An Examination of the Neural Unreliability Thesis of Autism.

John S. **Butler** [1,2,3,6,#], Sophie **Molholm** [1,4], Gizely N. **Andrade** [1] and John J. **Foxe** [1,3,4,5,#]

[1] The Sheryl and Daniel R. Tishman Cognitive Neurophysiology Laboratory, Department of Pediatrics, Albert Einstein College of Medicine & Montefiore Medical Center, Bronx, NY 10461, USA

[2] Trinity Centre for Bioengineering, Trinity College, University of Dublin, Ireland

[3] Trinity College Institute of Neuroscience, Trinity College, University of Dublin, Ireland

[4] The Dominick P. Purpura Department of Neuroscience, Rose F. Kennedy Intellectual and Developmental Disabilities Research Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

[5] The Ernest J. Del Monte Neuromedicine Institute, Department of Neuroscience, University of Rochester Medical Center, Rochester, NY 14642, USA.

[6] School of Mathematical Sciences, Dublin Institute of Technology, Kevin Street, Dublin 8, Ireland.

[#] Correspondence: john.s.butler@dit.ie or john_foxe@urmc.rochester.edu

SUPLEMENTARY MATERIALS

APPARATUS AND STIMULI

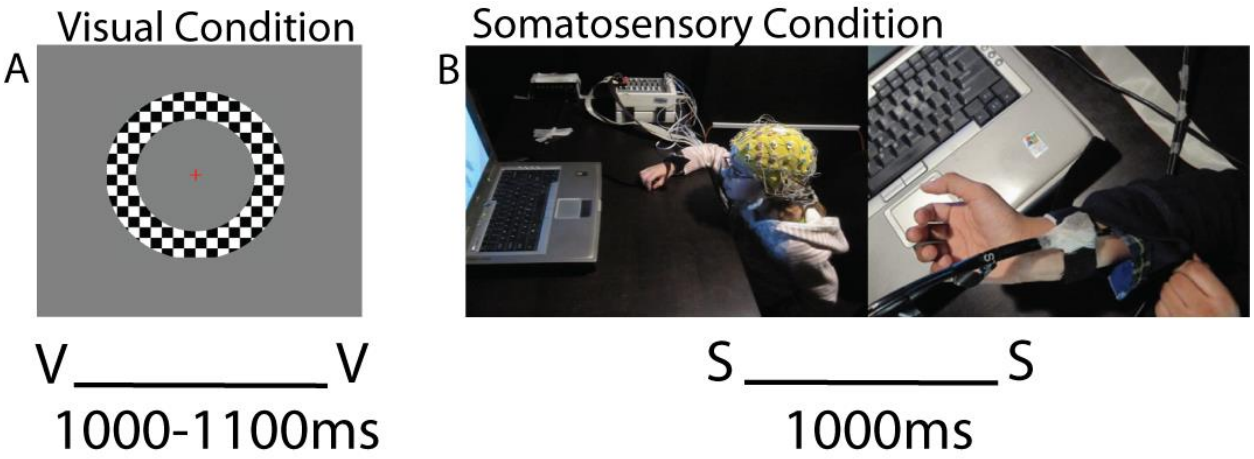

Figure S1 Stimuli and task A) Participants performed a change detection task to ensure fixation in which they were asked to respond to a color change (from red to green, lasting 33ms) of the fixation cross. The presentation of the checkerboard stimuli was temporally unrelated to this central fixation task. The visual stimuli were presented in a block of 100 stimuli at an ISI of 1050±50ms B) Participants watched a video of their choice. The somatosensory stimuli were presented in a block of 500 stimuli at an ISI of 1000ms

Evoked Single Trial Data

Figure S2 shows single trial data from representative NT and ASD participants for the visual condition over the central occipital area. The figure illustrates the similarity of data of the ASD and NT participants.

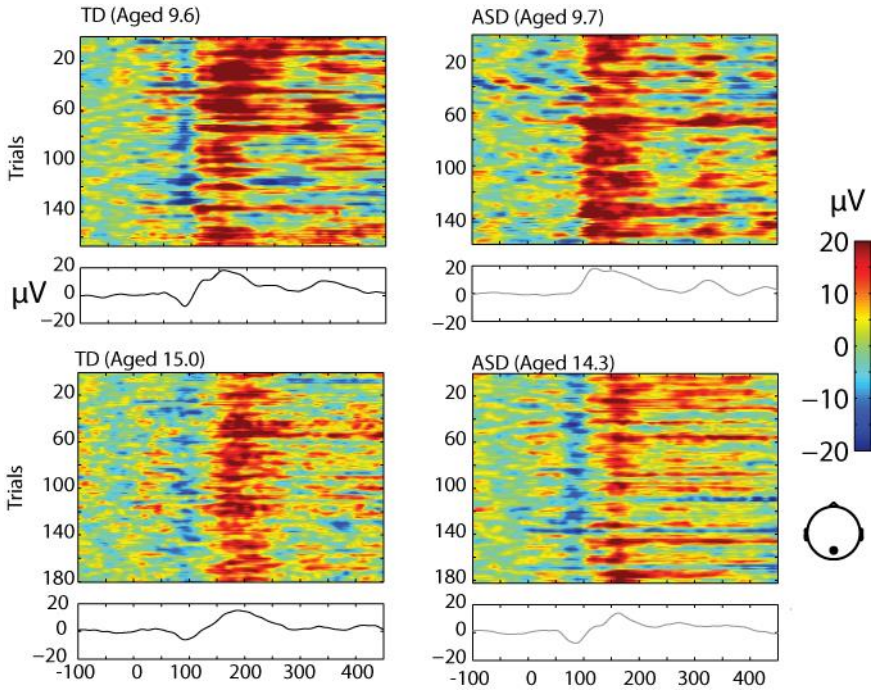

Figure S2 Representative Participants average evoked response and single trial data for two ASD and two age matched NT participants for the visual condition at the central occipital electrode site. Amplitude values are differentiated with the color scale shown to the right. Time is plotted on the x-axis from -100 to 450 ms. Trials are plotted on the y-axis. The participant averages of the trials are plotted below each color plot.

Correlations between SNR and inter-trial coherence for delta, theta and alpha bands across the whole epoch

To investigate the relationship between average and single trial measures across the whole epoch (Figure S3) of the reliability of the evoked response Pearson's correlation coefficients were computed between the SNR and mean ITC values in the Delta (1-4Hz), Theta (4-8Hz) and Alpha (8-14Hz) bands for the visual at three occipital electrode sites and the somatosensory conditions at the left and frontal central electrode sites.

The analysis revealed significant correlation relationships between SNR and ITC values in the Delta and Theta bands (Figure S3 Table S1). For the visual condition, the right occipital site for ASD and NT groups revealed a significant correlation of SNR with both Delta and Theta ITC values. At the left occipital site, the analysis revealed significant correlations between SNR with both Delta and Theta ITC values for the ASD group, while the NT data did not reach significance. Similarly, for the somatosensory condition the analysis revealed significant correlation of the SNR with both Delta and Theta ITC values across both groups.

Table S1 correlation r values showing the relationship between SNR with single trial inter-trial coherence over the whole epoch for the visual and somatosensory conditions.

