Brainstem BOLD response to visual and acoustic stimuli

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Abstract— **Understanding the fundamental roles of brainstem function resulting in proper motor control is critical to motor-rehabilitation after brain injuries. In particular, vestibular and reticular formation nuclei are thought to be associated with spasticity in chronic stroke patients. We used two kinds of stimuli in 10 healthy subjects to activate these nuclei while collecting high-resolution (1.5-mm) fMRI across the majority of brainstem. Optokinetic stimuli evoked illusory self-motion to activate the vestibular nuclei. Acoustic-startle stimuli were sets of loud tones designed to activate of the reticular formation. We summarized the response represented in a form of activation volume, mean percent signal change, and the phase delay (time lag) following the stimulus. We observed patterns of significant activations in the brainstem but did not find significant differences between the stimulus. We conclude that more sensitive measurement techniques are needed to reliably detect vestibular and reticular formation nuclei responses.**

I. Introduction

The brainstem modulates critical functions involved in sensorimotor control [1] and is composed of many clusters of nuclei [2]. Following stroke, the normal functions of these nuclei are disrupted by reduced cortical inhibition, resulting in impairments such as spasticity. One clear aspect of spasticity is hyperreflexia, a common movement disorder [3]. It is unclear specifically which dysfunctional nuclei are primarily responsible for hyperreflexia. Miller et al. stimulated stroke survivors with acoustic-startle (AS) bursts, measuring electromyographic (EMG) activity in the sternocleidomastoid muscle as an indirect measure of activity in lateral vestibular nuclei (LVN) [4]. They found that the level of asymmetry of the response to the acoustic startle burst was correlated to clinical measures of spasticity, which are reflective of hyperreflexia. However, it is possible that the AS bursts may have activated the reticular formation (RF) [5]. Functional neuroimaging of the brainstem could help resolve this debate, but few studies have attempted neuroimaging of the vestibular nuclei (VN) and reticular formation (RF).

Functional magnetic resonance imaging (fMRI), with whole-brain coverage and millimeter resolution, is likely the optimal tool to investigate human functional brainstem activity, but there remain challenges. With a complex vascular structure and small size, the brainstem is more vulnerable than rest of the brain to physiological artifacts such as cardiac pulsatility and respiration. Another challenge is that the hemodynamic response in the brainstem differs from the more well-researched cortex [6]. Once activity is identified, there is not yet an atlas for functional neuroimaging that clearly defines the boundaries of the nuclei. Despite these challenges, there is some evidence that functional brainstem activity in LVN and RF can be measured. In particular, Wildenberg et al. [7] measured VN activation using an optokinetic stimulus [8], to induce the sensation of self-motion. They observed a single $2mm^3$ region of activity in the LVN after multiple comparison correction. Thus, functional imaging of brainstem nuclei remains preliminary and more work is needed.

Our goal was to observe and differentiate functional activation in LVN and RF using 3T fMRI in healthy individuals. We aimed to reproduce the Wildenberg et al. study using an optokinetic stimuli, but also used an AS stimulus hypothesized to excite both VN and RF. We used a high-resolution fMRI sequence optimized for subcortical imaging and used a stimulus alternation frequency designed to avoid physiological confounds. Our goal is to enable clinical assessments of brainstem function.

II. Materials and Methods

A. Participants

We recruited 11 neurologically intact participants with normal or corrected-to-normal vision (mean age 46.36, SD 14.41, 4 males). After obtaining informed consent according to the University of Texas at Austin Institutional Review Board, participants underwent two sessions of fMRI scanning. All participants experienced the optokinetic stimuli and 10 experienced the AS stimuli (Figure 1). We dropped one subject due to technical issues with their fMRI session.

B. Experimental setup

The optokinetic stimulus was a two-dimensional moving and rotating checkerboard (Fig. 1) intended to elicit a feeling of self-motion that stimulates the lateral vestibular network in healthy adults [9]. Auditory stimuli designed to elicit an Acoustic Startle Response (ASR) were based on previous work [5]. Loud (100 dB) pulses (50-ms duration, 1kHz) were played through a binaural headset at a pseudorandom time points within a minimum of interval of 1s. Both stimuli were presented as blocked alternations consisting of 15 seconds of stimulation followed by 15 seconds of rest. Fig. 1 (left) illustrates the protocols. We ran both stimuli on the same individual on different days. Functional images were obtained

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using a T2*-weighted, spiral-trajectory sequence with TR/TE of 1.5s/38ms to obtain cubic 1.5-mm voxels [10]. An illustration of the stimuli and coverage is shown in Fig. 1 (middle). For each subject, we also collected a structural volume using a T1 MPRAGE sequence with a resolution of $1mm^3$, which was used to permit co-registration of the data obtained from the two experiments.

