Normal Movement Selectivity in Autism

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SUMMARY

It has been proposed that individuals with autism have difficulties understanding the goals and intentions of others because of a fundamental dysfunction in the mirror neuron system. Here, however, we show that individuals with autism exhibited not only normal fMRI responses in mirror system areas during observation and execution of hand movements but also exhibited typical movement-selective adaptation (repetition suppression) when observing or executing the same movement repeatedly. Movement selectivity is a defining characteristic of neurons involved in movement perception, including mirror neurons, and, as such, these findings argue against a mirror system dysfunction in autism.

INTRODUCTION

Impaired social interaction is one of the three core symptoms of the autism spectrum disorders (ASD) (DSM-IV-TR, 2000). This impairment has been attributed to a dysfunction of the human mirror neuron system (Fecteau et al., 2006; Iacoboni and Dapretto, 2006; Oberman and Ramachandran, 2007; Rizzolatti et al., 2009; Williams et al., 2001), which is thought to play a central role in our ability to perceive the intentions and goals of others (Rizzolatti and Craighero, 2004). Evidence supporting this hypothesis comes from neuroimaging studies reporting weaker mirror system responses in ASD individuals, compared with typical control individuals, during movement observation, execution, and imitation tasks. However, previous studies have not assessed the selectivity of cortical activity in mirror system areas for particular movements.

Movement selectivity is a fundamental characteristic of neurons involved in movement perception, including mirror neurons. Accurate perception and interpretation of an observed movement requires the ability to distinguish it from other movements by representing it with a unique neural response. Indeed, movement selectivity is a defining feature of monkey mirror neurons; different subsets of mirror neurons respond to particular preferred movements, whether observed or executed, so that their activity distinguishes between different movements and also between different intentions/goals (Fogassi et al.,

2005; Gallese et al., 1996; Kohler et al., 2002; Umilta et al., 2001). In previous investigations, we (Dinstein et al., 2007) and others (Chong et al., 2008; Hamilton and Grafton, 2006; Kilner et al., 2009; Lingnau et al., 2009; Shmuelof and Zohary, 2005) have shown that human mirror system areas contain movement-selective neural populations that adapt when hand movements are observed and/or executed repeatedly. Do mirror system areas of individuals with ASD also exhibit such movement-selective responses? The mirror system dysfunction hypothesis would predict not.

Here, we applied an fMRI adaptation protocol to test whether high functioning individuals, meeting clinical criteria for autism, exhibit movement-selective cortical responses equivalent to those of typical controls. Subjects passively observed images of hand postures in one experiment and actively executed the same hand postures in a second experiment. The autism and control subjects exhibited similarly robust cortical responses during the observation and execution of hand movements. Moreover, the profile of movement selectivity in mirror system areas was equivalent in both groups; anterior intraparietal sulcus (aIPS) exhibited both motor and visual adaptation (reduced fMRI responses for repeated versus nonrepeated movements) and ventral premotor (vPM) cortex exhibited motor adaptation. These results argue against a mirror system dysfunction in autism.

RESULTS

The movement observation experiment assessed adaptation in the visual domain by comparing fMRI (BOLD) responses during observation of a repeating hand posture with responses during observation of different hand postures. Similarly, the movement execution experiment assessed adaptation in the motor domain by comparing fMRI responses during repeated versus nonrepeated execution of hand movements (see Experimental Procedures). According to the mirror system hypothesis, individuals with autism should exhibit not only weaker mirror system responses during observation and execution of movements, but also weaker adaptation during repeated observation and execution of movements.

Whole-brain SPM analyses of the observation and execution experiments showed similar results in the autism and control groups. Typical visual areas responded during movement observation (Figure 1, green), typical motor system areas responded during movement execution (Figure 2, blue), and mirror system
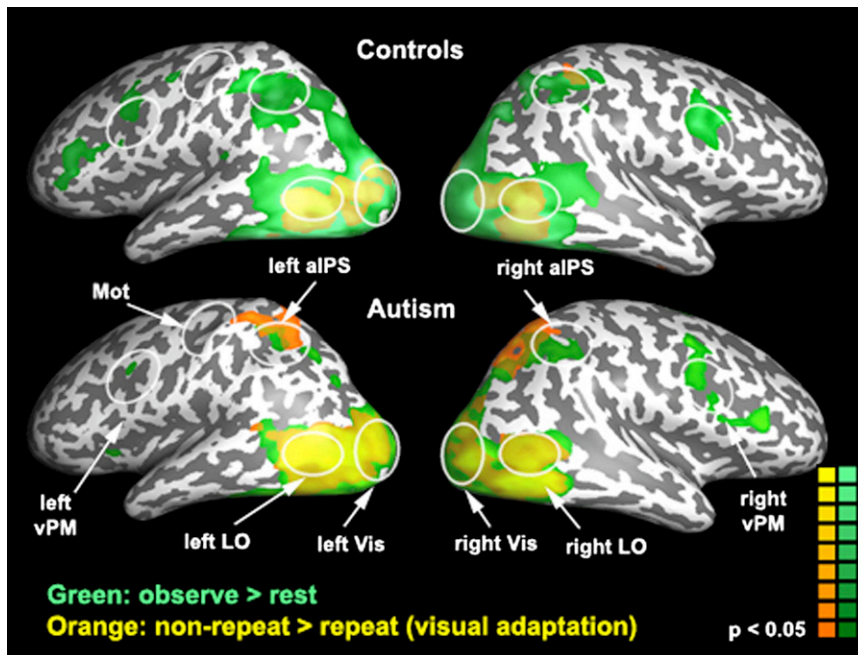
Figure 1. Cortical Responses during Movement Observation Experiment

Green: Brain areas exhibiting significantly stronger responses during observation than rest.

