Specificity of Inferior Olive Response to Stimulus Timing

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Liu T, Xu D, Ashe J, Bushara K. Specificity of inferior olive response to stimulus training. J Neurophysiol 100: 1557–1561, 2008. First published July 16, 2008; doi:10.1152/jn.00961.2007. The inferior olive is the sole source of the climbing fiber system, one of the two major afferent systems of the cerebellum; however, its exact role remains unknown. A longstanding hypothesis is that the inferior olive with its unique intrinsic rhythmic firing properties mediates motor timing. However, direct evidence linking the inferior olive to timing behavior has been difficult to demonstrate in animal or human studies likely due to the inhibition of inferior olive responses by self-produced movement. Here we used event-related functional magnetic resonance imaging (fMRI) and a perceptual task that dissociates the temporal from nontemporal attributes of sensory input. Subjects were asked to attend to rhythmically occurring identical visual stimuli and to detect a change in their timing, spatial orientation, or color. Inferior olive activation was seen only when perceiving a change in stimulus timing. These results are consistent with animal studies demonstrating that the inferior olive is especially sensitive to “unexpected” sensory events and further provide evidence supporting the specificity of the inferior olive response to stimulus timing. The results are consistent with the view that the inferior olive and the climbing fiber system mediate the encoding of temporal information required for both motor and nonmotor cognitive processes.

INTRODUCTION

The olivocerebellar system originates exclusively from the inferior olive as climbing fibers that project to the Purkinje cells and the deep cerebellar nuclei (Palay and Chan-Palay 1974). Despite decades of research and detailed knowledge of the microanatomy and circuitry of the olivocerebellar system, its function remains a subject of continuing debate (Bloedel and Brachti 1998; De Zeeuw et al. 1998; Gibson et al. 2004). Timing of movement and movement error detection are two of the major functions that have long been proposed for the olivocerebellar system based on its unique morphologic and electrophysiologic properties (Armstrong 1974; Braitenberg 1967; Gellman et al. 1985; Llinas et al. 1974; Llinas and Sasaki 1989; Rushmer et al. 1976). The timing hypothesis was initially advanced by Braitenberg, who proposed that the climbing fibers control the timing of excitation of Purkinje cells by modulating the conduction velocity of parallel fibers which serve as delay lines (Braitenberg 1967; Braitenberg and Armstrong 1974). This hypothesis was based primarily on the morphological features of the cerebellar cortex but lacked electrophysiological support. Given the known length of the parallel fibers (<10 mm) and their conduction velocity (5 mm/ms), the predicted delays would be too short for the 100 ms scale of movement timing (Eccles et al. 1967). Nonetheless the timing hypothesis re-emerged from further studies demonstrating that the olivocerebellar system is capable of generating rhythmic and synchronous discharges (Llinas et al. 1974). The capability of the olivary neurons to fire in synchrony with one another has been attributed to electrotonic coupling mediated via dendro-dendritic gap junctions which themselves are under inhibitory control from the deep cerebellar nuclei (Llinas et al. 1974; Sotelo et al. 1974). The changeability of electro-tonic coupling is believed to be an important feature of the olivocerebellar system that allows the generation of multiple patterns of synchronously firing inferior olivary neurons (Llinas et al. 1974). It is proposed that despite the relatively slow neuronal firing rate of the inferior olive, it is capable of controlling both the timing and spatial aspects of fast movement by re-patterning the synchronized firing of neuronal ensembles and their population codes rather than by the conventional single neuron frequency codes (Welsh et al. 1995).

The hypothesis that the olivocerebellar system detects errors or mismatches between intended and achieved movement has been driven primarily from early animal studies showing that the inferior olive neurons are highly sensitive to unexpected external sensory stimuli (Armstrong 1974; Eccles et al. 1972; Gellman et al. 1985; Oscarsson 1980; Rushmer et al. 1976). Unexpected sensory events during movement (such as perturbations during locomotion or visual signals representing retinal image slip during eye movement) were shown to increase the inferior olive discharges (Andersson and Armstrong 1987; Barmack and Simpson 1980; Kim et al. 1987; Winkelman and Frens 2006). These findings were interpreted from the perspective of motor control to indicate that the inferior olive signals unexpected events and therefore errors in movement. However, the inferior olive was also shown to respond to passive displacement of the animal’s limb in the absence of active movement (Gellman et al. 1985; Gibson et al. 2004; Rushmer et al. 1976). Indeed certain phases of movement were shown to inhibit the inferior olive responsiveness to sensory input (Gellman et al. 1985; Gibson et al. 2004; Rushmer et al. 1976). These observations could not be readily accommodated by the movement error detection hypothesis (Gellman et al. 1985; Gibson et al. 2004; Rushmer et al. 1976).

