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Heterogeneity of neural mechanisms of response to pivotal response treatment

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Abstract

We investigated the mechanisms by which Pivotal Response Treatment (PRT) improves social communication in a case series of 10 preschool-aged children with Autism Spectrum Disorder (ASD). Functional magnetic resonance imaging (fMRI) identified brain responses during a biological motion perception task conducted prior to and following 16 weeks of PRT treatment. Overall, the neural systems supporting social perception in these 10 children were malleable through implementation of PRT; following treatment, neural responses were more similar to those of typically developing children (TD). However, at baseline, half of the children exhibited hypoactivation, relative to a group of TD children, in the right posterior superior temporal sulcus (pSTS), and half exhibited hyperactivation in this region. Strikingly, the groups exhibited differential neural responses to treatment: The five children who exhibited hypoactivation at baseline evidenced increased activation in components of the reward system including the ventral striatum and putamen. The five children who exhibited hyperactivation at baseline evidenced decreased activation in subcortical regions critical for regulating the flow of stimulation and conveying signals of salience to the cortex—the thalamus, amygdala, and hippocampus. Our results support further investigation into the differential effects of particular treatment strategies relative to specific neural targets. Identification of treatment strategies that address the patterns of neural vulnerability unique to each patient is consistent with the priority of creating individually tailored interventions customized to the behavioral and neural characteristics of a given person.

Keywords

Autism; fMRI; Treatment; Outcome; Biological motion

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Conflict of interest Pamela Ventola, Daniel Yang, Hannah Friedman, Devon Oosting, Julie Wolf, Denis Sukhodolsky, and Kevin A. Pelphrey declare that they have no conflict of interest.

Informed consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, and the applicable revisions at the time of investigation. Informed consent was obtained from the parents of all patients and verbal assent was obtained from all of the children.

Background

Given the universality of social deficits in Autism Spectrum Disorder (ASD), dysfunction in brain systems subserving social perception has become a key focus in research on ASD (e.g., Kaiser et al. 2010; Jou et al. 2011a; Jou et al. 2011b; Dawson et al. 2005; Pitskel et al. 2011; Bolling et al. 2011). This work has identified clear brain-based biological markers for social dysfunction in ASD. For example, in comparing the activation to biological motion versus scrambled motion among children with ASD, unaffected siblings (UAS), and typically developing children (TD), Kaiser et al. (2010) identified activity unique to children with ASD in a circuit known to be involved in social perception. Children with ASD, relative to TD and UAS, showed reduced differential activation to biological versus scrambled motion in the left ventrolateral prefrontal cortex (vlPFC), right amygdala, right posterior superior temporal sulcus (STS), ventromedial prefrontal cortex (vmPFC), and the bilateral fusiform gyri (FFG). These and other brain-based biological markers can now be utilized to advance the treatment of ASD. Experimental therapeutics is an approach by which intervention studies generate information about the underlying mechanisms of a disorder and explicate how the treatment leads to clinical change (e.g., Insel 2014). Such an approach supports a strong translational connection between research and clinical practice, and as such, studies can elucidate how a treatment is working, for whom it works best, and why it works at a biological-systems level, thereby highlighting the potential for individualized medicine.

Pivotal Response Treatment (PRT; Koegel et al. 1987) is one of a very few empirically validated treatments for children with ASD. It builds upon principles of Applied Behavioral Analysis (ABA) but was developed specifically to target the core social and communication deficits in autism. Extensive research suggests that maintaining motivation and engagement in children with developmental delays and ASD is crucial in developing social and communication skills (Dunlap 1984; Dunlap and Egel 1982; Dunlap and Koegel 1980; Koegel and Egel 1979; MacMillan 1971). Thus, motivation is one of the key aspects of PRT and is achieved using techniques such as child choice, natural reinforcement, reinforcement of attempts, and inclusion of maintenance tasks (Koegel et al. 1987). A detailed explication of this intervention approach can be found in a recent handbook (Koegel and Koegel 2012). Research on PRT has shown it to be effective in fostering language and social communication skills in individuals with ASD (Koegel and Frea 1993; Ventola et al. 2014), symbolic play (Stahmer 1995), speech intelligibility (Koegel et al. 1998), and self-initiated queries (Koegel et al. 2003). The approach has been used successfully with individuals of all ages, from infants to adults (Steiner et al. 2013; Doggett et al. 2013; LeBlanc et al. 2007).

Despite the extensive clinical research demonstrating the efficacy of PRT, very few studies have examined the neural correlates of treatment response. Voos et al. (2013) demonstrated the efficacy of PRT in a short-duration model (16-weeks) as illustrated in a case series of two high-functioning children with ASD. Both children showed significant gains on behavioral measures of social communication and adaptive skills, as measured by the Autism Diagnostic Observation Schedule (ADOS; Lord et al. 1999), Clinical Evaluation of Language Fundamentals-Fourth Edition (CELF-4; Semel et al. 2003), and Vineland-II. Additionally, they used pre- and post-treatment fMRI measurements to identify the neural

correlates of successful response to PRT. Both children showed increased activation from baseline to treatment endpoint during a social perception task (coherent versus scrambled biological motion) in key brain regions underpinning social functioning in TD children. However, this study was limited only to two children.

Using a distinct, yet similar approach, the Early Start Denver Model (ESDM; Princiotta and Goldstein 2013), Dawson and colleagues (2012) used EEG to measure neural correlates of response to 2 years of treatment in an Early Start Denver Model (ESDM) program. Children with ASD were randomized to receive treatment under the ESDM or referral to community intervention for 2 years. After the intervention, EEG activity was measured during the presentation of faces versus objects. Age-matched TD children were also assessed. The ESDM group exhibited greater improvements in autistic symptoms, IQ, language, and adaptive and social behaviors than the community intervention group. The ESDM and TD groups showed a shorter Nc latency and increased cortical activation (decreased α power and increased θ power) when viewing faces, whereas the community intervention group showed the opposite pattern (shorter latency and greater cortical activation when viewing objects). Greater cortical activation while viewing faces was associated with improved social behavior. Unfortunately, this study did not include a baseline time point. Thus, it is not possible to evaluate whether and/or how the groups differed prior to the onset of treatment.

