Developmental Science

Developmental Science 19:5 (2016), pp 757–769 DOI: 10.1111/desc.12333

PAPER

Functional brain organization for number processing in pre-verbal infants

Laura A. Edwards,[1,2] Jennifer B. Wagner,[3] Charline E. Simon[4] and Daniel C. Hyde[4]

1. *Graduate School of Education, Harvard University, USA*
2. *Boston Children's Hospital, Harvard Medical School, USA*
3. *Department of Psychology, College of Staten Island, City University of New York, USA*
4. *Department of Psychology, University of Illinois Urbana-Champaign, USA*

Abstract

Humans are born with the ability to mentally represent the approximate numerosity of a set of objects, but little is known about the brain systems that sub-serve this ability early in life and their relation to the brain systems underlying symbolic number and mathematics later in development. Here we investigate processing of numerical magnitudes before the acquisition of a symbolic numerical system or even spoken language, by measuring the brain response to numerosity changes in pre-verbal infants using functional near-infrared spectroscopy (fNIRS). To do this, we presented infants with two types of numerical stimulus blocks: number change blocks that presented dot arrays alternating in numerosity and no change blocks that presented dot arrays all with the same number. Images were carefully constructed to rule out the possibility that responses to number changes could be due to non-numerical stimulus properties that tend to co-vary with number. Interleaved with the two types of numerical blocks were audio-visual animations designed to increase attention. We observed that number change blocks evoked an increase in oxygenated hemoglobin over a focal right parietal region that was greater than that observed during no change blocks and during audio-visual attention blocks. The location of this effect was consistent with intra-parietal activity seen in older children and adults for both symbolic and non-symbolic numerical tasks. A distinct set of bilateral occipital and middle parietal channels responded more to the attention-grabbing animations than to either of the types of numerical stimuli, further dissociating the specific right parietal response to number from a more general bilateral visual or attentional response. These results provide the strongest evidence to date that the right parietal cortex is specialized for numerical processing in infancy, as the response to number is dissociated from visual change processing and general attentional processing.

Research highlights

- Investigated functional brain organization for numerical processing in pre-verbal infants using fNIRS.
- The brain response to number dissociates from the broader visual-attentional response, suggesting a specialized brain mechanism for number in early infancy.
- The brain response to number was restricted to right parietal cortex, suggesting a strict lateralization before the acquisition of a symbolic number system.

Introduction

Humans have the ability to mentally represent numerical magnitudes, or numerosity, non-verbally from early infancy, and this ability appears to persist throughout the lifespan (Feigenson, Dehaene & Spelke, 2004; Xu & Spelke, 2000; Izard, Sann, Spelke & Streti, 2009). For many, exposure to modernized culture and education adds a symbolic number system, counting routine, and increasingly sophisticated mathematical abilities (see Carey, 2009; Le Corre & Carey, 2007; Wynn, 1992). Functional neuroimaging studies with adults and children suggest

Address for correspondence: Daniel C. Hyde, Department of Psychology, University of Illinois Urbana-Champaign, 621 Psychology Building, 603 East Daniel Street, Champaign, IL 61820, USA; e-mail: dchyde@illinois.edu

that a specialized brain system underlies both non-verbal and symbolic/verbal numerical abilities (Venkatraman, Ansari & Chee, 2005; Piazza, Pinel, Le Bihan & Dehaene, 2007). However, it is unclear from both behavioral and brain-imaging work whether this specialized organization for number arises through experience, education, and/or instruction with a symbolic number system, or whether such organization is present before such experiences could explain it. One difficulty with studying early brain organization for numerical abilities, however, has been technological limitations in measuring the brain response in infants and young children before numerical language acquisition and symbolic number system experience. As a result, while functional organization for number has been extensively studied in the adult brain, relatively little research has investigated it very early in development. Here we used functional near-infrared spectroscopy (fNIRS), an emerging technology for studying early brain development, to investigate how the infant brain processes numerical quantities before formal education, symbolic number instruction, or spoken language emerges.

Functional neuroimaging studies with children and adults show that comparing, calculating over, or even passively viewing numbers engages a network of prefrontal and parietal brain regions including bilateral regions of the intraparietal sulcus (IPS), left angular gyrus (AG), bilateral superior parietal lobe (SPL), and prefrontal cortex (PFC) (see Dehaene, 1997; Dehaene, Piazza, Pinel & Cohen, 2003; Nieder & Dehaene, 2009, for reviews). Of particular interest in this network are bilateral regions in and around the IPS as, in contrast to other more general-purpose nodes of the numerical cognition network, these regions appear to have a specialized role in numerical abilities (Nieder & Dehaene, 2009; Dehaene *et al*., 2003). Some portion of these regions responds selectively to number regardless of format (dots, digits, words) or sense modality (auditory spoken vs. visual symbol) in children and adults (Piazza, Izard, Pinel, Le Bihan & Dehaene, 2004; Pinel, Piazza, Le Bihan & Dehaene, 2004; Pinel, Dehaene, Riviere & Le Bihan, 2001; Temple & Posner, 1998). Activity in these regions appears to be relevant to numerical cognition as it correlates with mathematics abilities, and damage or temporary impairment to these regions impairs numerical abilities (Ansari, Grabner, Koschutnig, Reishofer & Ebner, 2011; Bugden, Price, McLean & Ansari, 2012; Cappelletti, Barth, Fregni, Spelke & Pascual-Leone, 2007; Dormal, Andres & Pesenti, 2008; Price, Mazzocco & Ansari, 2013). Based on this and other evidence, activity in these IPS regions has been hypothesized to represent abstract numerical quantity (see Dehaene *et al*., 2003).

Developmental neuroimaging studies, however, suggest that the functional organization of brain regions involved in numerical processing may change in several important ways over the lifespan (e.g. Cantlon, Brannon, Carter & Pelphrey, 2006; Ansari & Dhital, 2006). For example, most studies with children and adults show bilateral IPS activation to both symbolic and non-symbolic numbers in both incidental viewing and active tasks, but studies with younger children show less left parietal activity (Piazza *et al*., 2004; Cantlon *et al*., 2006). One cross-sectional study showed stronger effects of number comparison in the left IPS for adults compared to 9–11-year-old children (Ansari & Dhital, 2006). Another study showed that left IPS involvement in non-symbolic numerical magnitude processing increased over childhood (Cantlon *et al*., 2006). Furthermore, individual differences in left IPS activity correlate with individual differences in symbolic arithmetic ability around 8 years of age (Bugden *et al*., 2012) and changes in left IPS but not right IPS are correlated with changes in elementary school mathematics (Emerson & Cantlon, 2015). What is clear from this literature with children is that activation becomes increasingly bilateral with age. Evidence to date, however, is almost exclusively drawn from children and adult samples where organization, specialization, and lateralization is confounded with symbolic number and mathematics skills, the acquisition of which begins at younger ages than are typically feasible to study using fMRI.

To our knowledge, only two published studies inform our understanding of the underlying functional brain organization for number processing before the acquisition of symbolic number systems (Hyde, Boas, Blair & Carey, 2010; Izard, Dehaene-Lambertz & Dehaene, 2008). One study contrasted event-related potentials (ERPs) in 3-month-old infants to changes in the numerosity of a set of objects with those to changes in the shape of the individual items in the set. Using a source localization algorithm on scalp ERP data, they estimated that the response to number originated primarily from right fronto-parietal regions and dissociated from a more ventral response to shape changes (Izard *et al*., 2008). A second study employing fNIRS, a technique with much better spatial resolution than ERPs, found that only the right parietal cortex of 6-month-olds was sensitive to numerosity, as indicated by an increase in oxygenated hemoglobin when infants were presented with non-symbolic numerical changes in contrast to shape changes (Hyde *et al*., 2010).

