

Inferior Olive Response to Passive Tactile and Visual Stimulation with Variable Interstimulus Intervals

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Abstract The unique anatomical and electrophysiological features of the inferior olive and its importance to cerebellar function have been recognized for decades. However, understanding the exact function of the inferior olive has been limited by the general lack of correlation between its neural activity and specific behavioral states. Electrophysiological studies in animals showed that the inferior olive response to sensory stimuli is generally invariant to stimulus properties but is enhanced by unexpected stimuli. Using functional magnetic resonance imaging in humans, we have shown that the inferior olive is activated when subjects performed a task requiring perception of visual stimuli with unpredictable timing (Xu et al. *J Neurosci* 26 (22):5990–5995, 2006, Liu et al. *J Neurophysiol* 100 (3):1557–1561, 2008). In the current study, subjects were scanned while passively perceiving visual and tactile stimuli that were rendered unpredictable by continuously varying interstimulus intervals (ISIs). Sequences of visual stimuli and tactile stimuli to the right hand were presented separately within the same scanning session. In addition to

the activation of multiple areas in the cerebellar cortex consistent with previous imaging studies, the results show that both tactile and visual stimulation with variable ISIs were effective in activating the inferior olive. Together with our previous findings, the current results are consistent with the electrophysiological studies in animals and further support the view that the inferior olive and the climbing fiber system primarily convey the temporal information of sensory input regardless of the modality.

Keywords fMRI · Tactile · Visual · Temporal · Inferior olive · Cerebellum

Introduction

The inferior olivary complex is the largest group of nuclei in the brain stem (approximately 6 mm in diameter and 13 mm in vertical length) located bilaterally in the ventrolateral aspect of the medulla oblongata and is the sole source of the climbing fiber system; it is one of the two afferent systems of the cerebellum. A distinctive feature of the olivary neurons is their tendency to fire spontaneously (at 1–10 Hz) and their synchrony with the activating groups of Purkinje cells [3, 4]. Axons of the inferior olive neurons project to the contralateral cerebellar cortex as climbing fibers with each olivary neuron generating approximately ten climbing fibers each, forming one-on-one synapse with a Purkinje cell [5].

The climbing fibers response to sensory stimulation was shown to be independent of the stimulus intensity and duration and did not correlate with stimulus presentation frequencies higher than 10 Hz, a property attributed to the prolonged action potential of olivary neurons followed by a relatively long (100 ms) refractory period [6–10]. The

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inferior olive neurons were also shown to be particularly responsive to unexpected stimuli such as perturbations during movement [5, 6, 11–14]. These unique response properties have been interpreted differently to support different theories regarding the function of the climbing fiber system: as an unexpected event detector, movement error detector or a system that encodes the timing of the sensory events [15–18].

Using functional magnetic resonance imaging (fMRI) at 3 Tesla in normal human subjects, we have shown that the inferior olive is activated when subjects are perceived sequences of visual stimuli with multiple interstimulus intervals (ISIs, complex rhythms) but not isochronous sequences. There was also no inferior olive activation during motor performance of complex or isochronous sequences [1]. This finding was consistent with electrophysiological animal studies showing decreased responsiveness of the olivary neurons to sensory input during self-produced movement, which may explain the failure of prior functional imaging studies to demonstrate inferior olive activation [19–21]. We have further shown that the inferior olive response was specific to the unexpected change in the timing of visual stimuli (but not the unexpected change in their color or spatial orientation) [2]. However, thus far, the inferior olive fMRI activation has been limited to the visual modality using active behavioral tasks that require perception and a motor response to stimulus timing. The inferior olive response to passive visual and tactile stimulation has not been demonstrated in humans, although this was the main method used in electrophysiological studies in animals [12, 22, 23]. The aim of the current study is to test the efficacy of passive stimulation using visual and tactile stimuli with unpredictable timing in activating the inferior olive.

Materials and Methods

Thirteen subjects (eight men and five women; mean age \pm SD 39.83 \pm 14.27 years) participated in the study. All subjects were right-handed and had normal or corrected to normal visual acuity. All subjects gave a written informed consent according to the guidelines approved by the Minneapolis Veterans Affairs Medical Center and the University of Minnesota human subjects' committees.

Paradigm

Subjects were scanned during tactile and visual stimulation in separate runs but in the same scanning session (two runs for each condition). Each run consisted of six blocks (each 32 s) of tactile or visual stimulation with variable ISIs interleaved with six blocks of rest (each 18 s) in a block (box-car) design.

For both tactile and visual stimulation, six different ISIs were used to construct temporal sequences (rhythms) that were presented pseudo-randomly such that no rhythm was repeated in a stimulation block. Each block contained eight different rhythms, with each rhythm consisting of six ISIs (e.g., CEADBF, AECDFB, where $A=1,200$ ms, $B=1,000$ ms, $C=800$ ms, $D=600$ ms, $E=400$ ms and $F=200$ ms). Thus the timing of each stimulus was unpredictable.