	Visual						**Somatosensory**			
R	Left Occipital		Central Occipital		Right Occipital		Left Parietal		Frontal Central	
	NT	ASD	NT	ASD	NT	ASD	NT	ASD	NT	ASD
Delta (1-4Hz)	0.407†	0.627**	0.429†	0.267	0.605**	0.642**	0.5*	0.34	0.489*	0.36
Theta (4-8Hz)	0.388†	0.662**	0.346	0.455*	0.516*	0.482*	0.682*	0.512*	0.333	0.321
Alpha (8-14Hz)	0.272	0.425	0.2	0.377	0.023	0.275	0.145	0.03	0.496*	0.337

† p<0.1 * p<0.05 ** p<0.01

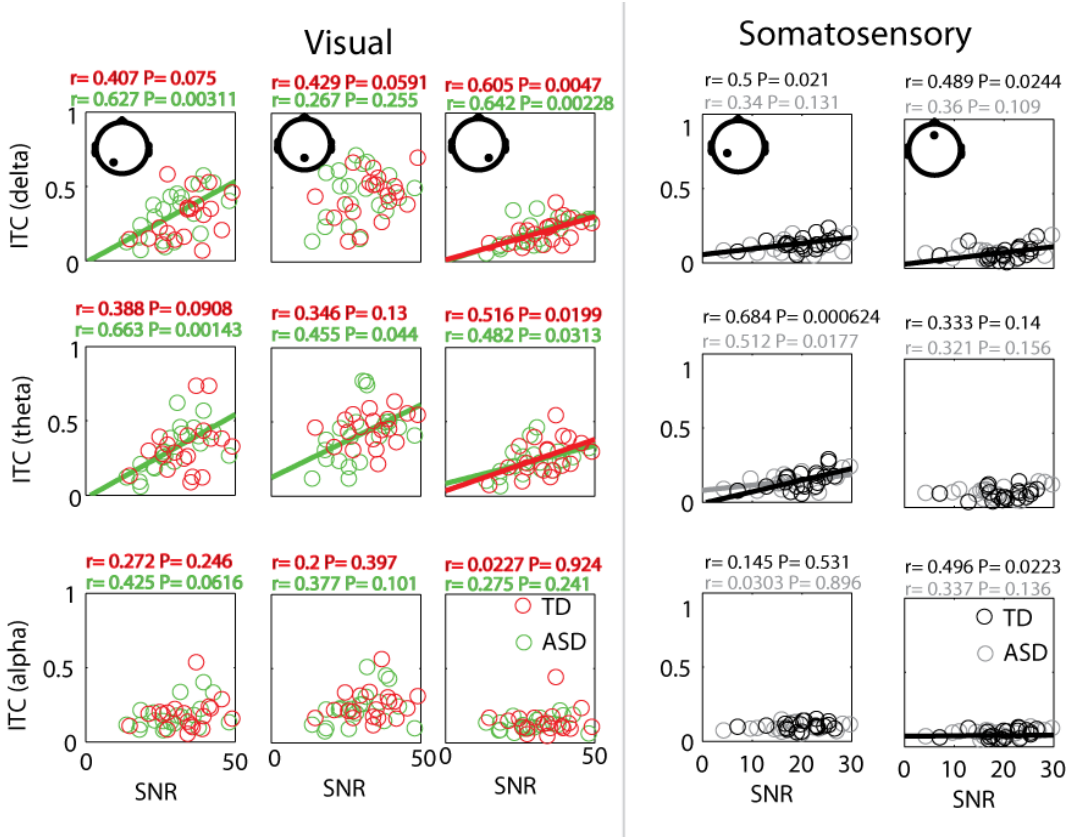

Figure S3 Scatter plots of ITC values in delta (1-4Hz), theta (4-8Hz) and alpha (8-14Hz) bands as a function SNR at three occipital electrode sites (columns). (A) In the visual condition, red and black circles represent data obtained from ASD and NT participants, respectively. (B) In the somatosensory grey and black circles symbols represent data obtained from ASD and NT participants, respectively. Lines indicate the least square fitted lines for correlations with p-values less than 0.05.

MATCHED GROUPS UNCORRECTED ANALYSIS SINGLE TRIAL ANALYSIS

EEG Spectrum Analyses

The single trial (k) data was submitted to the EEGLAB function newtimef to the compute the power spectrum $F_k(f, t)$ at frequency (*f)* over a 250ms sliding time window centered at time (t) using a Morlet wavelet with linearly increasing wavelet cycles from 1 cycle at 4 Hz to 3 cycles at 40Hz from -280 ms to 472ms with 4ms steps (Delorme & Makeig, 2004). . From this, event-related spectral perturbations (ERSP) was calculated

$$ERSP(f,t) = \frac{1}{n}\sum_{k=1}^{n} |F_k(f,t)|^2.$$

Finally, the ERSP was baseline-normalized across the frequency range, the mean baseline log power spectrum from -280 to 0ms was subtracted from each spectral estimate.
The inter-trail coherence (ITC) is a frequency-domain measure of the consistency of the phase of the evoked response ranging between 0 and 1, and serves as the primary metric of inter-trial reliability here (Delorme & Makeig, 2004; Mercier et al., 2013; Mercier et al., 2015; Tallon-Baudry et al., 1996). A result near 0 implies low reliability in the phase of the evoked response across trials and 1 implies a perfectly reliable response across epochs. Using the same notation as the ERSP, ITC is calculated by

$$ITC(f,t) = \frac{1}{n}\sum_{k=1}^{n} \frac{F_k(f,t)}{|F_k(f,t)|}$$

where || is the complex norm.

Results: Figures S4 and S4 shows the group mean ITC and ERSP values and false discovery rate corrected comparison, respectively, for the large matched NT group (top row) and the ASD group (middle row), and the corrected group comparison plot (bottom row) for the visual condition at three occipital electrode sites (S4A and S5A) and for the somatosensory condition at a left central parietal site and frontal central site (S4B and S5B).

ITC data: From Figure S4, the similarity of the ITC values across the two groups can be observed. *Visual response* Figure S4A shows the largest visual ITC values at ~100ms, coinciding with the largest evoked peak at the central occipital sites. Corrected comparison of the ITC values between the groups resulted in no differences.

