The hemodynamic response in children with Simplex Autism

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ABSTRACT

Background: Numerous functional magnetic resonance imaging (fMRI) studies of the brain-bases of autism have demonstrated altered cortical responses in subjects with autism, relative to typical subjects, during a variety of tasks. These differences may reflect altered neuronal responses or altered hemodynamic response. This study searches for evidence of hemodynamic response differences by using a simple visual stimulus and elementary motor actions, which should elicit similar neuronal responses in patients and controls.
Methods: We acquired fMRI data from two groups of 16 children, a typical group and a group with Simplex Autism, during a simple visuomotor paradigm previously used to assess this question in other cross-group comparisons. A general linear model estimated the blood-oxygen-level-dependent (BOLD) signal time course, and repeated-measures analysis of variance tested for potential cross-group differences in the BOLD signal.
Results: The hemodynamic response in Simplex Autism is similar to that found in typical children. Although the sample size was small for a secondary analysis, medication appeared to have no effect on the hemodynamic response within the Simplex Autism group.
Conclusions: When fMRI studies show BOLD response differences between autistic and typical subjects, these results likely reflect between-group differences in neural activity and not an altered hemodynamic response.

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1. Introduction

Fluctuations in the blood-oxygen-level-dependent (BOLD) signal have been shown to couple tightly with neural activity (Logothetis et al., 2001). Thus, functional magnetic resonance imaging (fMRI) and functional connectivity MRI (fcMRI) can be used as indirect measures of neural activity. However, atypical subjects, such as children with ASD (autistic spectrum disorders), may have a quantitatively different relationship between the blood-oxygen-level-dependent (BOLD) signal, which is measured by fMRI, and neuronal responses, i.e., different neurovascular coupling.

This is important because recent fMRI studies (e.g., Mostofsky et al., 2009; Muller et al., 2003) have shown differences in the BOLD signal for motor, parietal, cerebellar, and prefrontal cortical regions of the brain during complex visuomotor tasks. Further, recent fcMRI studies have reported under-connectivity between anterior and posterior regions of the brain (e.g., Cherkassky et al.,

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2006); aberrant connectivity in frontal, parietal, and occipital regions of the brain (e.g., Noonan et al., 2009); and reduced long range functional connection between regions of the brain comprising the default-mode network (e.g., Kennedy and Courchesne, 2008; Monk et al., 2009; Weng et al., 2010).

fMRI and fcMRI comparisons of ASD and typical subjects typically assume a hemodynamic response time course that, independent of differences in neural activity, is the same between ASD and typical subjects (e.g., Gomot et al., 2008; Kaiser et al., 2010). However, little data exist to show that the hemodynamic response is generally similar between people with and without ASD. In order to interpret properly the current autism fMRI literature, it is important to demonstrate that the basic hemodynamic response is similar between ASD and typical cohorts at typical sample sizes. A key first step would be to examine the hemodynamic response of people with and without ASD during a simple task, where the demands of the task are not likely to be affected by an ASD diagnosis (Church et al., 2011, 2012; Harris et al., 2011). The value of this step is predicated on the assumption that the two cohorts would perform the task the same way and have similar neuronal activity that could then be measured by fMRI. The limitations to this assumption are discussed in Section 4.

We compared the temporal dynamics of the BOLD signal between children with and without Simplex Autism (defined below) during a simple visuomotor task (Miezin et al., 2000) in which we would expect no significant differences in the underlying neural activity. The same paradigm has been used to test the hypothesis that typical adults and children have the same fundamental relationship between neural activity and the BOLD signal (Kang et al., 2003). If similar BOLD time courses are observed in multiple vascular distributions when the task is sufficiently simple that neuronal processing is expected to be equivalent in autistic and control children, then differences in BOLD time courses that are observed in fMRI autism studies of similar sample sizes more likely reflect differences in neural activity than differences in the hemodynamic response.

2. Materials and methods

2.1. Participants

Simplex Autism refers to well-characterized ASD individuals with no affected first-degree relatives. Sixteen typical and 16 Simplex Autism children (ages 9–14 years) were recruited using a variety of means, including recruitment from the local community through flyers and advertisements, and through other research collaborations. Demographics are shown in Table 1. Subjects were screened out for a history of focal neurological deficit, strabismus, or vision not corrected to normal acuity with glasses. All subjects (typical and ASD) had no first-degree relatives with an ASD. In addition, typical children could not have any first-degree relatives with Attention-Deficit/Hyperactivity Disorder (ADHD). Simplex Autism participants had (1) community MD or PhD clinical diagnoses of Autistic Disorder, Asperger's Disorder, or Pervasive Developmental Disorder Not Otherwise Specified

Table 1

Demographics of participants included in the fMRI study. Psychotropic medications included selective serotonin reuptake inhibitors, psychostimulants, and antipsychotic medications. Independent samples t-tests were conducted to determine whether the groups were significantly different on age, intelligence quotient (IQ), the vocabulary and block design scaled scores, and the Social Responsiveness Scale (SRS), a quantitative measure of autistic traits. While age was not significantly different between Simplex Autism and typical cohorts ($P = 0.28$), IQ was significantly greater for the typical than the Simplex Autism cohort ($P = 0.016$). SRS was significantly greater for the Simplex Autism than the typical cohort ($P < 0.0001$). The vocabulary ($P = 0.019$) but not block design ($P = 0.15$) subscale score was significantly greater in the typical cohort. One typical subject did not perform the block design subtest, and was not included in the t-test comparison.

	Demographics	
	Simplex Autism subjects	Typical subjects
Male/female	13m/3f	13m/3f
Age	11.9 ± 1.89	12.6 ± 1.80
IQ	104.9 ± 12.97	115.7 ± 10.64
Vocabulary scaled score	10.25 ± 3.26	13.00 ± 2.37
Block design scaled score	10.88 ± 3.05	12.23 ± 2.12
SRS	102 ± 21.7	17.8 ± 9.56
Medicated/un-medicated	8/8	0/16

(PDD-NOS), and (2) consensus research ASD diagnoses as measured by the Autism Diagnostic Observation Schedule (Lord et al., 2000) and Autism Diagnostic Interview Revised (Lord et al., 1994). Five typical and two Simplex Autism subjects were assessed in previous studies using the Wechsler Intelligence Scale for Children (WISC-IV). The other children were assessed using the Wechsler Abbreviated Scale of Intelligence (WASI). All children performed the vocabulary subtest of the corresponding assessment, and all but one typical child performed the block design subtest of the corresponding assessment. Informed consent and assent were obtained using procedures approved by the Washington University Human Research Protection Office.

2.2. MRI protocols

MRI data were acquired using a Siemens 3T Trio scanner (Erlangen, Germany) with standard 12-channel head coil. An iMac Macintosh computer (Apple, Cupertino, CA) running Psyscope X software (Cohen et al., 1993) was used to control the stimulus display. Responses were recorded using a fiberoptic key-press device held in the subject's hands. An LCD projector was used to project stimuli onto a screen at the head of the bore. Subjects viewed the stimuli through a mirror attached to the head coil.