Together, the two studies of functional brain organization for numerical processing in infants provide evidence that number processing is lateralized (and then becomes bilateral later in life) and specialized in infancy (Hyde *et al.*, 2010; Izard *et al.*, 2008). However, these conclusions should be considered tentative for several reasons. With regard to lateralization, the methods employed in both studies are limited in spatial resolution. ERPs have notoriously poor spatial resolution and, in general, source localization algorithms such as that used by Izard and colleagues are thought of as better confirmatory than exploratory measures (see Luck, 2005, for a discussion). NIRS has better spatial resolution than ERPs, but Hyde and colleagues (2010) used only a four-channel fNIRS system, severely restricting the measurements to a small portion of the posterior brain (only two scalp sites) in each hemisphere. It is possible that only right-lateralized activity was seen because areas responsive to number in left-lateralized regions fell outside of the two left hemisphere channels. As has been noted by others in discussion of this particular study (Aslin, 2013), more widespread measurement of the posterior brain would be needed to make strong conclusions about the localization, laterality, and the specificity of the observed effect.

More importantly, it is still unclear from previous studies investigating the brain organization for number processing in infants whether observed number responses reflect a number-specific response or more general attentional responses to change. This concern has been heightened by recent behavioral work showing that when a visual stream changing in number is pitted against a visual stream of the same number, young infants actually prefer to attend to stimuli that change in number (Libertus & Brannon, 2010; Libertus, Starr & Brannon, 2014). This behavioral finding, although consistent with the idea that infants represent and compare numerosity in an approximate manner, provides an alternative explanation for why parietal responses to number changes may be larger than responses to control conditions in parietal regions: neural differences may reflect more interest or attentional processing to number changes. Neither of the two published studies claiming number-specificity in the infant brain reported efforts to employ nor particular data to evaluate the effectiveness of visual attention controls across numerical and non-numerical conditions (Hyde *et al.*, 2010; Izard *et al.*, 2008). To be clear, control conditions combined with controls over non-numerical continuous aspects of stimuli ensure that the observed responses (both brain and behavioral) reflect sensitivity to number somewhere in the brain. However, without further control conditions that dissociate visual-attentional processing from numerical processing, it is unclear whether observed brain responses to number change (compared to no change or change on some other dimension) actually reflect numerical specificity of the infant brain or more general attentional processing differences between conditions.

To address these issues, we measured the brain response to non-symbolic number changes over a majority of the surface of the posterior parietal and occipital cortex using a 24-channel fNIRS system in infants before the acquisition of spoken language or symbolic number systems. Specifically, we asked whether any posterior parietal or occipital brain regions are selective for numerosity in young infants by comparing the brain response to blocks of sequentially presented images of dot arrays that changed in numerosity (8 dots, 16 dots, 8 dots, 16 dots…) with blocks of images of dot arrays all containing the same number (8 dots, 8 dots, 8 dots…) (see Hyde & Spelke, 2011, or Libertus & Brannon, 2010). Blocks were presented briefly (12.5 seconds) and were separated by blocks of colorful, audio-visual animations for the purpose of avoiding habituation, maintaining interest or attention equally across numerical conditions (change and no change), and engaging (or re-engaging) attention over the course of the experiment. We reasoned that if the IPS, or any other brain region, was truly specialized for number, we would observe a greater response in that region during experimental blocks showing numerical changes compared to blocks of similar numerical stimuli that varied equally in change of non-numerical visual parameters but contained no numerical change under conditions of equal visual/behavioral attention, and compared to audio-visual stimuli that were much more interesting (but did not contain a number change). We further reasoned that if numerical processing is strongly lateralized before symbolic number systems and language emerge, then we would observe significant responses to number change in the right hemisphere only (Hyde *et al.*, 2010). Finally, we reasoned that specialized responses to number should further dissociate from occipital and other parietal regions responsive to general attentional differences between stimuli that would likely show a greater response to colorful, audio-visual stimuli compared to the silent numerical conditions involving the sequential presentation of gray and white images of dots.

Method

Participants

A total of 13 infants (M_{age} = 6.64 months; SD = 0.62) were included in the final analysis. All had reached a gestational age of at least 37 weeks at birth, had no reported pre- or post-natal medical/neurological issues, and no known genetic disorders. An additional seventeen were excluded from the final analysis due to insufficient behavioral looking (see details below), two were excluded due to poor signal associated with excessive/dark hair, one was excluded due to improper fit of the headgear, one was excluded due to equipment failure, and one was excluded for retaining an insufficient number of artifact free blocks per condition after pre-processing of the data (see details below). Institutional Review Board approval from Boston Children's Hospital was obtained, and the parent or legal guardian gave written informed consent before the study began.

Data acquisition set-up

Hemodynamic responses were measured from the scalp at 10 Hz using a 24-channel Hitachi ETG-4000 continuous wave NIRS system. Measurement optodes were arranged in two 3 × 3 chevron arrays containing a total of 10 incident and eight detecting optodes spaced 3 cm apart (see Figure 1). Incident fibers emitted light at wavelengths of 695 and 830 nm. Optode arrays were held in place by custom-designed neoprene headgear. Bilateral arrays (each forming 12 measurement channels) embedded within the headgear were positioned over the occipital and parietal regions of each hemisphere based on scalp measurements to obtain landmarks from the International 10–20 EEG system to allow us a reasonable estimate to the underlying brain areas associated with each data channel (e.g. Okamoto, Dan, Sakamoto, Takeo, Shimizu *et al*., 2004). Specifically, the middle optode in the left hemisphere chevron was centered between 10–20 points P3 and O1, and the middle optode in the right hemisphere chevron array was centered between 10–20 points P4 and O2 (see Figure 1).

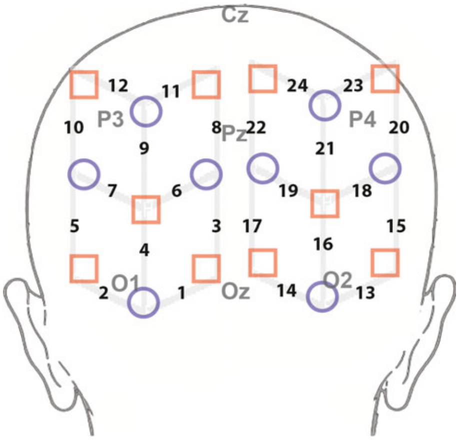

Figure 1 *Schematic of head probe. Red squares represent sources, blue circles represent detectors, bolded black numbers represent data channel labels, and bolded gray text represents 10–20 scalp landmarks (relative to which the probe was placed). Scalp positioning and relation to 10–20 landmarks are only an estimate based on the average head size of infants in this age range.*

Design and procedure

Subjects were seated in a dimly lit room on the lap of their parent or guardian, approximately 65 cm from a video monitor. The optical head probe was initially placed on the infant head, after which a signal quality check was performed to ensure that we were obtaining good measurements from at least 18 out of the 24 measurement channels (75%). Signal quality evaluation was done automatically using the default settings of the Hitachi NIRS system based on the intensity being received by the detectors. If more than six channels were shown to be poor (indicating poor contact and/or measurement artifact), the head probe was adjusted (by moving hair out of the way or securing it more tightly) until all but six or fewer channels were showing good signals. After the signal quality was adjusted to be above threshold, the experiment began, with infants viewing blocks of stimuli as fNIRS measurements were taken.