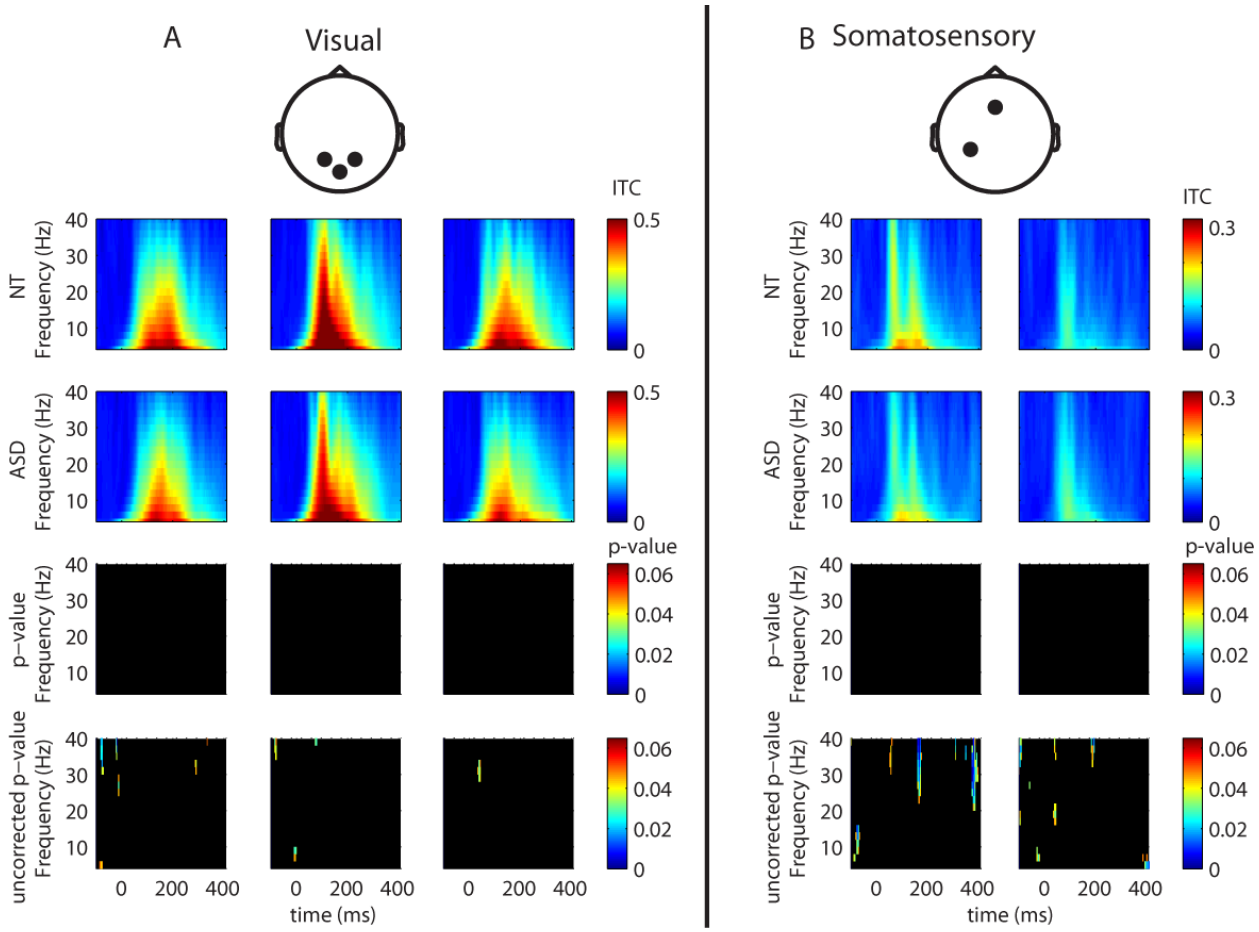

Figure S4 Mean Inter trial Coherence for NT (top row) and ASD (middle row) groups for (A) visual condition at three occipital electrode sites (columns) and (B) somatosensory at frontal and left central sites. Time is plotted in the x-axis from −100 to 450ms. Frequencies are plotted in the y-axis. The bottom row shows non-parametric statistical comparisons between the NT and ASD group. Bottom rows depict the uncorrected and false discovery rate corrected p values.

Somatosensory response Figure S4B illustrates the somatosensory ITC values, which were largest at the left parietal site for both groups, with the largest ITC values coinciding with the largest evoked peak, at ~80ms. Overall, the ITC data shows highly similar reliability of the evoked response for the ASD and NT groups for the visual and somatosensory conditions.

ERSP data: Examination of Figure S5A reveals that the visual ERSP responses were similar between the ASD and NT groups. In the visual evoked response, both groups showed an increase in ERSP from ~50 to ~180ms between 4 and 40 Hz followed by a decrease in power from ~180 to 400ms between 4 and 40 Hz at the occipital electrode sites (Figure S5A). Examination of Figure 5B reveals that the somatosensory ERSP responses were highly similar

between the ASD and NT groups, with an increase in ERSP at from ~50 to ~160ms between 4 and 40 Hz followed by a decrease in power from ~160 to 400ms between 4 and 40 Hz. Statistical comparison of ERSP values between the two groups revealed no significant differences. The results here illustrate highly similar response for specific channels, in two supplementary videos the evoked and ITC and ESRSP data and the FDR corrected comparison for both groups is presented for each of the 64 channels for the visual (Supplementary Video 1 –Visual Condition) and somatosensory (Supplementary Video 2 –Somatosensory Condition) condition.

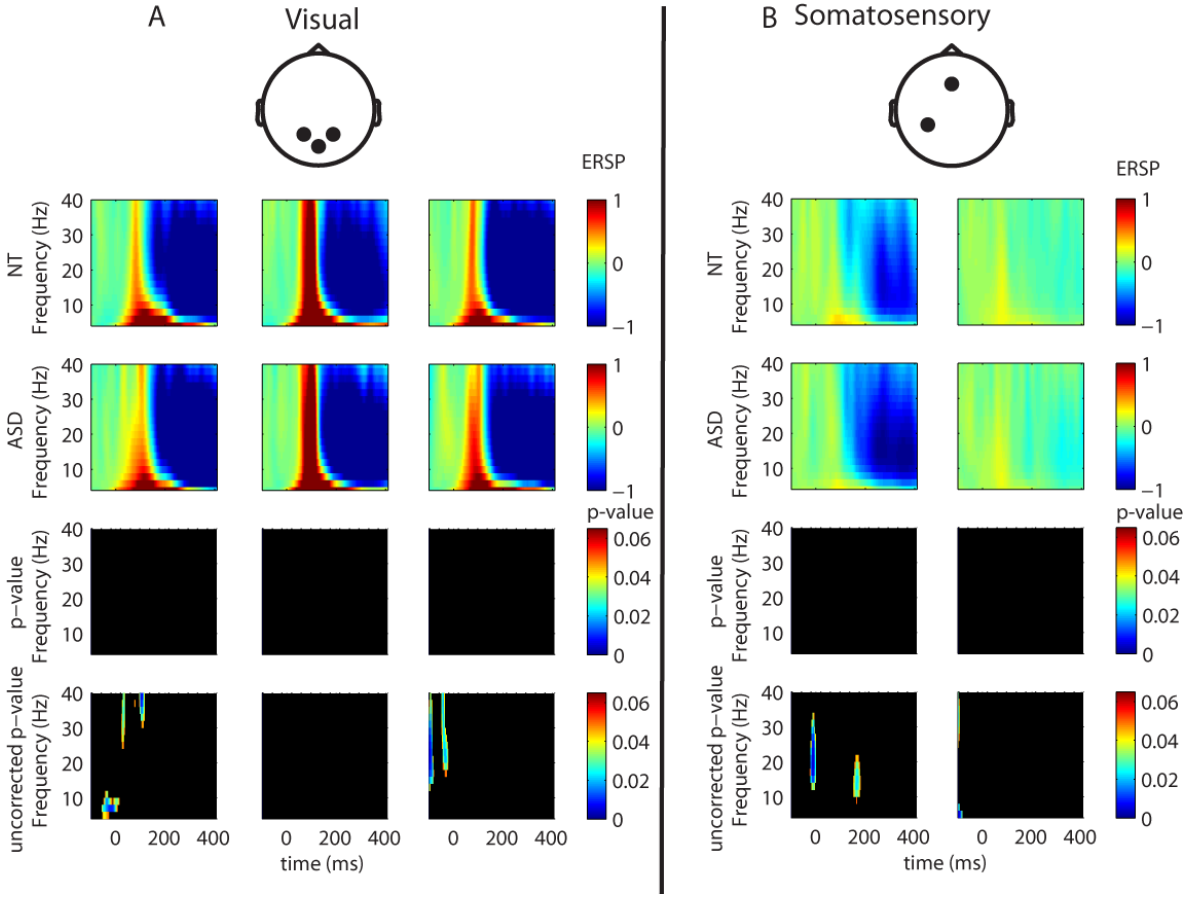

Figure S5 Mean Event-Related Spectral perturbations for NT (top row) and ASD (middle row) groups for (A) visual condition at three occipital electrode sites (columns) and (B) somatosensory at frontal and left central sites. Time is plotted in the x-axis from −100 to 450 ms. Frequencies are plotted in the y-axis. The bottom row shows non-parametric statistical comparisons between the NT and ASD group. False discovery rate corrected p values less than 0.025 are depicted.