The heterogeneity of ASD leads to challenges for an ‘experimental therapeutics’ approach. Although all children with ASD share some clinical features that are central to the disorder, there is significant variability in clinical presentation as well as co-morbid or co-occurring symptoms and behaviors that make treatment research particularly challenging. Emerging, in part, from the variability in ASD, are multiple theories about the origin of social deficits. For example, as illustrated in Fig. 1a, the social motivation hypothesis (Dawson et al. 2005) suggests that reduced social drive/motivation leads to inattention to key aspects of social information and consequent failure of developmental specialization in experience-expectant brain systems, such as the face and action perception systems. Diminished social motivation in ASD might stem from deficits in forming representations of and categorizing the reward value of social information. This is consistent with the work of Scott-Van Zeeland and colleagues (2010) who found diminished neural responses to both social and monetary rewards in ASD, with a more pronounced reduction in response to social rewards. Specifically, deficits in frontostriatal response during social, but not monetary, rewarded learning were observed as well as a relationship between ventral striatum activity and social reciprocity.

Alternatively, as illustrated in Fig. 1b, the intense world hypothesis (Markram, et al. 2007; Markram and Markram 2010a, b), posits that the deficits seen in ASD stem from hyperreactivity of specific neural systems that are involved in gating information flow and selective attention. They suggest that the hyperactivity leads to hyper-perception, hyper-attention, and hyper-memory, which together are debilitating. Such hyperactivation in brain circuits, especially in the amygdala, thalamus, frontal and temporal regions, may cause the world to be perceived as overly intense, even harsh and unpleasant, to which individuals respond by withdrawing attention particularly from social situations, which can be complex and difficult to predict, or by engaging in repetitive and self-soothing behaviors (Markram et

al. 2007). One of the suggestions based on the intense world hypothesis therefore is to surround the child with a highly predictable and calm environment for the first years of life.

These two theories make very similar predictions at a behavioral level: decreased attention to social information, a lack of development of “social” expertise (as defined in Fig. 1), and resulting significant social deficits. However, as described above, the neural-systems level pathways to these behavioral deficits likely differ. The social motivation hypothesis implicates a primary dysfunction in reward processing circuits, whereas the intense world hypothesis proposes abnormalities in brain regions involved in selective attention and regulating the flow of information to the cortex. Mirroring demonstrations of multiple genetic causes of ASD, it is likely that the mechanisms specified in each theory operate to varying degrees in each individual case. In the current study, which is a preliminary report on the topic, we sought to understand the neural-systems-level mechanisms by which PRT improves social communication in a case series of preschool-aged children with ASD. We used functional magnetic resonance imaging (fMRI) to identify brain responses during a well-validated biological motion perception task. Our design and analysis approach allowed us to distinguish, at a baseline scan prior to treatment onset, children characterized as hyperreactive (reflecting the intense world explanation of ASD social symptoms) versus hyporeactive (reflecting the social motivation explanation).

Methods

Participants

Participants included ten preschool-aged children (2 female, 8 male) diagnosed with an autism spectrum disorder (ASD) using “gold-standard” diagnostic procedures. All children entered the study with a prior diagnosis of ASD, and the diagnosis was confirmed by a highly experienced licensed clinical child psychologist. Diagnostic impressions were informed by the ADOS Module 3 (Lord et al. 1999) and the Autism Diagnostic Interview-Revised (ADI-R; Rutter et al. 2003). Clinicians administering these measures were research reliable. All children met criteria for a classification of either autism spectrum or autism on the ADOS and autism on the ADI-R, and final diagnosis was made based on expert clinical judgment. Five typically developing (TD) children were also included as a control group. The children were matched on age and cognitive abilities. Refer to Table 1 for characterization information.

Treatment approach

PRT is a naturalistic, behaviorally-based treatment approach. It involves specific components (child choice, child attending, clear opportunity, contingent reinforcement, natural reinforcement, reinforcement of attempts, and interspersed maintenance/acquisition tasks) designed to increase the child’s social motivation. Additionally, the approach is highly naturalistic, so for the context of the current study, the sessions were play-based and used materials such as craft supplies, balls, blocks, and ‘play-doh.’

For each child, treatment lasted 16 weeks, with a total of 7 h of treatment per week. Sessions were held in the clinic as well as in the child’s home. Five hours per week were direct

intervention with the child, and 2 h per week consisted of parent guidance. The treatment targeted pivotal areas, including social initiation and responsivity, with the premise that improvements in these areas should lead to more widespread and generalized improvements in multiple areas of development and represent core changes in social motivation. A more detailed description of PRT can be found in the original instruction manual (Koegel et al. 1989) and in an updated guide (Koegel and Koegel 2012).

Fidelity of implementation

All clinicians were trained in PRT by faculty from the University of California Santa Barbara (UCSB), the research institution where PRT was developed. The lead clinician sent two separate videotaped sessions (of different children) to the trainer to ensure maintenance of treatment fidelity. Both videos met the standard fidelity criteria. To ensure that the bachelors-level clinicians were correctly implementing PRT during their sessions, they met with the licensed clinician for 2 h per week. During these meetings, clinicians discussed the children's progress, current presentation, and specific activities for the treatment sessions that would be motivating and foster skill development. Additionally, the lead clinician observed sessions live and via videotape at least once weekly for each participant.