High-resolution structural images were acquired using a sagittal MP-RAGE T1 weighted sequence (TE = 3.08 ms, TR = 2.4 s, TI = 1000 ms, flip angle = 8°, 176 slices at 1 mm isotropic resolution/voxel). Functional images were acquired using a BOLD contrast sensitive, gradient echo, echo-planar sequence (TE = 27 ms, flip angle = 90°, 4 mm × 4 mm × 4 mm isotropic resolution/voxel). MR acquisition was 2.5 s/frame and consisted of 32 contiguous, axial slices, centered on the hemispheric divide and parallel to the AC–PC plane. The first four frames in each run were discarded to allow stabilization of longitudinal magnetization. Each functional run lasted approximately

three minutes (72 frames). Four runs were acquired per subject.

2.3. Behavioral paradigm

The task, a jittered event-related design known to generate highly reproducible activation in sensorimotor and visual cortex for both adults and children, has been described in detail previously (Kang et al., 2003; Miezin et al., 2000). Briefly, subjects pressed a button at the onset and offset of a visual stimulus presented for 1.26 s. The visual stimulus was a radial counterphase-flickering checkerboard subtending ∼11° of the visual field surrounding the fovea. Right and left index fingers were used for onset and offset respectively. Approximately 30 stimulus presentations appeared per 72-frame run. Accuracy, measured as a percentage of onset and offset omissions, and median reaction time (RT) per subject were evaluated using two-sample two-tailed *t*-tests.

2.4. fMRI data analysis

BOLD data from each subject were pre-processed to remove noise and artifacts (Kang et al., 2003; Miezin et al., 2000). Head motion per BOLD run was quantified using total root mean square (RMS) linear and angular displacement measures. For the main analysis, and in order to match the amount of head movement between the two cohorts, two of the four runs per subject were chosen, so that average total RMS head movement was not significantly different between the two groups. The total RMS is derived from measurements in six directions relative to the first frame acquired across each run. We attempted to match best this total RMS between each typical and each ASD subject. As a result, we did not control for the order of the runs. Results were primarily analyzed from these motion-matched, selected pairs of runs. In addition, as a secondary analysis, results from each individual run and the combined set of four BOLD runs regardless of motion difference across groups were analyzed.

An anatomical average of each subject's cross-aligned BOLD data was registered to his/her MP-RAGE. Spatial normalization was performed using a 12-parameter affine warping of the individual MP-RAGE images to an atlas-representative target as described previously (Kang et al., 2003; Snyder, 1996). The atlas-representative target itself was constructed by 12-parameter affine co-registration of a group of MP-RAGE images representing two groups of 13 young children (ages 7–9) and 12 young adults (ages 21–30). To measure the accuracy of atlas transformations, eta (η) values were derived from the correlation of similarity between the atlas and the morphed MP-RAGE images (Snyder, 1996). η^2 is the measure of variance in the source image that is accounted for by variance in the target image (Kang et al., 2003).

To investigate putative differences in the shape of the BOLD time course in Simplex Autism, no assumptions were made about its underlying shape. Preprocessed data were smoothed using a 2 mm full-width half-max kernel, and analyzed using an implementation of the general linear model (GLM). Both a constant offset term and a linear trend term were included in the GLM for each BOLD run. Seven time points (1 TR/MR frame = 2.5 s apart) for each stimulus trial were modeled in the GLM. Significant differences between the two cohorts were tested using a voxelwise group by time course (2 groups × 7 MR frames), sphericity corrected, repeated measures ANOVA (Kang et al., 2003; Miezin et al., 2000), which accounts for correlations in the design (Ollinger et al., 2001a,b). The statistical maps were corrected for multiple comparisons using the Monte Carlo method (to achieve significance with $P < 0.05$, 24 contiguous voxels with a Z score >3.5 are needed) (Kang et al., 2003; McAvoy et al., 2001). In-house software (FIDL), was used to perform these analyses. Activated regions were identified in the statistical map generated from the main effect of time course. Effects of diagnosis were examined using the group by time course interaction statistical map. The group by time course interaction measures shape differences between the BOLD signals from the two cohorts. As alluded to above, five additional group by time repeated measures ANOVAs were performed to confirm the lack of a significant group by time interaction: one ANOVA combining all four runs per subject, and one ANOVA for each individual run per subject.

To assess potential differences further, ROIs were delineated from the main effect of time course map via a peak-finding algorithm as described elsewhere (Kang et al., 2003; Miezin et al., 2000). Time courses, averaged over the activated voxels, were derived per ROI and subject. Time courses for each ROI were entered into a group by time repeated measures ANOVA for subsequent analysis.

3. Results

Behavioral performance was examined in terms of both accuracy and RT. No differences in onset ($T_{30} = 1.197$, $P = 0.241$) and offset ($T_{17.057} = -1.630$, $P = 0.121$) RTs were observed between Simplex Autism and typical cohorts. No significant differences were found between Simplex Autism and typical cohorts for onset and offset omissions (all $P > 0.09$). Omissions and incorrect responses represented less than 6% of all trials per subject.

3.1. Primary analysis: two runs per subject matched for differences in motion between Simplex Autism and typical cohorts

For the primary analysis, where two runs per subject were chosen in order to control for head movement, no significant differences in head movement were observed between the Simplex Autism and typical children ($T_{30} = 0.482$, $P = 0.633$). Mean RMS values for each group were under 1 mm (0.57 mm for Simplex Autism, 0.51 mm for typical). Mean η^2 values were similar between Simplex Autism (0.9902) and typical (0.9909) groups, and means were not statistically different ($T_{21.98} = 1.734$, $P = 0.097$).

After correcting for multiple comparisons, the voxelwise ANOVA revealed significant main effects of time course (Fig. 1a, top), but no group by time course interactions (Fig. 1a, bottom). A subset of participants

(12 Simplex Autism and 12 typical participants), matched for both motion ($P = 0.33$) and full-scale IQ (Simplex Autism FSIQ = 111 ± 8, typical FSIQ = 113 ± 9, $P = 0.53$), were also examined to ensure that IQ differences did not mask any group by time interactions. Matching for IQ revealed no

Monte-Carlo corrected significant group by time course interactions (Fig. 1b, bottom).