INDIVIDUAL PARTICIPANT ANALYSIS TIME FREQUENCY ANALYSIS

To further illustrate the similarities of the time frequency data across the groups the significance of deviations from baseline power was assessed for each participant using a bootstrap method (Delorme and Makeig 2004). For each participant a surrogate data distribution was constructed by selecting spectral estimates for each trial from randomly selected latency windows in the pre-stimulus interval then averaging these, this was conducted 200 times. From the distribution $p<0.01$ significance were defined. These values were used to threshold the post-stimulus ITC and ERSP.

Figure S6 and S7 depicts the number of NT (top row) and ASD (middle row) participant's and the difference between the groups (bottom row) with significant ITC and ERSP for the visual (left side) and somatosensory (right side) conditions. The bottom row depicts the subtraction of the number of significant NT participants from the number of significant ASD participants, with blue representing more significant ASD participants than NT participants and red representing more NT participants with significant data than ASD participants. Figure S6 and S7 show that similarly high number of participants' exhibit significant activity for both groups. The ITC and ERSP time periods and frequencies of the largest number of participants, almost 100%, agree with the plots showing the values of ITC and ERSP (Figure S4 and S5). This further emphasis that the individual participant data is clean and that there is a high reliability across trials within participants for both groups. In two supplementary videos single participant significance ITC and ERSP data and the subtraction of the groups is presented for each of the 64 channels for the visual (Supplementary Video 3 – Individual Participant Visual Condition) and somatosensory (Supplementary Video 4 – Individual Participant Somatosensory Condition) condition.

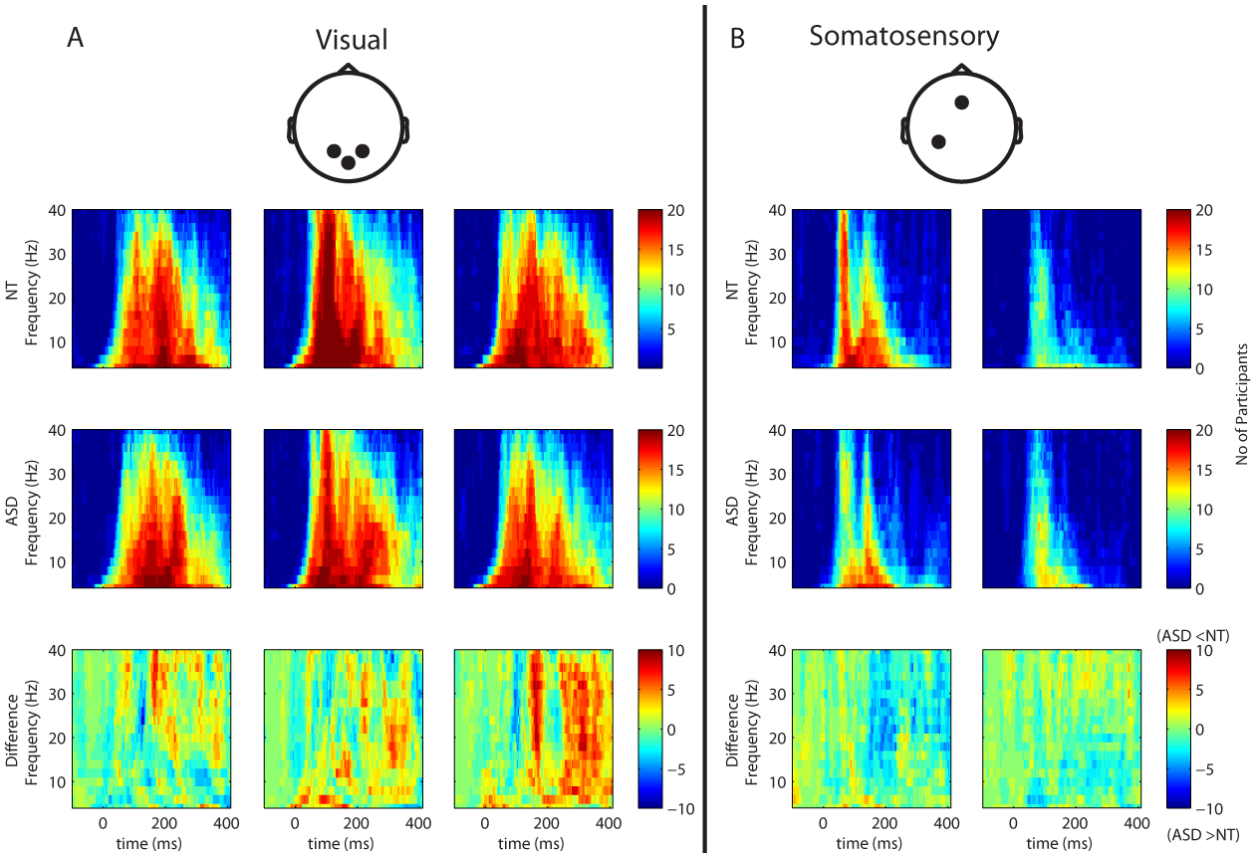

Figure S6. Number of NT (top row) and ASD (middle row) participants and the difference (bottom row) in the visual (left) and somatosensory (right) conditions with ($P < 0.01$) significant post stimulus activity for Inter trial Coherence (ITC). The bottom row depicts the subtraction of the number of significant NT participants from the number of significant ASD participants. Time is plotted in the x-axis from −100 to 450 ms. Frequencies are plotted in the y-axis. With the number of significant participants depicted on the color bar.

