

Using near-infrared spectroscopy to assess neural activation during object processing in infants

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Abstract. The capacity to represent the world in terms of numerically distinct objects (i.e., object individuation) is a milestone in early cognitive development and forms the foundation for more complex thought and behavior. Over the past 10 to 15 yr, infant researchers have expended a great deal of effort to identify the origins and development of this capacity. In contrast, relatively little is known about the neural mechanisms that underlie the ability to individuate objects, in large part because there are a limited number of noninvasive techniques available to measure brain functioning in human infants. Recent research suggests that near-IR spectroscopy (NIRS), an optical imaging technique that uses relative changes in total hemoglobin concentration and oxygenation as an indicator of neural activation, may be a viable procedure for assessing the relation between object processing and brain function in human infants. We examine the extent to which increased neural activation, as measured by NIRS, could be observed in two neural areas known to be involved in object processing, the primary visual cortex and the inferior temporal cortex, during an object processing task. Infants aged 6.5 months are presented with a visual event in which two featurally distinct objects emerge successively to opposite sides of an occluder and neuroimaging data are collected. As predicted, increased neural activation is observed in both the primary visual and inferior cortex during the visual event, suggesting that these neural areas support object processing in the young infant. The outcome has important implications for research in cognitive development, developmental neuroscience, and optical imaging. © 2005 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1852551]

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1 Introduction

The capacity to individuate objects—to determine whether an object currently in view is the very same object, or a different object, than seen before—is one of our most basic cognitive abilities. This capacity enables infants to represent the world in terms of numerically distinct objects that persist in space and time, and forms the foundation for more complex thought and behavior. Given the importance of object individuation to human cognition, a great deal of effort has been expended to identify the origins and development of this capacity (e.g., Aguiar and Baillargeon,¹ Baillargeon and Graber,² Bonatti et al.,³ Meltzoff and Moore,⁴ Spelke et al.,⁵ Tremoulet et al.,⁶ Van de Walle et al.,⁷ Wilcox,⁸ Wilcox and Baillargeon,^{9,10} Wilcox and Chapa,¹¹ Wilcox et al.,¹² Wilcox and Schweinle,^{13,14} Xu,¹⁵ and Xu and Carey¹⁶). Most of these studies have used visual attention (i.e., looking time) methods to assess object individuation and have focused on the extent

to which infants use featural information (e.g., shape, color, size) to signal the presence of distinct objects. In contrast, comparatively little is known about the neural mechanisms that support this capacity in human infants. One reason for this gap in knowledge is that there are a limited number of noninvasive techniques available to measure brain functioning in infants. Brain-imaging methods that can be used to identify the neural areas involved, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), are difficult to apply in awake infants. Other techniques, such as electroencephalography (EEG), event-related potentials (ERP), and magnetoencephalography (MEG), provide important information about the timing of neural responses but are limited in identifying the location from which the responses are generated. This has limited investigators in their efforts to systematically explore the functional development of object-processing pathways. There is, therefore, a critical need to identify new strategies to enable a delineation of the spatial and temporal relation between behavior and

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brain function in object processing in infants. One method that is currently under development is near-IR spectroscopy (NIRS), an optical imaging technique that measures changes in cerebral blood flow. This procedure is noninvasive, can be used during behavioral tasks, and provides temporal and spatial information about neural activation, making it ideal for infant research. The goal of the research presented here is to determine the extent to which NIRS is sensitive to changes in neural activation during an object processing task.

1.1 NIRS: A Measure of Brain Functioning

In NIRS, near-IR light is projected through the scalp and skull into the brain, and the intensity of light diffusely reflected is recorded. The modulation of the recorded intensity by localized changes in the optical properties of the brain is used as a measure of neural activation. There are currently two NIRS techniques that have applicability to address questions about the neural basis of object processing. One technique, often referred to as event-related optical signal (EROS), assesses the amount of near-IR light that is modulated by neural tissue during stimulus presentation (see Gratton et al.¹⁷ and Gratton and Fabiani¹⁸). This approach is based on the fact that the light-scattering properties of neural tissue change when neurons are active. Although the physiological mechanisms remain to be fully understood (it appears that ion movement across the neuronal membrane is involved), different patterns of light scattering are temporally well correlated with electric field changes.^{19–21}

The second technique, and the one used in this research, utilizes changes in blood volume and hemoglobin oxygenation (i.e., hemodynamics) as an index of neural activation (see Meek,²² Grinvald et al.,²³ Strangman et al.,²⁴ Villringer and Chance,²⁵ and Villringer and Dirnagl²⁶). This technique has been used with medically at-risk infants in the clinical setting,^{27–34} and more recently, its applicability in the experimental setting has been explored.^{35–37} The rationale for this approach rests on the concept that neural activation in response to a stimulus results in increased energy demands in the area activated. To accommodate the demand for energy, cerebral blood flow (CBF) increases to the activated brain areas bringing oxygen and glucose. Changes in blood flow lead to an increase in blood volume and can be assessed by measuring local concentrations of oxyhemoglobin (oxygenated blood) and deoxyhemoglobin (deoxygenated blood). Typically, during cortical activation local concentrations of oxyhemoglobin (HbO₂) increase, whereas concentrations of deoxyhemoglobin (HbR) decrease.^{38–42} However, some researchers^{32,39,40,42–45} have reported an increase, rather than a decrease, in HbR. Although it is not entirely clear why an increase in HbR is sometimes observed, in infants it is assumed to be a less mature hemodynamic response. From the summated changes in HbR and HbO₂, total hemoglobin (HbT) can be computed and is found to increase following brain activation. To capitalize on these changes, the low tissue absorption of near-IR light between approximately 650 and 950 nm is utilized. At these wavelengths, light is differentially absorbed by oxygenated and deoxygenated blood.^{25,46} Hence, measuring the light intensity modulation during stimulus presentation, and comparing it to the light intensity during a baseline event in which no stimulus is presented, provides important information about the hemodynamic response to

brain activation (i.e., relative concentrations of HbO₂ and HbR). Evidence that there is a linear relationship between hemodynamics and neural activity⁴⁷ and that NIRS produces results consistent with other imaging techniques (fMRI and PET) used simultaneously,^{48–50} provides converging evidence that NIRS can provide a reliable measure of brain function. Finally, because hemodynamic responses to stimulation are much greater than changes in the light-scattering properties of neural tissue, resulting in a greater SNR, the hemodynamic technique is currently a more robust measure of neural activation than EROS.