Using event-related fMRI, we have shown significant activation of the inferior olive when human subjects perceived nonrepeating complex temporal sequences of visual stimuli (complex rhythms) compared with simple isochronous sequences (Xu et al. 2006). These results suggest that the inferior olive is sensitive to stimulus timing and provide an alternative interpretation of the inferior olive sensitivity to unexpected sensory stimuli shown in animal studies. However, such an
interpretation can't be confirmed by the available data because the temporal and nontemporal characteristics of sensory stimuli that activate the inferior olive have not been systematically studied. The aim of this study was to dissociate the temporal from nontemporal attributes of sensory input to test the specificity of the inferior olive response to the stimulus timing.

METHODS

Subjects

Twelve healthy right-handed subjects [4 women and 8 men, age: (mean ± SD) 22.6 ± 3.0] participated in the study as paid volunteers after giving written informed consent according to guidelines approved by the University of Minnesota and the Minneapolis VA Institutional Review Boards.

Behavioral tasks

We used mixed epoch/event-related fMRI design (Visscher et al. 2003). Epoch (block)-related analysis was used to identify areas activated during attention to temporal and nontemporal attributes of visual stimuli. Event-related analysis was used to identify activations related to the perception of unexpected changes in the temporal and nontemporal attributes of single visual stimuli. Subjects performed an oddity detection (odd ball) paradigm while attending to the timing, spatial orientation or color of visual stimuli in three separate tasks: timing detection (time), spatial orientation detection (tilt), and color detection (color). In all tasks, non-odd visual stimuli were identical and consisted of a vertically oriented bar (stimulus duration = 200 ms, vertical visual angle = 4°) appearing rhythmically at 1 Hz at the center of a computer monitor against a black background. In the time task, subjects were instructed to attend to the stimulus timing and to respond by pressing a button when a change in the timing of stimulus presentation is perceived (odd stimuli occurred 550 ms prematurely). In the tilt task, subjects attended to the orientation of the stimulus and responded when an odd stimulus is perceived (the bar tilts from the vertical position by rotating at the center to the right or left by 2.5°). In the color task, subjects attended to the stimulus color and responded when the bar’s color changes from green (RBG: 99, 255, 0) to yellow/green (RBG: 255, 251, 0). The parameters of odd stimuli were determined by a prescanning behavioral study and correspond to 95% correct response rate to maximize the number of odd stimuli detected correctly during scanning. The visual display was projected through a back-lit screen and viewed via a mirror attached to the head coil. Subjects responded to odd stimuli by pressing a button with the right index finger.

Functional images were acquired while subjects performed a total of three runs. The total scanning time for each run was 5 min and 24 s. Each run began with rest period of 18 s followed by six blocks (36 s each) of stimulus presentation interleaved with rest periods of 18 s. A task cue (time, color, or tilt) lasting 4 s was presented before each block. Two blocks of each of the three tasks (time, color, or tilt) were presented in each run. The order of blocks (tasks) within each run was counterbalanced pseudorandomly within the subject and among subjects. During each block, a total of 34 stimuli were presented (interstimulus interval was 1 s) of which 3 odd stimuli were presented pseudorandomly such that odd stimuli were separated by ≥6 normal (non-odd) stimuli. This enabled the subjects to judge the change in odd stimuli particularly when performing the timing detection condition. Odd stimuli of a given condition (time, color, or tilt) were only presented in blocks corresponding to that condition (subjects attended to 1 stimulus attribute in each block).

Image acquisition

Blood-oxygenation-level-dependent (BOLD) contrast functional images were acquired with a 3-T MRI scanner (Magnetom Trio, Siemens, Erlangen, Germany) using a gradient echoplanar (T2*) sequence with the following parameters: echo time, 30 ms; repeat time, 2,000 ms; flip angle, 90°; field of view, 200 × 200 mm; in-plane resolution, 3 × 3 mm; slice thickness, 3 mm with 1-mm inter slice gap. Thirty-four axial slices covering the whole brain, cerebellum, and brain stem were obtained. A high-resolution anatomical T1 image was obtained with the following parameters: echo time, 4.7 ms; repeat time, 20 ms; flip angle, 22°; field of view, 256 × 256; in-plane resolution, 1 × 1 mm; slice thickness, 1 mm.