Using a peak-finding algorithm, 67 ROIs were found that showed significant time courses in the main effect analysis. Of these 67 ROIs, 19 were chosen to sample
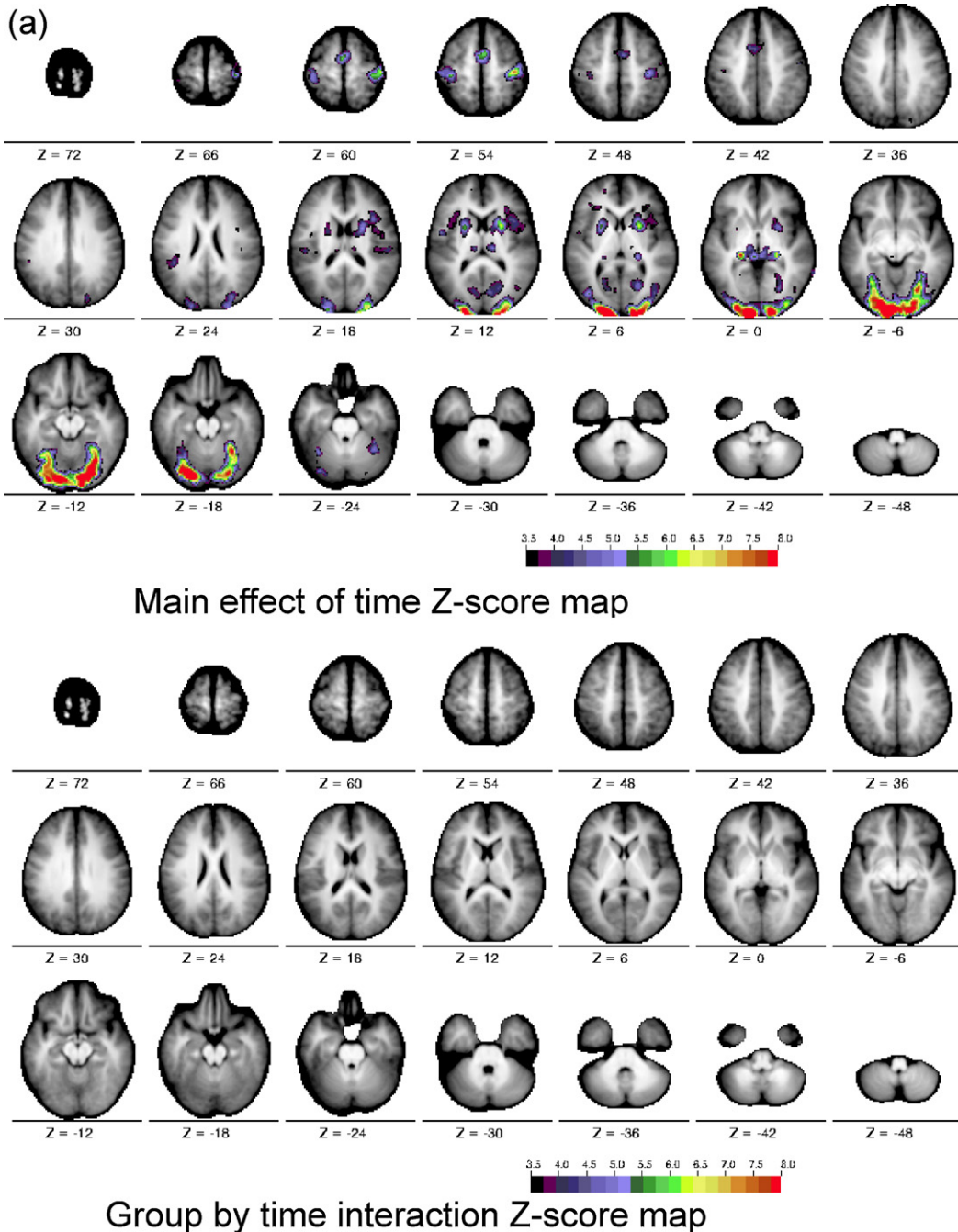
Fig. 1. (a) Statistical maps for the motion matched scans across the two groups (cohorts). The images show the Z score maps for the main effect of time course (top) and the interaction of time course by group (bottom). The statistical maps are corrected for multiple comparisons using the Monte Carlo method. The colors on the map represent Z scores from 3.5 (black) to 8 (red). (b) Statistical maps for motion and IQ matched scans across the two groups (cohorts). The images show the Z score maps for the main effect of time course (top) and the interaction of time course by group (bottom). The statistical maps are corrected for multiple comparisons using the Monte Carlo method. The colors on the map represent Z scores from 3.5 (black) to 8 (red).

Main effect of time Z-score map

Group by time interaction Z-score map
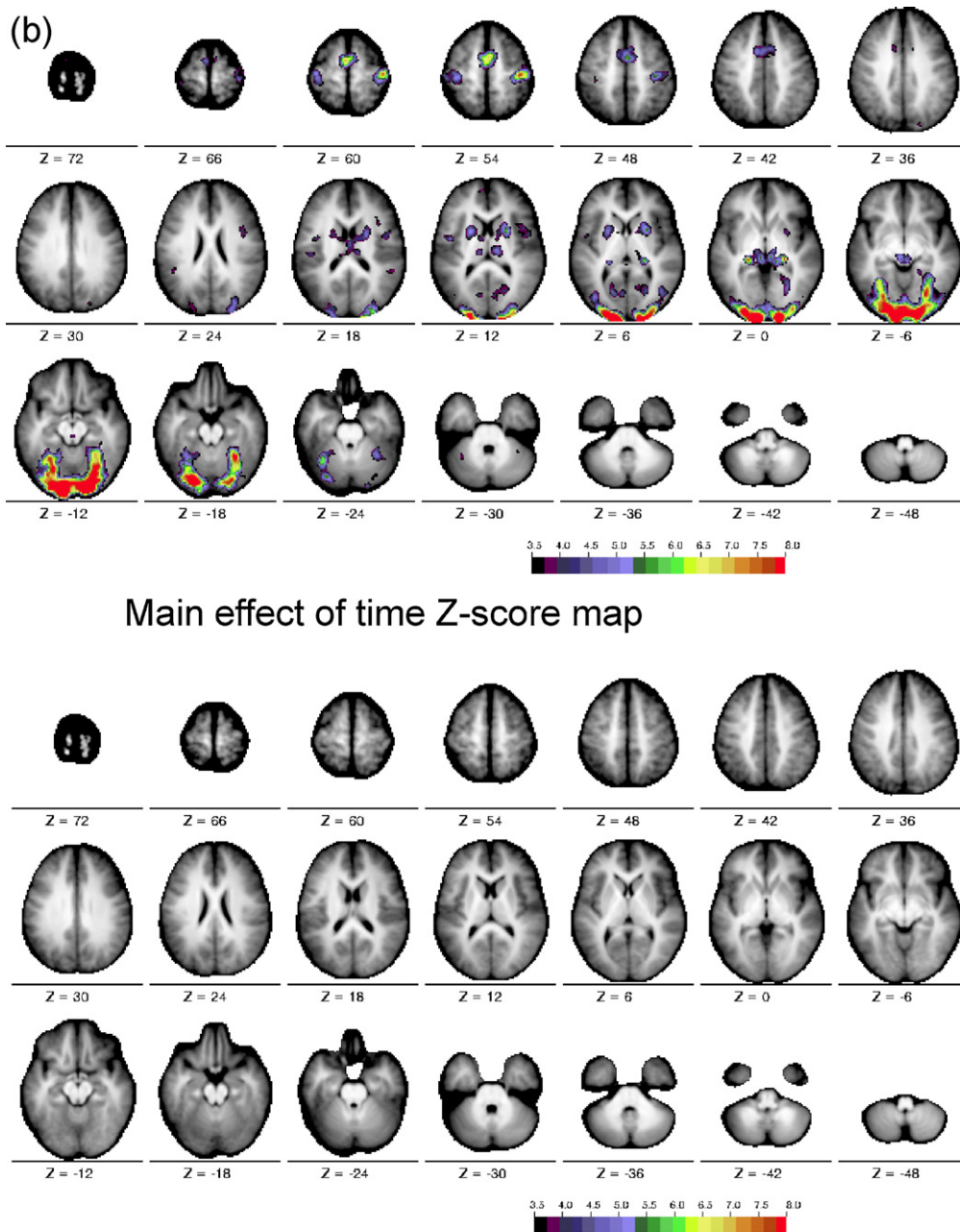
Fig. 1. (*Continued*).