The use of NIRS has several distinct advantages over other, more traditional brain-imaging techniques. One advantage is that, relatively speaking, NIRS has good temporal and spatial resolution. Brain signals can be routinely observed^{40,51,52} with a temporal sampling resolution of 0.01 s, which is faster than that typically observed with fMRI. While the hemodynamic response to brain activation occurs on a 1-s time scale, the better temporal resolution offered by NIRS will, for instance, enable better distinction of signal contamination arising from systemic physiological signals and motion artifacts, better resolution of the hemodynamic onset, and potentially, enable direct measures of fast neuronal signals. In addition, the effects are localized within 1 to 2 cm of the area activated.³⁷ Compared to electrophysiological techniques (EEG, ERP, MEG), where source localization is very difficult, spatial resolution is quite good. A second advantage is that NIRS is totally noninvasive and nonionizing. Hence, it is safe to use with infants repeatedly and for extended periods of time. A third advantage is that it is relatively inexpensive, portable, and with the appropriate training, relatively straightforward to use. This makes NIRS particularly attractive to researchers in the experimental setting. One potential disadvantage of using NIRS is that, because near-IR light diffuses rapidly when entering neural tissue, it is unsuitable for investigating neural activation in structures deeper than approximately 2 to 3 cm below the surface of the brain. However, if the neural structures of interest fall on or near the surface of the cortex, as they do in our research, NIRS is an ideal neuroimaging technique.

1.2 Narrow-Screen Task: Behavioral Measure of Object Processing

One visual attention paradigm that is particularly sensitive to developmental changes in infants' capacity to individuate objects is the narrow-screen task.^{8–12,53} In this task, infants sit on a parent's lap facing a puppet-stage apparatus. Infants participate in a two-phase procedure that consists of a familiarization phase and a test phase. In the familiarization phase, infants are presented with a familiarization event on the stage of the apparatus, in which two featurally distinct objects (e.g., a ball and a box) emerge successively to opposite sides of a wide yellow screen. The two objects move in the same depth plane (i.e., along the same axis), so that it would not be possible for them to pass each other behind the screen without colliding. The yellow screen is wide enough to hide both objects, side-by-side, at the same time. The purpose of the familiarization trials is to acquaint the infants with the objects they will see in the test trials. In the test phase, infants are presented with a test event (Fig. 1) that is identical to the

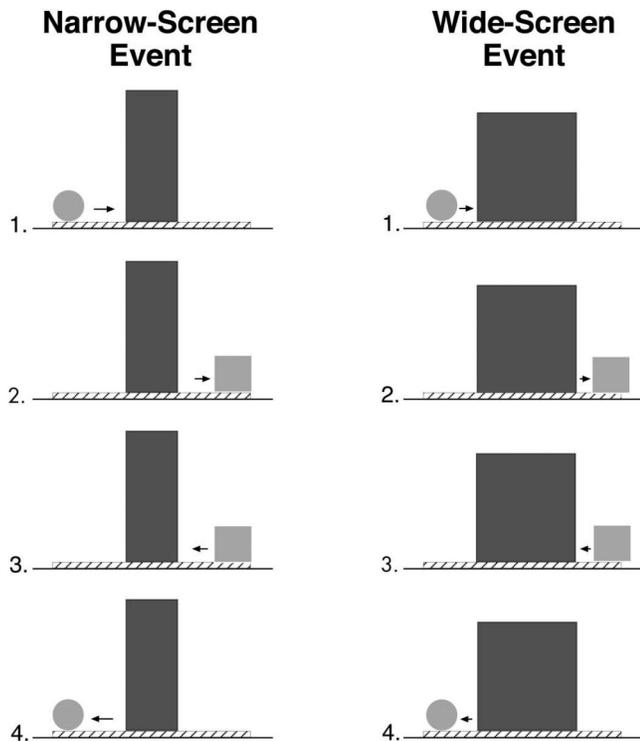


Fig. 1 Narrow- and wide-screen test events from Wilcox and Baillargeon.¹⁰ Steps 1 to 4 were repeated until the end of the trial. The ball and box varied on many feature dimensions, including shape, color, and texture.

familiarization event except that the yellow screen is replaced with a blue screen that is either sufficiently wide (wide-screen condition) or too narrow (narrow-screen condition) to hide both objects simultaneously. If infants (1) perceive the different-features event as involving two separate and distinct objects, (2) recognize that both objects can fit behind the wide but not the narrow screen, and (3) find the narrow- but not the wide-screen event unexpected, then the infants in the narrow-screen condition should look reliably longer at the test event. Note that this hypothesis was generated based on evidence that young infants are sensitive to size violations. For example, infants aged 2.5 to 6.5 months detect when an object is too small to fit through an opening, behind a screen, or in a container.^{54–56}

Wilcox and Baillargeon^{9,10} reported that when the objects varied on many feature dimensions (e.g., shape, pattern, color) infants 4.5 to 11.5 months of age looked reliably longer at the narrow- than wide-screen test event, suggesting that early in the first year, infants use featural differences to signal the presence of distinct objects. Data obtained in control experiments in which (1) the objects were made sufficiently small to fit behind either the wide or the narrow screen or (2) the same object was seen to each side of a wide or a narrow screen support this interpretation of the data.^{8–11} Finally, research utilizing other visual attention tasks^{9,14,57–61} and recent data obtained in a reaching task⁶² provide converging evidence for the conclusion that young infants can use featural information as the basis for individuating objects.

1.3 Neural Pathways That Support Object Processing

Where might we expect to observe neural activation in infants during an object individuation task that requires processing of featural information? The outcome of neurobehavioral, -anatomical, and -physiological studies in nonhuman primates indicates that there are two main routes for visual object processing.^{63–70} One pathway originates from the parvocellular layers of the lateral geniculate nucleus (LGN) and projects from the primary visual cortex to the inferior temporal cortex. This pathway, the ventral route, is important for the analysis of form, color, and pattern information. The other pathway originates from the magnocellular layers of the LGN and projects from the primary visual cortex to the posterior parietal cortex. This pathway, the dorsal route, is important for the analysis of motion, depth, and location information. More recent research using neuroimaging techniques with human^{71–74} and nonhuman^{75–79} primates provides converging evidence to support the conclusion that the inferior temporal cortex mediates processing of visual features important for the recognition and identification of objects, whereas the posterior parietal cortex mediates processing of the spatiotemporal properties of objects.

Of particular relevance to this research is the ventral pathway. This pathway is most likely to be involved in the processing of objects that differ on many feature dimensions, like those used by Wilcox and Baillargeon.¹⁰ Studies exploring the neural basis of object processing in infant monkeys suggest that the ventral route is operationally functional soon after birth, although it does undergo significant development during infancy.^{80–85} What has been left open to speculation is the extent to which the ventral pathway supports featurally based object processing in the human infant.