Orange: Brain areas exhibiting visual adaptation, as reflected in significantly stronger responses during nonrepeat blocks (when observing different hand movements) than repeat blocks (same hand movement repeatedly).

White ellipses outline the general location of the ROIs, which were selected separately for each subject.

More importantly, both subject groups exhibited movement-selective responses during observation and execution of movements. The two subject groups exhibited smaller fMRI responses in several cortical regions including bilateral early visual cortex, lateral occipital cortex, and anterior intraparietal sulcus, during blocks where the same movement was observed repeatedly in comparison to blocks where different movements were observed (Figure 1, orange). Both subject groups also exhibited smaller fMRI responses in several regions including left primary motor and somatosensory cortex, bilateral cingulate motor area, bilateral anterior intraparietal sulcus, and left ventral premotor cortex during executed movement repeats versus non-repeats (Figure 2, orange).

A region of interest (ROI) analysis revealed similar results across the two groups. We sampled cortical responses from two commonly reported mirror system areas, aIPS and vPM cortex, as well as from several control areas that are not believed
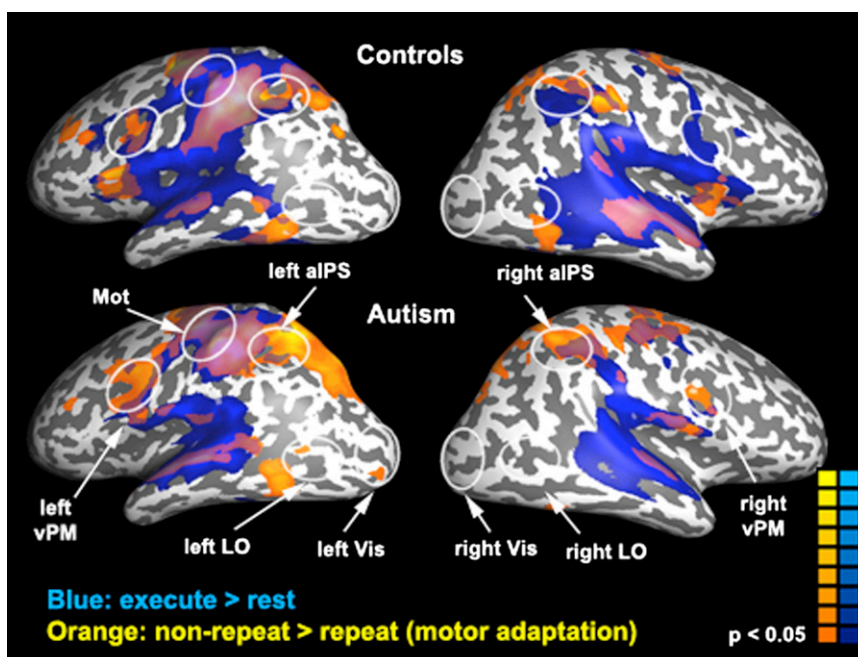
areas (aIPS and vPM) responded during both observation and execution. A direct voxel-by-voxel comparison between the two subject groups showed no significant differences between the groups in mirror system areas in either experiment. There were, however, significantly stronger responses in the control group in medial visual areas and a left dorsal lateral occipital area during the movement observation experiment and in an area below the right aIPS (ipsilateral to the hand used for execution) during the movement execution experiment (see Figure S1 available online).

Figure 2. Cortical Responses during Movement Execution Experiment

Blue: Brain areas exhibiting significantly stronger responses during execution than rest.

Orange: Brain areas exhibiting motor adaptation, as reflected in significantly stronger responses during nonrepeat blocks (when executing different hand movements) than repeat blocks (same hand movement repeatedly).

White ellipses outline the general location of the ROIs, which were selected separately for each subject.
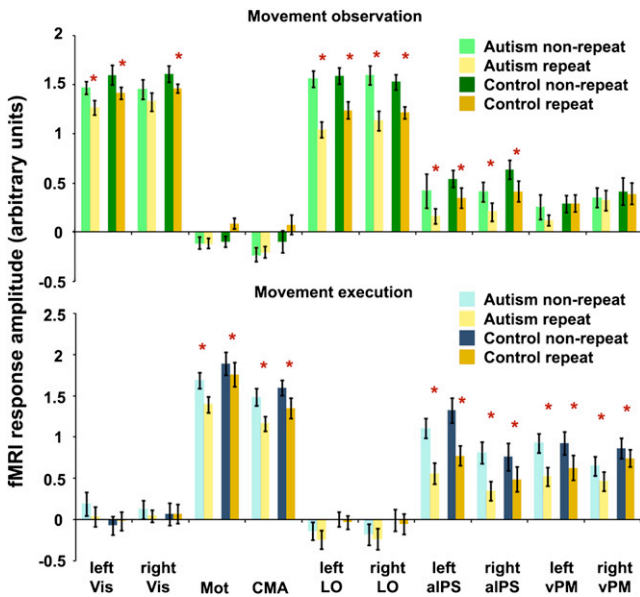
Figure 3. Region of Interest Analysis of the Movement Observation Experiment (Top) and the Movement Execution Experiment (Bottom)

Green: Mean response amplitudes when observing different (nonrepeating) hand movements, for the autism (light) and control (dark) groups.

Blue: Mean response amplitudes when executing different (nonrepeating) hand movements, for the autism (light) and control (dark) groups.