varying vascular distributions. Average time courses were computed for the 19 delineated ROIs for Simplex Autism and typical cohorts. Fig. 2 shows time courses for regions fed by anterior and middle cerebral arteries. Fig. 3 shows time courses for regions fed by the perforating branches from the posterior cerebral artery. Fig. 4 shows time courses for regions fed by the posterior cerebral artery. Fig. 5 shows time courses for regions fed by the superior cerebellar artery. The individual time courses were entered

into group by time repeated measures ANOVAs for subsequent analyses (Table 2, motion matched subjects; Table 3, motion and IQ matched). No significant diagnosis by time course interaction effects were found (all *P* values >0.1).

The Simplex Autism cohort was then split into two groups, those subjects that had been on one or more specific class of medications and those subjects not taking these medications. Each group has 8 subjects. The medications include stimulants, anti-psychotics, and selective
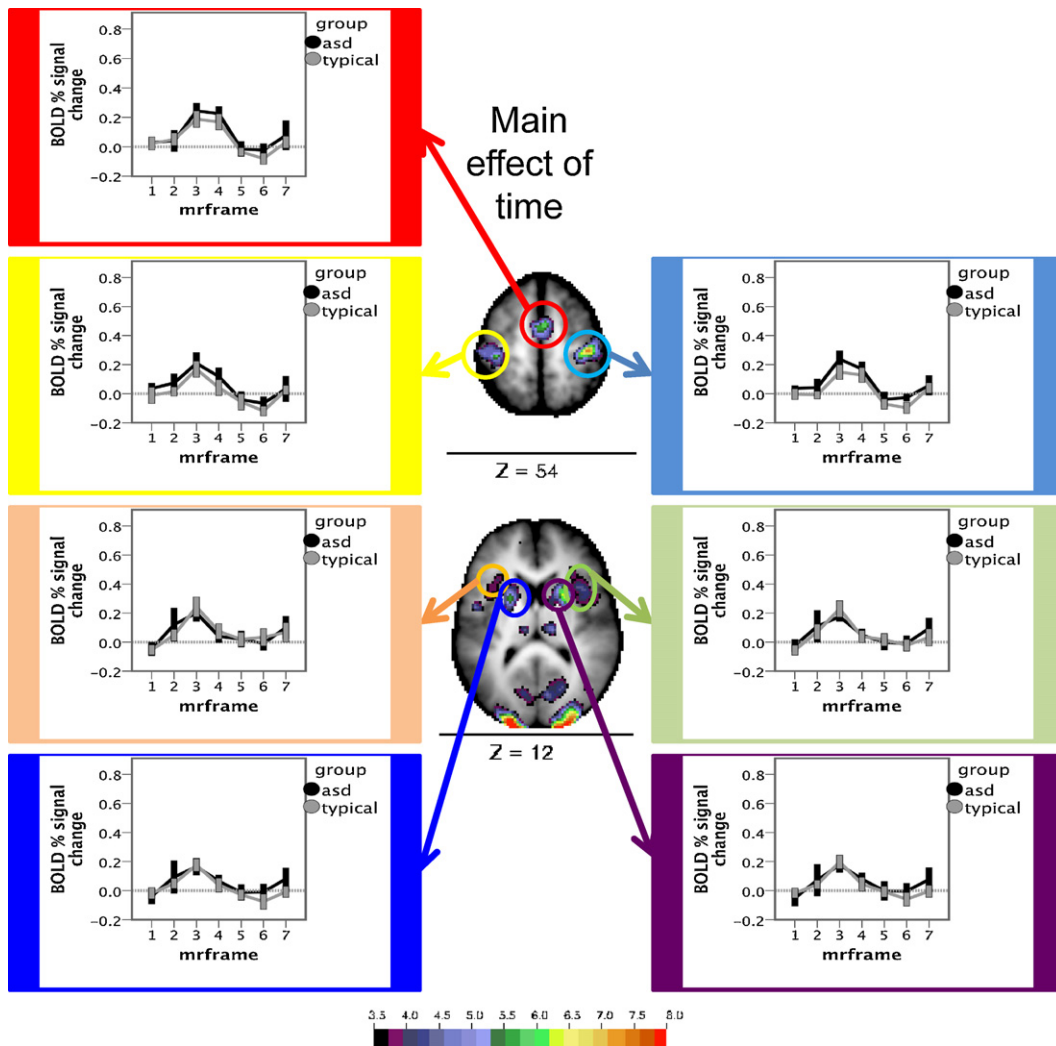
Fig. 2. Time courses and Monte Carlo corrected statistical *Z*-map for regions served by the anterior cerebral and middle cerebral arteries. The map depicts the *Z* statistics derived from the main effect of time course. The mean time courses for Simplex Autism (dark) and typical (light) cohorts are depicted for each of the chosen ROIs. Error bars represent 1 standard error of the mean. Color bar ranges from black (*Z* = 3.5) to red (*Z* = 8).

serotonin reuptake inhibitors (SSRIs). The 19 main effect ROIs were entered into group (medicated/not) by time course repeated measures ANOVAs, and no significant effects were observed (all *P* values >0.1).

3.2. Examination of group by time interactions found in the primary analysis at a liberal threshold

For further assessment, the time course × diagnosis interaction map was examined at an extremely liberal, uncorrected threshold of *Z* > 1.9. Two ROIs in visual cortex were chosen from the statistical *Z* map for this liberal time course × group interaction image (Fig. 6). Time courses were extracted from these ROIs and analyzed for group by time interactions using a time course by group repeated measures ANOVA (Table 4). One ROI shows a significant

group by time interaction (Fig. 6, top); the other region shows a group by time interaction trend (Fig. 6, bottom).