Formal fidelity of implementation was assessed for two randomly coded treatment sessions for each subject. Two randomly selected five-min segments per session were used for this fidelity assessment. The standard fidelity assessment published by the developers of the approach was used, and per convention, fidelity was defined as demonstrating the treatment components (child choice, child attending, clear opportunity, contingent reinforcement, natural reinforcement, reinforcement of attempts, and interspersal of maintenance/acquisition tasks) in 80 % of opportunities (Koegel et al. 1998; Koegel and Koegel 2012). The scoring was dichotomous; if the therapist demonstrated the component, a checkmark was used, and if not, a minus was used. All therapists maintained the defined treatment fidelity across the duration of the study.

Measures

Differential Ability Scales-II (DAS-II), Early Years Battery (Elliott 2006)—The DAS-II is a standardized assessment of cognitive abilities that measures verbal, nonverbal, and spatial reasoning abilities.

Autism Diagnostic Interview-Revised (ADI-R; Koegel et al. 2003)—The ADI-R is a comprehensive diagnostic parent-report interview that focuses on language/communication, reciprocal social interactions, and restricted, repetitive, and stereotyped behaviors and interests.

Autism Diagnostic Observation Schedule (ADOS; Lord et al. 1999)—The ADOS is a semi-structured diagnostic assessment that allows clinicians to observe and assess social, communication, and repetitive behaviors associated with ASD.

Social Responsiveness Scale-Second Edition (SRS-2; Constantino 2012)—The Social Responsiveness Scale-Second Edition is a 65-item parent report scale designed

specifically for use in ASD to quantitatively measure an individual's ability to engage in reciprocal social behavior in a naturalistic social setting. Each item on the scale inquires about an observed aspect of reciprocal social behavior that is rated on a scale from 0 (never true) to 3 (almost always true). Behaviors are assessed over five domains: Social Awareness, Social Cognition, Social Communication, Social Motivation, and Autistic Mannerisms. The total score generated serves as an index of severity of social deficits.

Child Behavior Checklist (CBCL; Achenbach and Rescorla 2001)—The CBCL is a parent-report measure of broad behavioral and emotional symptomatology. For the school-age version (ages 6–18), there are eight empirically-based syndromes scales as well as six diagnostically-oriented (DSM-5; American Psychiatric Association 2013) scales: Affective Problems, Anxiety Problems, Attention Deficit/Hyperactivity Problems, Oppositional/Deviant Problems, Somatic Problems, and Conduct Problems. For the preschool version (ages 1.5–5 years), there are seven empirically-based syndromes scales as well as five diagnostically-oriented (DSM-5) scales: Depressive Problems, Anxiety Problems, Pervasive Developmental Problems, Attention Deficit/Hyperactivity Problems, and Oppositional Deviant Problems. For the current study, parents of children ages 4 and 5 completed the preschool version, and parents of children aged 6 completed the school-age version.

Child Symptom Inventory-4; (CSI-4; Gadow and Sprafkin 2002)—The CSI-4 is a parent-report behavior rating scale whose items correspond to the symptoms of disorders defined by the DSM-IV. It contains 97 items that screen for 15 emotional and behavioral disorders. The CSI-4 contains symptom categories for the following DSM-IV disorders: Attention Deficit Hyperactivity Disorder-Inattentive Type, Attention Deficit Hyperactivity Disorder-Combined Type, Oppositional Defiant Disorder, Conduct Disorder, Generalized Anxiety Disorder, Social Phobia, Separation Anxiety Disorder, Schizophrenia, Autistic Disorder, Asperger's Disorder, Obsessive Compulsive Disorder, Major Depressive Disorder, Dysthymic Disorder, Specific Phobia, and Motor / Vocal Tics.

Experimental design

Before and after treatment, children were scanned while viewing coherent and scrambled point-light displays of biological motion created from motion capture data. The coherent biological motion displays featured an adult male actor performing movements relevant to early childhood experiences, such as playing pat-a-cake (Klin et al. 2009). The scrambled motion animations were created by randomly selecting 16 points from the biological motion displays and plotting their trajectories on a black background. Thus, the coherent and scrambled displays contained the same local motion information, but only the coherent displays contained the configuration of a person (Johansson 1973). During the MRI scan, stimuli were presented using E-Prime 2.0 software (Psychological Software Tools). Six coherent biological motion clips (BIO) and six scrambled (SCR) motion clips were presented (See Fig. 2) once each in an alternating-block design (time per block, 24 s). The experiment began and ended with a 20-s fixation period (total time, 328 s). The movies were presented without audio. The child was asked to watch the videos and was reminded to remain still and alert.

Imaging data acquisition

Scanning was performed on a Siemens MAGNETOM Trio, A Tim System 3 T scanner at the Yale Magnetic Resonance Research Center. The BOLD fMRI images were acquired with 164 volumes (TR=2,000 ms, TE= 25 ms, flip angle =60 deg, matrix =64×64, voxel size =3.44×3.44 mm×4.00 mm, number of slices =34) using a 32-channel head coil. Structural MRI images were acquired using a T1-weighted MPRAGE sequence (TR=1,900 ms, TE=2.96 ms, flip angle =9 deg, matrix =256×256, voxel size =1×1×1 mm, number of slices =160).

fMRI data processing

The data were processed using the FEAT (FMRI Expert Analysis Tool) in FSL (Jenkinson et al. 2012), using a standard processing pipeline implemented on the Yale University High-Performance Computing clusters. The pipeline consists of: 1) motion correction using MCFLIRT, 2) interleaved slice timing correction, 3) BET brain extraction, 4) spatial smoothing with FWHM 5 mm, and 5) high-pass temporal filtering (100 s). The first 10 and the last 8 volumes were fixations and were dropped from analysis. The remaining 146 volumes were analyzed. Registration of EPI data was performed using both the subject's structural scan and then the MNI152 standard brain.