Orange: Mean response amplitudes when hand movements were repeated, for the autism (light) and control (dark) groups.

Error bars represent SEM. Asterisks indicate statistically significant adaptation (p < 0.05, Bonferroni corrected).
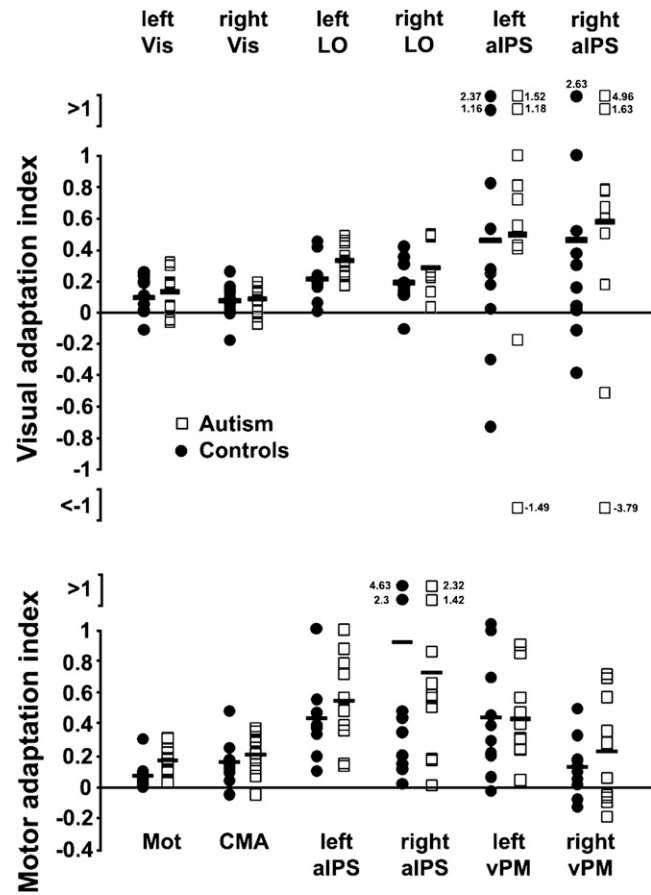

Figure 4. Adaptation Index for Individuals from the Autism Group (Open Squares) and Control Group (Filled Circles)

Top: Visual adaptation index in visual and mirror system ROIs.

Bottom: Motor adaptation index in motor and mirror system ROIs. The index was computed as the difference between nonrepeat and repeat responses divided by the absolute nonrepeat response (see Experimental Procedures). Solid lines denote the average across either the autistic or control subjects.

to contain mirror neurons. These included primary motor and somatosensory cortex (Mot), early visual cortex (Vis), cingulate motor area (CMA), and lateral occipital cortex (LO). All regions of interest were sampled separately from left and right hemisphere except for Mot, which was sampled only in the left hemisphere (movements were executed with the right hand), and CMA, which was sampled bilaterally due to its medial location that makes it difficult to separate responses from left and right hemispheres. These ten ROIs were defined individually for each of the subjects using a set of functional and anatomical criteria (see Experimental Procedures, Table S2, and Figure S2). The ROI sizes were generally smaller in the autism group, but significantly smaller only in left LO (p < 0.05, two-tailed t test, uncorrected to increase sensitivity, Figure S2). Note that the size of the selected ROIs depended on the statistical significance of the functional responses, which were a function of response amplitude and variability. Subjects with greater response variability would, therefore, be expected to exhibit fewer significantly activated voxels leading to smaller ROIs despite similar response amplitudes (see variability results below). The ROIs were defined based on their overall response to observation and execution and not based on a comparison between responses during repeat and nonrepeat blocks. There was, therefore, no statistical bias for the ROIs to exhibit adaptation.

The fMRI response amplitudes of the autism and control groups (Figure 3) were indistinguishable in the movement execution and movement observation experiments in all ROIs (p > 0.05, two-tailed t test, uncorrected to increase sensitivity). More importantly, both the autism and control groups exhibited similar magnitudes of adaptation when comparing responses during movement repeats and nonrepeats in several visual and motor areas (Figure 3). Significantly smaller visual responses to repeats were found in left and right LO, left and right aIPS, and in left Vis (p < 0.05, two-tailed paired t test, Bonferroni corrected). Significantly smaller motor responses to repeats were found in left Mot, bilateral CMA, left and right aIPS, and left and right vPM (p < 0.05, two-tailed paired t test, Bonferroni corrected). Importantly, right and left aIPS exhibited smaller responses to repeats in both the visual and motor domains. The robustness of the adaptation effects can be seen in the single subject adaptation indices, which showed similar response reductions among individuals of both groups (Figure 4) with consistent visual and motor adaptation in left and right aIPS for the majority of subjects. The autism group exhibited significantly stronger visual adaptation in left LO during the observation experiment
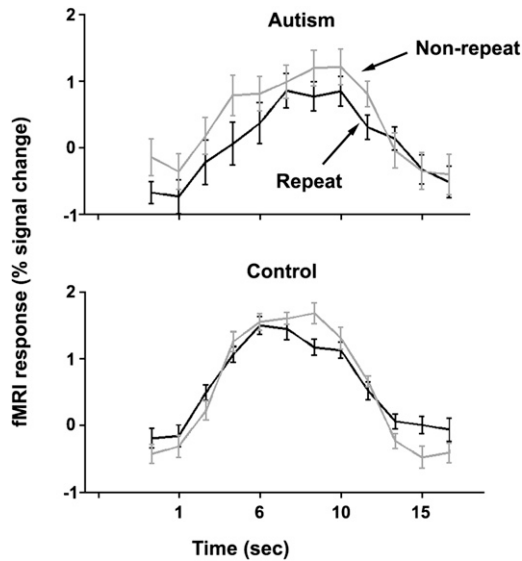
Figure 5. Within-Subject Variability

Variance across blocks. Average fMRI responses in left visual areas from a typical autistic subject (top) and control subject (bottom) during movement observation blocks. Responses from blocks where different hand movements were presented (gray) and blocks where the same hand movement was presented repeatedly (black) were averaged separately. Error bars (SEM across blocks) are larger for the autistic than the control subject.