3.3. Secondary analyses: all four runs combined per subject, and each run per subject

To help ensure that the lack of observed differences were not due to the selection of specific runs, all four runs acquired per subject were concatenated and analyzed using a diagnosis by time (2 × 7) repeated measures voxelwise ANOVA. After correcting for multiple comparisons, the voxelwise ANOVA revealed significant main effects of time course (Fig. 7, top), and a small group by time course interaction (Fig. 7, bottom). The region showing a significant interaction overlaps with the region showing an uncorrected interaction in the motion-controlled analysis.
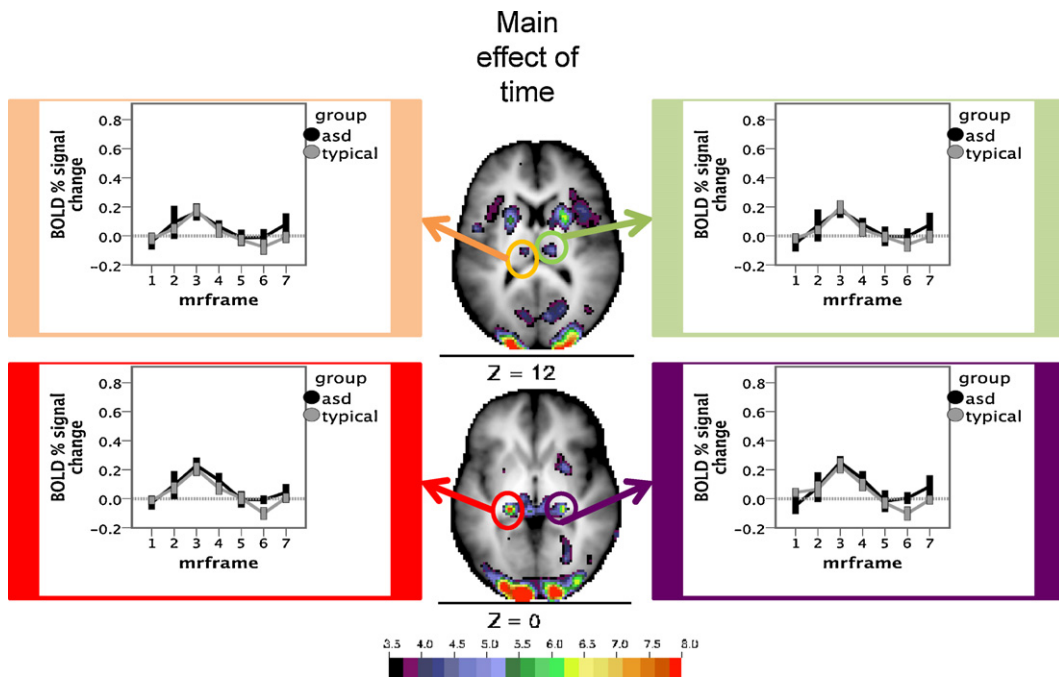
Fig. 3. Time courses and Monte Carlo corrected statistical Z-map for regions served by the perforating branches from the posterior cerebral artery. The map depicts the Z statistics derived from the main effect of time course. The mean time courses for Simplex Autism (dark) and typical (light) cohorts are depicted for each of the chosen ROIs. Error bars represent 1 standard error of the mean. Color bar ranges from black ($Z = 3.5$) to red ($Z = 8$).

We also ran a group by time course (2×7) repeated measures ANOVA for each of the four runs the subjects performed. Because a single run contains fewer trials, the estimates derived from each subject's GLM are weaker, and the ability to detect significant differences is limited. Therefore, the main effect and interaction images from each run were binarized at a threshold of $Z > 1.9$ and summed into a conjunction image. As shown in the main

effect conjunction image, all 19 regions of interest show consistent significant activation for each of the four runs (Fig. 8, top). However, the group by time interaction conjunction image shows a consistent activation for a region in right visual cortex, which is the same region identified in the other secondary analyses (Fig. 8, bottom).

Regardless of whether two motion-matched runs, all four runs, or a conjunction of each individual run is

Table 2
The results from the ROI time course by group repeated measures ANOVAs performed on the selected main-effect ROIs. X, Y, and Z coordinates correspond to the Talairach standard space. Degrees of freedom, F, P, and partial eta squared values are listed for the interaction of group diagnosis and time course.

| Region	Time course × group repeated measures ANOVAs						
	X	Y	Z	Df	F	P	Partial η^2
Right motor	39	−23	55	3.86, 115.76	0.18	0.94	0.006
SMA	−1	−4	57	3.85, 115.53	0.17	0.95	0.005
Left motor	−38	−28	59	3.75, 112.48	0.14	0.96	0.005
Right insula	40	8	10	3.80, 114.09	0.35	0.83	0.012
Left insula	−38	8	11	3.86, 115.65	0.36	0.83	0.012
Right putamen	23	4	9	3.44, 103.31	0.56	0.67	0.018
Left putamen	−22	3	9	3.16, 94.78	0.28	0.85	0.009
Right thalamus	12	−22	11	3.81, 114.28	0.45	0.76	0.015
Left thalamus	−9	−23	12	3.58, 107.50	0.43	0.76	0.014
Right LGN	19	−33	1	3.93, 117.89	1.08	0.37	0.035
Left LGN	−20	−31	0	3.78, 113.51	0.46	0.75	0.015
Right fusiform	28	−71	−10	3.94, 118.27	0.80	0.52	0.026
Left fusiform	−27	−65	−11	3.69, 110.74	0.68	0.60	0.022
Right visual	19	−87	−12	3.99, 119.71	1.52	0.20	0.048
Left visual	−23	−88	−12	3.75, 112.51	1.77	0.14	0.056
Right visual	15	−96	−5	3.59, 107.59	2	0.11	0.063
Left visual	−13	−91	−9	3.98, 119.52	1.09	0.37	0.035
Right cerebellum	36	−51	−25	3.57, 107.17	0.98	0.42	0.032
Left cerebellum	−32	−53	−20	3.93, 117.99	0.59	0.67	0.019
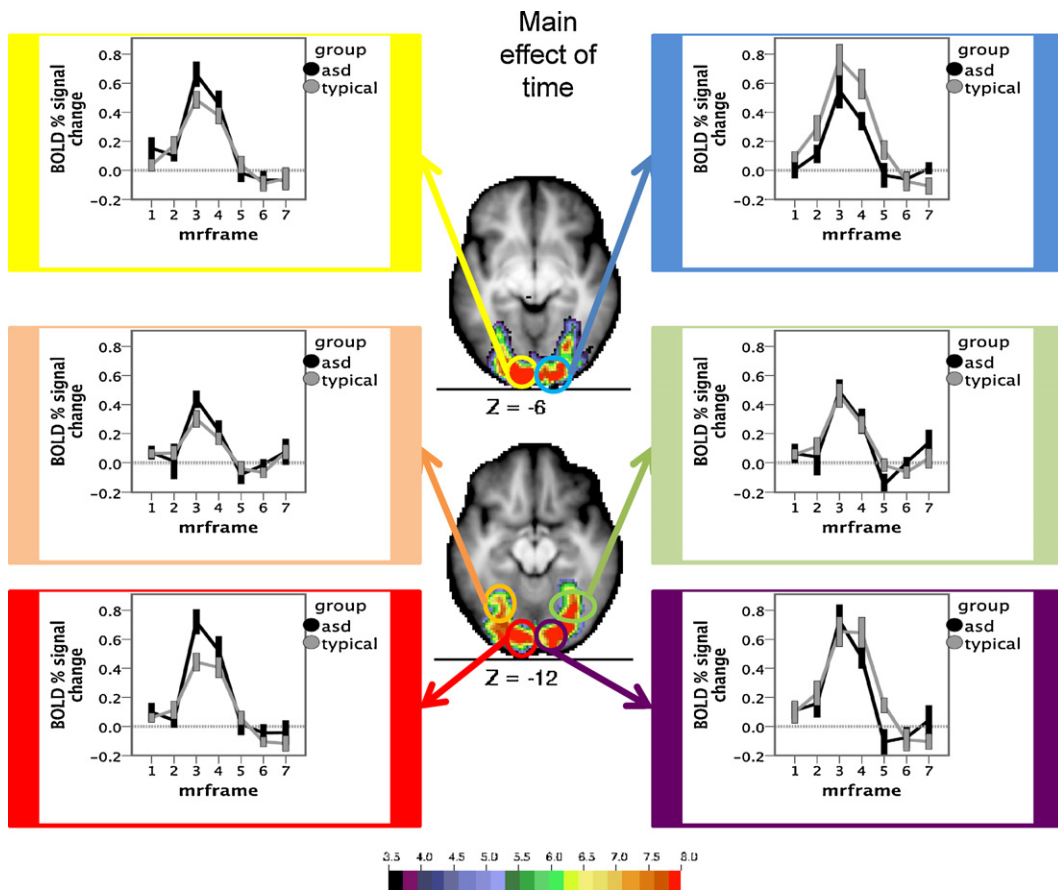
Fig. 4. Time courses and Monte Carlo corrected statistical *Z*-map for regions served by the posterior cerebral artery. The map depicts the *Z* statistics derived from the main effect of time course. The mean time courses for Simplex Autism (dark) and typical (light) cohorts are depicted for each of the chosen ROIs. Error bars represent 1 standard error of the mean. Color bar ranges from black (*Z* = 3.5) to red (*Z* = 8).
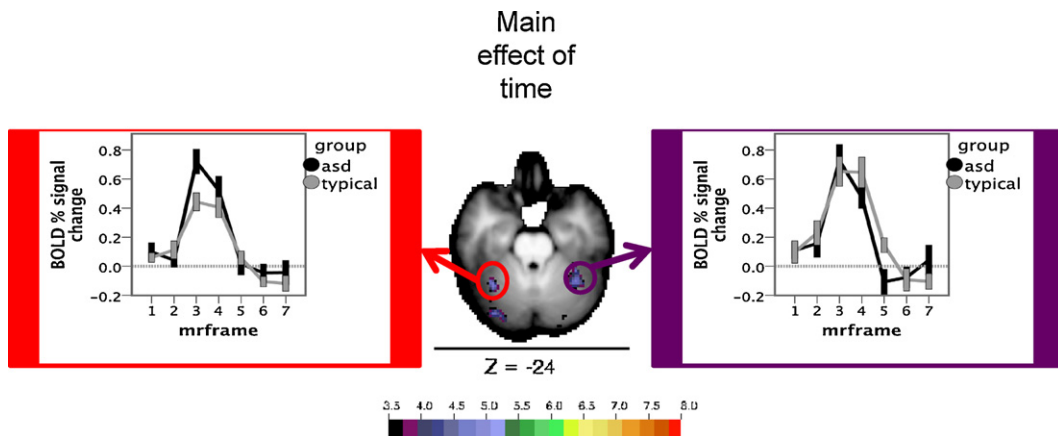