Artifact removal was performed with FIX (FMRIB's ICA-based Xnoiseifier) (Salimi-Khorshidi et al. 2014) in FSL, which involves three steps. In the first step, an experienced researcher (DY) examined the outputs from the MELODIC to hand-label the artifact-ridden components. This was done across all ASD participants on both pre-treatment and post-treatment scans to ensure that every single independent component (IC) among the ASD participants could be carefully examined. On average, there were 66.05 ICs per scan ($SD=11.08$) and among them, 24.20 ICs were identified as artifacts ($SD=12.73$). In the second step, we compared the hand-labeling results to those generated by the standard training data provided by FIX, to corroborate the identification of artifact components. From these, customized training data were built. Finally, we ran MELODIC again on the raw data for ASD and TD participants. Because MELODIC uses a random seed, it produced somewhat different ICs results. We applied the customized training data to the new MELODIC outputs to clean up the artifacts. In this final step, on average, there were 62.68 ICs per scan ($SD=11.19$) and among these, 25.96 ICs ($SD=25.67$) were identified as artifacts and removed. This artifact removal procedure was completed before the participants were classified as hyperactive vs. hypoactive participants, and the researchers were blind to whether they could be hyperactive or hypoactive.

The GLM analysis was implemented using FILM pre-whitening, convolution with the default gamma function in FEAT and temporal derivatives. Thresholding at the subject level was implemented through cluster analysis, voxel-level threshold $Z>1.96$, $p<.05$, two-sided, and cluster-level threshold $p<.05$. The subject-level parameter estimates were used for the higher-level fMRI analyses.

sMRI data processing

The T1-weighted MPRAGE structural scan was analyzed using FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>), which provides automatic volumetric segmentation of 40 structures, such as amygdala, thalamus, and hippocampus. This automatic segmentation technique is robust to anatomical variability and comparable in accuracy to manual labeling (Fischl et al. 2002).

Statistical analysis strategies

Our primary goal was to understand the neural responses to PRT using fMRI. Toward this goal, we used repeated-measures ANOVA in FSL/FEAT. This particular experimental design is also known as a single-group paired difference or paired *t*-test (for details: see <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM>) and is well-suited for a design that involves pretest and posttest. Specifically, the difference between pre-test and posttest on a group level was modeled, while each subject's mean effect was also estimated and controlled. Thus, the results of neural changes with PRT on the group level do not confound any individual subject's mean level of response. Finally, because of a small sample size, we employed fixed-effects modeling.

For region-of-interest analyses, given the well-validated biological motion fMRI task we used (e.g., Kaiser et al. 2010), we chose to use the right pSTS as a ROI across all participants with ASD. This ROI was based on the right banks of the STS in the Desikan-Killiany atlas (Desikan et al. 2006) in FreeSurfer, converted to MNI152 space. In addition, for the children exhibiting hypoactivation, we also included the ventral striatum, a component of the reward system, based upon the social motivation hypothesis (Kohls et al. 2012; Scott-Van Zeeland et al. 2010). This ROI was based on the ventral striatum in the Oxford-GSK-Imanova structural striatal atlas in FSL (Jenkinson et al. 2012). For the children with hyperactivation, we included the amygdala, thalamus, and hippocampus based upon the intense world hypothesis (Markram, et al. 2007; Markram and Markram 2010a, b). These three ROIs were based on the Harvard-Oxford subcortical structural atlas in FSL (Jenkinson et al. 2012). Within all of these ROIs, we conducted additional fine-grained analyses with the goal of distinguishing, via contrast masking in FSL, between results driven by positive or negative BOLD changes in response to PRT. More details are described in the succeeding Results sections.

Results

Clinical gains

The children made significant gains in social communication skills following treatment, as exemplified in Fig. 3. While all of the children made substantial improvements in their social communication skills, the magnitude of the gains varied between the children (Ventola et al. 2014, for more detail).

Group identification at baseline

Using a singleton-versus-group approach (see <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM> for details), each child with ASD was compared to a group of five TD children on the pre-

treatment BOLD response to biological motion versus scrambled motion in the right pSTS. A child with ASD with significantly weaker-than-normal (or stronger-than-normal) activation in pSTS region prior to treatment was categorized as hypoactive or hyperactive, respectively. Through this process, five of the children with ASD were identified as hyperactive, while the other five were identified as hypoactive. Their pre-treatment brain responses are shown in Fig. 4.

Group difference (pre-treatment): Cognitive abilities and clinical symptomatology

As shown in Table 2, the hyperactive and hypoactive groups did not differ in cognitive abilities or severity of ASD symptoms.

The groups were further compared on symptoms of psychopathology using the CSI-4 and CBCL. As indicated by Tables 3 and 4, the hyperactive (vs. the hypoactive) children tended to have greater anxiety and difficulties with attention and self-regulation, as evidenced by elevation of scores in the oppositional defiant disorder and conduct disorder domains. (See Ventola et al. 2014 for more details regarding clinical outcome.)

Group differences prior to treatment onset: Volumetric ROI analysis

In addition to clinical symptomatology, we further compared the two groups on the brain volumes of key structures that are thought to be key for the intense world hypothesis and social motivation theory. We predicted that amygdalae would be enlarged in the hyperactive vs. hypoactive group. As shown in Table 5, this prediction was supported for the left, but not right, amygdala. When tested against the TD group, the hyperactive group showed significantly enlarged left and right amygdalae, $p < .05$. Two primary structures connected to the amygdala, namely the thalamus and hippocampus, were also tested. The right hippocampus, but not the thalamus, was marginally enlarged in the hyperactive group relative to the hypoactive group, $p < .10$.

Whole brain analysis: brain mechanisms supporting response to PRT treatment

Hypoactive group—Using a repeated-measures ANOVA and the coherent (BIO) versus scrambled (SCR) biological motion contrast we compared the pre-treatment versus post-treatment brain responses within the ‘hypoactive’ children ($n=5$). As shown in Fig. 5, there were widespread increases in BIO > SCR activation following treatment. As predicted, the regions of significant increase included the right pSTS region as well as the ventral striatum. There were no regions of decreased BIO > SCR activation, post-treatment versus pre-treatment, in this group.