Figure 6. Within-Subject Variability

Standard deviation across blocks. Average SD across blocks in the movement observation experiment (top) and movement execution experiment (bottom) for repeat blocks (white, autism; medium gray, controls) and nonrepeat blocks (light gray, autism; dark gray, controls). Asterisks indicate significantly larger SD in the autism group ($p < 0.05$, randomization test, see Experimental Procedures). Error bars represent SEM across individuals.

and significantly stronger motor adaptation in left Mot during the execution experiment. All other ROIs, including mirror system areas, showed no significant difference in the amount of visual or motor adaptation ($p > 0.05$, randomization test, see Experimental Procedures and Figure S3). Equivalent results were also found when contracting the selected ROIs to a fixed size of 100 functional voxels such that ROI size was matched in all subjects of both groups (Figure S6).

We further characterized the responses of both subject groups by assessing the variability of the responses in each subject individually. The responses of a typical autistic subject were less reliable/consistent across blocks (strong response to some blocks and weak response to others) than those of a typical control subject (error bars in Figure 5). To quantify this difference in response reliability, or, in other words, the within-subject variability, we performed two complementary analyses. In the first analysis, we computed the average standard deviation (SD) across time points and blocks for each subject in each condition (i.e., averaging the error bars of Figure 5, see Experimental Procedures) and then compared the SDs across individuals of the two subject groups (Figure 6). Subjects with autism exhibited significantly larger SDs in right Vis and right vPM during the movement observation experiment and in left Mot, CMA, left aIPS, left vPM, and right vPM during the movement execution experiment ($p < 0.05$, randomization test, see Experimental Procedures and Figure S4). Larger within-subject variability in the autism group was equally evident in the responses to both repeat and nonrepeat blocks.

In a second analysis, we examined how well the GLM of each experiment fit the fMRI response time courses from each subject
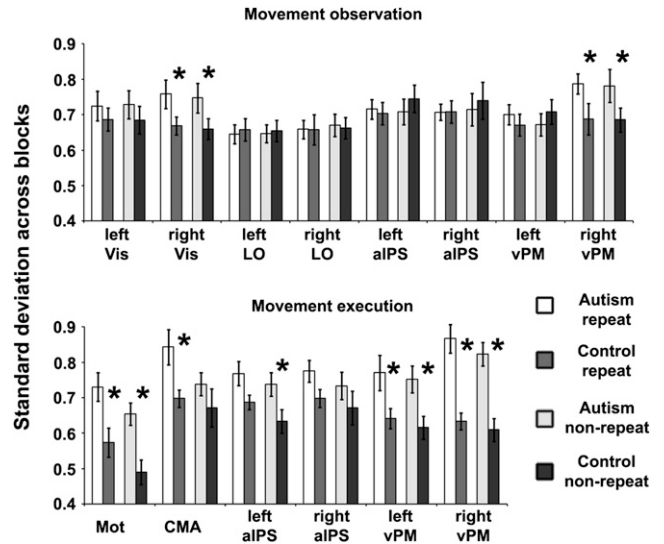
in each ROI. The GLM contained the expected hemodynamic responses based on the timing of the task blocks and assuming that the responses to successive blocks of a given condition were identical. When a particular brain area responds reliably, there is a good fit between the model and the brain activity such that a large proportion of the variance in the measured time-courses can be accounted for by the model. When brain responses are more "noisy" in timing or amplitude, the fit with the GLM is worse. Model fits were worse for individuals with autism than for controls in several ROIs (Figure 7). Significantly larger within-subject variability (poorer fit) was evident in left and right Vis during the movement observation experiment and in left Mot, left aIPS, and right vPM during the movement execution experiment ($p < 0.05$, randomization test, see Experimental Procedures and Figure S5). Importantly, autistic and control subjects exhibited almost identical hemodynamic responses, on average (Figure S7). Noisier responses in the autism group were, therefore, due to variability in the amplitude and timing of their neural responses and not because of general differences in the shape or duration of their hemodynamic responses.

DISCUSSION

The results presented here argue against a mirror system dysfunction in autism. Individuals diagnosed with autism exhibited robust responses in commonly reported mirror system areas aIPS and vPM both during observation (Figure 1, green) and execution (Figure 2, blue) of hand movements, which were equivalent to those of the control group. More importantly, autistic subjects exhibited visual and motor adaptation in right
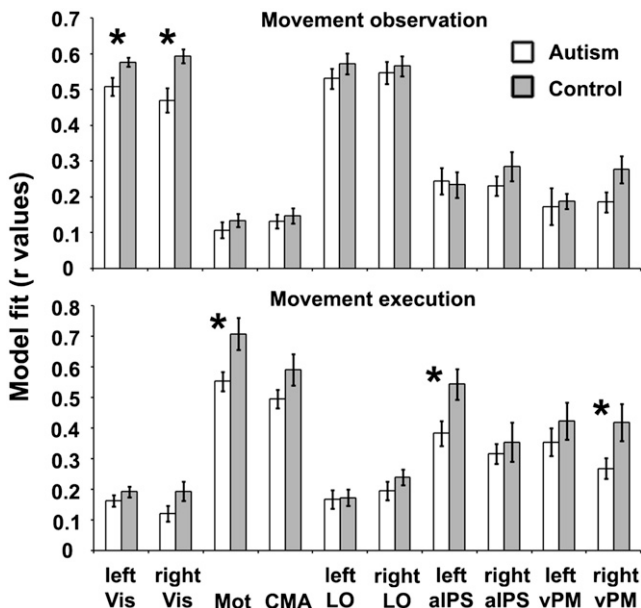
Figure 7. Within-Subject Variability

