Increased response variability in autistic brains?

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One of the key ideas regarding atypical connectivity in autistic brains is the hypothesis of noisier networks. The systems level version of this hypothesis predicts reduced reliability or increased variability in the evoked responses of individuals with autism. Using magnetoencephalography, we examined the response of individuals with autism spectrum disorder versus matched typically developing persons to passive tactile stimulation of the thumb and index finger of the dominant (right) hand. A number of different analyses failed to show higher variability in the evoked response to the thumb or to the index finger in the autism group as compared with typicals. Our results argue against the hypothesis that the brain networks in autism are noisier than normal. *NeuroReport* **20:1543–1548 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Autism spectrum disorders (ASD) are a family of pervasive developmental disorders that are widely accepted to be underscored by abnormalities in brain development. Recent studies on humans using behavioral and neuroimaging techniques [1–5], as well as studies using animal models [6], have shown anatomically characterized abnormal trajectories of brain development and functional impairments in several brain regions including the frontal lobe, medial temporal structures, and the cerebellum. However, because ASDs are developmental disorders rather than the result of acquired injury or disease, their basis is likely to be distributed in neural networks rather than in single structures of the brain. A deviant trajectory of brain development, therefore, would be expected to affect brain circuits across the brain and the interaction of brain regions during behavior.

Past studies on autism have found differences in functional connectivity between typically developing, or TD, persons and individuals with ASD, while they performed cognitive tasks, such as in the covariance of blood oxygen level dependent signals during sentence comprehension [5], and reduced functional connectivity between frontal and parietal brain areas during an executive task [4]. Other studies, using fMRI, of social cognition [7], working memory [8], and visuomotor coordination [9] all have shown evidence for differences in functional connectivity between control and autistic brains.

Thus, there is an emerging consensus that abnormality in neural circuitry is a central phenotype of autism. Several theories have been suggested regarding the specific nature of the abnormality. One appealing candidate is a noisy cortical network [10]; that is to say, persons with autism (ASD) have unreliable neural circuits. This proposal is based on the idea that there is an imbalance in the inhibition/excitation ratio and reduced cortical inhibition [11] in the brains of those with ASD. This idea, although influential and capable of providing a powerful functional basis for the symptomatology of ASD, requires independent empirical verification.

An intuitively appealing and plausible interpretation of the noisy network hypothesis is increased variability in the cortical response to external stimulation in autistic brains. That is to say, the autistic brain's response to sensory stimulation is more variable. As individuals with ASD frequently manifest any of a variety of abnormal responses to sensory experiences (e.g. seeking out or avoiding particular types of stimulation; apparent insensitivity to pain; extreme reactivity to being touched, etc), it is plausible that increased cortical variability in response to sensory stimulation could be present. In this study, we computed several measures of variability to empirically test this interpretation of the noisy cortical network hypothesis.

Methods

Participants

Magnetoencephalography (MEG) signals from 17 persons with a clinical diagnosis of ASD (mean ± SEM = 18.7 ± 0.7 years old) and 18 TD individuals (TDs, 19.0 ± 1.2 years old) were recorded. All individuals in the autism group met our research criteria for an ASD, as determined by using the Autism Diagnostic Observation

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Schedule [12] and Autism Diagnostic Interview, Revised [13] administered by trained clinicians. Five individuals in the autism group were clinically classified as pervasive developmental disorder-not otherwise specified, one as Asperger syndrome, and the remaining 11 as autistic disorder. Three persons with autism and four TDs were female. Potential participants were excluded when there was evidence of brain injury, seizure disorder, or neurotropic infection or disease, or if they had a history of identified severe psychopathology such as bipolar disorder, schizophrenia, or behavior problems severe enough to make accurate and reliable testing difficult. All participants were right handed as determined by the Edinburgh Handedness Inventory [14]. All individuals in the Autism group were high functioning: full scale IQs and verbal IQs derived from the Wechsler Abbreviated Scale of Intelligence [15] were greater than 85 (mean ± 1 SEM: full scale IQ: 97 ± 5; verbal IQ: 96 ± 5; performance IQ: 97 ± 5). TD individuals were volunteers without a history of ASD or other major developmental or psychiatric illness. Prior informed consent was obtained from all participants, or participants and their parents, under a protocol approved by the University of Texas Health Science Center-Houston and the University of Houston.