Hyperactive group—Via repeated-measures ANOVA, we compared the BIO > SCR response within hyperactive children ($n=5$) pre- vs. post-PRT. As shown in Fig. 6, the hyperactive children exhibited the predicted decrease in activation post-treatment in the right pSTS region, the amygdala, thalamus, and hippocampus. There were no regions of increased activation post-treatment relative to pre-treatment.

Region-of-interest analysis: changes with PRT treatment

Hypoactive group—Within the ROIs, we analyzed whether the increased activation in the BIO vs. SCR contrast in the hypoactive group was primarily driven by changes in the response to the BIO or SCR stimuli, or both, via contrast masking. For example, by masking the increased activation in the BIO vs. SCR contrast with increased activation in BIO alone, we were able to identify the regions associated with BIO vs. SCR changes primarily driven by increased activation in BIO. The increased activation in BIO alone refers to an increase post-treatment vs. pre-treatment, relative to an implicit baseline across the time series of the scan (i.e., ‘grand mean scaling’ in FSL). As indicated in Table 6, PRT reduced the hypoactive children’s ventral striatum responses to less socially meaningful stimuli, and to a lesser extent, increased the ventral striatum responses to more socially meaningful stimuli. In addition, PRT increased the response to biological motion in the hypoactive group in the right pSTS. Effect sizes are depicted in Fig. 7.

Hyperactive group—Within the ROIs, we analyzed whether the decreased activation in the BIO vs. SCR contrast in the hyperactive group were primarily driven by changes in the response to the BIO or SCR stimuli, or both, via contrast masking. For example, by masking the decreased activation in the BIO vs. SCR contrast with decreased activation in BIO alone, we were able to identify the regions associated with BIO vs. SCR changes primarily driven by decreased activation in BIO. The decreased activation in BIO alone refers to a decrease post-treatment vs. pre-treatment, relative to an implicit baseline across the time series for each voxel (i.e., ‘grand mean scaling’ in FSL). As described in Table 7, the effects of PRT were associated with changes in responses to BIO as well as SCR motion stimuli, in the right pSTS, amygdala, thalamus, and hippocampus. Effect sizes are depicted in Fig. 8.

Functional connectivity analysis

Hypoactive group—We conducted whole-brain functional connectivity analyses in our subject groups using the left and right ventral striatum, respectively, as an anatomically defined seed region. The results associated with the two seed regions were highly similar. For simplicity, we report only the analysis of the right ventral striatum. As shown in Fig. 9, results indicated significantly increased functional connectivity from the right ventral striatum to the right pSTS, with PRT versus baseline, $Z > 1.96$, Cluster $P < .05$, repeated-measures ANOVA (post vs. pre, for hypoactive group). There was also increased functional connectivity to a midline brain cluster, including the precentral cingulate, and paracingulate gyri, as well as another cluster in the right inferior frontal gyrus, corresponding to the expected location of a central node in the human mirror neuron system (Iacoboni and Dapretto 2006).

Hyperactive group—We conducted whole-brain functional connectivity analyses in our two groups using the left and right amygdalae as anatomically defined seed regions. For the right amygdala, there were no significant findings. For the left amygdala, as shown in Fig. 10, results indicated significantly decreased functional connectivity to the left prefrontal regions (including the inferior frontal gyrus and orbitofrontal cortex), with PRT versus baseline, $Z > 1.96$, Cluster $P < .05$, repeated-measures ANOVA (pre vs. post, for hyperactive group).

Discussion

We aimed to understand the neural mechanisms of response to an empirically validated behavioral treatment approach. Clinically, all 10 children with ASD made significant and meaningful improvement in social communication skills (Ventola et al. 2014). Additionally, the neural systems supporting social perception were malleable through the implementation of PRT; following treatment, neural responses to a well-validated task of social perception were more similar to those of TD children. As such, we demonstrated a potential biomarker for treatment response that can be further elucidated with genetics studies. Using this potential biomarker, as this work continues, we will look to assess treatment outcomes at the neural-systems-level, identify neuroimaging-derived biomarkers that may serve as moderators of treatment response, and inform the ability to predict treatment response towards an individualized medicine approach.

At baseline, two distinct subgroups emerged within our sample: one group of children exhibited decreased, or hypoactivity, in the right pSTS at the start of treatment, and the other exhibited increased, or hyperactivity, in the right pSTS at the start of treatment, relative to TD children. These differences in neural markers corresponded to differences in overall symptoms of anxiety and attention and even broad measures of behavioral regulation. The hyperactivation group had significantly greater parent-reported symptoms of both anxiety and attention problems and greater difficulties with compliance/behavioral control than the hypoactivation group. The groups did not otherwise differ; they were consistent on cognitive abilities, level of autistic symptomatology, and other comorbid psychiatric symptoms. Strikingly, the neural mechanisms of treatment response differed between these two distinct groups of children. The group of children who exhibited hypoactivation at the start of treatment evidenced increased activation in the ventral striatum and putamen, two regions involved in reward circuitry. The group of children who exhibited hyperactivation at the start of treatment evidenced decreased activation in subcortical regions responsible for regulating the flow of stimulation and conveying signals to the cortex, namely the thalamus.

These results are consistent with the widespread evidence on the heterogeneity of ASD, both at the clinical and neural-systems level. Given this inherent variability, differing theories have emerged to explain the nature and underlying mechanisms of ASD. No theory thus far can explain the manifold symptoms in all children on the autism spectrum, and consistent with this, our results fit two divergent theories. The neural profile and response to treatment for the hypoactivated group appears consistent with the social motivation hypothesis (Dawson et al. 2012). These children had decreased activity in the cortical regions associated with social perception, and then following treatment, the neural pattern, as measured using a well-validated cognitive paradigm (Kaiser et al. 2010), showed increased activation in reward circuitry as well as in cortical regions responsive to social information. These findings closely mirror the theoretical framework and prior work on this hypothesis (Kohls et al. 2012; Scott-Van Zeeland et al. 2010).