Goodness of fit. Average goodness of fit between the expected fMRI responses and the measured fMRI responses in the movement observation experiment (top) and movement execution experiment (bottom) for the autism group (white) and control group (gray). Asterisks indicate significant goodness-of-fit difference between groups (p < 0.05, randomization test, see Experimental Procedures). Error bars represent SEM.

and left aIPS, which were indistinguishable in magnitude from those of the control subjects (Figures 3 and 4). We interpret these visual and motor adaptation effects as evidence of distinct neural populations that respond selectively to particular preferred movements and that adapt (decrease their response) when a movement is repeatedly observed or executed (Grill-Spector and Malach, 2001). Such responses would be expected from neural populations that respond selectively to particular movements, including mirror neurons. These experiments, therefore, targeted a key feature of movement perception not addressed by previous studies—the ability of neural populations in mirror system areas to differentiate between different hand movements. Distinguishing between movements is a critical step for effectively mapping an observed movement onto the specific motor neuron population that encodes its execution and for determining the correct interpretation of the observed person's intentions as hypothesized by mirror system theories (Dinstein, 2008; Dinstein et al., 2008).

In a previous study, we found similar movement-selective visual and motor adaptation in areas vPM and aIPS of control subjects (Dinstein et al., 2007). In that study, we asked subjects to play the rock-paper-scissors game against a videotaped opponent while freely choosing their executed movement on each trial. We compared repeated versus nonrepeated observed and executed movements to assess visual and motor adaptation respectively. These adaptation effects were very similar in both distribution and amplitude to those reported here, albeit using a different experimental design. This replicability confirms that

visual and motor adaptation is a robust and reproducible phenomenon across different experimental protocols and demonstrates its successful use in assessing response selectivity in a population of autistic subjects. Future use of fMRI adaptation protocols in autism research offers many possibilities for precise characterization of neural population selectivity in different cortical systems of individuals with autism.

Previous studies that have examined mirror system responses in autism during observation, execution, and imitation of movements have yielded inconsistent findings. Although some studies have reported that individuals with autism exhibit weak fMRI (Dapretto et al., 2006), EEG (Martineau et al., 2008; Oberman et al., 2005), MEG (Nishitani et al., 2004), and TMS-induced corticospinal excitability (Theoret et al., 2005) responses, other fMRI (Williams et al., 2006), EEG (Oberman et al., 2008; Raymaekers et al., 2009), and MEG (Avikainen et al., 1999) studies have reported that individuals with autism exhibit equivalent responses to those of controls. There are numerous methodological issues that could have led to the disparate reports cited above. For example, it is difficult to control the behavior of subjects in an MRI scanner. When subjects are asked to imitate a movement, delays in the timing or length of the movement may greatly impact the estimated resulting brain response (e.g., if autistic subjects always imitate the movement later/slower than controls, their estimated brain response will seem weaker). Rather than trying to reconcile the results above, we simply suggest that the fact that individuals with autism can exhibit equally strong mirror system responses as those of controls argues against the claim of a generally dysfunctional mirror system in autism.

A far stronger argument against a mirror system dysfunction in autism lies in the finding that individuals with autism exhibit movement-selective adaptation equivalent to that of controls. Previous mirror system studies have used several different experimental tasks to assess mirror system responses, including passive observation and active imitation protocols using meaningless hand movements, hand-object interactions, symbolic hand movements, or emotional facial expressions. These different tasks recruit numerous neural populations (in mirror system and other cortical areas) that might include mirror neurons, but also include many other neural populations involved in vision, motor planning, motor execution, working memory, and emotion. Mirror neurons make up only about 10% of the neurons that respond during movement observation or execution in monkey mirror system areas (Fogassi et al., 2005; Gallese et al., 1996; Kohler et al., 2002; Umilta et al., 2001). Current neuroimaging techniques (fMRI, EEG, and MEG) sum over the responses of millions of neurons, thereby making it difficult to discern which of the many overlapping neural populations generated the responses in the autism and control groups. Because of this limitation, neither previous mirror system studies of autism nor the current adaptation study are capable of isolating the responses of mirror neurons alone. Nevertheless, by assessing visual and motor adaptation in mirror system areas, we have isolated the responses of movement-selective neural populations important for movement perception, rather than summing across the responses of other neural populations that coexist in these areas (Dinstein, 2008). If mirror system

theories of movement perception are indeed correct, one would expect subpopulations of mirror neurons to be "tuned" to the movement they encode. This means that mirror system areas would be expected to contain circuits of visual, mirror, and motor neurons that would be intimately interconnected by their selectivity/preference for a particular movement. The fact that these movement-selective neural circuits respond normally (adapt in a movement-selective manner) in individuals with autism suggests that the functional integrity of their mirror system areas is intact. Characterizing neural selectivity offers a far more detailed assessment of the mirror system's functional integrity, which was not possible in previous fMRI studies that summed over the responses of all neural populations within these areas.