Stimuli

Pneumatically driven mechanical taps (1.8 kg/cm^2) of 40 ms duration (20 ms rise time) were individually applied to the right thumb or right index finger (4D Neuroimaging Inc., San Diego, California, USA). This is a benign tactile stimulus that elicits a mild sensation on the skin. Participants were told that a pressure pulse will be delivered and that all they had to do was to close their eyes, relax, and stay still. A training block containing five stimuli before the actual recording familiarized participants with the stimuli.

Procedure

Participants lay supine on a comfortable bed and kept their eyes closed. Fiducial markers were placed on their forehead and in the ears. The locations of the fiducial markers were recorded into the computer by means of a digitizer (stylus pen). The digitizer was slowly rolled over the participant's scalp and the shape of his or her head was thus recorded.

Magnetoencephalography recordings

All MEG recordings used a whole-head neuromagnetometer containing an array of 248 gradiometers (Magnes WH3600, 4D Neuroimaging Inc.). The instruments were placed in a magnetically shielded and sound attenuated room (Vacuumschmelze Gmbh & Co., KG, Hanau, Germany). There were 2000 epochs of stimulation of the index finger (D2) and 700 epochs of stimulation of the thumb (D1) in separate blocks. A single epoch lasted 575 ms and included a 120 ms prestimulus baseline. Data were acquired with a 1.0-Hz high-pass cutoff at a sampling rate of 290 Hz. Portions of the signal that were correlated to sensors placed far away from the head were likely to be noise and were subtracted out. Epochs remaining were used for analysis.

Magnetoencephalography analysis

We measured the variability in the evoked response to tactile stimulation in autistic and control brains. Before our analysis, epochs containing exaggerated moments such as eye blinks (peak to peak deflections > 2pT) were discarded. The following analyses were computed on the remaining epochs. For each participant in our sample, the sensor in the contralateral somatosensory cortex that exhibited the largest evoked response relative to baseline was automatically selected using a program that we developed and designed. For this sensor, we computed across all trials and times (40–260 ms after the onset of stimulus; 290 Hz sampling rate) for each body part the (i) mean evoked response, (ii) variance in the evoked response, and (iii) the ratio of the variance to the mean of the response (coefficient of variation or CV).

Sensor selection

Our approach was inspired by the region of interest or ROI approach advocated by Kanwisher and colleagues [16] in fMRI data analysis. We developed and designed a program to select the sensor that exhibited the largest evoked response relative to baseline. The need to select a sensor for each individual participant instead of using a particular sensor for all participants emerged for the following reasons. MEG sensors are placed in a helmet-shaped device called a dewar. Unlike the electrode cap in EEG, which is fastened to the participant's head, the dewar does not touch the participant's head. Although the participant's head has to be placed inside the dewar in a proper way, we still cannot claim that the same sensors map onto the same anatomical brain region from participant to participant. This problem is exacerbated by the fact that head size varies appreciably across participant. Thus, it is difficult to achieve anatomical or morphological equivalence across participants in somatic MEG recordings [17]. Finally, even if morphological equivalence across participants were somehow established, there are variations in functional anatomy that cannot be accounted for. Taking into account these issues, we selected the sensor in the contralateral hemisphere to the stimulus that showed the highest functional or physiological evoked response relative to baseline. The sensor thus selected, whose exact location in the array varied across participant, was largely (though not entirely) used for the present analysis. Details on sensor selection are provided next.