Fig. 5. Time courses and Monte Carlo corrected statistical *Z*-map for regions served by the superior cerebellar artery. The map depicts the *Z* statistics derived from the main effect of time course. The mean time courses for Simplex Autism (dark) and typical (light) cohorts are depicted for each of the chosen ROIs. Error bars represent 1 standard error of the mean. Color bar ranges from black (*Z* = 3.5) to red (*Z* = 8).

Table 3
The results from the ROI time course by group repeated measures ANOVAs performed on the selected main-effect ROIs, for the IQ matched groups. *X*, *Y*, and *Z* coordinates correspond to the Talairach standard space. Degrees of freedom, *F*, *P*, and partial eta squared values are listed for the interaction of group diagnosis and time course.

Region	Time course × group repeated measures ANOVAs: IQ matched						
	X	Y	Z	Df	F	P	Partial η^2
Right motor	39	−23	55	3.72, 81.9	0.57	0.67	0.025
SMA	−1	−4	57	3.82, 84.1	0.5	0.81	0.022
Left motor	−38	−28	59	3.05, 67.18	0.74	0.53	0.033
Right insula	40	8	10	3.28, 72.14	0.82	0.5	0.036
Left insula	−38	8	11	3.3, 72.49	0.79	0.52	0.035
Right putamen	23	4	9	2.96, 65.07	0.86	0.46	0.038
Left putamen	−22	3	9	2.75, 60.42	0.63	0.59	0.028
Right thalamus	12	−22	11	6, 132	0.29	0.94	0.013
Left thalamus	−9	−23	12	4.14, 91.08	0.17	0.96	0.008
Right LGN	19	−33	1	6, 132	1.03	0.41	0.045
Left LGN	−20	−31	0	4.49, 98.68	1.08	0.37	0.047
Right fusiform	28	−71	−10	4.04, 88.94	1.41	0.24	0.06
Left fusiform	−27	−65	−11	4.05, 89.09	2.11	0.085	0.088
Right visual	19	−87	−12	6, 132	1.54	0.17	0.065
Left visual	−23	−88	−12	3.86, 84.76	1.75	0.15	0.074
Right visual	15	−96	−5	3.94, 86.65	1.49	0.21	0.063
Left visual	−13	−91	−9	3.89, 85.67	1.68	0.17	0.071
Right cerebellum	36	−51	−25	6, 132	1.43	0.21	0.061
Left cerebellum	−32	−53	−20	3.66, 80.62	0.93	0.45	0.041

analyzed, the only observed difference between Simplex Autism and typical cohorts is located in a very small portion of right visual cortex. Other regions from multiple vascular distributions show no differences in the hemodynamic response between Simplex Autism and typical cohorts.