1.4 Presented Research

The purpose of the presented research is to identify the extent to which two areas in the ventral pathway—the primary visual and the inferior temporal cortex—respond to a visual event involving two featurally distinct objects. Neural activation, as measured by relative changes in cerebral blood flow, was assessed during a wide-screen ball-box event similar to the one depicted in Fig. 1. Because we were primarily concerned with demonstrating that neural activation can be observed in young infants during an object-processing task, and there are already a number of behavioral studies indicating that 4.5- to 11.5-month-old infants interpret the ball-box event as involving two distinct objects, regardless of whether it is seen with a narrow or a wide screen (Wilcox and Baillargeon;^{9,10} see also Wilcox and Schwienle¹³), we did not test infants in a narrow-screen condition. Based on neuroimaging data recently reported by Taga et al.,³⁷ we expected to observe increased neural activation in the primary visual cortex in response to the ball-box event. In addition, if the inferior temporal cortex is sufficiently mature to support featurally based object processing in the young infant, then increased neural activation should also be observed in this region of the brain.

2 Method

2.1 Participants

Participants were seven 6.5-month-old infants, five male and two female (M age=6 months, 12 days, range=5 months, 14

days to 6 months, 25 days). Three additional infants were tested but eliminated from the sample because of large motion artifacts in the signals ($N=2$) or failure to obtain adequate signals because of obstruction by hair ($N=1$). (See Sec. 2.4 for the criteria used to eliminate data based on motion artifacts.) Infants' names were obtained from birth announcements in the local newspaper and commercially produced lists. Parents were contacted by letter and follow-up phone calls. Parents were offered reimbursement for their travel expenses but were not compensated for their participation. Informed consent was obtained from the parents before testing began.

2.2 Apparatus and Stimuli

The apparatus consisted of a wooden cubicle 213 cm high, 105 cm wide, and 43.5 cm deep. The infant sat on a parent's lap facing an opening 51 cm high and 93 cm wide in the front wall of the apparatus. The floor of the apparatus was covered with cream-colored contact paper, the side walls were painted cream, and the back wall was covered with lightly patterned contact paper. A platform 1.5 cm high, 60 cm wide, and 19 cm deep and covered with lightly patterned contact paper lay 4.5 cm from the back wall and centered between the left and right walls. To allow for smooth and quiet movement of the objects, a strip of blue felt lay lengthwise down the center of the platform. A slit in the back wall enabled the experimenter to reach into the apparatus and move the ball and the box. The slit was 6.5 cm high, 52.5 cm long, and was located 10 cm above the apparatus floor; cream-colored fringe helped conceal the slit. The box used in the box-ball event was 10.25 cm square, made of Styrofoam, covered with red felt, and decorated with silver thumbtacks. The ball was 10.25 in diameter, made of Styrofoam, and painted green with evenly spaced yellow, blue, and red dots. The screen was 21.5 cm high and 30 cm wide and made of blue cardboard. During the experiment the infant's head was approximately 78 cm from the objects on the platform.

A muslin-covered shade was lowered in front of the opening in the front wall of the apparatus at the end of each trial and remained lowered until the beginning of the next trial. Two muslin-covered wooden frames, each 213 cm high and 68 cm wide, stood at an angle on either side of the apparatus. These frames isolated the infant from the experimental room. To illuminate the stage, four 20-W fluorescent bulbs were affixed to the inside walls of the apparatus (one on each wall). No other lighting was used.

Two experimenters worked together to produce the ball-box event. The first wore a black glove and manipulated the objects. The second raised and lowered the shade that covered the front opening of the apparatus. The numbers in parentheses (see the following) indicate the time taken to produce the actions described. A metronome attached to the back of the apparatus blinked once per second to help the first experimenter adhere to the event scripts (because we did not want the event to have an auditory component, the metronome was set to blink, rather than to tick, once per second).

Prior to the start of each trial, the first experimenter gently tilted the box to the left and to the right, once to each side per second, at the left edge of the platform (infants' point of view). The screen stood upright at the center of the platform; the ball was hidden behind the right side of the screen. When

the computer signaled the start of the trial, the first experimenter moved the box behind the left edge of the screen (2 s) and, after an appropriate interval, the ball emerged from behind the right edge of the screen and moved to the right end of the platform (2 s); the ball paused (1 s) and then the event was seen in reverse. The entire 10-s box-ball cycle was then repeated until the end of the trial. When in motion, the objects moved at a rate of 12 cm/s.

The amount of time infants spent looking at the ball-box event was recorded, and looking time data were time-locked to the neuroimaging (i.e., NIRS) data. Looking behavior was monitored by two observers who watched the infant through peepholes in the cloth-covered frames on either side of the apparatus. Each observer held a button box connected to a Dell computer and depressed a button when the infant attended to the event. Each trial was divided into 100-ms intervals, and the computer determined in each interval whether the two observers agreed on the direction of the infants' gaze. Interobserver agreement was measured for six of the infants (only one observer was present for one of the infants) and was calculated for each test trial on the basis of the number of intervals in which the computer registered agreement, out of the total number of intervals in the trial. Agreement averaged 96% per test trial per infant.

Infants were presented with four test trials that were 30 s in duration. Because analysis of the neuroimaging data requires baseline recordings of the measured intensity of refracted light, infants were also presented with a 10-s silent pause, during which time no visual or auditory event was presented, prior to each trial. A final 10-s silent pause followed the last trial. We chose 10 s as our pause interval because infants often become fussy with longer intervals and prior research indicates that 10 s is sufficient for blood flow to return to baseline levels. Finally, because failure to visually attend to the event could result in a decrease in hemodynamic response in the primary visual and/or inferior temporal cortex, we inspected the looking time data for data blocks, or trials, in which the infant (1) cumulated less than 20 s looking time or (2) looked away from the display for more than 5 consecutive seconds. There were no trials that failed to meet the behavioral criteria.

2.3 Instrumentation

The neuroimaging equipment contained three major components: (1) two fiber optic cables that delivered near-IR light to the scalp of the participant (i.e., emitters); (2) four fiber optic cables that detected the diffusely reflected light at the scalp and transmitted it to the receiver (i.e., detectors); and (3) an electronic control box that served both as the source of the near-IR light and the receiver of the reflected light. The signals received by the electronic control box were processed and relayed to a Dell Inspiron 7000 laptop computer. A custom computer program recorded and analyzed the signal.

Prior to event presentation, infants were fitted with custom-made headgear. The headgear consisted of two emitter probes sewn into a terrycloth headband. Each probe emitted light at two wavelengths, 690 and 830 nm. The former is more sensitive to deoxygenated blood, whereas the latter is more sensitive to oxygenated blood. One probe was positioned directly above the inion. This is the location in the primary visual cortex that Taga et al.³⁷ reported as optimal for recording activation in response to visual stimulation. The other

probe was positioned directly above, and slightly behind, the left ear (T3 using the International 10/20 system for EEG recording). This location in the human inferior temporal cortex is thought to be analogous to the area in the monkey inferior temporal cortex that mediates object recognition and identification.^{71,73,75} To measure the exiting light, two 1-mm detector optic fibers were positioned equidistant, in the horizontal plane, from each emitter probe. Interdetector distance was 2 cm. Each detector recorded both wavelengths of light. Each triad (one emitter and two detectors) was embedded in a 5.5×1.5-cm strip of 0.2-cm-thick nonelastic rubberized material (similar to a computer mouse pad) and then sewn into the headband, which was elasticized. Hence, the distance between the components of each triad did not vary by head circumference, whereas the distance between each triad did vary. The distance between the center of the two triads ranged from approximately 11 to 12 cm, depending on head circumference. Given that IR light diffuses quickly after passing through the skull and entering neural tissue,⁴⁹ it is unlikely that light released by the emitter of one triad would be registered by the detector of the other triad. Finally, to help ensure that infants would be comfortable wearing our headgear, when the infant's appointment was scheduled we instructed parents to periodically place a headband and/or hat on their infant's head on the days prior to the experimental test session.