The children in the hyperactivated group, however, countered the social motivation hypothesis; the neural profile and response to treatment for this group was consistent with the intense world hypothesis (Markram and Markram 2010a, b). These children exhibited

increased activation in cortical regions associated with social perception, and then following treatment, their neural pattern, using the same well-validated cognitive paradigm, showed decreased activation in the same cortical regions and also in subcortical regions associated with relaying information (e.g., thalamus). It seemed PRT might have worked to help these children regulate the intensity of information flow, which therefore improved social functioning. Our clinical findings supported this idea as well; this group of children exhibited significantly higher levels of anxiety and attention difficulties/hyperactivity than the hypoactivated group, consistent with the idea of difficulty regulating information flow. The intense world hypothesis, however, is not without critique. Many point to how sensory symptoms vary widely in ASD and that these sensory processing atypicalities are seen in ASD but are not central to the disability. Additionally, there is much caution around the clinical treatment implications for the theory, as children with ASD should not be shielded from social interactions, and their environments should not be filtered, as the theory may suggest. Indeed, our work suggests that even children exhibiting hyperreactivity to complex sensory information can benefit from a therapy that targets social interaction.

ASD is a heterogeneous disorder. Despite this heterogeneity, though, all children in our study made substantial clinical gains; the clinical endpoint was similar, as was the endpoint for cortical activation. Both groups “normalized” activation in cortical regions linked to social perception, but the hyperactivated group decreased activation and the hypoactivated group increased activation following treatment. Additionally, all exhibited changes in subcortical circuitry. We hypothesize that subcortical changes, based on the neural characteristics of the given individuals, led to cortical changes. As the study continues, and we collect data at more frequent time periods, we will be better able to elucidate the temporal relationship and therefore the direction of this relationship between cortical and subcortical regions.

Though this work is preliminary with relatively small groups that limit generalizability, it nonetheless illustrates the heterogeneity of ASD and its multifactorial nature, and it suggests the existence of multiple neural biomarkers. Therefore, PRT may exert its effect in different ways for different children, but it is effective in promoting social communication skill development and importantly, leads to changes at the level of neural systems.

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Fig. 1.
Two theories of social deficits and their treatment via PRT. **a** The Social Motivation Hypothesis; **b** The Intense World Hypothesis

Fig. 2.
fMRI stimuli: biological and scrambled motion

Fig. 3.
Change (improvement) in SRS raw score pre- to post-treatment. Error bars indicate standard error of the mean

Fig. 4.

Comparison of the BOLD responses to biological motion versus scrambled motion prior to treatment from three groups. Top panel: As expected, TD children exhibited activity in the right pSTS during social perception. Bottom panel, left (*right*): children with significantly stronger (*weaker*) activation in this right pSTS prior to treatment, versus the TD children, were categorized as hyperactive (*hypoactive*). $Z > 1.96$, cluster $P < .05$

Fig. 5.

In the children exhibiting hypoactivation at pre-treatment ($n=5$), **a** PRT increased BIO > SCR activity in the right pSTS; **b** PRT increased subcortical activity in social reward circuitry (ventral striatum). Repeated-measures ANOVA, threshold: voxel-level $Z > 1.645$, cluster-level $P < .05$, MNI152 coordinate

Fig. 6.

In the hyperactive children ($n=5$) PRT: **a** decreased BIO > SCR activity in the right pSTS; **b** PRT decreased BIO > SCR activity in the thalamus. Repeated-measures ANOVA, threshold: voxel-level $Z > 1.645$, cluster-level $P < .05$, MNI152 coordinate

Fig. 7.

Hypoactive group's brain BIO > SCR changes with PRT within the right pSTS and ventral striatum. Error bars indicate 10 and 90 % of the ordered percent signal change values of the voxels within the mask, representing a robust range

Fig. 8.

Hyperactive group's brain BIO > SCR changes with PRT within the right pSTS, amygdala, thalamus, and hippocampus. BOLD: biological motion > scrambled motion. Error bars indicate 10 and 90 % of the ordered percent signal change values of the voxels within the mask, representing a robust range

Fig. 9.

For the hypoactivation group, increased functional connectivity from the right ventral striatum (the seed region) to the right pSTS, after PRT. Repeated-measures ANOVA experimental design (post vs. pre), threshold: voxel-level $Z > 1.96$, cluster-level $P < .05$, BOLD: BIO > SCR

Fig. 10.

For the hyperactivation group, decreased functional connectivity from the left amygdala (the seed region) to the left prefrontal regions, after PRT. Repeated-measures ANOVA (pre vs. post), threshold: voxel-level $Z > 1.96$, cluster-level $P < .05$, BOLD: BIO > SCR

Table 1

Participant demographic and characterization data

	Group	Age (years)	Gender	Hyper/Hypo Activation	DAS-II verbal reasoning standard score	DAS-II nonverbal reasoning standard score	DAS-II spatial reasoning standard score	DAS-II global conceptual ability
Child 1	ASD	4.5	Male	Hyper-	107	109	137	122
Child 2	ASD	5.2	Male	Hypo-	105	117	130	121
Child 3	ASD	4.5	Female	Hypo-	108	127	119	122
Child 4	ASD	5.5	Male	Hypo-	117	111	105	114
Child 5	ASD	5.8	Male	Hyper-	153	101	109	127
Child 6	ASD	4.9	Female	Hypo-	111	117	114	117
Child 7	ASD	4.8	Male	Hyper-	98	89	104	96
Child 8	ASD	6.2	Male	Hyper-	123	122	121	128
Child 9	ASD	7.0	Male	Hyper-	95	94	100	95
Child 10	ASD	6.4	Male	Hypo-	105	101	109	106
Child 11	TD	5.2	Female	N/A	129	109	112	121
Child 12	TD	6.7	Male	N/A	113	102	100	106
Child 13	TD	5.1	Male	N/A	103	111	95	104
Child 14	TD	6.3	Female	N/A	107	104	129	117
Child 15	TD	7.7	Female	N/A	-	-	-	-