Each block began with a fixation screen under manual control of the experimenter monitoring the gaze of the infant participant. Blocks were not initiated until the infant was visually attending to the screen. Once the participant was looking, experimental blocks of 10 images consisting of 8 or 16 dots of varying sizes and positions were presented for 12.5 seconds. Blocks were purposely kept short (12.5 s) relative to similar studies in the behavioral literature (~60 seconds) to avoid behavioral habituation, or boredom, and to encourage continual and equal visual attention across both conditions for the duration of the experiment (Starr, Libertus & Brannon, 2013; Libertus *et al*., 2014). Within blocks, images were presented for 1000 ms and were separated by a blank screen that jittered in duration between 150 and 350 ms. Numerical blocks contained either images of 8 dots only (no change blocks), or alternating 8 and 16 dot images (number change blocks). The block types were presented in fixed, alternating order, beginning with the no change blocks. In between each block of numerical stimuli, an animation consisting of an image of an object or animal appearing in sync with an exciting sound effect was presented for 10 seconds. This was intended to capture (or recapture) the attention of the infant, alleviate boredom, and to allow any brain region selective for number to return to baseline while activating general visual attention regions. Subjects viewed a maximum of 20 numerical blocks (10 of each type) and 19 audio-visual animations, but testing was halted if the infant became inattentive or fussy. A gray screen with a white fixation cross in the middle preceded all number blocks. Attention animations started 1 second after the end of every numerical stimulus block and ended, on average, 4.38 (*SD* = 2.33) seconds before the next numerical stimulus block began, as numerical stimuli blocks were under manual control and only began when the infant was looking at the fixation screen that followed the visual animation.

Stimuli

The numerical dot arrays consisted of non-overlapping white dots on a gray 650 by 650 pixel background with a centered, small white fixation cross. Dot generation was carried out using an automated program designed to produce stimuli controlled over non-numerical parameters for studies of numerical cognition (Hyde *et al.*, 2010; Hyde & Spelke, 2011, 2012; Izard *et al.*, 2008; Piazza *et al.*, 2004; Piazza *et al.*, 2007). Specifically, our dot arrays were constructed to control, over the entire set, for systematic covariance between number and other non-numerical properties of the dot arrays (such as total area, item size, density, position, etc.). To do this, we generated a large set of dot arrays where all dots *within* a single image were the same size. However, images varied pseudo-randomly in parameters such as individual dot size, position, density, and total area. Given that not all of the non-numerical stimulus parameters can be controlled at once, half of the images in each condition included a range of dot sizes that were, on average, equated for individual item size (no change images: dot size range 6–12 mm in diameter, *M* diameter = 9 mm; *SD* = 2 mm; number change images: dot size range 6–12 mm in diameter, *M* diameter = 9 mm, *SD* = 2 mm) while the other half of the images contained fixed dot sizes for each number that were approximately equated on total area/density (no change images: *M* cumulative area = 3518 mm^2, *SD* = 0, *M* density = .008 items per mm^2; number change images: *M* cumulative area = 3569 mm^2, *SD* = 52 mm^2, *M* density = .008 items per mm^2). A random subset of the images created was then chosen and organized pseudo-randomly into a fixed presentation list for the study (200 total images: 100 item size controlled and 100 total area controlled) with the constraint that every block (10 images) included 5 images controlled for item size and five images controlled for total area. These controls ensured that the non-numerical stimulus parameters of images were not a predictive or reliable cue to number over the experiment, as the relationship between the number of items and non-numerical stimulus parameters was continually and randomly changing from image to image.

Behavioral coding

Video recordings of study sessions were coded offline for looking behavior to stimuli for the purposes of determining which blocks were attended and which blocks were not, as well as determining whether an infant's looking was sufficient to include them in the analysis. No differences in total duration of looking (no change: *M* = 104 seconds, *SD* = 14.1 seconds; number change: *M* = 104.8 seconds, *SD* = 15.5 seconds, *t*(12) = –0.37, *p* = .72) or average proportion of looking per block (no change: *M* = .778, *SD* = .11, number change *M* = .780, *SD* = .11; *t*(12) = –0.16, *p* = .87) were observed between the no change and number change conditions. Clear differences, however, were observed between behavioral looking to the number conditions and the attention condition. Specifically, both total looking (number: *M* = 145.6 seconds, *SD* = 17.9; attention: *M* = 163.4 seconds, *SD* = 8.4, *t* (12) = –4.96, p < .0005) and average proportion of looking per block (number: *M* = .802, *SD* = .10; attention: *M* = .900, *SD* = .05, *t*(12) = –5.37, *p* < .0005) were larger for attention blocks than for number blocks.[1]

Blocks where infants viewed at least four (out of the 10 total) numerical images were considered acceptable blocks for further fNIRS data processing. To be included in our analysis, we required that in at least three of those acceptable blocks for each condition, the infant looked minimally at the first four images presented. This was done in an effort to maintain some level of consistency in the timing of the hemodynamic response between trials and subjects. Data from infants who failed to meet this criterion were excluded from further analysis (see Subjects section). No differences were observed in the average number of images viewed per block (out of 10 possible; no change: *M* = 7.22, *SD* = 1.12; number change: *M* = 7.28, *SD* = 1.20; *t*(12) = –0.31, *p* > .75) or the average number of blocks included in no change and number change conditions (no change: *M* = 9.08, *SD* = 1.26; number change: *M* = 9.00, *SD* = 1.63; *t* (12) = 0.20, *p* > .84) after reduction of fNIRS data based on looking behavior.

fNIRS pre-processing

All fNIRS data pre-processing was conducted using freely available Homer2 software version 1.5.2 (Huppert,

---

[1] For comparisons of number and attention blocks, we equated block length by only analyzing the first 10 seconds of each block, as actual length was different between them (number blocks = 12.5 s.; attention blocks = 10 s.). For the analysis of total looking between number and attention blocks, the last block for each condition was dropped so as to equate the number of blocks.