For each individual sensor, we first ensemble averaged all epochs. Then, a threshold was set to distinguish spontaneous activity or noise from evoked response: the

top 2.5% and bottom 2.5% of all values of the prestimulus baseline signal constituted the threshold. Poststimulus activity that crossed the prestimulus baseline threshold was interpreted as evoked response. The area of the poststimulus response that crossed the threshold was calculated, and this served as a measure of the total stimulus-evoked activation at the given sensor. The sensor that had the largest total evoked activity measured in the above manner and that was located in contralateral somatosensory cortex was then automatically selected for further analysis.

Variability in the evoked response

For each individual participant, body part (RD1, RD2), and selected sensor, we computed the mean and standard deviation in the evoked response time series across all epochs. We also computed the ratio of signal standard deviation to mean, a measure analogous to the coefficient of variation (CV). CV is a measure of the dispersion of a distribution. Our measure is different from CV in the way that we calculated the mean. The mean of our distribution had some time points which were 0 femtoTesla or fT. The CV at these time points $\rightarrow \infty$. To overcome this issue, we took the absolute value of the mean and then added a 200 fT baseline to all time points. A modified CV was calculated using this modified mean.

We also computed the mean and variance in the evoked response for the five most active sensors and the five least active sensors to investigate how the variance was spatially distributed. For each of these sensors, the same measures were computed.

Entropy

A second measure of variability in response that accounts for linear as well as nonlinear components of the response is entropy [18]. Entropy is a measure of uncertainty of an outcome. For the case in which there are n possible outcomes, a uniform distribution in which all the outcomes have equal probability ($1/n$) has maximum entropy [19].

Again for the selected sensor, we computed the entropy for each participant separately across all trial and times. At each time point, the values were divided into bins where the width W of each bin was calculated as $W = 2$ (IQR) $N^{-1/3}$, where IQR is the interquartile percentage and N is the number of data points.

Statistics

Statistical analysis used SPSS 15 for Windows (SPSS Inc., Chicago, Illinois, USA). A two-way mixed-model analysis of variance, with time as the within-subjects factor (40–250 ms poststimulus) and group as the between-subjects factor, was used to examine differences in the variability and entropy of the evoked responses of the TD and ASD groups. Student's t-tests (two-tailed) were used to examine group differences in age and IQ. Linear correlations between variance and IQ were computed and analyzed for significance.

Results

We investigated variability in the MEG signal recorded in response to tactile stimulation of the thumb (D1) and index finger (D2) of the dominant (right) hand. All measures were separately computed for each participant, and then classified according to group (ASD or TD).

RD2

A statistical comparison of the mean evoked responses (Fig. 1, top row) showed no significant difference in mean response amplitude between ASDs and TDs [$F(1,27) = 1.739$, mean square error (MS_e) = 7016.20, $P = 0.198$]. Figure 1, middle and bottom rows, respectively, show the standard deviations and CVs for TDs (black) and ASDs (gray). As Fig. 1b, c, e and f show, there is little evidence for greater variability in the response of autistic brains to the tactile stimulus. Indeed, variability in the evoked response in ASDs was not greater than that in TDs [$F(1,27) = 2.6$, $MS_e = 81446.26$, $P = 0.118$]. Upon normalizing for differences in mean response, that is, by comparing group CVs, marginally smaller CVs in ASDs were observed [$F(1,27) = 2.936$, $MS_e = 2.17$, $P = 0.098$], suggesting a trend toward lower variability in the evoked response of autistic, not typical, brains. Group $\times$ time interactions were significant on none of the three measures.