C. Data analysis

We performed preprocessing including slice timing correction, movement correction within each run and between runs, registration to each subject's own native space and then co-registered to MNI152 standard space using the ANTs resliced by FreeSurfer [11]. We estimated between TR motions with temporal smoothing of 5 TRs to improve SNR; this approach compensates for the lower SNR obtained at these higher spatial resolutions. We discarded the runs in each subject where more than 0.75mm/TR occur, the remaining runs were averaged across 7~10 runs containing 80 TRs and 100 TRs for vection and ASR respectively. We did not apply spatial smoothing due to the need to localize small nuclei. We determined the significance of each voxel by ranking its signal against a null distribution generated using a non-parametric permutation of 1000 iterations, reordering stimulus and rest blocks for each iteration. We created a customized region-of-interest (ROI) to cover the brainstem in the MNI152 space described in Fig. 1 (b). Vestibular nuclei are located inferior and lateral to the 4th ventricle while reticular formation (RF) is superior to the ponto-medullary junction and surrounds the neuraxis [12]. We chose the entire VN instead of LVN due to the small size of the LVN. Voxels were filtered with an uncorrected significance level of $p \leq 0.05$. Additionally, we analyzed the whole brainstem response after excluding the cortico-spinal tract located anterior of the pons to inspect the overall response of the brainstem. The common coverage of the brainstem available in all subjects is described in Fig 3, which includes the whole VN but likely does not encompass the entire RF. We quantified the brainstem volume using automatic segmentation with FreeSurfer [13].

D. Measurements

Our main outcome measures were: 1) number of significantly activated voxels forming a connected structure within each ROI (nVox); 2) mean strength of activity on each side of the brainstem (% change); and 3) mean phase of the response (rad) representing the time delay of the fMRI response to the stimuli. We modeled the response to our block-design stimuli as a sinusoid at 1/30 Hz. We ran bootstrap analysis to provide a null distribution observed across our data in each stimulus. Within subject comparisons between the two stimuli were made using a paired t-test ($\alpha < 0.05$).

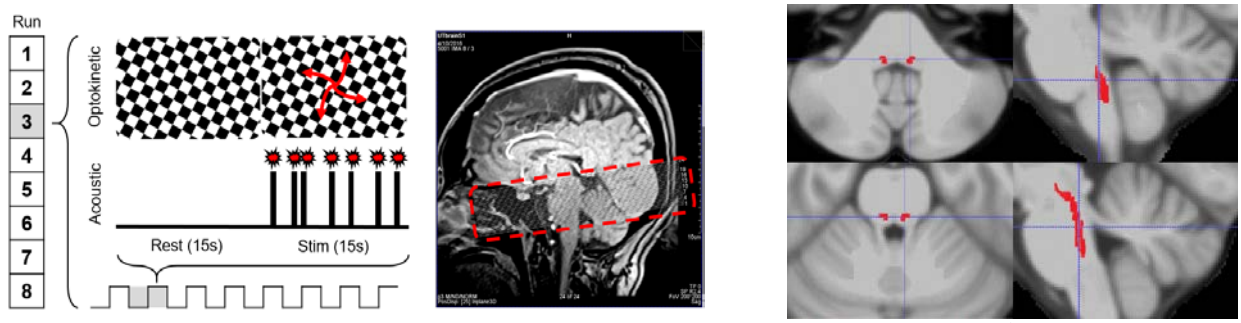
E. Linear regression modeling

We examined additional covariates using a linear regression model. Our linear regression model contained dependent variables of activity strength (number of voxels) and average response amplitude, and with covariates of age and brainstem volume in each subject's native space.