2.4 Analysis of the Neuroimaging Data

The NIRS data were analyzed, for each neural area separately, in the following way. The raw signals from the two detectors from each neural area were digitized at 200 Hz for each of the eight channels, converted to optical density units,⁴² digitally low-pass-filtered at 10.0 Hz to reduce measurement noise in the optical signal, and decimated to 20 samples/s. We performed principle component analysis (PCA) of the spatial covariance of this preprocessed data to design spatial filters to reduce systemic physiology and motion artifacts that were common to the eight channels of data. The PCA procedure followed is described in Zhang et al.⁸⁴ We used these filters to remove ~85% of the covariance of the data. This required filtering two or three principle components from the data, depending on the degree of motion in each data set. The number of principle components to filter from the data was chosen based on the corresponding significance of the hemodynamic response during the last 5 s of the 30-s stimulus presentation. Note that this filter did not discriminate the data based on wavelength or concentrations, as described in Zhang et al.⁸⁴ In this way, the designed filters focused primarily on motion artifacts, which are correlated across wavelength, where the hemodynamic response would not be correlated in the same way. These filtered data, for each of the two wavelengths (690 and 830 nm), were then converted to relative concentrations of oxygenated (HbO₂) and deoxygenated (HbR) blood using the modified Beer-Lambert law.⁴⁹ Changes in HbO₂ and HbR, as well as changes in total blood flow (HbT), were analyzed using 45-s time epochs composed of the following components: the 5 s immediately prior to the onset of the ball-box test event, the entire 30-s test event, and the 10 s immediately following the test event. The optical signals during the 45-s epoch were grand averaged over the seven participants and four test trials. However, trials were removed from the grand

average when motion artifacts were detected (i.e., the entire epoch, including all channels, were removed when motion artifacts were detected). Motion artifacts were identified by a change in the filtered intensity of greater than 5% in 1/20 s during the 30 s that the stimulus was presented. Using this criterion, one participant contributed only three trials, and another only two trials. Finally, data were included only from those detectors that registered a significant change ($p < 0.05$) from baseline in the signal during stimulus presentation. When a change in signal was observed in one detector, but not the other, the assumption was made that the latter detector was improperly placed relative to brain activation in the region of interest.

3 Results

3.1 Looking Time Data

The infants' looking times during the test trials were averaged and a grand average was computed [$M = 28.53$, standard deviation (SD) = 1.35]. The infants looked almost continuously throughout the test trial, suggesting that they found the ball-box event engaging. The fact that the infants found the event so engaging may have contributed to the robust nature of the neuroimaging results (see later).

3.2 Neuroimaging Data

An example of raw optical data at 690 and 830 nm from the primary visual cortex and the associated single-subject, four-trial block average is shown in Fig. 2 using different amounts of principle component filtering to illustrate the effect of the PCA on the raw data and the resultant block average, and to demonstrate that this stimulus produces a robust hemodynamic response that is observable trial by trial in the raw data. The typical hemodynamic response is for HbO₂ to increase and HbR to decrease following stimulus presentation, as we see in Fig. 2. This results in an increase in absorption (i.e., optical density) at 830 nm and typically a decrease at 690 nm, as we see in Figs. 2 and 3. The first row of Fig. 2 shows the raw, unfiltered data and reveals strong motion-induced fluctuations in the data prior to the first trial and during the 10-s interval between trials when the subject was not engaged and tended to move. The subsequent rows show the data filtered by one, three, and four principle components. The first component filters 40% of the covariance across the channels, removing a significant portion of the motion artifacts and producing a more typical response in HbO₂ and HbR that plateaus from 10 s to just after the stimulus ends at 30 s. Filtering the second and third components removes 73 and 85% of the variance, respectively, without significantly altering the hemodynamic response. The fourth component removes a summed total of 91% of the variance and significantly alters the hemodynamic response as well as decreasing the significance of the hemodynamic response. Thus, it is important to not overfilter the spatial covariance of the data as this can lead to a reduction of the hemodynamic response function. For all data sets included in this study, we typically filtered two or three components, removing approximately 80 to 90% of the variance.

The grand averaged hemoglobin concentration response curves are shown in Fig. 3, where time of 0 s is when the test event began. Relative changes in HbO₂, HbR, and HbT from