RD1

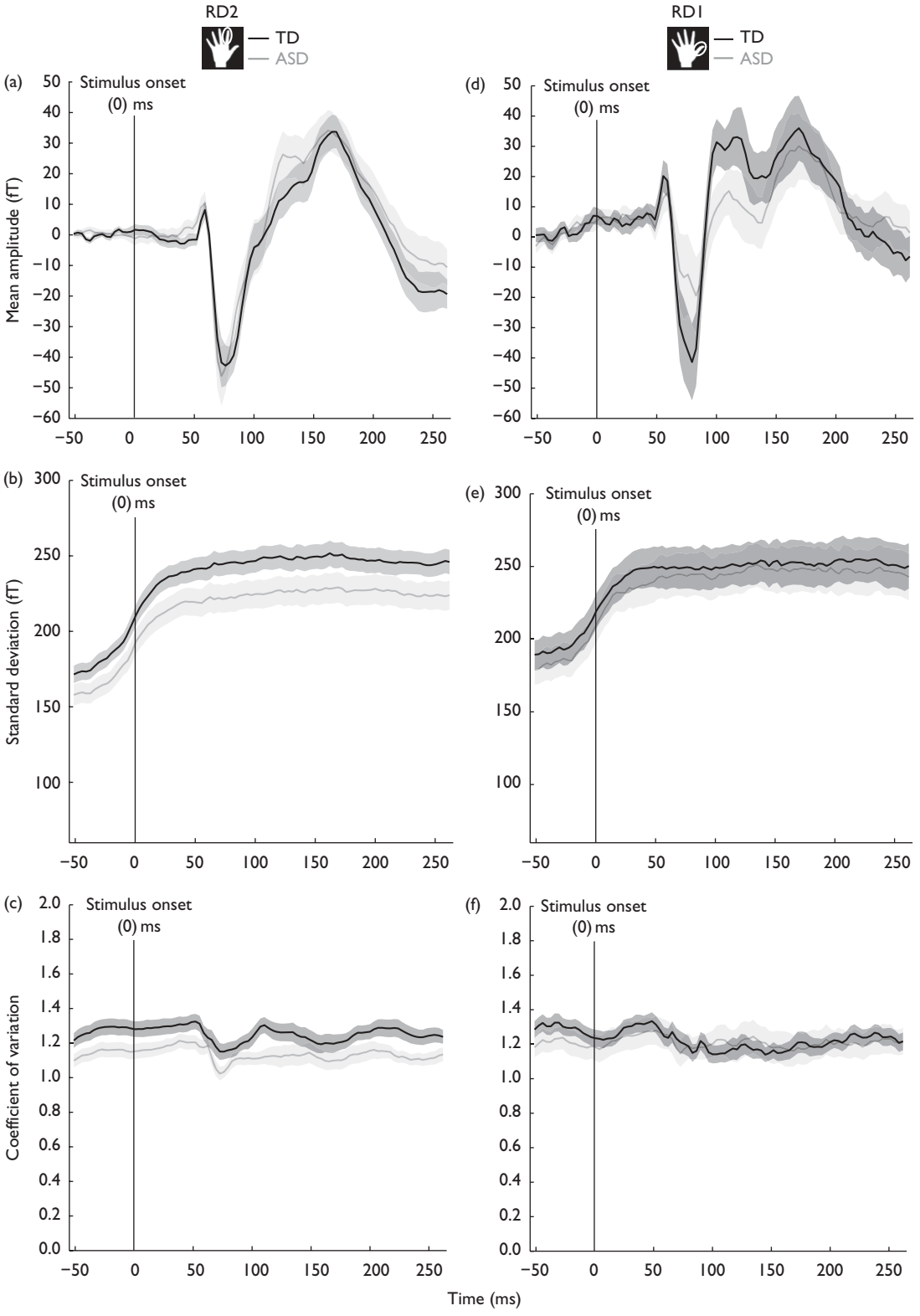
We repeated the sequence of computations for RD1, and the results were largely similar to those for RD2 (Fig. 1d, e and f). Again, the evoked response to stimulation of RD1 in ASDs was no less variable than that of TDs in terms of standard deviation [$F(1,33) < 1$], or CV [$F(1,33) < 1$]. There was no difference in response amplitude between the two groups [$F(1,33) < 1$]. No group $\times$ time interactions were significant.