Diamond, Franceschini & Boas, 2009) in combination with custom scripts run in MATLAB (R2014a). Data pre-processing began by identifying and eliminating noisy channels. We started by submitting raw intensity signals obtained from each channel for each subject to an automated channel pruning algorithm (too weak = mean light intensity over experiment < .2; too strong = mean light intensity over experiment > 4; or if the signal to noise ratio was too low = mean intensity/standard deviation of intensity < 2). Next, we normalized raw intensity signals and converted them to optical density units using the automated Homer2 algorithm. A principal component analysis (PCA) was then used over the optical density data to further filter out fluctuations in the signal common to all channels (as is the case with signal changes due to non-brain physiological signals such as heart beat and respiration as well as large motion artifacts), with the constraint that no more than 90% of the common variance across channels was removed (for a review of method see Cooper, Selb, Gagnon, Phillip, Schytz *et al.*, 2012; for similar applications in infant data see Wilcox, Boas, Bortfeld, Woods & Wruck, 2005 and Wilcox, Haslup & Boas, 2010). The signal from each channel was then bandpass filtered between .01 and 1 Hz. We then subjected the filtered signal to an automated motion detection algorithm, where motion was defined as a mean signal change of 30% between samples (200 ms time window) (Cooper *et al.*, 2012; Huppert *et al.*, 2009; Scholkmann, Spichtig, Muehlemann & Wolf, 2010). Two-second time windows around samples containing detected artifact were marked as bad and eliminated from further analysis. Individual experimental blocks were defined as 1 second before block onset to the end of the block (−1–12.5 seconds). Any block containing a motion artifact (as defined above) within this time frame was eliminated from further analysis. Signals were then converted to oxy- and deoxy-hemoglobin concentration using the modified Beer-Lambert Law (Obrig, Neufang, Wenzel, Kohl, Steinbrink *et al.*, 2000; Strangman, Boas & Sutton, 2002; Strangman, Franceschini & Boas, 2003). Remaining artifact-free blocks were averaged separately for each condition (no change, number change, and non-numerical attention animation blocks), for each subject from each channel relative to the mean response from −1 to stimulus onset for data analysis (a baseline period) and averaged across subjects for visualization purposes.

Subjects with fewer than two artifact-free blocks of fNIRS for each condition were eliminated from further analysis (one subject). Remaining subjects retained, on average 7.62 (*SD* = 2.57) blocks in the no change condition, 8.08 (*SD* = 2.75) blocks in the number change condition, and 17.46 (*SD* = 3.53) blocks in the attention condition. No differences were observed in the number of blocks retained after fNIRS data pre-processing between the no change and number change conditions ($t(12) = -0.92$, $p > .37$). The number of blocks retained was significantly greater for the attention condition compared to both the no change ($t(12) = -19.05$, $p < .001$) and change conditions ($t(12) = -26.84$, $p < .001$), as nearly twice as many blocks of the attention condition were presented compared to blocks of each of the numerical conditions separately. This was done in an effort to make dot arrays (number of blocks summed over both no change and change conditions) and attention blocks equally likely over the entire course of the experiment.

Statistical analysis

We focused our analysis on the oxygenated hemoglobin (oxyHb) response, rather than deoxygenated hemoglobin (deoxyHb) response or total hemoglobin (total Hb) response, for two reasons. First, oxyHb is thought to be a more reliable measure with a higher signal to noise ratio than deoxyHb, especially in infants (Devor, Ulbert, Dunn, Narayanan, Jones *et al.*, 2005; Kameyama, Fukuda, Uehara & Mikuni, 2004; Strangman *et al.*, 2003; Tong & Frederick, 2010; Watanabe & Kato, 2004). Second, previous fNIRS research on numerical cognition in infants has shown that oxyHb is sensitive to number change in parietal regions (Hyde *et al.*, 2010). Plots including deoxyHb, and totalHb can be found in the Appendix.

We compared relative levels of oxyHb in response to the different conditions to test several focused hypotheses. To test our primary hypothesis, that a subset of posterior brain regions would respond selectively to numerosity, we identified temporal data clusters where the hemodynamic response to number change blocks was greater than both the no change number condition and attention-grabbing audio-visual condition. As further evidence that number-selective regions dissociate from a general attentional response, and as a check to the quality of our NIRS signals across the probe, we also identified temporal data clusters where the hemodynamic response to our attention condition was greater than the response to both the no change and number change conditions, an indication of areas sensitive to general visual processing or audio-visual attention.

Significance testing was carried out using temporal cluster-based non-parametric permutation tests (see Cohen, 2014, or Maris & Oostenveld, 2007, for reviews). To do this we first identified the largest temporal cluster showing the pattern of interest (number change > no change & number change > attention OR attention > number change & attention > no change) for each optical data channel (24 total). We used repeated, one-tailed paired samples *t*-tests ($p < .05$) at each time sample during the experimental block (from 0 to 12.5 seconds; 126 samples) as pre-cluster threshold (see Table 1) (Bullmore, Suckling, Overmeyer, Rabe-Hesketh, Taylor *et al.*, 1999). We chose to use a directional (one-tailed) threshold because we were only interested in identifying responses patterning in one direction, where differences in the other direction would not be psychologically or physiologically meaningful (Kimmel, 1957; Ruxton & Neuhäuser, 2010).[2] Directional hypotheses were derived a priori based on previous literature showing that number-sensitive brain regions display a greater hemodynamic response to conditions involving number change compared to conditions involving the same number (e.g. Cantlon *et al.*, 2006; Piazza *et al.*, 2004; Hyde *et al.*, 2010). Next, we determined statistical significance of the largest temporal cluster of data showing the predicted pattern by comparing it to the distribution of maximum cluster sizes for that channel obtained through 5000 random permutations of the actual data. Specifically, for each permutation, we randomly assigned condition labels (no change, number change, attention) to the averages for each subject at each channel, re-ran the temporal cluster identification algorithm (using the same pre-cluster threshold parameters outlined above), identified temporal clusters showing differences in the direction of interest from the permutation, and extracted the maximum cluster size for that permutation. We calculated the observed cluster-corrected significance level (*p*-value) for the actual data by dividing the number of random permutations of the data that produced a larger maximum cluster at that same site by the total number of permutations conducted (5000) (Cohen, 2014). We only considered significant, temporal clusters on channels showing a cluster-corrected significance level of $p < .025$ (one-tailed) in the direction of interest. Here we chose a directional, one-tailed test because our null hypothesis included both the possibility that there were no actual differences between conditions or that conditions patterned opposite to the predicted pattern. We chose a more conservative statistical significance level to ensure a comparable probability of falsely rejecting the null hypothesis to more traditional two-tailed tests with a significance level of $p < .05$.

Table 1 *Summary of findings*

Channel number	Max. cluster size (samples)	Time window (seconds)	Cluster statistic (*p*-value)
Number-specific response			
20	18	7.1–8.8 s	$p = .02$
Visual-attentional response			
3	55	7.1–12.5 s	$p = .001$
6	74	5.2–12.5 s	$p = .0004$
16	32	9.4–12.5 s	$p = .001$
17	67	5.9–12.5 s	$p = .0002$
19	65	6.1–12.5 s	$p = .002$
22	13	2.5–3.7 s	$p = .02$

Note: This table contains temporal clusters where the response of interest (either the number change blocks or the attention blocks) was significantly greater than that to the other conditions after cluster correction for multiple sequential comparisons using non-parametric permutation testing.

Non-parametric permutation tests for cluster significance were chosen over more traditional methods of NIRS analysis for four main reasons (see Cohen, 2014, or Maris & Oostenveld, 2007, for extensive discussion of statistical issues; see Gervain, Mehler, Werker, Nelson, Csibra *et al.*, 2011, for review of traditional analysis approach). First, our probe contained a large number of channels, presenting the need to statistically correct for multiple comparisons. Correction for multiple comparisons is often completely avoided or inappropriately addressed[3] in traditional approaches (Gervain *et al.*, 2011). Permutation tests provide a reasonable solution for multiple comparisons by correcting based on information present in the actual results rather than the number of tests conducted (Cohen, 2014). Second, normality cannot necessarily be assumed with infant brain imaging data, and non-parametric permutation tests do not require that the data be normally distributed. Third, it was highly likely that the timing of the brain response would be different across different regions of posterior cortex given the breadth of brain coverage of our probe. Regional nuances in the brain response would likely be lost with traditional approaches that typically select a single time window or fixed duration time windows over which to analyze data from all channels. Permutation testing allows for the analysis of the full range of acquired data rather than restricting to

---

[2] We chose to use a pre-cluster threshold of $p < .05$ (one-tailed) given that stricter thresholds tend to bias clusters defined as large differences over a smaller number of time samples, where hemodynamic differences tend to be slower (and, as a result, likely to show more moderate differences between conditions over a longer period) (see Maris & Oostenveld, 2007 for a discussion of thresholds).