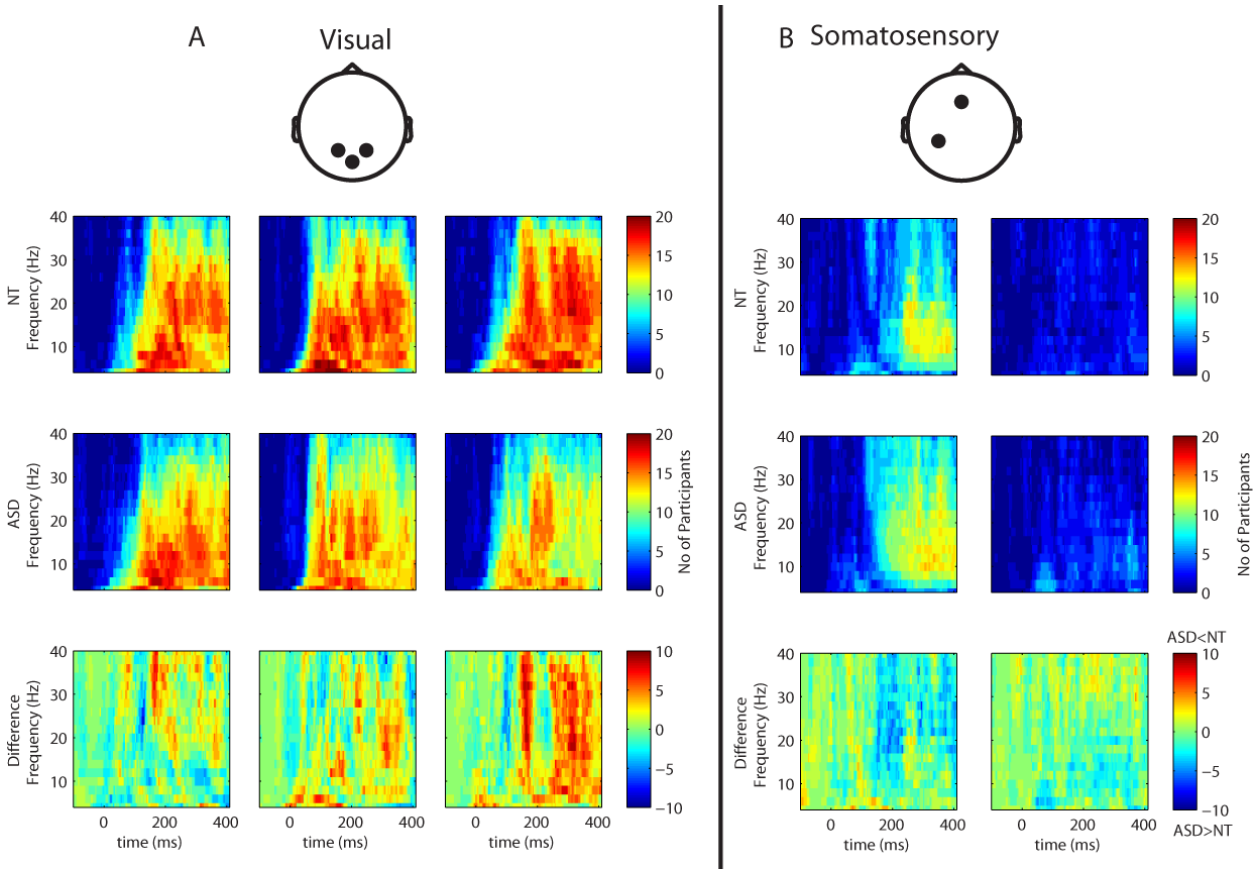

Figure S7 Number of NT (top row) and ASD (middle row) participants and the difference (bottom row) in the visual (left) and somatosensory (right) conditions with ($P < 0.01$) significant post stimulus activity for Event related Spectral Perturbation (ERSP). The bottom row depicts the subtraction of the number of significant NT participants from the number of significant ASD participants. Frequencies are plotted in the y-axis. With the number of significant participants depicted on the color bar.

CURRENT SOURCE DENSITY ANALYSIS

To account for possible differences due to volume conductance (Milne 2011), the single trial data was transformed using a second ordered spatial filter (Butler, Molholm et al. 2011), the current source density (CSD), and then ITC and ERSP analysis as described above was performed on NT and ASD groups (Figure S11). The CSD time-frequency data was highly consistent between the groups.

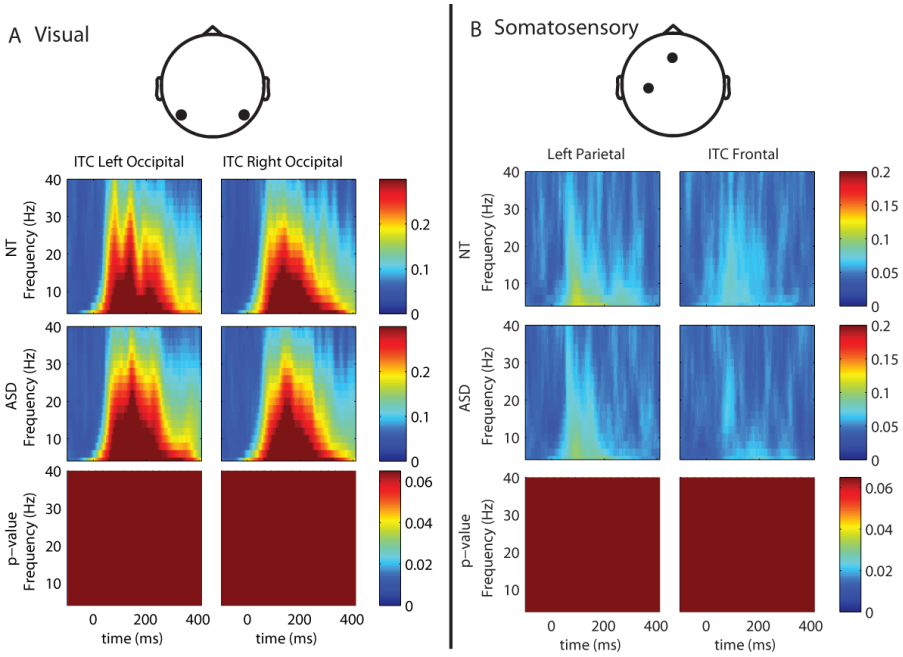

Figure S8 Mean Inter trial of the Current Source Density for electrode pairs for the NT (top row), ASD (middle row) and FDR corrected comparison between the groups (bottom row). The left panel displays the ITC for left and right occipital electrodes for the visual condition. The right panel displays the ITC for the left parietal and frontal central electrode for the somatosensory condition. Time is plotted in the x-axis from −100 to 450 ms. Frequencies are plotted in the y-axis.

BAYES FACTOR ANALYSIS

Statistical comparison of ITC and ERSP values across groups

To compare the ITC and ERSP the data were submitted to a point-wise unpaired *t* tests between the NT and ASD groups for all frequencies at each time point and are presented in statistical cluster plots.

Bayes Factor Analysis comparison of ITC and ERSP values across groups

The Jeffreys, Zellner and Siow (JZS) Bayes factor was computed using the default effect size of 0.707 (Rouder, Speckman, Sun, Morey, & Iverson, 2009). A JZS Bayes factor can be read such that a JZS Bayes factor greater than three favors the null hypothesis three times more than the alternative hypothesis, while a JZS Bayes factor less than one third is the favors the alternative three times more than the null, values between one third and three do not favor either.

Results

From Figure S9, the similarity of the ITC values across the NT (top row) and ASD (middle row) groups for the visual and somatosensory conditions. Figure S9 shows the largest visual ITC and values at ~100ms, coinciding with the largest evoked peak at the central occipital sites. Uncorrected unpaired t-test comparison of the ITC values between the groups resulted in very little statistical differences (third row). The bottom row shows the Bayes factor. The results suggest that there is substantial evidence in favour of the null hypotheses corresponding with the maximum ITC peak.