4. Discussion

Through this study of BOLD time courses, we found that the hemodynamic response appears comparable between Simplex Autism and typical cohorts. Head motion can
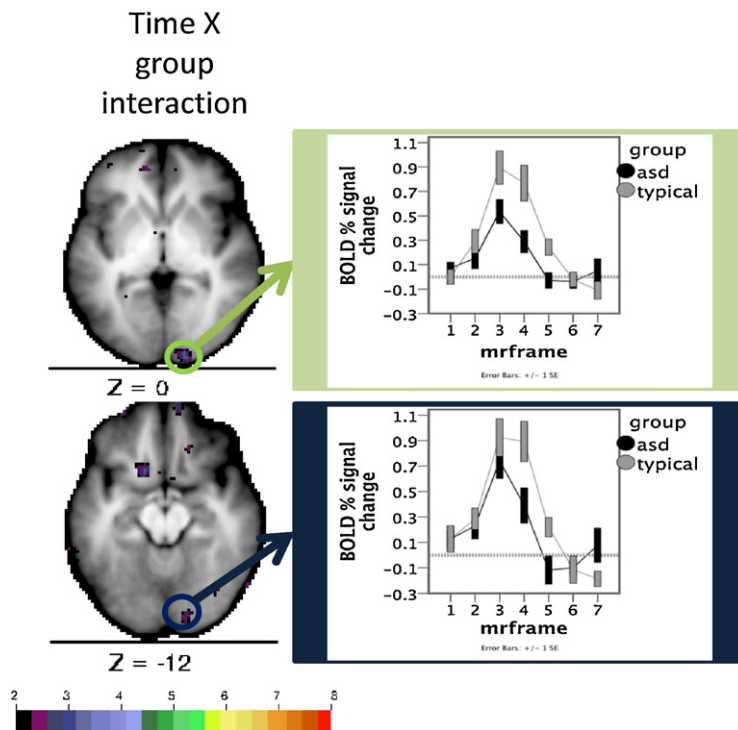

Fig. 6. Time courses and statistical *Z* map for time course × group interaction using a quality control low *Z* value threshold. The map depicts the uncorrected *Z* values derived from the time course × group interaction. The mean time course for Simplex Autism (dark) and typical (light) cohorts are depicted for each of the chosen ROIs. Error bars represent 1 standard error of the mean. Color bar ranges from black (*Z* = 2) to red (*Z* = 8).
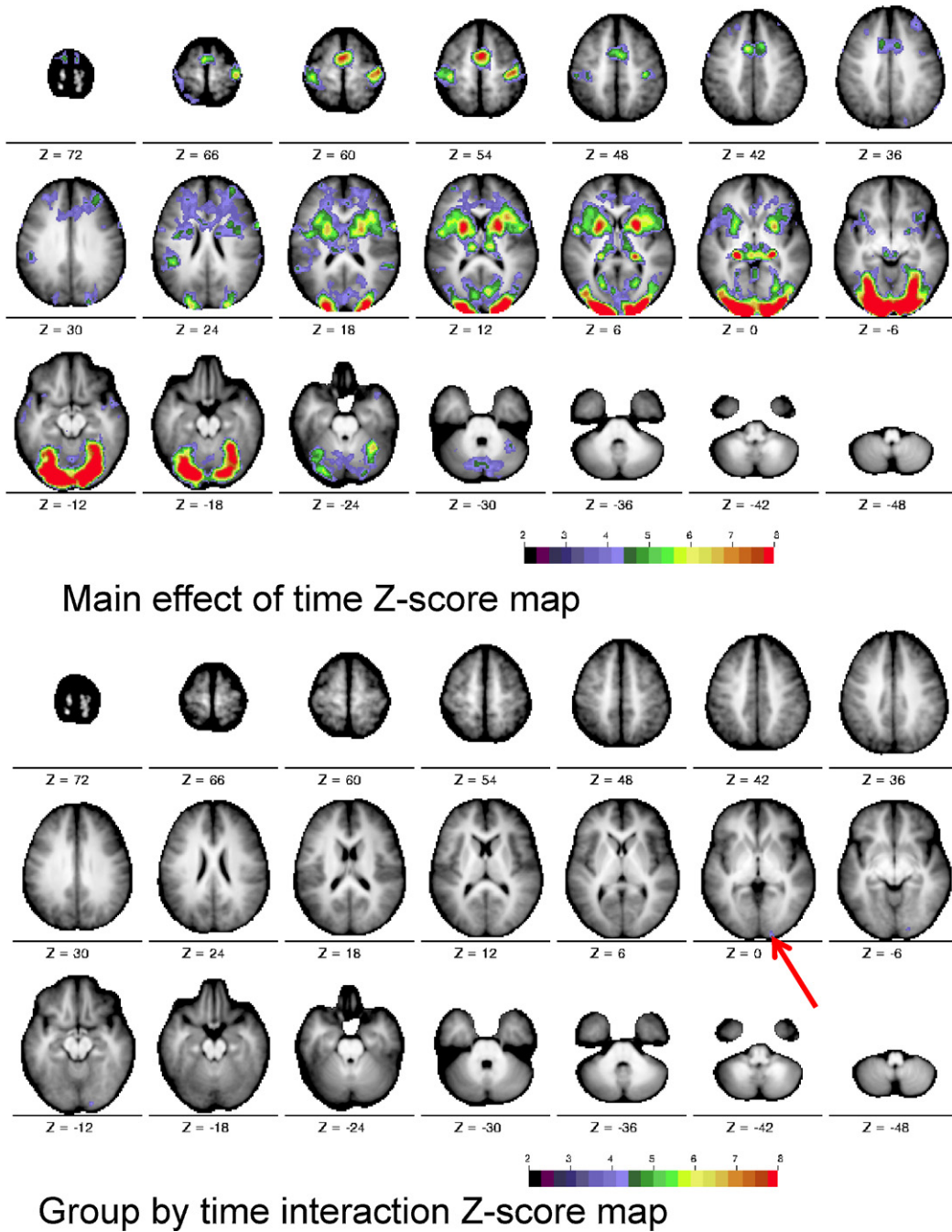
Fig. 7. Statistical maps for the analysis of all four runs per subject. The Monte Carlo corrected statistical *Z*-score maps were derived from the main effect of time course (top) and group by time course interaction (bottom). Color bar ranges from black (*Z* = 2) to red (*Z* = 8).

potentially induce artifacts in fMRI data, which can create false positive and false negative observations. When head motion was best matched between the two groups, no significant group effects were found. When groups were IQ and motion matched, no significant group by time interactions in the BOLD response were observed.