The results shown so far were on the functionally most active sensor. To test the generality of our findings, we also measured and averaged the signal from the five sensors in contralateral cortex that exhibited the largest evoked response relative to baseline. Again, mean variability in evoked response was not statistically (and was smaller numerically) greater in ASDs as compared with TDs for RD2 [$F(1,27) = 2.764$, $P = 0.108$] or RD1 [$F(1,33) = 1.279$, $P = 0.267$]. Overall, the findings suggest that the somatic evoked response in autistic brains was no more variable than that in the brains of neurotypicals.

Discussion

We tested for variability in the evoked response to tactile stimulation in the brains of individuals with autism. On the basis of the current theory [10], we predicted that the variability in the evoked MEG responses of

Fig. 1



Mean evoked response, standard deviation, and coefficient of variation of the group mean evoked response to tactile stimulation of the index finger (left panel) and thumb (right panel) in autism spectrum disorders (ASD) versus neurotypicals [typically developing (TDs)]. Light regions indicate confidence interval. (a) and (d) show the grand mean evoked responses, (b) and (e) show the standard deviation of the response, and (c) and (f) show the coefficients of variation of persons with autism (gray) and neurotypicals (black) for index finger (RD2) and thumb (RD1) stimulation, respectively.

the autism group would be greater than that in TD persons. However, we found no such difference. Not only that, if there was a trend at all, it was in the opposite direction: the responses of the control group were modestly, though not significantly, more variable than those of the autism group.

One important point to mention is that ASDs are a set of heterogeneous disorders and our autism sample consisted of a small number of participants with a diagnosis of pervasive developmental disorder-not otherwise specified, autism, or Asperger syndrome. One might argue that restricting our analysis to only those individuals that have a diagnosis of autistic disorder might yield a different outcome. However, we still did not find an increase in variability in our sample of 11 persons with a diagnosis of autistic disorder compared with TDs. Rather, we found a small trend toward reduced variability, in line with the results from the larger, heterogeneous sample.

To confirm this finding, we looked at response variability in a number of different ways. For instance, we calculated and compared the entropy of the responses of the ASDs versus TDs. A distribution of responses with higher entropy can be thought of as a distribution that has a higher degree of variability. However, our analysis again failed to reveal any differences in entropy between the two groups. An altogether different interpretation of variability is that of timing: the noisier the circuit, the more variable is the trial-to-trial latency of the response of the circuit. Thus, a noisier brain network in autism could be reflected in a shorter, wider response peak. We examined this possibility as well: specifically, we compared the full-width at half-maximum of MEP components M70 and M150 in both groups but did not find any difference. In summary, a number of analyses failed to reveal evidence that the somatic circuitry of autistic brains is noisier than that of control brains.

The noisy network hypothesis stems from the suggestion of an imbalance between excitation and inhibition in autistic brains. Indications for reduced GABAergic inhibition and abnormal glutamatergic transmission in ASD come from genetic [20,21] and anatomical studies [3,22]. A straightforward interpretation of this purported imbalance is an unstable, unreliable brain network [10], which was tested at the systems level here.

Our finding does not preclude a cellular-level interpretation of the hypothesis. That is to say, noise could mean synaptic noise. A noisy, unreliable synapse means a greater proportion of excitatory postsynaptic potential failures after presynaptic transmission, and/or a broader distribution of excitatory postsynaptic potentials amplitudes in response to the release of a single quantum of neurotransmitter at the presynaptic terminal [23–25]. This is an interesting idea but one that is not easy to verify experimentally.

Furthermore, it is possible that had we tested the groups using a different type of stimulation, one for example that involved an active cognitive or affective response, we might have found evidence of greater variability. Likewise, using a stimulus that the participant was known to respond to abnormally might have elicited a more variable response. These possibilities are not ruled out by the present findings; however, the finding of no differences in a task that used a very simple tactile stimulus shows at the least that increased variability in evoked response is not commonplace in autistic brains.

Conclusion

Contrary to the system level version of the hypothesis that autistic brains are noisier than control, our analysis of MEG response to tactile stimulation failed to find increased variability in autistic brains as compared with neurotypical ones. The search for an autism phenotype that robustly links abnormalities in functional connectivity and underlying biochemistry is ongoing.

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