[3] A common form of correction for multiple comparisons in traditional fNIRS analysis approaches is Bonferroni correction. Bonferroni correction involves dividing the significance values (*p*-values) by the number of tests conducted. When the number of tests one wants to correct for are small, Bonferroni correction may be appropriate. However, as the number of tests increases, such as in the case with our 24-channel dataset, Bonferroni correction becomes overly strict (see Cohen, 2014).

particular time windows and/or channels. Fourth, and relatedly, our approach allowed for a largely automated analysis, reducing the number of investigator decisions that had to be made along the way (such as when and exactly where to extract data). For example, rather than the investigator defining how much of a difference she/he thinks should be required before conditions should be considered significant (defining a time window over which to average and analyze the data), cluster-based permutation testing identifies and tests whether observed differences over the entire range of data are significant (probability of observing that cluster size if the null hypothesis was true). As such, we believe the permutation testing approach to be more objective than traditional approaches to fNIRS data analysis and more appropriate for our particular experiment (Cohen, 2014; Maris & Oostenveld, 2007).

Results

Number selectivity

To determine whether any regions we measured from selectively responded to numerosity, we analyzed the response to number change blocks as compared to no change blocks and audio-visual animation blocks. After cluster correction for multiple comparisons, only one data channel (channel 20, see Figures 1 and 2) out of 24 showed a greater oxyHb response to number change blocks than to both the no change blocks and the attention blocks (Figure 3). This channel fell within the upper right quadrant of the right hemisphere of our probe set, and differences emerged around 7–9 seconds after block onset (see Table 1 for exact time window of significant difference and statistics). Based on the placement of the optical probe relative to scalp landmarks (e.g. Okamoto *et al*., 2004), this channel fell within the right parietal region of our probe, and its location was consistent with number-related IPS activity seen in children and adults (Cantlon *et al*., 2006; Piazza *et al*., 2004). No other channels showed a sustained response indicative of number specialization after cluster correction for multiple comparisons.

General visual-attentional response

As a manipulation check to confirm the validity of the NIRS measurements across our head probe and to further investigate the relationship between number-specific responses and more general visual-attentional responses, we tested whether some regions were more responsive to the attention-orienting animations compared to both types of number blocks (attention animations > number change & attention animations > no change). After cluster correction for multiple comparisons, six channels (3, 6, 16, 17, 19, 22) showed a greater response to visual-attentional-orienting stimuli compared to both no change and number change experimental blocks (see Figure 3 for example; Table 1 for statistics). All of these channels were distinct from the one showing a number-selectivity profile; a majority of

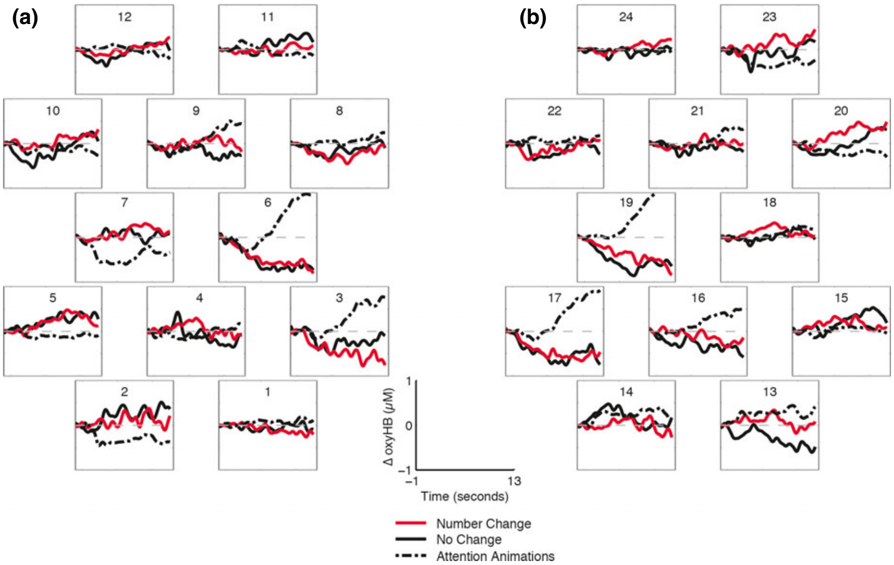

Figure 2 *Average hemodynamic (oxyHb) response to experimental conditions at each data channel. (a) Left hemisphere channels. (b) Right hemisphere channels. Response relative to pre-stimulus presentation baseline (−1–0 seconds).*

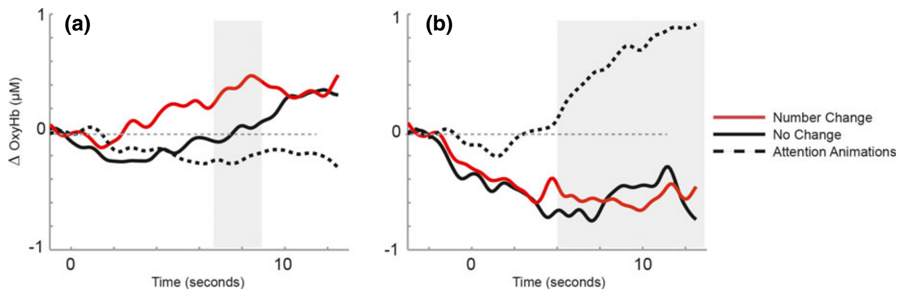

Figure 3 *Average hemodynamic (oxyHb) response in two specific channels. (a) Channel 20 data. Shaded area represents approximate time window where the response to number change blocks was significantly greater than both the no change and attention blocks after cluster correction for multiple comparisons. (b) Channel 17 data. Shaded area represents approximate time window where the response to attention animations was significantly greater than both no change and number change blocks after cluster correction for multiple comparisons. Response relative to pre-stimulus presentation baseline (−1–0 seconds).*

these channels fell in the lower central occipital region of the probe and a smaller subset within mid-parietal regions, consistent with areas of general visual and attentional processing (see Figures 1 and 2).

Discussion

We observed that one right parietal channel (out of 24 posterior channels) responded selectively to numerosity in the infant brain (see Figure 2 and Table 1). The location of this channel relative to our scalp placement of the probe is consistent with previous neuroimaging studies with infants using limited methodologies and, more generally, consistent with right parietal activity in and around the IPS seen in fMRI studies of numerical cognition in older children and adults (Cantlon *et al.*, 2006; Dehaene *et al.*, 2003; Hyde *et al.*, 2010; Piazza *et al.*, 2004). Our results significantly extend previous work by employing a method with cortical surface coverage, including widespread coverage of bilateral posterior occipital and parietal regions, which far exceeds the coverage and spatial resolution of any previous functional neuroimaging studies of numerical cognition in infants to date. Our results also uniquely show that the response in at least one portion of number-sensitive parietal regions is not simply a result of increased interest or attention to the number change relative to control conditions, as the response to number change was (a) greater than that to no change under conditions of equal visual attention and (b) spatially dissociated from more general visual attentional responses to stimuli much more engaging. As such, our findings have several implications for understanding continuity and change in functional brain organization for number processing.