III. Results

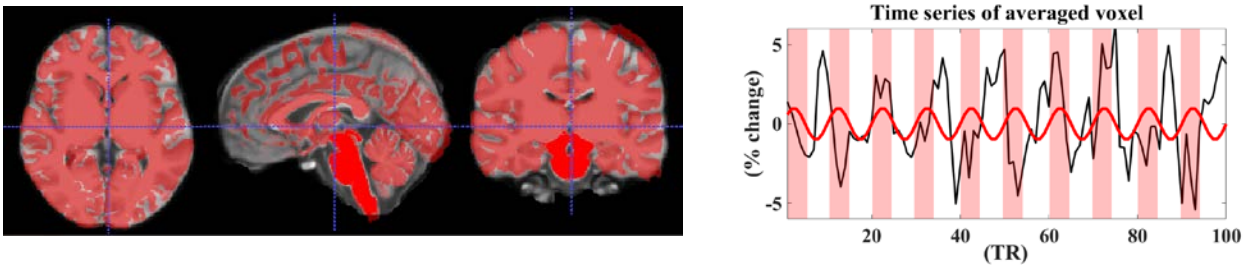
A. Response to vection stimuli

Over the whole brainstem, we observed 3576 mm^3 activated voxels, 0.66% change in mean response, and a 3.5 rad mean delay. In the VN, we found an average of 22.3 mm^3 of activation with mean amplitude 0.32%. In the RF, we had 14.9 activated voxels, 0.24% amplitude. Results are summarized in Table I and the group response is visualized in Fig 3. In two of the younger subjects, we observed bimodal phase distributions (Fig 4). The average motion/TR across the subjects was 0.47 ± 0.17 mm.



(a) (b)

Figure 1. (a) Experimental design – the acoustic burst/visual optokinetic stimuli are presented during the Stim block (15 seconds) and Rest block (15 seconds). Slice orientation and limited FOV during fMRI scanning are presented. (b) The vestibular network (VN) (top) and reticular formation (RF) (bottom) were qualitatively masked in the MNI152 space.



(a) (b)

Figure 2. (a) Co-registration (ANTs) and segmentation (b) Representative data showing fMRI response to a sinusoidal stimulus input.

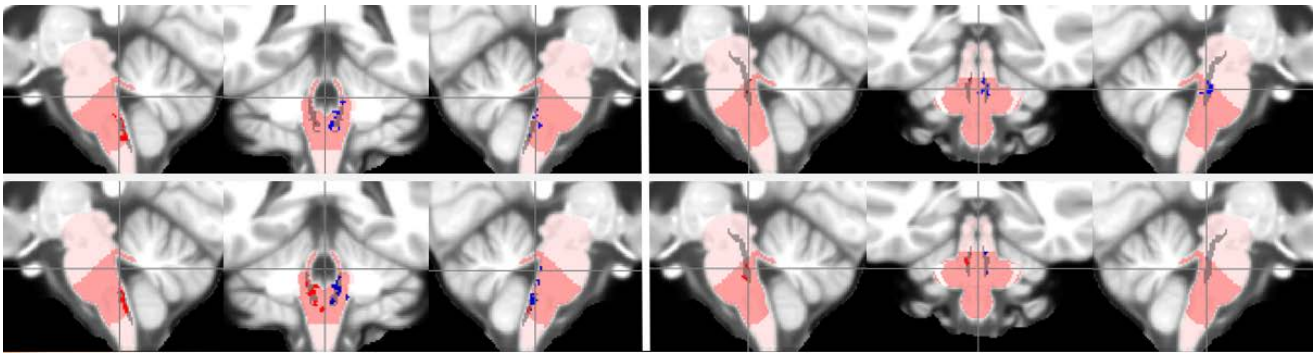


Figure 3. The common coverage within the brainstem is in dark red. The average response of all subjects within condition is represented from Top left (vestibular network with vection stimuli), top right (reticular formation with vection stimuli), bottom left (vestibular network with acoustic stimuli) and bottom right (reticular formation with acoustic stimuli). In each brainstem, red represents left side response and blue represents right side response.

TABLE I. MEASUREMENTS FOR OPTOKINETIC STIMULUS (CI 95%)