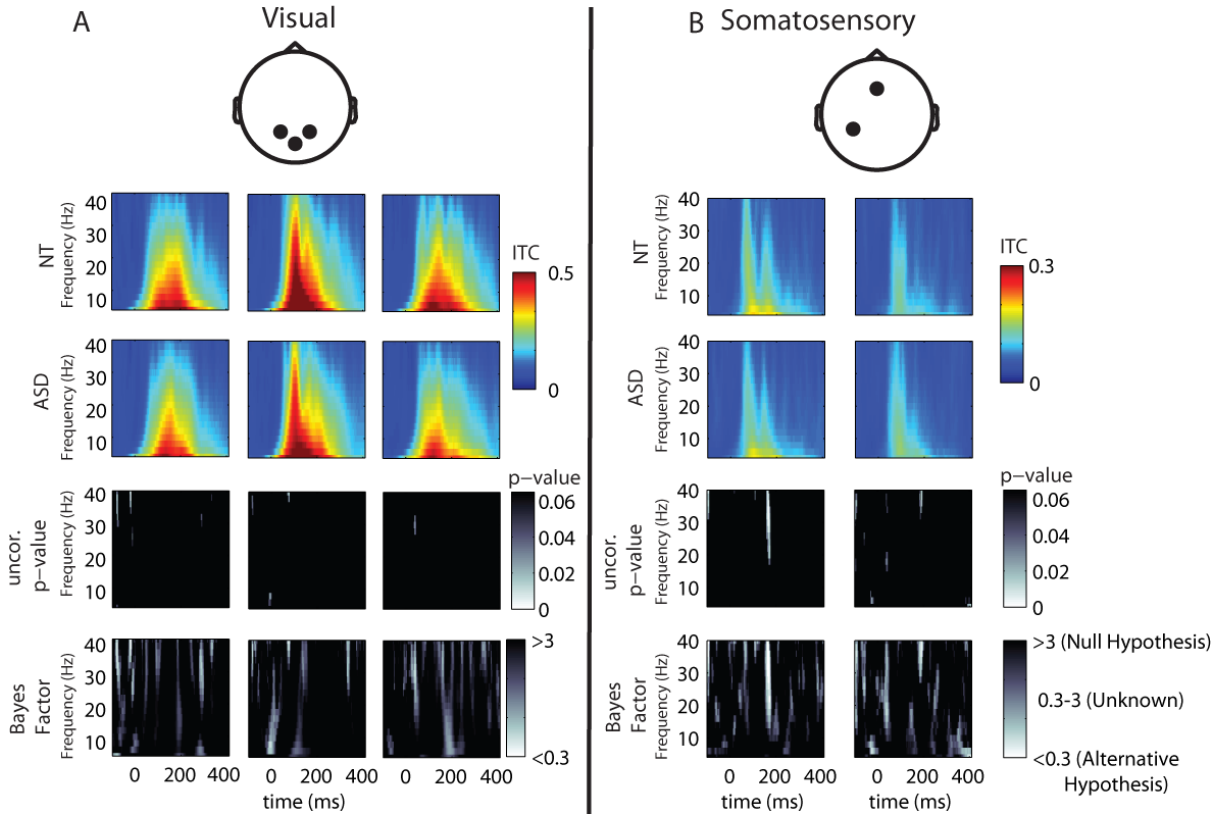

Figure S9 Mean Inter Trial Coherence (ITC) for NT (top row) and ASD (second row) groups for the visual condition at left, central and right occipital electrode sites and somatosensory condition at left parietal and frontal central electrode sites (columns). Time is plotted in the x-axis from −100 to 450 ms. Frequencies are plotted in the y-axis. The third row shows unpaired t-tests comparisons between the NT and ASD group. Uncorrected p values less than 0.05 are depicted. The bottom row shows the Bayes factor where black is evidence in favour of the null hypotheses, white favours the alternative and grey is unknown.

Figure S10 shows the similarity of the ERSP (right) values across the NT (top row) and ASD (middle row) groups for the visual and somatosensory conditions. The largest values ERSP values, coincide with the largest evoked peak. The ERSP values show desynchronization over left parietal site after 200ms. Uncorrected unpaired t-test comparison of the ERSP values between the groups resulted in very little statistical differences (third row). The bottom row shows the Bayes factor. The results suggest that there is substantial evidence in favour of the null hypotheses corresponding with the largest ERSP values.

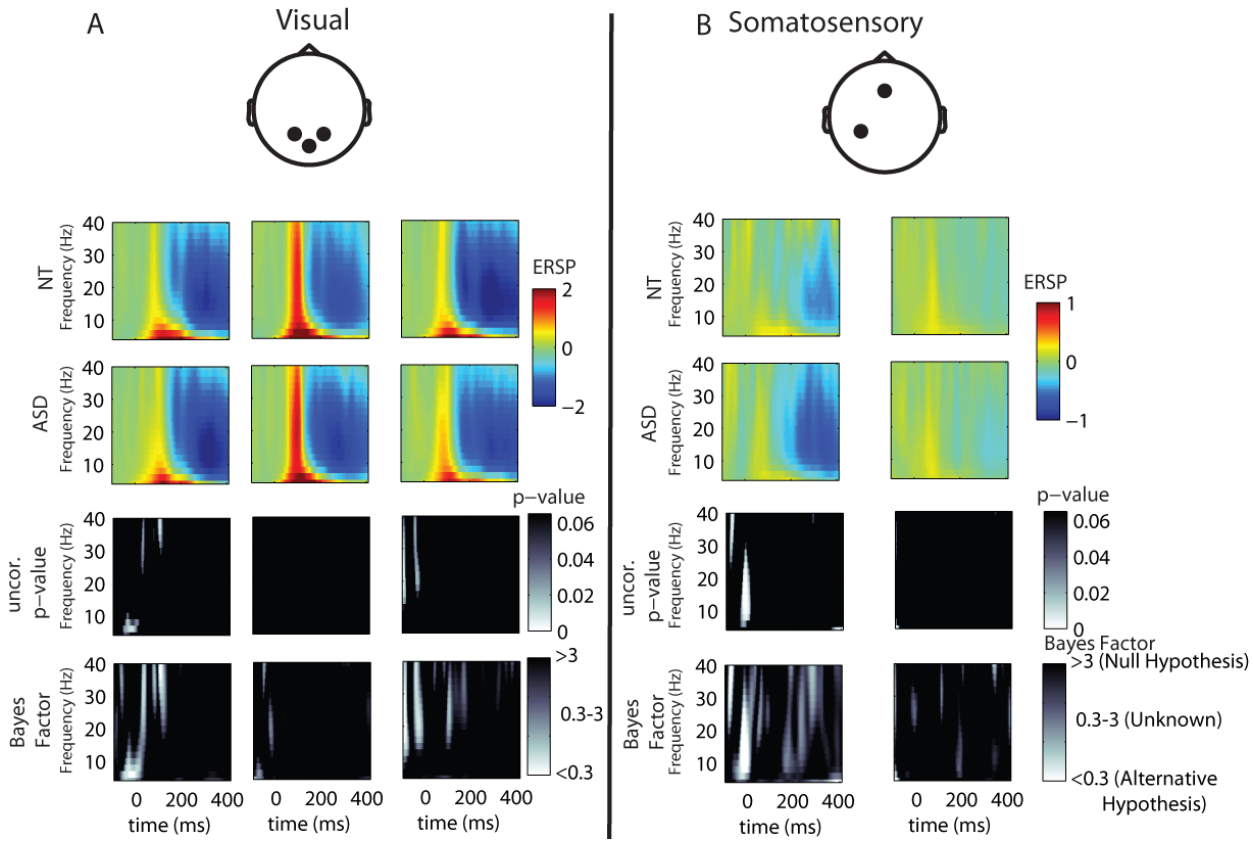

Figure S10 Mean Event Related Spectral Perturbation (ERSP) for NT (top row) and ASD (second row) groups for the visual condition at left, central and right occipital electrode sites and somatosensory condition at left parietal and frontal central electrode sites (columns). Time is plotted in the x-axis from −100 to 450 ms. Frequencies are plotted in the y-axis. The third row shows unpaired t-tests comparisons between the NT and ASD group. Uncorrected p values less than 0.05 are depicted. The bottom row shows the Bayes factor where black is evidence in favour of the null hypotheses, white favours the alternative and grey is unknown.