In the case of brain specialization for number, our data suggest developmental continuity in right parietal cortex. A single focal parietal channel showed a number-selective functional response pattern. Non-numerical stimulus parameters that often co-vary with number, such as item size, total area, spacing, and luminance varied equally across conditions and, thus, were not predictive of number over our set of stimuli.[4] We also observed that this number response was not likely due to differences in attentional processing between the number change and no change conditions because the greater response to number change held when behavioral visual attention to stimuli was equated and the response to changes in the number of white dots on a gray background produced a larger hemodynamic response in this parietal region compared to the response to colorful, dynamic, and multimodal stimuli designed to relieve infants of boredom and regain their attention. Finally, we observed a double dissociation, with a single channel that responded more to numerosity and a non-overlapping set of channels that responded more to attentional animations. These novel aspects of our study provide further evidence that responses to number can be distinguished from general attention responses. The contrast of number change blocks with these two controls (no change blocks and attention blocks) suggests a parietal region specialized for number similar to what has been seen in older children and adults

---

[4] To reduce the complexity of the design, we only used an increasing number change (8 dots to 16 dots) relative to the no change condition context (8 dots). Given this decision and the focus of our current experiment, we cannot distinguish an interpretation of the functional response to the number change condition as a response to increasing number from an interpretation of the response to numerosity more broadly (regardless of the direction of change). Nevertheless, we believe either interpretation is consistent with the conclusions drawn regarding specialization for number processing in the right parietal lobe of infants.

(Cantlon *et al.*, 2006; Piazza *et al.*, 2004). Furthermore, it suggests that this specialization occurs before education, instruction, or exposure to a symbolic number system could likely cause it.

In terms of brain lateralization for number, our data suggest a developmental change. We observed that only a single right-lateralized channel responded selectively to number and further dissociated from a general attentional response. We found no evidence of a left hemispheric response to number. While a lack of response in the left hemisphere does not preclude the possibility of a left parietal response to number in infancy, several aspects of our data suggest that this is not the case. The lack of a left parietal response is not due to differential signal quality across hemispheres, as we observed a bilateral visual attentional response to interesting audio-visual animations. The lack of a left parietal response is also not due to a limited coverage of the posterior brain as in a previous study (Hyde *et al.*, 2010), as we measured from a majority of the posterior cortical surface. It appears, then, that numerical processing may start off lateralized and become bilateral only over later development.

Lateralization of brain functions is common throughout all levels of the animal kingdom and, in some instances, it is thought to serve evolutionarily advantageous purposes (see Frasnelli, Vallortigara & Rogers, 2012). The case of lateralization for number has been made in other species as well (Rugani, Kelly, Szelest, Regolin & Vallortigara, 2010). Several recent experiments with avian species, for example, suggest a leftward bias for dividing space and for rudimentary types of enumeration or counting (indicative of right hemisphere lateralization) (e.g. see Rugani, Vallortigara, Vallini & Regolin, 2011). These biases in the behavior of non-human animals broadly follow those seen in adults' associations between number and space and are thought to have implications as broad as how we organize numbers spatially in our mind and in symbolic mathematics (e.g. Dehaene, 1997). Furthermore, the novel evidence presented here combined with the work with non-human animals suggests that initial right hemispheric lateralization and specialization for number in infants may be evolutionary ancient.

Several open questions, such as when and why numerical processing becomes bilateral, still remain. Previous work with older children and adults suggests that increased specialization of left parietal regions for number is correlated with symbolic number and mathematics proficiency (e.g. Bugden *et al.*, 2012; Emerson & Cantlon, 2015). One possible hypothesis is that this change occurs as a direct result of the acquisition of a symbolic number system. To date, however, neuroimaging studies have been unable to test 2–4-year-old children, the age at which symbolic number systems are typically acquired, to assess this possibility. Further puzzling is the fact that changes in lateralization appear to continue into late childhood, well beyond the age at which symbolic number knowledge is first acquired. This suggests a possible second hypothesis: that changes in lateralization are not entirely a function of the acquisition of symbolic number systems or a change in representation per se, but a change in how numbers are processed. That is, changes in lateralization might also reflect a semantic shift from quantity-based representations of symbolic numbers in the right hemisphere to memorized linguistic symbols. Future studies should investigate the driving forces behind functional organization changes in the brain and their relation to cognitive development in the domain of number.

Acknowledgements

We would like to thank the Advanced Multimodal Neuroimaging Training Program (NIH Blueprint for Neuroscience Research T90DA022759/R90DA023427) for their funding of L.A.E during a portion of this work. We also thank Charles Nelson and the Laboratories of Cognitive Neuroscience at Boston Children's Hospital for their support during this study.

References

Ansari, D., & Dhital, B. (2006). Age-related changes in the activation of the intraparietal sulcus during nonsymbolic magnitude processing: an event-related functional magnetic resonance imaging study. *Journal of Cognitive Neuroscience*, **18** (11), 1820–1828.

Ansari, D., Grabner, R.H., Koschutnig, K., Reishofer, G., & Ebner, F. (2011). Individual differences in mathematical competence modulate brain responses to arithmetic errors: an fMRI study. *Learning and Individual Differences*, **21** (6), 636–643.

Aslin, R.N. (2013). Questioning the questions that have been asked about the infant brain using NIRS. *Cognitive Neuropsychology*, **29** (1–2), 7–33.

Bugden, S., Price, G.R., McLean, D.A., & Ansari, D. (2012). The role of the left intraparietal sulcus in the relationship between symbolic number processing and children's arithmetic competence. *Developmental Cognitive Neuroscience*, **2** (4), 448–457.

Bullmore, E.T., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E. *et al.* (1999). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Transactions on Medical Imaging*, **18** (1), 32–42.

Cantlon, J.F., Brannon, E.M., Carter, E.J., & Pelphrey, K.A. (2006). Functional imaging of numerical processing in adults and 4-year-old children. *PLoS Biology*, **4** (5), e125.

Cappelletti, M., Barth, H., Fregni, F., Spelke, E.S., & Pascual-Leone, A. (2007). rTMS over the intraparietal sulcus disrupts numerosity processing. *Experimental Brain Research*, **179** (4), 631–642.

Carey, S. (2009). *The origin of concepts*. New York: Oxford University Press.

Cohen, M.X. (2014). *Analyzing neural time series data: Theory and practice*. Cambridge, MA: MIT Press.

Cooper, R.J., Selb, J., Gagnon, L., Phillip, D., Schytz, H.W. *et al.* (2012). A systematic comparison of motion artifact correction techniques for functional near-infrared spectroscopy. *Frontiers in Neuroscience*, **6**, 1–10.

Dehaene, S. (1997). *The number sense*. New York: Oxford University Press.

Dehaene, S., Piazza, M., Pinel, P., & Cohen, L. (2003). Three parietal circuits for number processing. *Cognitive Neuropsychology*, **20** (3–6), 487–506.