A further important test of mirror system integrity is cross-modal adaptation. Cross-modal fMRI adaptation has been reported in mirror system areas as subjects observe a movement they have just executed or execute a movement they have just observed (Chong et al., 2008; Kilner et al., 2009; Lingnau et al., 2009). Such adaptation is a signature of mirror neuron populations responding repeatedly to their preferred movement regardless of whether it is being observed or executed. The current study was not designed to assess cross-modal adaptation, although future studies could do so by building on the results reported here.

In further analyses, we noticed that individual autistic subjects exhibited larger block-by-block response variability/unreliability than individual control subjects (Figure 5, error bars). It is well known that different individuals with autism exhibit distinct and unique behavioral symptoms. Such behavioral variability may be expected to generate between-subject cortical response variability and, indeed, several studies have reported that brain responses during different motor and visual tasks are more variable across autistic individuals than across control individuals (Hasson et al., 2009; Humphreys et al., 2008; Muller et al., 2003; Muller et al., 2001). Here, however, we describe a different type of variability; variability in the brain responses of single subjects across different blocks of an experiment. This is a measure of the consistency or reliability of a single subject's neural responses across different trials/blocks of the experiment (within-subject variability). Despite exhibiting equivalent cortical response amplitudes on average, individuals with autism exhibited significantly larger within-subject variability than controls in early visual and ventral premotor areas during movement observation and in several motor areas during movement execution (Figures 6 and 7). This difference in response variability was not due to a general difference in the hemodynamic response, which was nearly identical in the two groups (Figure S7).

There may be several sources for the greater within-subject response variability found in the autism group. One possibility is that individuals with autism behave more variably (with less consistency) throughout an experiment than control subjects. For example, subjects with autism may have exhibited "noisy" eye movements across blocks, which may have generated more variable visual system responses during the movement observation experiment. A more exciting (yet speculative) possibility is that larger within-subject response variability is a measure of increased intrinsic neural "noise," which may be a

general characteristic of neural networks in autism. Several theories have proposed that ASD may be caused by early development of abnormally connected, "noisy," and "hyperplastic" cortical networks (Markram et al., 2007; Rubenstein and Merzenich, 2003) that are more prone to epilepsy; a common comorbidity in autism (Tuchman and Rapin, 2002). These theories suggest that noisy neural responses may cause the environment to be perceived as inconsistent and noisy, making it difficult for the child to cope with the outside world, and driving him/her to develop autistic behavioral symptoms in response. Further studies assessing within-subject response variability, while controlling for within-subject behavioral variability, across age-, IQ-, and gender-matched subject groups are urgently needed to investigate this hypothesis.

Regardless of the source of fMRI response variability, our results clearly show that this variability is not equal across the two subject groups, as is commonly assumed when interpreting fMRI studies of autism. An implication of this difference in variability is that one should exercise caution when comparing activations using statistical parameter maps (SPM) across the two groups (as done in Figures 1 and 2). Differences in statistical significance (p values) may be caused by differences in either the average response amplitude or by differences in the variability of the response across trials/blocks. For example, observing a statistically significant "activation" in the control group SPM, which is absent in the autism group SPM, might not be due to a weaker response in the autism group. The responses might be of equal strength across groups, on average, but with larger variability in the autism group.

Finally, if the mirror system of ASD individuals responds in a normal movement-selective manner, why do these individuals have problems imitating and understanding the movements/intentions of others? First, it is unclear whether individuals with autism actually do have such behavioral impairments (Hamilton et al., 2007). But even if we accept that they do, this question further assumes that our ability to imitate and understand one another socially is dependent only on the activity of mirror neurons. There is little evidence to support such an assumption (see Dinstein et al., 2008; Hickok, 2009; Southgate and Hamilton, 2008). Even in monkey studies, where mirror neurons have been successfully isolated (Fogassi et al., 2005; Gallese et al., 1996; Umilta et al., 2001), there is no evidence for a causal relationship between mirror neuron activity and the ability of the monkey to understand the meaning of an observed movement. Proof of such a relationship would require showing that the removal (ablation, inactivation) of mirror neurons impairs the monkey's ability to understand the meaning of observed movements. As yet, this experiment has not been performed. There is also no evidence for a connection between mirror neuron activity and imitation of movements. This issue has not been studied in monkeys although there have been reports that macaque monkeys do imitate, at least during infancy (Ferrari et al., 2006). Numerous imaging studies have concluded that imitation and action understanding in humans are abilities that depend on mirror system responses. However, imaging studies do not test causality, but rather report brain responses that are associated with the performance of a particular task. Moreover, these studies clearly show that activities of numerous visual and motor

neural populations (not just mirror system areas) are correlated with imitation and action understanding tasks. There is, therefore, no concrete evidence to suggest that a dysfunction in mirror neurons would cause impairments in imitation or understanding the intentions of others. Similarly, there is no reason to expect that individuals with difficulties imitating or understanding actions necessarily have dysfunctional mirror neurons, rather than dysfunctions in numerous other neural populations that play integral roles in these abilities.

