score</td>
</tr>
<tr>
<td>ASD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No Significant ROI findings</td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus (L)</td>
<td>211</td>
<td>4.17</td>
</tr>
<tr>
<td>Precuneus (R)</td>
<td>192</td>
<td>4.09</td>
</tr>
<tr>
<td>TPJ (R)</td>
<td>120</td>
<td>2.88</td>
</tr>
<tr>
<td>ASD</td>
<td>Person Co-Player Defection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cluster Size</td>
<td>t-score</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula (L)</td>
<td>189</td>
<td>4.95</td>
</tr>
<tr>
<td>TPJ (R)</td>
<td>149</td>
<td>3.86</td>
</tr>
<tr>
<td>Temporal Pole (R)</td>
<td>158</td>
<td>3.51</td>
</tr>
<tr>
<td>Caudate (L)</td>
<td>125</td>
<td>4.81</td>
</tr>
<tr>
<td>Caudate (R)</td>
<td>143</td>
<td>4.34</td>
</tr>
</tbody>
</table>

Note: ASD = autism spectrum disorder; x, y, and z refer to the left-right, anterior-posterior, and inferior-superior dimensions, respectively; L = left, R = right. All results thresholded using Bonferroni-Holm correction for multiple comparisons with extent thresholding using Monte Carlo simulations.
Table 4. Group Comparison Results for ROI Analyses, Co-Player Defection Trials

<table>
<thead>
<tr>
<th>Brain Regions (hemisphere)</th>
<th>ASD &lt; TD</th>
<th>Computer Partner</th>
<th>Peak Voxel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula (R)</td>
<td>90</td>
<td>4.28</td>
<td>-48</td>
</tr>
<tr>
<td>Insula (L)</td>
<td>123</td>
<td>3.69</td>
<td>-42</td>
</tr>
<tr>
<td>TPJ (L)</td>
<td>143</td>
<td>4.34</td>
<td>-48</td>
</tr>
<tr>
<td>TPJ (R)</td>
<td>192</td>
<td>3.51</td>
<td>45</td>
</tr>
<tr>
<td>Caudate (L)</td>
<td>158</td>
<td>3.56</td>
<td>-15</td>
</tr>
<tr>
<td>Caudate (R)</td>
<td>156</td>
<td>4.39</td>
<td>12</td>
</tr>
</tbody>
</table>

**Note:** ASD = autism spectrum disorder; TD = typically developing; x, y, and z = left-right, anterior-posterior, and inferior-superior dimensions, respectively; L = left; R = right; TPJ = temporal parietal junction. All results thresholded using Bonferroni-Holm correction for multiple comparisons with extent threshold criteria using Monte Carlo simulations. There were no significant findings for ASD>TD contrasts.
Table 5: Total Group Correlations between Neural Activations, Cortisol and Play Behavior

<table>
<thead>
<tr>
<th>Partner and Contrast</th>
<th>Cortisol S1 Stress</th>
<th>Cortisol S2 Stress</th>
<th>Self-Play</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Human Defection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula (L)</td>
<td>$r = -0.37^+$</td>
<td></td>
<td>$r = 0.40$</td>
</tr>
<tr>
<td>Insula (R)</td>
<td></td>
<td>$r = 0.41^*$</td>
<td></td>
</tr>
<tr>
<td>TPJ (L)</td>
<td>$r = -0.48^*$</td>
<td>$r = -0.38^+$</td>
<td>$r = 0.43^*$</td>
</tr>
<tr>
<td>TPJ (R)</td>
<td>$r = -0.46^*$</td>
<td>$r = 0.44^*$</td>
<td></td>
</tr>
</tbody>
</table>

Note: Partial correlations controlled for baseline cortisol level during play with confederate. L = left; R = right; TPJ = temporal parietal junction. S1 = cortisol level during initial exposure to confederate, S2 = cortisol level at end of 20-min play with confederate. Self-Play = percentage of time engaged in self-play on playground with confederate. * $p < 0.05$, † = trend ($p = 0.06$).
Figure 1. Payoff Matrix for Peer Interaction Game. The Payoff Matrix for the modified Prisoner's Dilemma Game referred to as the Peer Interaction Game was displayed with a picture of the human confederate or a picture of a computer for each round. The payoff matrix Decision phase was displayed from 0-4 s followed by a 4 s Fixation phase, at which time the child made a decision to either cooperate (press button “1”) or defect (press button “2”) with the partner. (Many children did not understand the word “defect”; thus, the word “compete” was used in the task). Subsequently, the Outcome matrix was presented from 8-12 s revealing each player’s choice and the resulting payoff for that round. Finally, another Fixation phase from 12-16 s complete the round. Thus each trial lasted 16 s. Player A referred to “You” (participant) and Player B referred to “Partner.” The first number in each cell was the subject’s potential payoff and the number in parentheses (red) was the payoff for the partner. The cumulative total was shown on the right side of the matrix in yellow.
Figure 2. Coronal slice depicts significant group differences in the right insula for computer defection trials, ASD<TD. Color bar indicates t values. R=right, Montreal Neurological Institute coordinates. Corrected p<0.05, with Monte Carlo simulation extent threshold correction. Bar graph indicates mean percent signal change extracted from the right insula anatomical mask for the TD group (blue) and the ASD group (red). Error bars indicate standard deviation.
Figure 3: Coronal slices depict significant group differences in the A) left insula, B) left temporal parietal junction C) right temporal parietal junction for human defection trials, ASD<TD. Color bar indicates t values. R=right, Montreal Neurological Institute coordinates. Corrected p<0.05, with Monte Carlo simulated extent threshold correction. Bar graphs indicate mean percent signal change extracted from each region for the TD group (blue) and the ASD group (red). Error bars indicate standard deviation.
Figure 4. Scatterplots depict associations between BOLD signal during defection trials with a human co-player in the scanner and playground data for ASD participants (red) and TD participants (blue). A) Log cortisol during play with the confederate on the playground and left TPJ BOLD, B) Log cortisol during play with the confederate on the playground and right TPJ BOLD, C) Percentage duration of self-play and right insula BOLD, D) Percentage duration of self-play and left TPJ BOLD, E) Percentage duration of self-play and right TPJ BOLD TPJ=temporal parietal junction, L=left, R=right.
Figure 5. Significant correlations between the neural activations during human defection (insula and temporal parietal junction), physiological response (cortisol) and confederate play (self-play) patterns.