Data analysis

fMRI data were preprocessed and analyzed using Statistical Parametric Mapping (SPM2) software (Wellcome Department of Cognitive Neurology, London, http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB (Mathworks, Sherborn, MA). For each subject, the first 3 volumes of each run were discarded to allow for T1 equilibration. The remaining volumes were corrected for head motion, realigned to the first image and sinc-interpolated over time to correct for phase advance during volume acquisition. Images were then normalized to the Montreal Neurological Institute (MNI) echoplanar imaging (EPI) brain template with enlarged box to include the whole cerebellum and brain stem (Z = 85 to –70 mm). Data were resampled to 2 × 2 × 2 mm and spatially smoothed with a Gaussian kernel of 8-mm full-width at half-maximum to decrease spatial noise. The functional data were temporarily smoothed to remove slow BOLD signal drifts using a high pass filter with 128-s cut-off. Head motion parameters were added as covariates of no interest in the statistical analysis model. T1-weighted anatomical images were coregistered to the functional scans and transformed into the same normalized MNI brain template.

Attention to stimulus timing, spatial orientation, and color (time attention, tilt attention, and color attention) were modeled as separate epochs. Perception of odd stimuli in each condition (time perception, tilt perception, and color perception) were modeled as separate events. The term perception is used in this manuscript to describe these events since they were time-locked to the onset of odd stimuli. However, given the temporal resolution of fMRI methods used, motor preparation and motor response were not clearly separable from perception of odd stimuli (Hernandez et al. 2002; Liu et al. 2006). Non-odd stimuli that were followed by a motor response (false alarms), and task instruction cues were modeled as separate events but were not included in further statistical comparisons.

Statistical analysis was performed at two levels of a mixed effects model. In the first level analysis (fixed effects), epochs and events of interest were convolved with a canonical hemodynamic response function in a general linear model as implemented in SPM2 yielding a parameter estimate for each epoch and event for each voxel. Using linear contrasts of the parameter estimates, statistical parametric maps of t statistic for every voxel were obtained for each condition. These contrasts were used in the second level group analysis treating intersubject variability as random effect and thus allowing statistical inference at the population level (Penny and Holmes 2003). Statistical threshold of 3.09 P ≤ 0.001 (uncorrected for multiple comparisons) was used for cerebellar and brain stem activations. Homologous activations below statistical threshold are listed for comparison when appropriate.

RESULTS

During scanning, the subjects performed at a correct hit rate (motor response to odd stimuli) of (mean ± SD) 99.50 ± 0.64, 99.81 ± 0.31, and 99.73 ± 0.31% [F(2,33) = 0.31, P = 0.23] and false alarm rate (motor response to non-odd stimuli) of 0.15 ± 0.23, 0.12 ± 0.21 and 0.15 ± 0.23% [F(2,33) = 0.12, P = 0.89] for timing, color, and orientation detection condi-
Cerebellar and brain stem activations: during attention to stimulus timing, spatial orientation and color (A) and when perceiving a change in stimulus timing, spatial orientation, and color (B). Event-related activations in B were time-locked to the onset of odd stimuli; however, blood-oxygen-level-dependent responses related to the perception of odd stimuli were not separable from motor preparation and button-press-related responses as odd stimuli were always followed by a motor response. Cerebellar regions’ nomenclature is according to Schmahmann’s atlas (Schmahmann 1999).

We focused on activations within the cerebellum and brain stem (Please see supplementary data in Tables S1 and S2 for whole brain activations). Attention to timing, spatial orientation, and color activated multiple overlapping areas predominantly in the left cerebellar hemisphere (Table 1A). Direct subtraction [using the contrast (time attention minus color attention) and (time attention minus tilt-attention)] showed higher activation during attention to timing in lobule VI bilaterally (−38, −46, −22, z-score 3.35; and 14, −50, −6, z-score: 4.46) when compared with attention to color and higher activation in the left lobule IX (−14, −60, −52, z-score 2.87; \(P < 0.005\); uncorrected) when compared with attention to spatial orientation. No activations in the cerebellar cortex during attention to timing survived both subtraction of attention to stimulus orientation and subtraction of attention to color [using the contrast (time attention minus color attention minus tilt attention)].