Tactile stimuli were generated by an MRI-compatible piezo transducers applied to the tips of right thumb and index finger (Mag Design and Engineering, Sunnyvale, CA). Stimulation with this device causes 1 to 2 mm skin displacement and a “clicking” sensation. During tactile stimulation, subjects were instructed to close their eyes and remain immobile while paying attention to the right hand.

The visual display was projected through a backlit screen at the head of the scanner bed and viewed via a mirror attached to the head coil. During visual stimulation, subjects were instructed to fixate on a very dark non-blinking disk (luminance = 1.53 cd/m²) at the center of the screen during rest and to attend to visual stimuli during stimulation. Eye movements were not monitored; however, this is unlikely to affect the findings of the study. The visual stimuli were presented at the center of the screen with no peripheral stimuli to provoke eye movements. Visual stimuli (200-ms duration) consisted of a white disk (luminance = 129.30 cd/m²) on a black background (luminance = 0.22 cd/m²).

Functional MRI

Blood oxygenation level-dependent 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 192 \times 192, in-plane resolution 3 \times 3 mm, slice thickness 3 mm. Thirty-four axial slices covering the entire 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 \times 256, in-plane resolution 1 \times 1 mm, slice thickness 1 mm.

Data Analysis

Statistical parametric mapping (SPM5) software (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (MathWorks, Inc., Natick, MA) was used for fMRI data preprocessing and analysis. The first three volumes of each run were discarded to allow for T1 equilibration. The remaining 165 volumes in each run (total of 330 volumes/subject) were realigned to the first image

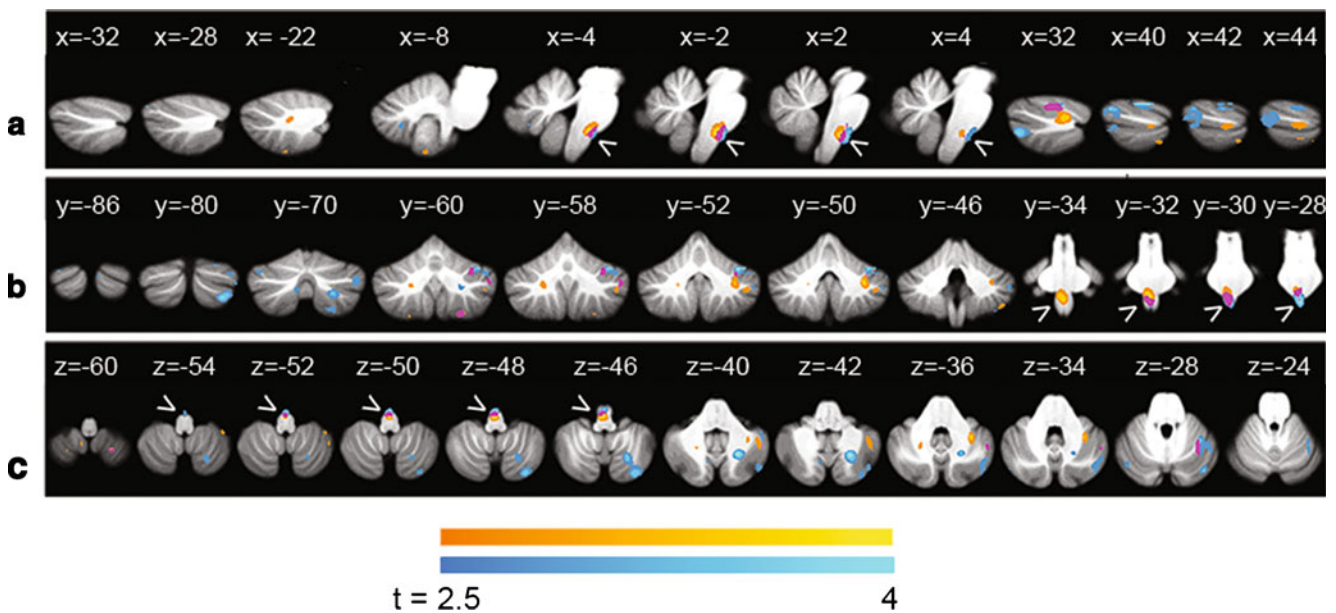


Fig. 1 Statistical parametric maps of cerebellum and brain stem during tactile (orange-yellow) and visual (blue) stimulation with variable ISIs. Areas of overlap are shown in purple. Maps are shown on sagittal **a**, coronal **b**, and axial templates **c**. Shown are stereotaxic coordinates (in

mm relative to the anterior commissure): *x*: (+) right, (–) left; *y*: (+) anterior, (–) posterior; and *z*: (+) superior, (–) inferior. Arrow heads Inferior olive

and normalized to the Montreal Neurological Institute (MNI) brain template with an enlarged box to include the entire cerebellum and brain stem ($Z=85$ to -70 mm). T1-weighted anatomical images were coregistered to the functional scans and transformed into the same MNI template. The functional data were spatially smoothed with a Gaussian kernel of 8 mm full width at half maximum to decrease spatial noise. The head motion parameters were added to the statistical analysis model as covariates of no interest.