56x61mm (150 x 150 DPI)
Supplementary Materials

Detailed Methods:

Peer Interaction Playground Paradigm: The naturalistic playground paradigm includes two research participants (a child with ASD, and a TD child) and a confederate child of the same age and gender who provided structure to the otherwise natural interaction. For the current investigation, four confederates were utilized (3 males, 1 female).

The 20-minute play is subdivided into periods of prescribed free and cooperative play facilitated by a confederate child on the playground communicating with the research staff via concealed audio technology (Corbett et al. 2010; Schupp et al. 2013). Interactions were video recorded using state-of-the-art equipment, which included four professional 70 Sony PTZ remotely operated cameras housed in glass cases and affixed to the four corners of the external playground fence. The cameras contain pan, tilt and zoom features allowing full capture of the playground. Wireless audio communication was established by Sennheiser body pack and Audio-Technica transmitters and receivers, which functioned as battery-operated microphones that were clipped to the shirt of each child and simultaneously recorded by an 8 channel mixing board.

The paradigm was divided into four 5-minute time (T) periods of intermittent free play and solicited play. The first period (T1) consisted of unsolicited free play. During the second period (T2), the confederate solicited interaction on the play equipment for cooperative play. During the third period (T3), the confederate was instructed to again engage in free play. During the fourth period (T4), the confederate again solicited the two participants to engage in a cooperative game involving toys. For the current study, only the play periods that involved solicited play by the confederate were analyzed (T2 and T4).
Observer XT Version 8.0 software was used for the collection and analysis of the interaction observational data (Noldus 2008). Data were analyzed based on a predefined list of operationalized behaviors (Corbett et al. 2010; Schupp et al. 2013). Reliability was calculated for a random sample of 25% of observations using the Noldus Observer software. Inter-rater reliability was calculated using Cohen’s Kappa at $K = 0.80$ while test-retest reliability was $K = 0.89$. Verbal and play (cooperative, self) interactions were calculated as percentage of time engaged. Observer (Noldus 2008) reliability calculations for the specific behaviors used in the current study were: cooperative play 91% and $K = .89$, self-play = .85 verbal interaction 90% and $k = .85$.

**Physiological Arousal During Play:** Salivary samples were obtained to measure the level of cortisol, a primary stress hormone, in order to assess physiological arousal experienced by the children in response to the social interaction at the beginning (20-min) and end (40-min) of play (there is a 20-min lag in the detection of cortisol in saliva which guides the collection of saliva in 20 minute intervals). Our established methods for home and protocol collection and analysis are fully described elsewhere (Corbett et al. 2010; Schupp et al. 2013). All playground interactions occurred during the afternoon between 3:00 and 4:30 pm to control for diurnal fluctuations of salivary cortisol.

**Cortisol Assay:** The salivary cortisol assay was performed using a Coat-A-Count® radioimmunoassay kit (Siemens Medical Solutions Diagnostics, Los Angeles, CA) modified to accommodate lower levels of cortisol in human saliva relative to plasma. Saliva samples, which had been stored at -20°C, were thawed and centrifuged at 3460 rpm for 15 minutes to separate
the aqueous component from mucins and other suspended particles. The coated tube from the kit was substituted with a glass tube into which 100 µl of saliva, 100 µl of cortisol antibody (courtesy of Wendell Nicholson, Vanderbilt University, Nashville, TN), and 100 µl of $^{125}$I-cortisol were mixed. After incubation at 4°C for 24 hours 100 µl of normal rat serum in 0.1% PO4/EDTA buffer (1:50) and precipitating reagent (PR81) were added. The mixture was centrifuged at 3460 rpm for 30 minutes, decanted, and counted. Serial dilution of samples indicated a linearity of 0.99. Interassay coefficient of variation was 10.4%.

**Additional fMRI Task Protocols:**

During training, the children played a simulated practice game with a familiar cartoon character. All of the children understood the value of money as a tangible reward and grasped the task concepts within the 30-minute training period. The participants earned money, the amount depending on both players’ choices (Figure 1). The total for each round was provided and a cumulative total summed as the game progressed. The child earned half of the cumulative total in the form of gift cards.

At the beginning of each round, the participant was shown a photograph of either the child confederate or of a computer. In reality, the other player was always the same preprogrammed computer strategy. Following the scan, participants were informed that they were exclusively playing with the computer. The participants reported that they believed they were playing with the different human and computer partners, but none were negatively affected by the deception.

**Image Acquisition:** High resolution structural, gradient echo, and echoplanar T2* with blood oxygen level-dependent (BOLD) contrast images were acquired on a 3T Phillips scanner. For the
functional runs we acquired 27 slices (4 mm thick, 1 mm slice gap) at a 30 degree oblique angle to the AC-PC line using the single-shot gradient recalled echo-echo planar imaging (GRE-EPI) sequence with TR 2000 ms, TE 32 ms, flip angle 90 degrees, FOV 22 cm, 64 x 64 matrix.

Functional images were collected in runs of 335 volumes. The initiation of the scan and task was synchronized using a TTL pulse delivered to the stimulus control software at the onset of the first scan.