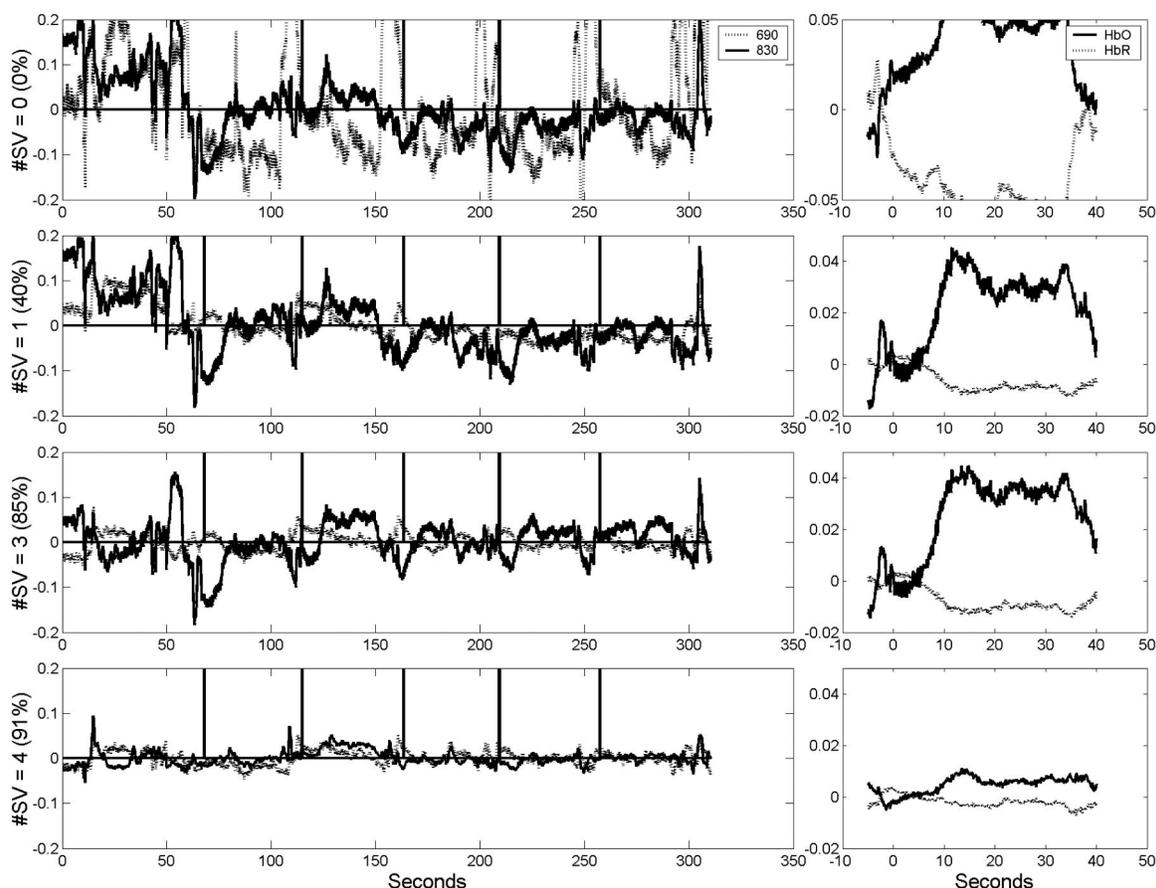


Fig. 2 Raw data (left column) and block-averaged response over four trials from a single subject illustrate the importance of filtering the spatial covariance of the data with a PCA to reduce motion artifacts in the data. The raw data from the primary visual cortex is displayed in optical density units at 690 and 830 nm over the 300-s duration of the experiment. The initiation of each 30-s trial is indicated by the thick vertical bar. The block-averaged data are displayed in relative concentration units of HbO₂ and HbR. The first row shows the raw data. The subsequent rows show the effect of filtering one, three, and four principle components.

10 to 30 s following the initiation of the event are compared to the baseline from -5 to 0 s. The oxy-, deoxy-, and total hemoglobin concentration responses are significantly different from 0 ($p < 0.01$). The visual cortex shows the typical re-

sponse of a decrease in HbR and an increase in HbO₂ and HbT. Interestingly, the temporal region shows an increase in HbR as well as HbO₂ and HbT. This uncharacteristic increase in HbR is not a deactivation of the temporal cortex since it occurs in parallel with an increase in HbT, indicative of an increase in CBF. Instead, it suggests that the temporal cortex has a stronger increase in the ratio of oxygen consumption change to blood flow change relative to that taking place in the visual cortex. Furthermore, this uncharacteristic increase in HbR is likely a result of immature neurovascular coupling that has been observed in other NIRS studies of the infant brain.^{28,32,34,43,44} Further research will be necessary to identify the physiological basis for the immature response observed in the inferior temporal cortex (e.g., a smaller increase in blood flow to the activated area and/or greater metabolic rates of the activated neural tissue), and the consequences this has for cognitive processing.

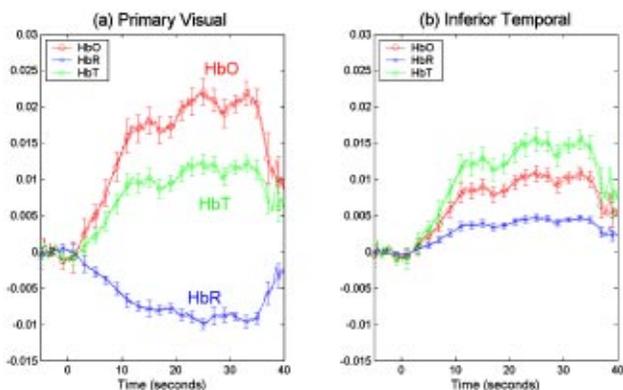


Fig. 3 Mean change in HbO₂, HbR, and HbT in response to the wide-screen ball-box event, in relative units (y axis). The 45-s trial epoch (see text) is displayed in the following way: -5 to 0 is baseline, 1 to 30 s is the 30-s ball-box event, and 31 to 40 s is poststimulus. Relative changes in HbO₂, HbR, and HbT during the time 10 to 30 s of this epoch were compared to 0. All observed changes were significant ($p < 0.01$).

4 Discussion

In the presented research, neural activation, as measured by relative changes in cerebral blood flow, was obtained in the primary visual cortex and the inferior temporal cortex during an event in which two featurally distinct objects, a ball and a box, emerged successively to opposite sides of a screen.

These results indicate that NIRS is a feasible method for assessing neural activation during visual object processing and suggest several directions for future research.

One direction is to determine the extent to which neuroimaging can provide a more direct measure of object individuation in infants. For example, to assess object individuation using the narrow-screen task, infants must be tested in two conditions: narrow and wide screens. Significantly longer looking to a narrow- than wide-screen test event is taken as evidence that infants (1) used the featural difference (e.g., shape, color) to individuate the objects, (2) recognized that both objects could fit behind the wide but not the narrow screen, (3) found full occlusion of both objects behind the narrow screen unexpected, and hence (4) demonstrated prolonged looking to the narrow-screen test event. Hence, interpretation of the behavioral data requires several inferences about the relation between cognitive processing and looking behavior during test events. Most visual attention tasks require inferences of this sort and, when supported by the outcome of appropriate control conditions, these inferences are considered valid. However, if successful performance on the narrow-screen task (i.e., longer looking to the narrow- than the wide-screen event) is associated with unique, well-defined patterns of neural activation, then neuroimaging data could be used to assess object individuation. Identification of a direct neural marker of object processing would be a major methodological advancement in the field of infant cognition and developmental neuroscience.

Another direction for future research is to identify the neural mechanisms that underlie developmental changes in infants' capacity to use featural information to individuate objects. The objects used by Wilcox and Baillargeon,^{9,10} like those used in the presented experiments, varied on many feature dimensions (e.g., shape, color, texture). However, there is evidence that infants are not equally sensitive to all types of featural information. By 4.5 months, for example, infants use shape differences, but it is not until 11.5 months that they use color differences, to individuate objects. These findings are particularly intriguing because both the ventral and dorsal pathways are involved in shape analysis: the ventral pathway extracts shape from contour, whereas the dorsal pathway extracts structure from motion.^{63–65,85,86} In contrast, only the ventral route is sensitive to color information.⁶⁶ Unfortunately, relatively little is known about the functional maturation of these two pathways. Infant performance on the narrow-screen task (or other object individuation tasks), and neuroimaging data collected during the task, could be used to dissociate the development of the object processing pathways that support infant use of shape and color information to individuate objects.