EXPERIMENTAL PROCEDURES

Subjects

Thirteen high-functioning male adults with autism (mean age 27.4, range 19 to 40 years old) and ten control subjects (five women and five men, mean age 27.4, range 21 to 35 years old) participated in this study. All subjects had normal or corrected-to-normal vision, provided written informed consent, and were paid for their participation in the study. Two control subjects and two autistic subjects were left-handed, but performed the movements with their nondominant right hand. The Committee on Activities Involving Human Subjects at New York University and the Institutional Review Board at Carnegie Mellon University and the University of Pittsburgh approved the experimental procedures, which were in compliance with the safety guidelines for MRI research. For each subject, we obtained a high-resolution anatomical volume, two runs of the movement observation experiment, and two runs of the movement execution experiment. Of the data acquired from autistic subjects, three data sets were excluded because of jerky head movements exceeding 2 mm. The presented analyses are, therefore, based on data collected from ten autistic and ten control subjects who completed the experiments successfully.

The diagnosis of autism was established using the Autism Diagnostic Interview Revised (ADI-R) (Lord et al., 1994), the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000) and expert clinical evaluation (Table S1). Autistic subjects had an average IQ (intelligence quotient) score of 110 (range 95-128), average ADI social score of 21 (range 15-27), average ADI communication score of 15 (range 8-22), average ADI stereotypy score of 6 (range 3-10), average ADOS social score of 9 (range 5-13), and average ADOS communication score of 5 (range 4-6). Potential subjects with autism were excluded if they had an associated neuropsychiatric or neurological disorder. Exclusion was based on neurological history and examination, chromosomal analysis, and/or metabolic testing.

The two subject groups were not matched on IQ or gender. However, this only increases our confidence in concluding that individuals with autism exhibited indistinguishable mirror system responses from those of the general control population.

Visual Stimuli and Motor Response

Stimuli were presented via an LCD projector and custom optics onto a rear-projection screen in the bore of the MRI scanner. Subjects were supine and viewed the screen through an angled mirror, which also prevented them from seeing their own hands. A rectangular foam tray was positioned above each subject's pelvis and attached to the bed in the scanner. Subjects executed movements with their hand resting on the upper surface of the tray. The executed movements were videotaped through a window from the console room.

Movement Observation Experiment

Subjects passively observed still images of six hand postures (rock, paper, scissors, thumbs-up, gun, and hang-loose). All subjects completed two runs of this experiment. Note that still images of movement end-points/postures rather than video clips of movements have been previously used to assess mirror system responses in subjects with autism and controls (Dapretto et al., 2006) and have been reported to elicit robust mirror system responses in typical subjects (e.g., Aziz-Zadeh et al., 2006; Carr et al., 2003). Images were presented in 9 s blocks of a single hand posture repeated six times (repeat blocks) or six different hand postures (nonrepeat blocks). All nonrepeat blocks contained the same hand postures presented in the same order. Repeat and nonrepeat blocks were presented in random order, but the same random order was used in both runs for each subject. Each image was presented for 750 ms followed by 750 ms of blank and the 9 s of visual stimulation were followed by 6 s of blank. The experiment contained 9 blocks of each type with 15 s of blank at the beginning and end for a total length of 5 min.

Movement Execution Experiment

Subjects executed the same six hand movements (rock, paper, scissors, thumbs-up, gun, and hang-loose) using their right hand, as instructed auditorily. All subjects completed two runs of this experiment. Instructions were presented in 12 s blocks of a single movement repeated six times (repeat blocks) or six different movements (nonrepeat blocks). Repeat and nonrepeat blocks were arranged randomly, but the same random order was used with all subjects. Each block contained 12 s of movement execution (2 s for each movement) followed by 6 s of rest. The experiment contained 10 blocks of each type with 15 s of rest at the beginning and the end for a total length of 6:30 min. Movement execution trials were slightly longer (2 s) than movement observation trials (1.5 s) to allow subjects enough time to execute the movements comfortably.

MRI Acquisition

Functional and anatomical images of the brain were acquired using identical Siemens (Erlangen, Germany) 3T Allegra MRI scanners located at the NYU Center for Brain Imaging and the Brain Imaging Research Center in Pittsburgh. Both scanners were equipped with the same Siemens birdcage head coil used for RF transmit and receive. Blood oxygenation level-dependent (BOLD) contrast was obtained using a T2*-sensitive echo planar imaging pulse sequence (repetition time of 1500 ms for movement observation experiment and 2000 ms for movement execution experiment, echo time = 30 ms, flip angle = 75°, 24 slices, $3 \times 3 \times 3$ mm voxels, field of view = 192 mm). High resolution anatomical volumes were acquired with a T1-weighted 3D-MPRAGE pulse sequence ($1 \times 1 \times 1$ mm).

Preprocessing, Movement Correction, Segmentation, and Inflation

fMRI data were processed with the Brain Voyager software package (R. Goebel, Brain Innovation, Maastricht, The Netherlands) and with custom software written in Matlab (Mathworks, Natick, MA, USA). The first two images of each functional scan were discarded. Preprocessing of functional scans included 3D motion correction and temporal high-pass filtering with a cutoff frequency of 6 cycles per scan. To minimize any residual head movement artifacts in data sets of both subject groups, after motion correction, the estimated head motion variables were removed (by orthogonal projection) from the fMRI time course of each voxel. Functional images were aligned with the high-resolution anatomical volume using trilinear interpolation, and the anatomical and functional images were transformed to the Talairach coordinate system (Talairach and Tournoux, 1988). The cortical surface was reconstructed from the high-resolution anatomical images, separately for each subject; the procedure included segmenting the gray and white matter and inflating the gray matter.