Devor, A., Ulbert, I., Dunn, A.K., Narayanan, S.N., Jones, S.R. *et al.* (2005). Coupling of the cortical hemodynamic response to cortical and thalamic neuronal activity. *Proceedings of the National Academy of Sciences of the United States of America*, **102** (10), 3822–3827.

Dormal, V., Andres, M., & Pesenti, M. (2008). Dissociation of numerosity and duration processing in the left intraparietal sulcus: a transcranial magnetic stimulation study. *Cortex*, **44** (4), 462–469.

Emerson, R.W., & Cantlon, J.F. (2015). Continuity and change in children's longitudinal neural responses to numbers. *Developmental Science*, **18** (2), 314–326.

Feigenson, L., Dehaene, S., & Spelke, E. (2004). Core systems of number. *Trends in Cognitive Sciences*, **8** (7), 307–314.

Frasnelli, E., Vallortigara, G., & Rogers, L.J. (2012). Left–right asymmetries of behaviour and nervous system in invertebrates. *Neuroscience & Biobehavorial Reviews*, **36** (4), 1273–1291.

Gervain, J., Mehler, J., Werker, J.F., Nelson, C.A., Csibra, G. *et al.* (2011). Near-infrared spectroscopy: a report from the McDonnell infant methodology consortium. *Developmental Cognitive Neuroscience*, **1** (1), 22–46.

Huppert, T.J., Diamond, S.G., Franceschini, M.A., & Boas, D.A. (2009). HomER: a review of time-series analysis methods for near-infrared spectroscopy of the brain. *Applied Optics*, **48** (10), D280–D298.

Hyde, D.C., Boas, D.A., Blair, C., & Carey, S. (2010). Near-infrared spectroscopy shows right parietal specialization for number in pre-verbal infants. *NeuroImage*, **53** (2), 647–652.

Hyde, D.C., & Spelke, E.S. (2011). Neural signatures of number processing in human infants: evidence for two core systems underlying numerical cognition. *Developmental Science*, **14** (2), 360–371.

Hyde, D.C., & Spelke, E.S. (2012). Spatiotemporal dynamics of processing nonsymbolic number: an event-related potential source localization study. *Human Brain Mapping*, **33** (9), 2189–2203.

Izard, V., Dehaene-Lambertz, G., & Dehaene, S. (2008). Distinct cerebral pathways for object identity and number in human infants. *PLoS Biology*, **6** (2), e11.

Izard, V., Sann, C., Spelke, E.S., & Streti, A. (2009). Newborn infants perceive abstract numbers. *Proceedings of the National Academy of Sciences of the United States of America*, **106** (25), 10382–10385.

Kameyama, M., Fukuda, M., Uehara, T., & Mikuni, M. (2004). Sex and age dependencies of cerebral blood volume changes during cognitive activation: a multichannel near-infrared spectroscopy study. *NeuroImage*, **22** (4), 1715–1721.

Kimmel, H.D. (1957). Three criteria for the use of one-tailed tests. *Psychological Bulletin*, **54** (4), 351–353.

Le Corre, M., & Carey, S. (2007). One, two, three, four, nothing more: an investigation of the conceptual sources of the verbal counting principles. *Cognition*, **105**, 395–438.

Libertus, M.E., & Brannon, E.M. (2010). Stable individual differences in number discrimination in infancy. *Developmental Science*, **13** (6), 900–906.

Libertus, M.E., Starr, A., & Brannon, E.M. (2014). Number trumps area for 7-month-old infants. *Developmental Psychology*, **50** (1), 108–112.

Luck, S.J. (2005). *An introduction to the event-related potential technique*. Cambridge, MA: MIT Press.

Maris, E., & Oostenveld, R. (2007). Nonparametric statistical testing of EEG and MEG data. *Journal of Neuroscience Methods*, **164** (1), 177–190.

Nieder, A., & Dehaene, S. (2009). Representation of number in the brain. *Annual Review of Neuroscience*, **32**, 185–208.

Obrig, H., Neufang, M., Wenzel, R., Kohl, M., Steinbrink, J. *et al.* (2000). Spontaneous low frequency oscillations of cerebral hemodynamics and metabolism in human adults. *NeuroImage*, **12** (6), 623–639.

Okamoto, M., Dan, H., Sakamoto, K., Takeo, K., Shimizu, K. *et al.* (2004). Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. *NeuroImage*, **21** (1), 91–111.

Piazza, M., Izard, V., Pinel, P., Le Bihan, D., & Dehaene, S. (2004). Tuning curves for approximate numerosity in the human intraparietal sulcus. *Neuron*, **44** (3), 547–555.

Piazza, M., Pinel, P., Le Bihan, D., & Dehaene, S. (2007). A magnitude code common to numerosities and number symbols in human intraparietal cortex. *Neuron*, **53** (2), 293–305.

Pinel, P., Dehaene, S., Riviere, D., & Le Bihan, D. (2001). Modulation of parietal activation by semantic distance in a number comparison task. *NeuroImage*, **14** (5), 1013–1026.

Pinel, P., Piazza, M., Le Bihan, D., & Dehaene, S. (2004). Distributed and overlapping cerebral representations of number, size, and luminance during comparative judgments. *Neuron*, **41** (6), 983–993.

Price, G.R., Mazzocco, M.M., & Ansari, D. (2013). Why mental arithmetic counts: brain activation during single digit arithmetic predicts high school math scores. *Journal of Neuroscience*, **33** (1), 156–163.

Rugani, R., Kelly, D.M., Szelest, I., Regolin, L., & Vallortigara, G. (2010). Is it only humans that count from left to right? *Biology Letters*, **6**, 290–292. rsbl20090960.

Rugani, R., Vallortigara, G., Vallini, B., & Regolin, L. (2011). Asymmetrical number-space mapping in the avian brain. *Neurobiology of Learning and Memory*, **95** (3), 231–238.

Ruxton, G.D., & Neuhäuser, M. (2010). When should we use one-tailed hypothesis testing? *Methods in Ecology and Evolution*, **1** (2), 114–117.

Scholkmann, F., Spichtig, S., Muehlemann, T., & Wolf, M. (2010). How to detect and reduce movement artifacts in near-infrared imaging using moving standard deviation and spline interpolation. *Physiological Measurement*, **31** (5), 649–662.

Starr, A., Libertus, M.E., & Brannon, E.M. (2013). Number sense in infancy predicts mathematical abilities in childhood. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 18116–18120.

Strangman, G., Boas, D.A., & Sutton, J.P. (2002). Non-invasive neuroimaging using near-infrared light. *Biological Psychiatry*, **52** (7), 679–693.

Strangman, G., Franceschini, M.A., & Boas, D.A. (2003). Factors affecting the accuracy of near-infrared spectroscopy concentration calculations for focal changes in oxygenation parameters. *NeuroImage*, **18** (4), 865–879.

Temple, E., & Posner, M.I. (1998). Brain mechanisms of quantity are similar in 5-year-old children and adults. *Proceedings of the National Academy of Sciences of the United States of America*, **95** (13), 7836–7841.

Tong, Y., & Frederick, B.B. (2010). Time lag dependent multimodal processing of concurrent fMRI and near-infrared spectroscopy (NIRS) data suggests a global circulatory origin for low-frequency oscillation signals in human brain. *NeuroImage*, **53** (2), 553–564.