SIMULATION ANALYSIS FOR TEMPORAL AND AMPLITUDE VARIABILITY: MODELING THE UNRELIABLE STIMULUS EVOKED RESPONSE

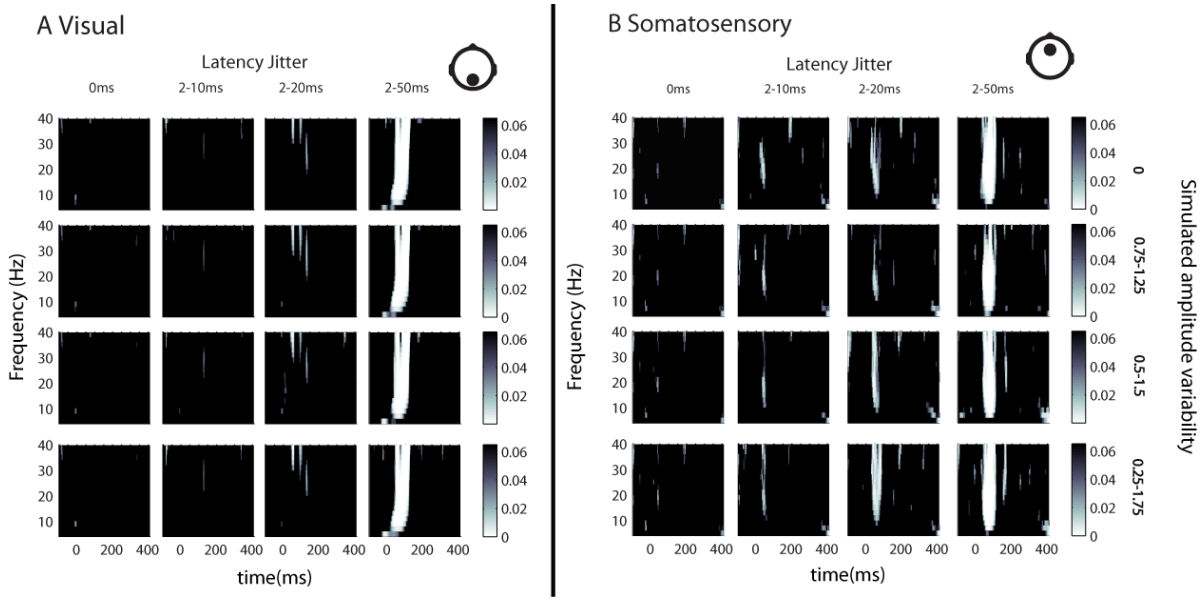

Figure S11 Uncorrected non-parametric statistical comparisons of the ITC values of the ASD group and simulated latency jitter and amplitude variability on the NT group for four latency jitter widths (columns; 0ms, 10ms, 20ms and, 50ms) and four amplitude variability (rows; 1.0, 0.75-1.25, 0.5-1.5, 0.25-1.75) for the visual condition at the central occipital electrode site (A) and for the somatosensory condition at the frontal central site (B). Significance is depicted for effects meeting a 0.05 alpha criterion. The color bar indicates significant differences, with black indicating an absence of significance. Time is plotted in the x-axis from −100 to 450 ms. Frequencies are plotted in the y-axis.

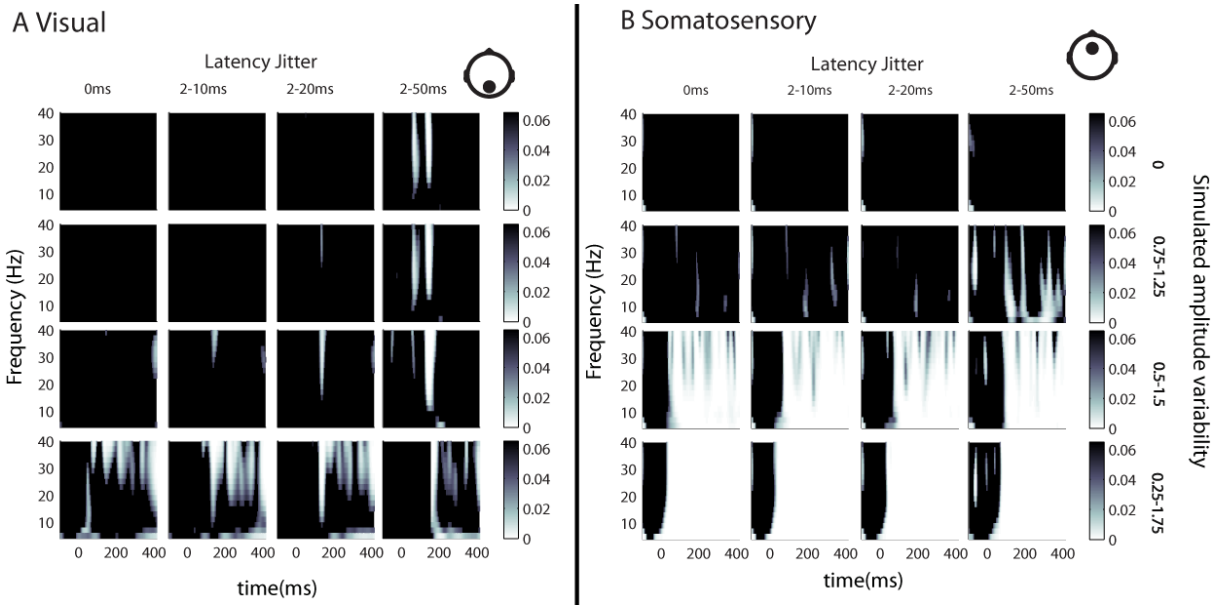

Figure S12 Uncorrected non-parametric statistical comparisons of the event-related spectral perturbations (ERSP) of the ASD group with the simulated data for four simulated latency jitter widths (columns; 0ms, 5ms, 10ms and, 25ms) and four amplitude jitters (rows; 1.0, 0.75-1.25, 0.5-1.5, 0.25-1.75) of the NT group for the visual condition at the central occipital electrode site (A) and for the somatosensory condition at the frontal central site (B). Significance is depicted for effects meeting a 0.05 alpha criterion. The color bar indicates significant differences, with black indicating an absence of significance. Time is plotted in the x-axis from −100 to 450 ms. Frequencies are plotted in the y-axis.

SNR AND ITC AS A FUNCTION OF NUMBER OF SWEEPS

Figure S13 illustrates the relationship of the number of sweeps and SNR and ITC on an individual participant level. The graphs shows that a small number of sweeps can result in over inflated ITC values as illustrated in the Alpha range of under inflated ITC values as illustrated in the theta range.