Multiple overlapping areas in both the medial and lateral regions of the cerebellar cortex were activated bilaterally when subjects detected a change in stimulus timing, spatial orientation and color (Table 1B, Fig. 1). It should, however, be noted that although the activations shown in Table 1B and Fig. 1 were time-locked to the onset of odd stimuli, BOLD responses related to the perception of odd stimuli were not separable from motor preparation and button-press-related responses as odd stimuli were always followed by a motor response. Direct subtraction [using the contrast (time perception minus color perception)] showed higher activation when perceiving a change in stimulus timing compared with stimulus color in left Crus I (−52, −60, −38, z-score: 3.22) and right lobule IX (6, −46, −44 z-score 3.6). There were no significant activations in the cerebellar cortex when perceiving a change in stimulus timing was compared with spatial orientation by direct subtraction using the contrast (time perception minus tilt perception).

In addition to cerebellar cortical areas, perception of change in stimulus timing activated the inferior olive bilaterally (Table 1B, Fig. 2). There was no inferior olive activation during attention conditions in any of the three tasks. The specificity of

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**Table 1. Cerebellar and brain stem activations**

<table>
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<tr>
<th>Region</th>
<th>Stimulus Timing</th>
<th>X, Y, Z (mm)</th>
<th>Z Score</th>
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<th>Stimulus Orientation</th>
<th>X, Y, Z (mm)</th>
<th>Z Score</th>
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<th>Stimulus Color</th>
<th>X, Y, Z (mm)</th>
<th>Z Score</th>
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<td>A. Attention</td>
<td>B. Perception of Change</td>
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<tr>
<td>L lobule IX / VIIIB</td>
<td>−14, −52, −56</td>
<td>4.31</td>
<td></td>
<td>−18, −54, −58</td>
<td>4.18</td>
<td>−18, −54, −56</td>
<td>3.43</td>
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<tr>
<td>R lobule IX / VIIIB</td>
<td>14, −46, −60</td>
<td>3.72</td>
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<td>−44, −54, −48</td>
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<td>L lobule VI</td>
<td>−48, −72, −28</td>
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<tr>
<td>L lobule V / Crus I</td>
<td>−20, −52, −32</td>
<td>4.66</td>
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<tr>
<td>R lobule VIIIB</td>
<td>10, 76, −46</td>
<td>3.21</td>
<td></td>
<td>20, −68, −24</td>
<td>4.17</td>
<td>24, −78, −48</td>
<td>4.58</td>
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<tr>
<td>R lobule VIIB</td>
<td>−16, −74, −50</td>
<td>3.57</td>
<td></td>
<td>−20, −74, −50</td>
<td>4.48</td>
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<tr>
<td>L lobule VIIIA</td>
<td>−20, −68, −52</td>
<td>3.53</td>
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<td></td>
<td>16, −66, −56</td>
<td>5.43</td>
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<tr>
<td>L lobule VIIIA / VIIIB</td>
<td>−28, −60, −58</td>
<td>3.81</td>
<td></td>
<td>−12, −68, −48</td>
<td>3.69</td>
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<tr>
<td>L VIIIA Lobule</td>
<td>−34, −50, −54</td>
<td>3.56</td>
<td></td>
<td>−28, −40, −42</td>
<td>3.84</td>
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<tr>
<td>L lobule IX</td>
<td>−14, −52, −60</td>
<td>4.05</td>
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<td>R Inferior Olive</td>
<td>2, −36, −52</td>
<td>3.89</td>
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<td>L Inferior Olive</td>
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Cerbellar and brain stem activations: during attention to stimulus timing, spatial orientation and color (A) and when perceiving a change in stimulus timing, spatial orientation, and color (B). Event-related activations in B were time-locked to the onset of odd stimuli; however, blood-oxygen-level-dependent responses related to the perception of odd stimuli were not separable from motor preparation and button-press-related responses as odd stimuli were always followed by a motor response. Cerebellar regions’ nomenclature is according to Schmahmann’s atlas (Schmahmann 1999).

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FIG. 1. Areas activated when detecting a change in stimulus timing, spatial orientation, and color. Statistical parametric maps of event-related activations time-locked to the onset of stimuli with odd timing (red), spatial orientation (blue), and color (green). Blood-oxygen-level-dependent (BOLD) responses related to the perception of odd stimuli were not separable from motor preparation and button press-related responses. Activations are shown on axial (A), coronal (B), and sagittal templates (C). Shown are stereotaxic coordinates (in mm relative to the anterior commissure): X: (+) right, (−) left; Y: (+) anterior, (−) posterior and Z: (+) superior (−) inferior. Arrows: inferior olive.
inferior olive response to perception of change in stimulus timing was further confirmed by direct subtraction of its response to the change in stimulus orientation and color [using the contrasts (time perception minus tilt perception) and (time perception minus color perception)]. This showed right inferior olive activation over and above response to change in stimulus orientation (2, −34, −54, z-score 2.95) and color (4, −32, −50, z-score 2.87, P < 0.005; uncorrected; Fig. 2). Using the contrast (time perception minus time attention), the right inferior olive showed significantly higher response to perception of change in stimulus timing when compared with attention to timing (2, −34, −52, z-score = 4.01). There was no inferior olive activation using the contrasts: (tilt perception minus tilt attention) or (color perception minus color attention).