Venkatraman, V., Ansari, D., & Chee, M.W. (2005). Neural correlates of symbolic and non-symbolic arithmetic. *Neuropsychologia*, **43** (5), 744–753.

Watanabe, A., & Kato, T. (2004). Cerebrovascular response to cognitive tasks in patients with schizophrenia measured by near-infrared spectroscopy. *Schizophrenia Bulletin*, **30** (2), 435–444.

Wilcox, T., Boas, D.A., Bortfeld, H., Woods, R., & Wruck, E. (2005). Using near-infrared spectroscopy to assess neural activation during object processing in infants. *Journal of Biomedical Optics*, **10** (1), 1010–1019.

Wilcox, T., Haslup, J.A., & Boas, D.A. (2010). Dissociation of processing of featural and spatiotemporal information in the infant cortex. *NeuroImage*, **53** (4), 1256–1263.

Wynn, K. (1992). Children's acquisition of the number words and the counting system. *Cognitive Psychology*, **24**, 220–251.

Xu, F., & Spelke, E.S. (2000). Large number discrimination in 6-month-old infants. *Cognition*, **74** (1), B1–B11.

Received: 18 August 2014
Accepted: 6 May 2015

Appendix

Appendix figures illustrate responses at each of the 24 channels for oxyhemoglobin (oxyHb), deoxyhemoglobin (deoxyHb), and total hemoglobin (totalHb) separately for (1) the no change condition (Figure A1), (2) the number change condition (Figure A2) and (3) the attention animations (Figure A3).

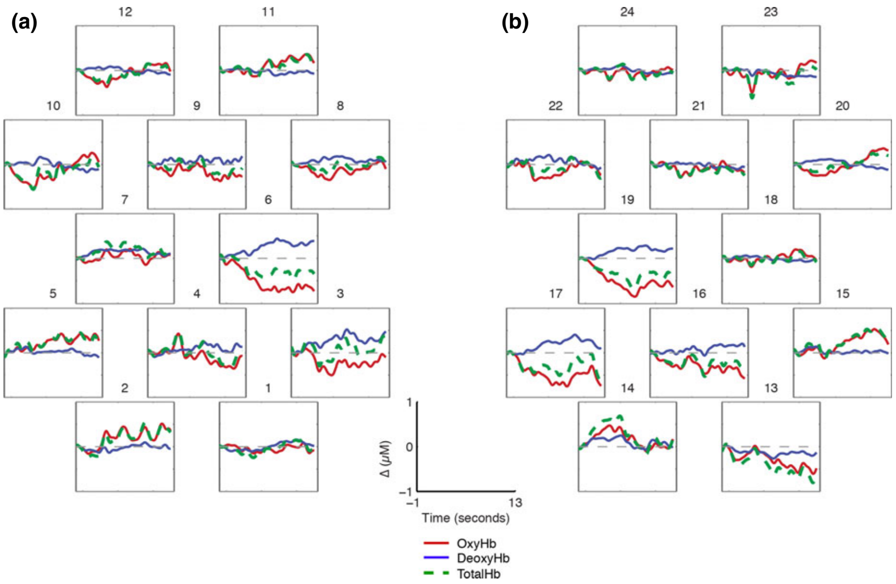

Figure A1 *OxyHb, DeoxyHb, & TotalHb for the no change condition. (a) Left hemipshere data channels. (b) Right hemipshere data channels. Response relative to pre-stimulus presentation baseline (−1–0 seconds).*

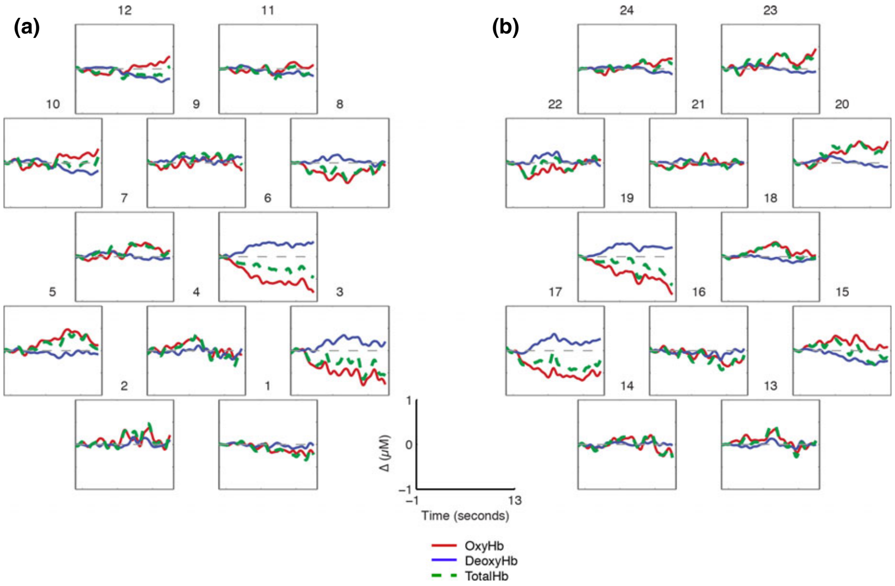

Figure A2 *OxyHb, DeoxyHb, & TotalHb for the number change condition. (a) Left hemipshere data channels. (b) Right hemipshere data channels. Response relative to pre-stimulus presentation baseline (−1–0 seconds).*

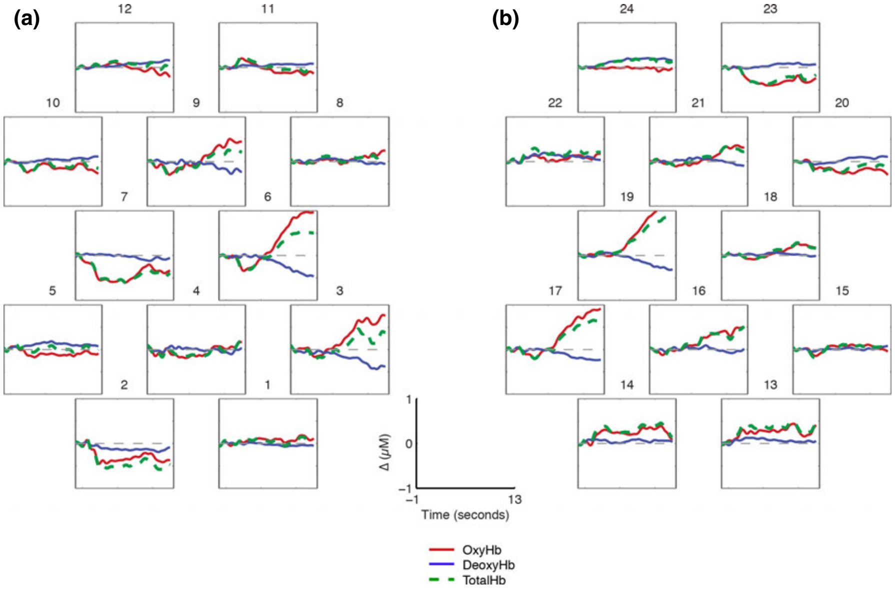

Figure A3 *OxyHb, DeoxyHb, & TotalHb for attention animations condition. (a) Left hemipshere data channels. (b) Right hemipshere data channels. Response relative to pre-stimulus presentation baseline (−1–0 seconds).*