Cognitive data was unavailable for Child 15

Table 2

Cognitive abilities and severity of ASD symptomatology prior to treatment onset and after treatment endpoint

Measures	Pre-treatment				Post-treatment			
	Hyperactive	Hypoactive	Diff. (<i>t</i> -value)	Cohen's <i>d</i>	Hyperactive	Hypoactive	Diff. (<i>t</i> -value)	Cohen's <i>d</i>
IQ (DAS-II, GCA)	113.60 (16.68)	116.00 (6.44)	−0.30	−0.19	–	–	–	–
ADOS module 3								
Social affect	13.40 (4.83)	11.40 (5.73)	0.60	0.38	8.80 (4.44)	10.00 (4.24)	−0.44	−0.28
RRB	3.80 (2.76)	4.20 (1.30)	−0.29	−0.19	2.00 (1.23)	3.60 (1.34)	−1.97	−1.24
CSS score	8.60 (1.52)	8.00 (1.87)	0.56	0.35	6.00 (2.45)	7.60 (2.79)	−0.96	−0.61
SRS Total raw	90.00 (20.75)	76.40 (29.58)	0.76	0.53	66.60 (29.98)	40.67 (12.66)	1.39	1.13
SRS Total t-score	77.00 (9.83)	72.20 (12.87)	0.61	0.42	65.80 (14.34)	54.67 (8.02)	1.21	0.96

Standard deviations appear in parentheses. Pre-treatment SRS $n=9$ and post-treatment SRS $n=8$, due to changes in study protocol. All subjects received the ADOS at both time points

Table 3

Group comparisons on the CBCL, DSM-Oriented Scales prior to treatment onset and after treatment endpoint

Domain	Pre-treatment				Post-treatment			
	Hyperactive	Hypoactive	Diff. (t-value)	Cohen's d	Hyperactive	Hypoactive	Diff. (t-value)	Cohen's d
Affective problems	0.26 (0.25)	0.03 (0.06)	1.49	1.27	0.29 (0.21)	0.00 (0.00)	2.41	1.95
Anxiety problems	0.43 (0.21)	0.16 (0.05)	2.88 *	1.77	0.59 (0.58)	0.10 (0.17)	1.39	1.15
ADD & ADHD	0.85 (0.20)	0.43 (0.12)	3.28 *	2.55	1.26 (0.25)	0.32 (0.17)	5.41 **	4.40
Oppositional Defiant	0.98 (0.73)	0.29 (0.34)	1.51	1.21	1.22 (0.69)	0.11 (0.19)	2.67	2.19

The item average was used so that the score could be comparable between the pre-school and school-age versions of the measure (the two versions have different numbers of items). Only shared domains were included. Pre-treatment n =8, post-treatment n =6 due to changes in study protocol ($n=2$) and failure for families to complete post-treatment forms ($n=2$)

*
 $p < .05$.

**
 $p < .01$

Table 4

Group comparisons on the CSI-4 prior to treatment onset and after treatment endpoint