**DISCUSSION**

Attention to temporal and nontemporal attributes of visual stimuli in the current study predominantly activated the left cerebellar hemisphere; a finding consistent with right (contralateral) cerebral hemispheric dominance during visual attention tasks (Fink et al. 1997; Le 1998). The results show overlapping activations during attention and perception of odd stimuli in the timing, spatial orientation, and color detection conditions with no evidence for modular specificity within the cerebellar cortex. Human lesion studies implicated both medial and lateral regions of the cerebellar cortex in timing mechanisms (Ivry et al. 1988). These studies suggested a medial/lateral dissociation such that medial lesions impaired the motor (or implementation) component of finger tapping timing task, while lateral lesions impaired the central timekeeper component resulting in “clock” variability as shown by variability scores according to Wing and Kristofferson model (Wing and Kristofferson 1973). It has further been postulated that the intermediate cerebellum (through its direct connections with the motor system) mediates the motor aspects of timing while the lateral cerebellar hemispheres mediate the cognitive aspects of temporal processing through its connections with frontoparietal association cortex (Lewis and Miall 2003; Middleton and Strick 2001). Previous functional imaging studies including our previous fMRI study in which the motor and perceptual aspects of timing were dissociated, showed activation of both medial and lateral cerebellar hemispheres and provided no support to the medial/lateral dissociation suggested by lesion studies (Dhamala 2003; Diedrichsen et al. 2007; Harrington et al. 2004; Ramnani and Passingham 2001; Schubotz et al. 2000; Xu et al. 2006). The current data further suggest that the lateral and medial cerebellar areas activated during perceptual and motor timing tasks are not specific to timing mechanisms but are also involved in attention to the nontemporal features of sensory stimuli.

As predicted, the inferior olive showed significant response when perceiving a change in stimulus timing. Although the inferior olive neurons have been shown to respond robustly to visual and somatosensory stimuli (Bloedel and Ebner 1984; Hoffmann et al. 1976; Simpson 1984), the activation of the inferior olive in the current study cannot be attributed solely to visual stimulation (Bloedel and Ebner 1984; Simpson 1984). Our results show significantly higher response to perception of change in stimulus timing when compared with attention to timing. During attention to timing condition, the rhythmically occurring non-odd visual stimuli were identical to the odd stimuli, which differed only in their time of occurrence. Thus the inferior olive response was specific to the perception of modulation in the temporal attributes of the stimulus. This is consistent with the results of our previous fMRI study showing inferior olive activation during perception of complex temporal sequences of visual stimuli compared with isochronous sequences (Xu et al. 2006). Our findings are in general agreement with electrophysiological studies that showed activation of the inferior olive and climbing fibers by unexpected sensory events (Bloedel and Bracha 1998; Gellman et al. 1983; Kim et al. 1987). In these studies, the sensitivity of inferior olive/climbing fibers to unexpected sensory events was interpreted as evidence supporting the view that the olivocerebellar system signals errors in movement (Bloedel and Bracha 1998; Kim et al. 1987). In the current study, the odd stimuli in all three conditions (timing, orientation, and color detection conditions) can be considered unexpected events; however, the inferior olive was activated only in the timing condition. It may be argued that motor preparation and button press in the current study were not clearly separable from the BOLD response related to the perception of odd stimuli given the temporal resolution of fMRI methods (Hernandez et al. 2002; Liu et al. 2006). However, based on our previous study, which showed inferior olive activation only during perception of stimulus timing without motor activity, the inferior olive activation in the current study is unlikely related to motor preparation or motor response to stimulus timing. It is also unlikely that the absence of inferior olive activation in the spatial orientation and color detection conditions is due to motor response-related “suppresion” of inferior olive activity. The motor responses occurred after the odd stimuli and the reaction time in the timing condition did not differ from that in the spatial orientation detection condition.

Our results provide evidence supporting the specificity of the inferior olive response to stimulus timing, a finding consistent with the view that the inferior olive and the climbing fiber system contribute to both motor and nonmotor cognitive processes by encoding temporal information.

**GRANTS**

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