Comparisons of medicated to non-medicated Simplex Autism subjects revealed no significant differences in BOLD activity. It should be noted that this observation is limited due to very small numbers in each cell. It is also possible that different medications could have differing or opposing effects on the hemodynamic response. Nevertheless, the data acquired show no evidence that the combined effect of medications commonly used in people with ASD impacts the hemodynamic response in this sample.
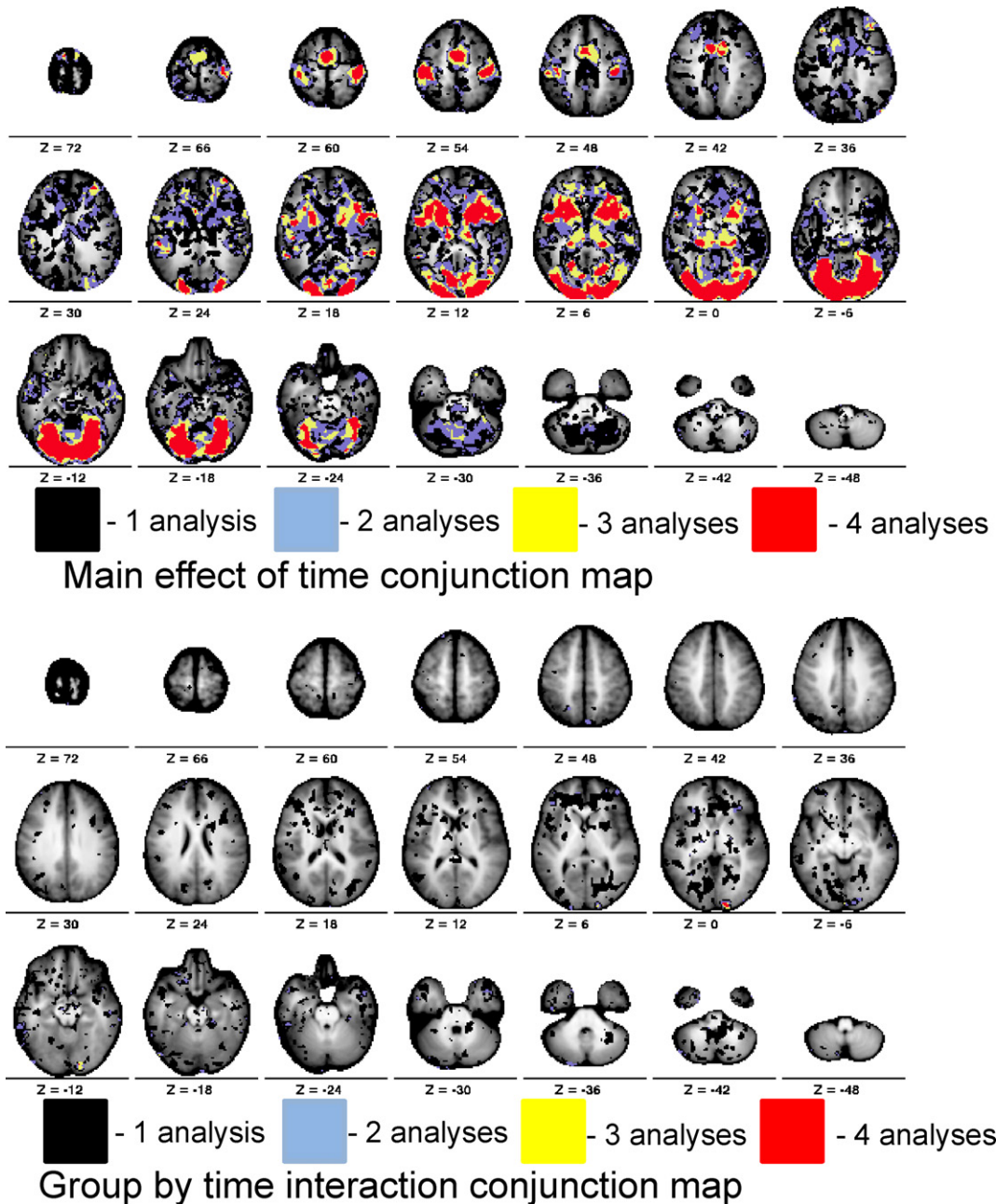
Fig. 8. Conjunction maps for the four analyses of each run per subject. The main effect of time and group by time interaction Z-score maps produced from each run were thresholded at a very liberal Z value (Z > 1.9) and summed. Summing the binarized maps produced a single conjunction map for the main effect of time (top) and interaction (bottom). Values on the map represent the number of analyses (1–4) which showed significant effects at a Z > 1.9. Color bar ranges from black (one analysis) to red (four analyses).

The present study represents a first step in testing whether the hemodynamic response in autism is distinguishable from typically developing children. By using a task sufficiently simple that we can assume that the two groups are performing the task similarly (Church et al., 2012; Harris et al., 2011), demonstrable differences in BOLD response could be interpreted as evidence for an altered hemodynamic response in autism. As stated above, our approach yielded no compelling evidence for Simplex Autism versus control differences in the shape of the task-evoked BOLD signal, in multiple comparisons-corrected analyses of motion-matched BOLD runs. There are several limitations, discussed immediately below.

When head motion was ignored or statistical thresholding was relaxed far below what we would consider appropriate in reporting an "fMRI effect," a small region

Table 4

The results from the ROI time course by group repeated measures ANOVA using the quality control low Z value threshold. These ROIs were extracted by using a peak-finding algorithm (described in Section 2), where all voxels had a $Z > 1.9$. Given X, Y, and Z coordinates correspond to the Talairach standard space. Degrees of freedom, F, and P, observed power, and partial eta squared values are depicted for the interaction of group diagnosis and time course.

Region	QC analyses: interactions at uncorrected significance						
	X	Y	Z	Df	F	P	Partial η^2
Right visual	15	−97	0	3.19, 95.73	4.3	0.006	0.13
Right visual	15	−91	−11	2.36, 70.68	2.8	0.06	0.085

located in right visual cortex appears to be significantly different between Simplex Autism and typical cohorts. One could argue that this difference may represent an altered hemodynamic response localized exclusively to a small portion of right visual cortex. However, it is possible that the difference observed in right visual cortex may relate to an unmeasured behavior. Visual fixation was only qualitatively assessed, and because people with autism may have trouble with oculomotor control (Goldberg et al., 2000, 2002; Luna et al., 2007; Minshew et al., 1999) and fixating a point (Mahone et al., 2006; Pruett et al., 2011), it is possible that small differences in visual fixation may have led to some small differences in visual cortical activity. Because lurking variables can potentially confound the interpretation of fMRI data, it is important to be cautious in estimating the statistical significance of an effect, in interpreting a single finding, and in rigorously examining the quality of the data (Church et al., 2012).

The data presented here only directly pertain to neural processing resources that are engaged in this particular task. Combined electrophysiological and fMRI recordings from mice have shown that different neurons, even within the same brain region (Enager et al., 2009), differ in their neurovascular coupling (Devonshire et al., 2012; Sloan et al., 2010). Therefore, it is possible that driving other populations of neurons might show differentials in fMRI responses.

Our claim, that the hemodynamic response is not altered in autism, is an operational claim, for our data do not measure neural activity directly. One interpretation of this operational claim is that the neurovascular coupling is comparable between autistic and typical cohorts. However, it is theoretically possible that some combination of altered neural activity and altered neurovascular coupling could negate each other, leaving no observed differences in BOLD activity in multiple vascular distributions. Combining MEG and/or EEG with fMRI acquisition might directly address this possibility, by providing convergent electrophysiological data that can dissociate effects of neural activity from effects of neurovascular coupling.

Because these limitations assume that both neural activity and neurovascular coupling are altered in autism, these limitations would seem to be more troubling if robust BOLD response differences were observed between the two groups. However, the data presented here show scant evidence of any meaningful difference in the hemodynamic response in ASD at these sample sizes for neural populations that are responsive to this task. A lack of significant

group by time course interactions does not indicate that the hemodynamic response is completely "normal" in Simplex Autism. However, it is encouraging to see that the hemodynamic response appears comparable in multiple vascular distributions during a simple straightforward task. This finding is important for autism fMRI/fcMRI research because it indicates that, for studies of a similar sample size, when strong autism versus control differences are seen with BOLD contrast in the regions investigated in this study, the observation is more likely not attributable to differential neurovascular coupling, but reflects differences in underlying neural activity.

Conflict of interest statement

Acknowledgments

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