fMRI data were analyzed using the general linear model (GLM) and the theory of Gaussian fields as implemented in SPM5. Two conditions (tactile and visual stimulation) were modeled as epochs using a box-car function. A parameter estimate for each condition was calculated for each voxel. Statistical parametric maps for each condition were obtained for each subject using appropriately weighted

contrasts of the parameter estimates (Fig. 1). Intersubject alignment of cerebellar and brain-stem maps was further improved using nonlinear normalization to a high-resolution atlas template of the human cerebellum and brain stem [spatially unbiased atlas template (SUIT)] [24]. A second level group analysis was then performed treating intersubject variability as random effect [25]. Statistical threshold of $p \leq 0.001$ (uncorrected for multiple comparisons) was used for random effect results. Homologous activations below statistical threshold are listed for comparison.

Results

We focused on activations within the cerebellum and brain stem (for whole brain activations, please see Table S1). As

Table 1 Cerebellar and brain-stem areas activated during tactile and visual stimulation with variable interstimulus intervals

Region	(A) Tactile		(B) Visual	
	<i>X, Y, Z</i> (mm)	<i>t</i> Value	<i>X, Y, Z</i> (mm)	<i>t</i> Value
Right lobule VI	32, –50, –35	3.21	40, –58, –25	4.40
Right crus I			40, –78, –29	3.86
Left crus I			–28, –86, –27	3.22
Right crus II			30, –80, –47	3.81
Right VIIb			36, –48, –57	3.12
Right lobule VIIa	42, –46, –53	3.21		
Right inferior olive	3, –32, –48	3.05	2, –29, –48	3.83
Left inferior olive	2, –32, –47	3.62	–2, –30, –49	4.49

Corrected *p* value at inferior olive activation was ≤ 0.05 after small volume correction (3-mm radius). Cerebellar regions' nomenclature is according to Schmahmann's atlas [35]. Lobules were identified using human cerebellum MRI atlas [36]

expected tactile stimulation activated multiple areas in cerebellar cortex including right anterior (lobule VI) and posterior (lobule VIIIb) lobes corresponding to the sensorimotor hand areas shown in previous studies (Table 1) [26, 27]. Tactile stimulation also activated the inferior olive (Table 1). Visual stimulation activated multiple areas in the cerebellar cortex in agreement with previous studies [28]. As expected from our previous studies, visual stimulation activated the inferior olive, overlapping the tactile activation. Conjunction analysis further identified the inferior olive ($-2 -30 -48$, $t=3.61$) and right lobule VI ($34, -63 -27$, $t=3.39$) as areas activated in common to both tactile and visual stimulation and without significant interaction between conditions [29]. Direct subtraction showed tactile-specific activation in right lobule VI ($38 -58 -41$, $t=2.99$) and lobule VIIIb ($42 -46 -51$, $t=2.90$) and visual-specific activation in right crus II ($48 -76 -41$, $t=3.22$).

Discussion

An important finding of this study is the activation of the inferior olive during tactile stimulation which has not been shown in previous functional imaging studies in humans. The overlapping tactile and visual activation of inferior olive, demonstrated in the current study, is consistent with the anatomical and electrophysiological findings in animals and likely represents the activation of the principal olive which is the largest of the three main subnuclei (the principal olive and the dorsal and medial accessory olives) of the inferior olivary complex. In humans, the principal olive constitutes 85% of the whole olivary complex, and its increase in size in primates is believed to parallel the expansion of the lateral cerebellar hemispheres which reciprocally project to the principal olive via the dentate nucleus [30, 31]. Spinal pathways mediating somatosensory projections to the inferior olivary complex primarily terminate in the much smaller dorsal and medial accessory olives (which project to the intermediate cerebellum/interpositus nucleus). However, all major subnuclei of the inferior olive including the principal olive were shown to respond to visual and several somatosensory submodalities including tactile and proprioceptive stimulation [12, 22].

The current study also demonstrates the feasibility of detecting inferior olive response using fMRI and a “passive” sensory stimulation paradigm in which subjects were not required to memorize or generate a motor response to the presented stimuli. Passive sensory stimulation is not influenced by the subjects performance, motor coordination, or effort and can potentially be standardized and utilized as a noninvasive method to investigate human disorders, such as essential tremor and autism, in which the inferior olive has been implicated [32–34].

The current results are consistent with our previous findings of inferior olive activation during perception of complex rhythms and perception of a single stimulus with unexpected timing. Both tactile and visual stimulation using variable ISIs were effective in activating the inferior olive. Stimulation with isochronous sequences was not applied in the current study; however, our previous studies showed no inferior olive activation when perceiving isochronous sequences [1]. Therefore, together with our previous results, the current results further demonstrate the sensitivity of the inferior olive to the temporal structure of sensory input regardless of the modality.

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Conflicts of interest For all authors, potential conflicts do not exist.

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