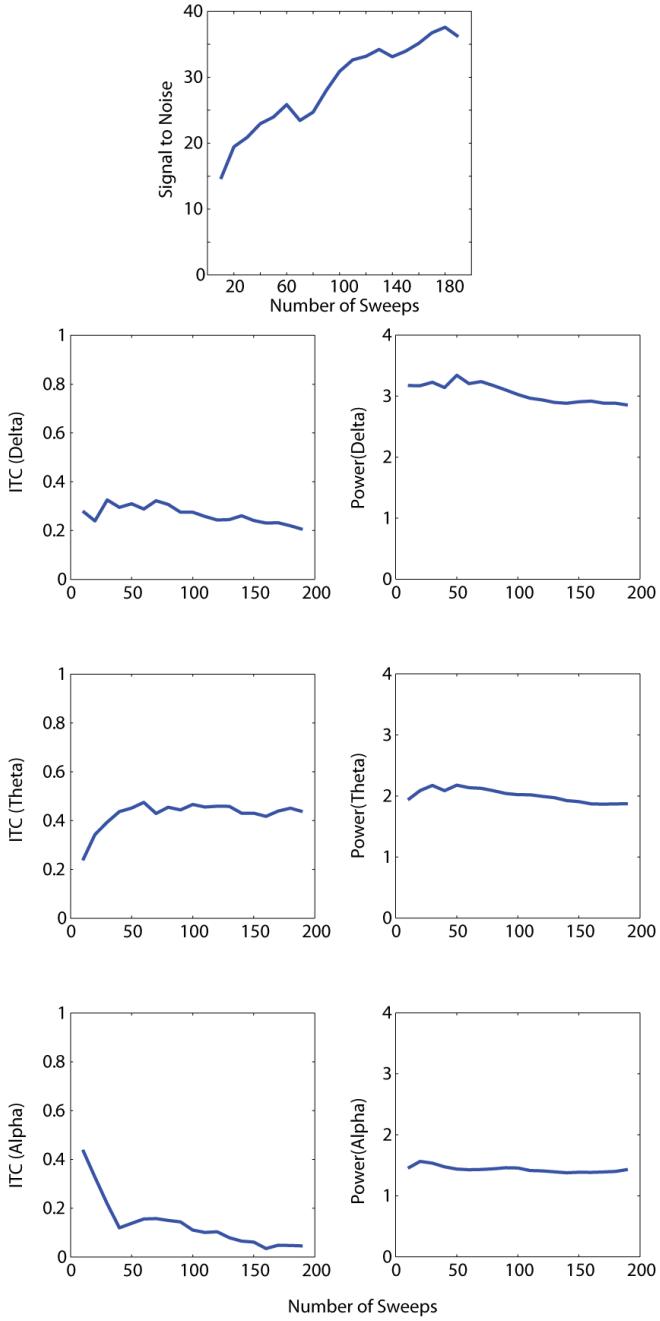

Figure S13 Representative Participant plots of signal-noise-ratio (SNR), ITC and Power Values at the central occipital electrode site as a function number of sweeps.

Correlations between SNR and FSIQ data

Correlation analysis revealed no relationship between Signal to Noise of the evoked response and Full Scale IQ (Figure S14) in the visual and somatosensory experiment for both the ASD and NT groups.

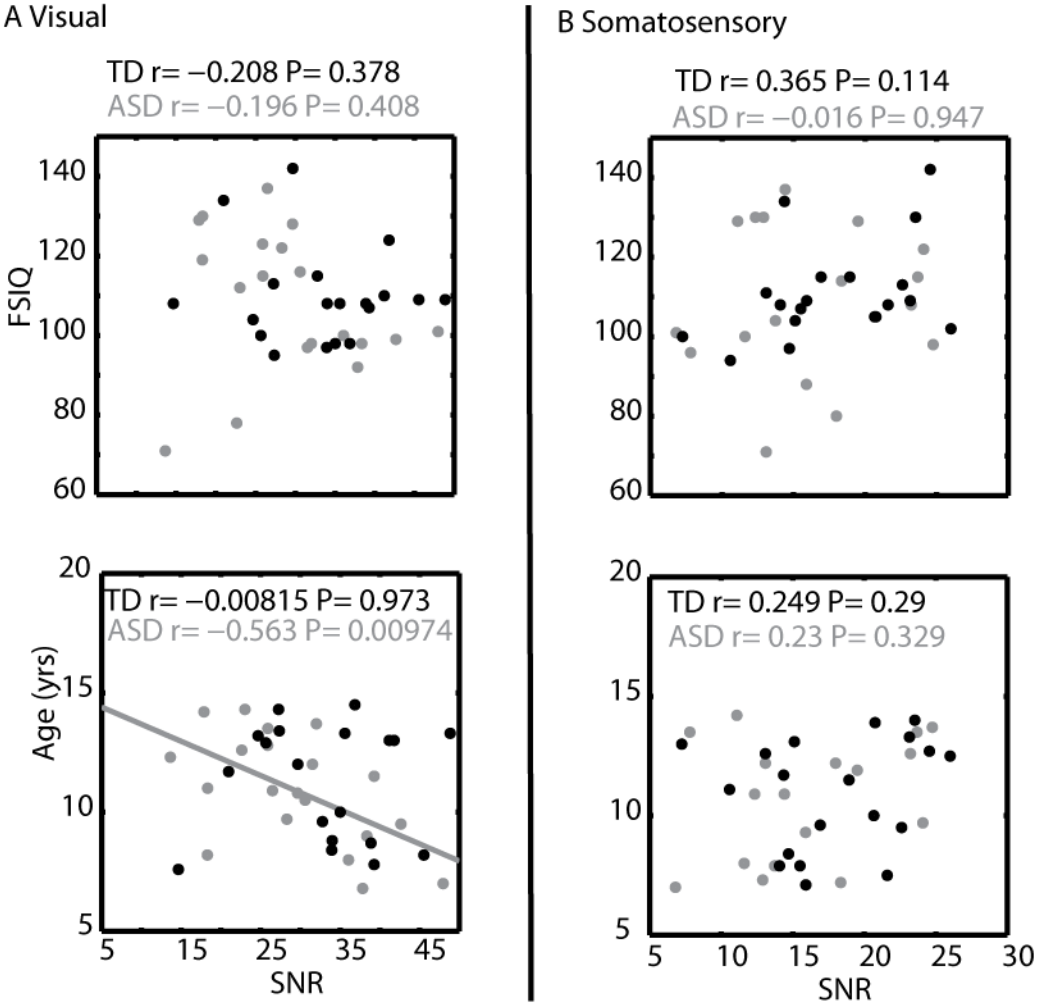

Figure S14 Scatter plots of FSIQ and Age scores as a function SNR for the visual condition. Grey and black circles symbols represent data obtained from ASD and NT participants, respectively.

Medication Tables

Table S2 Table of medications for the 20 ASD participants in the Somatosensory Condition

Medication	Number of participants
No Meds	14
Daytrana patch (methylphenidate)	1
Focalin XR 20mg (dexmethylphanidate), propranolol 10mg, strattera 60mg (atomoxetine)	1
fluoxetine 15mg	1
guanfacine 10mg	1
sertraline 75mg	1
Tenex (guanfacine) 1mg	1

Table S3 Table of medications for the 20 ASD participants in the Visual Condition

Medication	Number of participants
No Meds	11
concerta (methylphenidate)	2
Daytrana patch (methylphenidate)	1
Focalin XR 20mg (dexmethylphanidate), propranolol 10mg, strattera 60mg (atomoxetine)	1
fluoxetine 15mg	1
guanfacine 10mg	1
sertraline 75mg	1
Tenex (guanfacine) 1mg	1
Zoloft 25mg (sertraline)	1

References

Butler JS, Molholm S, Fiebelkorn IC, Mercier MR, Schwartz TH, and Foxe JJ. Common or redundant neural circuits for duration processing across audition and touch. *J Neurosci* 31: 3400-3406, 2011.
Delorme A, and Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* 134: 9-21, 2004.
Milne E. Increased intra-participant variability in children with autistic spectrum disorders: evidence from single-trial analysis of evoked EEG. *Frontiers in psychology* 2: 51, 2011.
Rouder JN, Speckman PL, Sun D, Morey RD, and Iverson G. Bayesian t tests for accepting and rejecting the null hypothesis. *Psychonomic bulletin & review* 16: 225-237, 2009.