Domain	Pre-treatment				Post-treatment			
	Hyperactive	Hypoactive	Diff. (t-value)	Cohen's d	Hyperactive	Hypoactive	Diff. (t-value)	Cohen's d
AD/HD, inattentive type	9.00(1.73)	9.75 (5.06)	-0.24	-0.20	9.00 (6.00)	7.33 (2.52)	0.44	0.36
AD/HD, hyperactive-impulsive type	15.33 (4.51)	5.25 (2.75)	3.71*	2.70	13.33 (4.51)	4.33 (2.52)	3.02*	2.46
Oppositional defiant disorder	15.33 (4.73)	3.00(2.16)	4.71*	3.35	12.67(10.02)	3.00 (2.65)	1.62	1.32
Conduct disorder	6.33 (4.04)	0.25 (0.50)	3.08*	2.11	7.33 (9.24)	0.00 (0.00)	1.38	1.12
Generalized anxiety disorder (1)	0.67 (0.58)	0.25 (0.50)	1.03	0.78	1.00(1.00)	0.33 (0.58)	1.00	0.82
Generalized anxiety disorder (2)	6.67 (3.06)	1.75 (0.50)	3.27*	2.24	5.00 (5.20)	1.33 (0.58)	1.22	0.99
Specific phobia	0.33 (0.58)	0.00 (0.00)	1.20	0.80	0.33 (0.58)	0.00 (0.00)	1.00	0.80
Obsessions	0.33 (0.58)	0.00 (0.00)	1.20	0.80	0.00 (0.00)	0.00 (0.00)	-	-
Compulsions	0.33 (0.58)	0.00 (0.00)	1.20	0.80	0.33 (0.58)	0.00 (0.00)	1.00	0.80
Posttraumatic stress disorder	0.00 (0.00)	0.00 (0.00)	-	-	0.00 (0.00)	0.00 (0.00)	-	-
Motor tics	0.33 (0.58)	0.25 (0.50)	0.21	0.15	0.33 (0.58)	0.33 (0.58)	0.00	0.00
Vocal tics	0.67(1.16)	0.00 (0.00)	1.20	0.82	0.33 (0.58)	0.00 (0.00)	1.00	0.80
Schizophrenia (1)	0.00 (0.00)	0.00 (0.00)	-	-	0.00 (0.00)	0.00 (0.00)	-	-
Schizophrenia (2)	0.67 (0.58)	0.00 (0.00)	2.39	1.63	0.67(1.16)	0.00 (0.00)	1.00	0.82
Major depressive disorder (1)	1.00(1.00)	0.00 (0.00)	2.07	1.41	0.67(1.16)	0.00 (0.00)	1.00	0.82
Major depressive disorder (2)	0.67 (0.58)	0.00 (0.00)	2.39	1.63	0.33 (0.58)	0.00 (0.00)	1.00	0.80
Dysthymic disorder (1)	1.00(1.00)	0.00 (0.00)	2.07	1.41	0.33 (0.58)	0.00 (0.00)	1.00	0.80
Dysthymic disorder (2)	1.00(1.00)	0.00 (0.00)	2.07	1.41	0.67(1.16)	0.00 (0.00)	1.00	0.82
Autistic disorder (1)	6.33 (1.16)	3.25 (2.99)	1.66	1.36	5.00(1.73)	2.33 (0.58)	2.53	2.07
Autistic disorder (2)	6.00(1.73)	4.75 (3.50)	0.56	0.45	4.67 (2.52)	2.67(1.16)	1.25	1.02
Autistic disorder (3)	5.33 (1.53)	2.50 (1.73)	2.24	1.73	4.67 (2.31)	1.00(1.00)	2.52	2.06
Autistic disorder total	17.67 (3.79)	10.50 (1.00)	3.73*	2.59	14.33 (6.43)	6.00 (2.00)	2.14	1.75
Asperger's disorder (1)	6.33 (1.16)	3.25 (2.99)	1.66	1.36	5.00(1.73)	2.33 (0.58)	2.53	2.07
Asperger's disorder (2)	5.33 (1.53)	2.50 (1.73)	2.24	1.73	4.67 (2.31)	1.00(1.00)	2.52	2.06
Social phobia	4.00 (2.65)	3.75 (1.71)	0.15	0.11	4.33 (2.08)	3.00 (0.00)	1.11	0.90
Separation anxiety disorder	3.00 (2.65)	0.00 (0.00)	2.35	1.60	5.50(2.12)	0.00 (0.00)	4.92*	3.67
Enuresis	1.00(1.73)	0.25 (0.50)	0.85	0.59	0.67(1.16)	0.00 (0.00)	1.00	0.82
Encopresis	1.00(1.73)	0.00 (0.00)	1.20	0.82	0.67(1.16)	0.00 (0.00)	1.00	0.82

To maximize the variance of the score to be compared between the groups, the items were coded as *Never* = 0, *Sometimes* = 1, *Often* = 2, *Very Often* = 3, and the total score is reported. $N=7$ due to changes in study protocol

Table 5
Brain volumes (in cubic mm) measured prior to treatment onset and after treatment endpoint

ROI	Pre-treatment				Post-treatment			
	Hyperactive	Hypoactive	Diff. (t-value)	Cohen's d	TD	Diff. (t-value) Hyperactive vs. TD	Cohen's d	Cohen's d
Left amygdala	1,738 (236)	1,422 (74)	2.85*	1.81	1,415 (174)	2.46*	1.56	1.54
Right amygdala	2,036 (355)	1,799 (288)	1.17	0.73	1,588 (150)	2.60*	1.64	1.71
Left thalamus Proper	6,194 (725)	5,978 (333)	0.61	0.38	5,983 (441)	0.56	0.35	1.46
Right thalamus Proper	6,560 (953)	6,181 (439)	0.81	0.51	6,223 (508)	0.70	0.44	1.10
Left hippocampus	4,332 (579)	4,012 (312)	1.09	0.69	3,553 (598)	2.09	1.32	0.37
Right hippocampus	4,171 (326)	3,844 (127)	2.10	1.32	3,763 (376)	1.83	1.16	0.80
Left VS/NAcc	1,122 (229)	994 (94)	1.16	0.73	871 (83)	2.31	1.46	0.09
Right VS/NAcc	935 (114)	853 (75)	1.35	0.85	875 (56)	1.06	0.67	1.54

The TD had structural MRI data at the pre-treatment time point only. VS/NAcc Ventral Striatum/Nucleus Accumbens, which was estimated using the Accumbens-area in FreeSurfer

* $p < .05$

Table 6

Hypoactive group's brain changes with PRT within the right pSTS and Ventral Striatum

Mechanism of change	Region	Hemi	<i>x</i>	<i>y</i>	<i>z</i>	Extent (voxels)
Increased response to BIO	Right pSTS	R	46	-36	6	39
	Ventral striatum	R	8	6	-2	25
Decreased response to SCR	Ventral striatum	L	-14	6	-8	34
		R	8	8	-4	36

Voxel size = $2 \times 2 \times 2 \text{ mm}^3$, Extent (voxels) = 5. The *x*, *y*, *z* coordinates listed are the location of the peak activation in each cluster, in MNI152 space (in mm). BOLD: biological motion > scrambled motion

Table 7

Hyperactive group's brain changes with PRT within the right pSTS, amygdala, thalamus, and hippocampus

Mechanism of change	Region	Hemi	x	y	z	Extent (voxels)
Decreased response to BIO	Right pSTS	Right	56	-42	4	259
	Amygdala	Right	26	0	-12	45
	Thalamus	Left	-10	-34	8	343
		Right	18	-28	-4	259
	Hippocampus	Left	-8	-40	4	35
		Right	22	-28	-8	100
Increased response to SCR	Right pSTS	Right	48	-42	12	81
	Amygdala	Right	22	-10	-12	17
	Thalamus	Left	-8	-34	8	185
		Right	8	-12	12	250
	Hippocampus	Right	28	-8	-28	24

Voxel size = $2 \times 2 \times 2$ mm. Extent (voxels) = 5. The x, y, z coordinates listed are the location of the peak activation in each cluster, in MNI152 space in mm. BOLD: BIO > SCR