Once the neural basis of shape and color processing has been identified, we can begin to explore the effect of experience on neural functioning. Wilcox and her colleagues^{11,12} recently identified two kinds of experiences that can increase infant sensitivity to color information. These findings raise two very important questions. The first question is whether the change in behavioral response Wilcox and her colleagues have observed is accompanied by a corresponding change in neural response. Evidence that the response of inferior temporal neurons is altered by recent experiences (e.g., Gross⁸⁷) leads us to be optimistic. The second question is whether the

effect of experience, observed both in behavioral and neural functioning, is transient or long term (e.g., once infants demonstrate increased sensitivity to color information, is this effect observed in the days and months following the experience).

Finally, it is imperative to explore the relation between cerebral hemodynamics and neural functioning in the developing infant brain. In the presented research, we focused on robust effects, mainly because the relation between cerebral hemodynamics and neural functioning in the infant is not fully understood. Interestingly, we found the typical increase in HbO₂ and associated decrease in HbR in the occipital cortex but an associated increase in HbR in the inferior temporal cortex. The typical response is universally observed in the mature, healthy human and animal brain. The combined increase in HbO₂ and HbR was observed previously in the immature brain by other optical studies,^{28,32,34,43,44} and suggests that the brain-activation-induced flow response is reduced relative to the blood volume and/or oxygen consumption increase. It is expected that as the brain matures, the increase in HbR will shift to a decrease. Following this transition in a longitudinal study will lend more insight into the neurovascular relationship and how it evolves in the maturing brain. Furthermore, it is important to understand the evolution of this relationship to better interpret the correlation of neural response and cognitive processing.

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References

1. A. Aguiar and R. Baillargeon, "Developments in young infants' reasoning about occluded objects," *Cogn. Psychol.* **45**, 267–336 (2002).
2. R. Baillargeon and M. Graber, "Where's the rabbit? 5.5-month-old infants' representation of the height of a hidden object," *Cogn. Develop.* **2**, 375–392 (1987).
3. L. Bonatti, E. Frot, R. Zangl, and J. Mehler, "The human first hypothesis: identification of conspecifics and individuation of objects in the young infant," *Cogn. Psychol.* **44**, 388–426 (2002).
4. A. Meltzoff and K. Moore, "Object representation, identity, and the paradox of early permanence: steps toward a new framework," *Inf. Behav. Develop.* **21**, 201–235 (1998).
5. E. S. Spelke, R. Kestenbaum, D. J. Simons, and D. Wein, "Spatiotemporal continuity, smoothness of motion and object identity in infancy," *Br. J. Develop. Psychol.* **13**, 113–143 (1995).
6. P. D. Tremoulet, A. M. Leslie, and G. D. Hall, "Infant individuation and identification of objects," *Cogn. Develop.* **15**, 499–522 (2001).
7. G. Van de Walle, S. Carey, and M. Prevor, "Bases for object individuation in infancy: evidence from manual search," *J. Cogn. Develop.* **1**, 249–280 (2000).
8. T. Wilcox, "Object individuation: infants' use of shape, size, pattern, and color," *Cognition* **72**, 125–166 (1999).
9. T. Wilcox and R. Baillargeon, "Object individuation in infancy: the use of featural information in reasoning about occlusion events," *Cogn. Psychol.* **37**, 97–155 (1998).
10. T. Wilcox and R. Baillargeon, "Object Individuation in young in-