Statistical Parameter Mapping

We performed a standard statistical parameter mapping (SPM) analysis (Friston et al., 1994) to assess brain activation associated with each experimental condition. In short, we constructed a general linear model (GLM) for the underlying neural response to each experimental condition. For example, the model for our movement observation experiment was a matrix that contained a row for each time point, where neural activity was modeled as either "on" = 1 or "off" = 0, and a column for each condition: repeat and nonrepeat. The expected neural activity model (each column of the model matrix) was convolved with a canonical hemodynamic impulse response function to create a model of the expected hemodynamic response (Boynton et al., 1996). We used linear regression to estimate response amplitudes (beta values) for each voxel and each condition. Response amplitudes were computed separately for each voxel in each subject and then a paired

t test was used to determine significant voxel-by-voxel response differences across conditions (i.e., treating intersubject differences as a random effect) (Friston et al., 1999). Only voxel clusters exceeding 15 mm³ are displayed in the statistical maps. Unless stated otherwise, the resulting cortical activation maps were rendered on a representative individual's cortical surface.

ROI Definition and Analysis

To assess whether cortical areas responding during movement observation and/or execution exhibited movement-selective adaptation, we defined ten ROIs individually for each subject using a combination of anatomical and functional criteria. We overlaid each subject's statistical parameter map for observation and execution versus rest on their high-resolution anatomical scan and chose all active voxels within a radius of 15 mm around particular anatomical landmarks (Figure S2). A false discovery rate (FDR) of 0.05 was used to threshold the statistical parameter maps of each subject. FDR is a method of correcting for multiple comparisons by controlling for the expected proportion of false positives among suprathreshold voxels (Genovese et al., 2002) rather than for the rate of false positives among all voxels as done by the stricter Bonferroni method. See Figure S2 for ROI size comparison and Table S2 for ROI coordinates and a list of the anatomical landmarks used. In two of the control subjects and in three of the autism subjects, the FDR analysis did not yield any significant voxels in right and left vPM. In these cases the ROIs were defined entirely based on anatomical criteria, by selecting gray matter voxels surrounding the junction between the precentral sulcus and the inferior frontal sulcus.

ROI analyses were carried out by averaging across voxels so as to compute a single response time course for each ROI in each individual subject. We used regression with a GLM to estimate fMRI response amplitudes, as described above, separately for each ROI in each subject individually. We then performed paired t tests to determine which ROIs showed significant response differences across subjects for selected pairs of conditions (e.g., observed repeat versus nonrepeat), and corrected for multiple comparisons using the Bonferroni method.

Adaptation Index

Visual and motor adaptation indices were computed for each subject and each ROI separately. The index was the difference between the average nonrepeat response and the average repeat response divided by the absolute value of the nonrepeat response: (nonrepeat – repeat)/[nonrepeat].

A randomization test was used to assess whether the adaptation indices of the two groups were statistically different from each other or not. Specifically, we generated a distribution of index differences, according to the null hypothesis that there was no difference between groups, by randomly assigning individuals to either subject group (i.e., randomly shuffling subject identities). The randomization was repeated 10,000 times separately for each ROI to characterize ROI-specific randomized distributions (Figure S3). For the adaptation difference between the autism and control groups in a particular ROI to be considered statistically significant, it had to fall above the 95th percentile or below the 5th percentile of the relevant distribution.

We also performed the same analysis using a similar adaptation index where instead of dividing by the absolute nonrepeat response we divided by the sum of the absolute repeat and nonrepeat responses. This index had the advantage of being normalized to 1 such that subjects with relatively large or small adaptation indices had a smaller affect on the mean of the group. This analysis revealed equivalent results leading to the same conclusions as those reported using the adaptation index described above.

Within-Subject Response Variability (Trial-Triggered Average)

Response variability was characterized, separately for each experimental condition, ROI, and subject, by computing the variance across blocks. Specifically, we extracted 15 and 18 s fMRI segments in the visual and motor experiments respectively, which began at the onset of each block. This resulted in 18 visual repeat, 18 visual nonrepeat, 20 motor repeat, and 20 motor nonrepeat segments for each ROI and each subject. Figure 5 shows the average visual repeat and nonrepeat segments taken from left visual cortex of a single autistic subject and a single control subject during the movement observation experiment. The error bars in Figure 5 represent the standard error of the mean (SEM) across blocks for each time point in the segment/block.

To assess variability across subjects, we computed the SD across blocks of each condition and averaged the SD across all time points in the block (i.e., all time points plotted in Figure 5). This resulted in a single numerical measure for each subject; the average SD across blocks of a particular condition. Figure 6 shows a comparison of average SDs across subjects of the two groups. Statistics were computed using a randomization test similar to that described for adaptation indices. We generated a distribution of SD differences, by randomly assigning individuals to either subject group, and determined whether the difference in SD of the actual two subject groups fell above the 95th percentile or below the 5th percentile of the identity-randomized difference distribution (Figure S4).

Within-Subject Response Variability (Model Fits)

Another method for assessing the response variability was to determine the goodness of fit between the general linear model and the measured fMRI response time-courses. Goodness of fit, r, was computed, separately for each subject and each ROI, as the square root of the variance accounted for by the estimated hemodynamic responses (Gardner et al., 2005). That is, the modeled hemodynamic response time course for each condition (repeat or nonrepeat) was multiplied with the appropriate response amplitude (beta weight) and the resulting time courses of the two conditions were summed. The r^2 was then computed by dividing the variance of the modeled time course by the variance of the measured time course. Statistics were computed using a randomization test similar to that described for adaptation indices (Figure S5). We generated a distribution of model fit differences, by randomly assigning individuals to either subject group, and determined whether the model fit difference of the actual two subject groups fell above the 95th percentile or below the 5th percentile of the identity-randomized difference distribution.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven figures and two tables can be found with this article online at doi:10.1016/j.neuron.2010.03.034.

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