- fants: further evidence with an event monitoring task," *Develop. Sci.* **1**, 127–142 (1998).
11. T. Wilcox and C. Chapa, "Priming infants to attend to color and pattern information in an individuation task," *Cognition* **90**, 265–302 (2004).
 12. T. Wilcox, C. Chapa, and R. Woods, "Multisensory exploration and object individuation in infants" (in preparation).
 13. T. Wilcox and A. Schweinle, "Object individuation and event mapping: infants' use of featural information," *Develop. Sci.* **5**, 132–150 (2002).
 14. T. Wilcox and A. Schweinle, "Infants' use of speed information to individuate objects in occlusion events," *Inf. Behav. Develop.* **26**, 253–282 (2003).
 15. F. Xu, "The role of language in acquiring kind concepts in infancy," *Cognition* **85**, 223–250 (2002).
 16. F. Xu and S. Carey, "Infants' metaphysics: the case of numerical identity," *Cogn. Psychol.* **30**, 111–153 (1996).
 17. G. Gratton, P. M. Corballis, E. Cho, M. Fabiani, and D. C. Hood, "Shades of gray matter: noninvasive optical images of human brain responses during visual stimulation," *Psychophysiology* **32**, 505–509 (1995).
 18. G. Gratton and M. Fabiani, "The event-related optical signal: a new tool for studying brain function," *Int. J. Psychophysiol.* **42**, 109–121 (2001).
 19. G. Gratton, M. Fabiani, P. M. Corballis, D. C. Hood, M. R. Goodman-Wood, J. Hirsch, K. Kim, D. Friedman, and E. Gratton, "Fast and localized event-related optical signals (EROS) in the human occipital cortex: comparisons with the visual evoked potential and fMRI," *Neuroimage* **6**, 168–180 (1997).
 20. D. M. Rector, G. R. Poe, M. P. Kristensen, and R. M. Harper, "Light scattering changes follow evoked potentials from hippocampal Schaeffer collateral stimulation," *J. Neurophysiol.* **78**, 1707–1713 (1997).
 21. R. A. Stepnoski, A. LaPorta, F. Raccaia-Behling, G. E. Blonder, R. E. Slusher, and D. Kleinfeld, "Non-invasive detection of changes in membrane potential in cultured neurons by light scattering," *Proc. Natl. Acad. Sci.* **88**, 9382–9386 (1991).
 22. J. Meek, "Basic principles of optical imaging and application to the study of infant development," *Develop. Sci.* **5**, 371–380 (2002).
 23. A. Grinvald, T. Bonhoeffer, D. Malonek, D. Shoham, E. Bartfeld, A. Arieli, R. Hildesheim, and E. Ratzlaff, "Optical imaging of architecture and function in the living brain," in *Memory: Organization and Locus of Change*, A. Grinvald, T. Bonhoeffer, D. Malonek et al., Eds., pp. 49–85, Oxford University Press, New York (1991).
 24. G. Strangman, D. A. Boas, and J. P. Sutton, "Non-invasive neuroimaging using near-infrared light," *Biol. Psychol.* **52**, 679–693 (2002).
 25. A. Villringer and B. Chance, "Non-invasive optical spectroscopy and imaging of human brain function," *Trends Neurosci.* **20**, 435–442 (1997).
 26. A. Villringer and U. Dirnagl, "Coupling of brain activity and cerebral blood flow: basis of functional imaging," *Cerebrovasc Brain Metab. Rev.* **7**, 240–276 (1995).
 27. Y. Chen, S. Zhou, C. Xie, S. Nioka, M. Delivoria-Papadopoulos, E. Anday, and B. Chance, "Preliminary evaluation of dual wavelength phased array imaging on neonatal brain function," *J. Biomed. Opt.* **5**, 194–200 (2000).
 28. S. R. Hintz, D. A. Benaron, A. M. Siegel, A. Zourabian, D. K. Stevenson, and D. A. Boas, "Bedside functional imaging of the premature infant brain during passive motor activation," *J. Perinat. Med.* **29**, 335–343 (2001).
 29. J. H. Meek, C. E. Elwell, D. C. McCormick, A. D. Edwards, J. P. Townsend, A. L. Steward, and J. S. Wyatt, "Abnormal cerebral haemodynamics in perinatally asphyxiated neonates related to outcome," *Arch. Dis. Child* **81**, F110–F115 (1999).
 30. J. H. Meek, M. Firbank, C. E. Elwell, J. Atkinson, O. Braddick, and J. S. Wyatt, "Regional hemodynamic responses to visual stimulation in awake infants," *Pediatr. Res.* **43**, 840–843 (1998).
 31. J. H. Meek, M. Noone, C. E. Elwell, and J. S. Wyatt, "Visually evoked haemodynamic responses in infants with cerebral pathology," *Pediatr. Res.* **45**, 909 (1999).
 32. K. Sakatani, S. Chen, W. Lichty, H. Zuo, and Y. Wang, "Cerebral blood oxygenation changes induced by auditory stimulation in newborn infants measured by near infrared spectroscopy," *Early Hum. Dev.* **55**, 229–236 (1999).
 33. J. S. Soul and A. J. du Plessis, "New technologies in pediatric neurology: Near-infrared spectroscopy," *Semin Pediatr. Neurol.* **6**, 101–110 (1999).
 34. P. Zaramella, F. Freato, A. Amigoni, S. Salvadori, P. Marangoni, A. Suppiej, B. Schiavo, and C. Lino, "Brain auditory activation measured by near-infrared spectroscopy (NIRS) in neonates," *Pediatr. Res.* **49**, 213–219 (2001).
 35. A. A. Baird, J. Kagan, T. Gaudette, K. A. Walz, N. Hershlag, and D. A. Boas, "Frontal lobe activation during object permanence: data from near-infrared spectroscopy," *Neuroimage* **16**, 1120–1126 (2002).
 36. M. Pena, A. Maki, D. Kovacic, G. Dehaene-Lambertz, H. Koizumi, F. Bouquet, and J. Mehler, "Sounds and silence: an optical topography study of language recognition at birth," *Proc. Natl. Acad. Sci.* **100**, 11702–11705 (2003).
 37. G. Taga, K. Asakawa, A. Maki, Y. Konishi, and H. Koizumi, "Brain imaging in awake infants by near-infrared optical topography," *Proc. Natl. Acad. Sci.* **100**, 10722–10727 (2003).
 38. M. Bartocci, J. Winberg, C. Ruggiero, L. L. Bergqvist, G. Serra, and H. Lagercrantz, "Activation of olfactory cortex in newborn infants after odor stimulation: a functional near-infrared spectroscopy study," *Pediatr. Res.* **48**, 18–23 (2000).
 39. Y. Hoshi and M. Tamura, "Dynamic multichannel near-infrared optical imaging of human brain activity," *J. Appl. Physiol.* **75**, 1842–1846 (1993).
 40. G. Jaszewski, G. Strangman, J. Wagner, K. K. Kwong, R. A. Poldrack, and D. A. Boas, "Differences in the hemodynamic response to event-related motor and visual paradigms as measured by near-infrared spectroscopy," *Neuroimage* **20**, 479–488 (2003).
 41. H. Obrig, T. Wolf, C. Döge, J. J. Hülsing, U. Dirnagl, and A. Villringer, "Cerebral oxygen changes during motor and somatosensory stimulation in humans, as measured by near-infrared spectroscopy," *Adv. Exp. Med. Biol.* **388**, 219–224 (1996).
 42. G. Strangman, M. A. Franceschini, and D. A. Boas, "Factors affecting the accuracy of near-infrared spectroscopy concentration calculations for focal changes in oxygenation parameters," *Neuroimage* **18**, 865–879 (2003).
 43. M. Bartocci, J. Winberg, G. Papendieck, T. Mustica, G. Serra, and H. Lagercrantz, "Cerebral hemodynamic response to unpleasant odors in the preterm newborn measured by near-infrared spectroscopy," *Pediatr. Res.* **50**, 324–330 (2001).
 44. S. Chen, K. Sakatani, W. Lichty, P. Ning, S. Zhao, and H. Zuo, "Auditory-evoked cerebral oxygenation changes in hypoxic-ischemic encephalopathy of newborn infants monitored by near infrared spectroscopy," *Early Hum. Dev.* **67**, 113–121 (2002).
 45. T. Kato, A. Kamei, S. Takashima, and T. Ozaki, "Human visual cortical function during photic stimulation monitoring by means of near-infrared spectroscopy," *J. Cereb. Blood Flow Metab.* **13**, 516–520 (1993).
 46. G. Gratton, A. Sarno, E. Maclin, P. M. Corballis, and M. Fabiani, "Toward noninvasive 3-D imaging of the time course of cortical activity: Investigation of the depth of the event-related optical signal," *Neuroimage* **11**, 491–504 (2000).
 47. G. Gratton, M. R. Goodman-Wood, and M. Fabiani, "Comparison of neuronal and hemodynamic measures of the brain response to visual stimulation: an optical imaging study," *Hum. Brain Mapp* **13**, 13–25 (2001).
 48. A. Kleinschmidt, H. Obrig, M. Requardt, K. D. Merboldt, U. Dirnagl, A. Villringer, and J. Frahm, "Simultaneous recording of cerebral blood oxygenation changes during human brain activation by magnetic resonance imaging and near-infrared spectroscopy," *J. Cereb. Blood Flow Metab.* **16**, 817–826 (1996).
 49. G. Strangman, J. P. Culver, J. H. Thompson, and D. A. Boas, "A quantitative comparison of simultaneous BOLD fMRI and NIRS recordings during function brain activation," *Neuroimage* **17**, 719–731 (2002).
 50. A. Villringer, S. Minoshima, C. Hock, H. Obrig, S. Ziegler, U. Dirnagl, M. Schwaiger, and A. Villringer, "Assessment of local brain activation. A simultaneous PET and near-infrared spectroscopy study," *Adv. Exp. Med. Biol.* **413**, 149–153 (1997).
 51. A. M. Franceschini and D. A. Boas, "Noninvasive measurement of neuronal activity with near-infrared optical imaging," *Neuroimage* **21**, 372–386 (2004).
 52. F. F. Jobsis, "Noninvasive infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters," *Science* **198**, 1264–1267 (1977).
 53. R. Woods and T. Wilcox, "Infants' use of luminance to individuate

- objects," presented at the biennial meeting of the Society for Research in Child Development, 2003, Tampa, FL.
54. A. Aguiar and R. Baillargeon, "8.5-month-old infants' reasoning about containment events," *Child Dev.* **69**, 636–653 (1998).
 55. E. S. Spelke, K. Breinlinger, J. Macomber, and K. Jacobson, "Origins of knowledge," *Psychol. Rev.* **99**(4), 605–632 (1992).
 56. M. M. Sitskoom and A. W. Smitsman, "Infants' perception of dynamic relations between objects: passing through or support?" *Dev. Psychol.* **31**(3), 437–447 (1995).
 57. S. Hespos, "Tracking individual objects across occlusion and containment events in 6.5-month-old infants," presented at the Int. Conf. on Infant Studies, July 2000, International Society for Infant Studies, Brighton, UK.
 58. A. M. Leslie and M. Glanville, "Is individuation by feature in young infants limited by attention or by working memory?" presented at the biennial meeting of the Society for Research in Child Development, April 2001, Minneapolis, MN.
 59. A. Needham, "Infants' use of featural information in the segregation of stationary objects," *Inf. Behav. Develop.* **21**, 47–76 (1998).
 60. A. Needham, "The role of shape in 4-month-old infants' segregation of adjacent objects," *Inf. Behav. Develop.* **22**, 161–178 (1999).
 61. A. Needham, "Object recognition and object segregation in 4.5-month-old infants," *J. Exp. Child Psychol.* **78**, 3–24 (2001).
 62. T. Wilcox, S. McCurry, and R. Woods, "Evidence for featurally-based object individuation in infants from a reaching task" (in preparation).
 63. M. Livingstone and D. Hubel, "Psychophysical evidence for separate channels for the perception of form, color, movement, and depth," *J. Neurosci.* **7**, 3416–3468 (1987).
 64. M. Livingstone and D. Hubel, "Segregation of form, color, movement, and depth: anatomy, physiology, and perception," *Science* **240**, 740–749 (1988).
 65. E. A. De Yoe and D. C. Van Essen, "Concurrent processing streams in monkey visual cortex," *Trends Neurosci.* **11**, 219–226 (1988).
 66. R. Desimone and L. G. Ungerleider, "Neural mechanisms of visual processing in monkeys," in *Handbook of Neuropsychology*, Vol. 2, F. Boller and J. Grafman, Eds., pp. 267–299, Elsevier, New York (1989).
 67. M. A. Goodale and A. D. Milner, "Separate visual pathways for perception and action," *Trends Neurosci.* **15**, 20–25 (1992).
 68. M. Mishkin, L. G. Ungerleider, and K. A. Macko, "Object vision and spatial vision: two cortical pathways," *Trends Neurosci.* **6**, 414–417 (1983).
 69. L. G. Ungerleider and M. Mishkin, "Two cortical visual systems," in *Analysis of Visual Behavior*, D. J. Ingle, M. A. Goodale, and R. J. W. Mansfield, Eds., pp. 549–586, MIT Press, Cambridge, MA (1982).
 70. D. C. Van Essen, C. H. Anderson, and D. J. Felleman, "Information processing in the primate visual system: an integrated systems perspective," *Science* **255**, 419–423 (1992).
 71. M. Boucart, M. E. Meyer, D. Pins, G. W. Humphreys, C. Scheiber, D. Gounod, and J. Foucher, "Automatic object identification: an fMRI study," *Brain Imaging* **11**, 2379–2383 (2000).
 72. V. P. Clark, R. Parasuraman, K. Keil, R. Kulansky, S. Fannon, J. M. Maisog, L. G. Ungerleider, and J. V. Haxby, "Selective attention to face identity and color studied with fMRI," *Hum. Brain Mapp* **5**, 293–297 (1997).
 73. K. Grill-Spector, T. Kushnir, T. Hendler, S. Edelman, Y. Itzhak, and R. Malach, "A sequence of object-processing stages revealed by fMRI in the human occipital lobe," *Hum. Brain Mapp* **6**, 316–328 (1998).
 74. M. Kraut, J. Hart, B. J. Soher, and B. Gordon, "Object shape processing in the visual system evaluated using functional MRI," *Neurology* **48**, 1416–1420 (1997).
 75. K. Tanaka, "Mechanisms of visual object recognition: monkey and human studies," *Curr. Opin. Neurobiol.* **7**, 523–529 (1997).
 76. K. Tanaka, "Mechanisms of visual object recognition studied in monkeys," *Spatial Vis.* **13**, 147–163 (2000).
 77. K. Tsunoda, Y. Yamane, M. Nishizaki, and M. Tanifuji, "Complex objects are represented in macaque inferotemporal cortex by the combination of feature columns," *Nat. Neurosci.* **4**, 832–838 (2001).
 78. G. Wang, M. Tanifuji, and K. Tanaka, "Functional architecture in monkey inferotemporal cortex revealed by in vivo optical imaging," *Neurosci. Res. (N Y)* **31**, 33–46 (1998).
 79. G. Wang, K. Tanaka, and M. Tanifuji, "Optical imaging of functional organization in the monkey inferotemporal cortex," *Science* **272**, 1665–1668 (1996).
 80. J. Bachevalier, M. Brickson, C. Hagger, and M. Mishkin, "Age and sex differences in the effects of selective temporal lobe lesion on the formation of visual discrimination habits in rhesus monkeys (*macaca mulatta*)," *Behav. Neurosci.* **104**, 885–899 (1990).
 81. J. Bachevalier and M. Mishkin, "An early and a late developing system for learning and retention in infant monkeys," *Behav. Neurosci.* **98**, 770–778 (1984).
 82. H. R. Rodman, J. P. Skelly, and C. G. Gross, "Stimulus selectivity and state dependence of activity in inferior temporal cortex in infant monkeys," *Proc. Natl. Acad. Sci.* **88**, 7572–7575 (1991).
 83. M. J. Webster, L. G. Ungerleider, and J. Bachevalier, "Connections of inferior temporal areas TE and TEO with medial temporal-lobe structures in infant and adult monkeys," *J. Neurosci.* **11**, 1095–1116 (1991).
 84. Y. Zhang, D. H. Brooks, M. A. Franceschini, and D. A. Boas, "Eigenvector-based spatial filtering for reduction of physiological interference in diffuse optical imaging," *J. Biomed. Opt.* (this issue).
 85. R. Desimone, S. Schein, J. Moran, and L. Ungerleider, "Contour, color and shape analysis beyond the striate cortex," *Vision Res.* **25**, 441–452 (1985).
 86. Kraut et al. (1987).
 87. C. G. Gross, "Representation of visual stimuli in inferior temporal cortex," *Phil. Trans. Biol. Sci.* **335**, 3–10 (1992).