Stats	ROI	Both	Left	Right
nVox (mm^3)	Bstem	3576.3 [1498.4, 6298.2]	1780.8 [800.7, 3124.0]	1795.6 [703.6, 3161.3]
	VN	22.3 [12.0, 32.0]	11.6 [3.0, 22.4]	10.8 [4.8, 17.8]
	RF	14.9 [1.8, 35.3]	2.6 [1.0, 4.4]	12.3 [0.4, 31.8]
Avg Response (% change)	Bstem	0.66 [0.53, 0.84]	0.65 [0.51, 0.84]	0.67 [0.53, 0.85]
	VN	0.32 [0.19, 0.45]	0.24 [0.08, 0.40]	0.28 [0.16, 0.39]
	RF	0.24 [0.12, 0.34]	0.24 [0.12, 0.35]	0.16 [0.05, 0.28]
Avg phase delay (rad)	Bstem	3.54 [2.82, 4.08]	3.52 [2.83, 4.07]	3.56 [2.90, 4.09]
	VN	3.38 [1.78, 4.68]	2.62 [1.00, 4.24]	3.51 [1.86, 5.02]
	RF	2.59 [1.33, 4.02]	2.96 [1.38, 4.45]	1.46 [0.07, 2.86]

B. Response to acoustic stimuli

Over the whole brainstem, we observed 3106 mm^3 activation, and 0.79% amplitude. In the VN, we found 46.8 mm^3 activation with 0.30% amplitude. In the RF, we had 10.6 mm^3 activation, and 0.19% amplitude. Results are summarized in Table II and visualized in Fig 3. Again, bimodal phase distributions were observed in the younger subjects. The average motion/TR was 0.39 ± 0.18 mm.

TABLE II. MEASUREMENTS FOR ACOUSTIC STIMULUS (CI 95%)

Stats	ROI	Both	Left	Right
nVox (mm^3)	Bstem	3106.6 [1710.8, 4852.3]	1532.9 [895.6, 2357.4]	1573.7 [870.1, 2490.8]
	VN	46.8 [20.2, 76.8]	24.6 [4.3, 53.5]	22.2 [8.8, 39.2]
	RF	10.6 [1.7, 23.1]	9.0 [0.0, 24.2]	1.6 [0.2, 3.3]
Avg Response (% change)	Bstem	0.79 [0.57, 1.03]	0.72 [0.54, 0.92]	0.86 [0.61, 1.13]
	VN	0.30 [0.17, 0.45]	0.28 [0.12, 0.45]	0.25 [0.16, 0.34]
	RF	0.19 [0.10, 0.29]	0.10 [0.00, 0.21]	0.09 [0.03, 0.19]
Avg phase delay (rad)	Bstem	3.21 [2.72, 3.72]	3.18 [2.62, 3.72]	3.24 [2.69, 3.73]
	VN	2.62 [1.35, 3.79]	2.30 [1.05, 3.77]	2.33 [1.09, 3.58]
	RF	2.16 [0.89, 3.41]	1.03 [0.16, 2.20]	1.13 [0.00, 2.52]

C. Comparison between the visual/acoustic stimulus

We compared between stimulus on measurements on 9 subjects with paired Wilcoxon-rank sum test. We found no significant differences towards the type of stimulus in any ROIs or subject (Table III).

TABLE III. NONPARAMETRIC P-VALUES OF OPTOKINETIC VS ASR

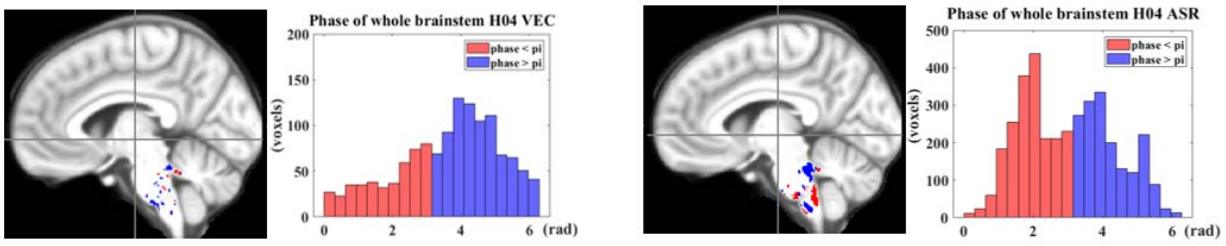
Stats	ROI	Both	Left	Right
nVox (mm^3)	Bstem	0.910	1.000	0.910
	VN	0.359	0.734	0.250
	RF	0.742	0.938	0.742
Avg Response (% change)	Bstem	0.496	0.820	0.359
	VN	0.734	0.734	0.570
	RF	0.547	0.109	0.547
Avg phase delay (sec)	Bstem	0.203	0.164	0.359
	VN	0.570	0.820	0.652
	RF	0.742	0.109	0.625

D. Regression model explaining the response level

Finally, we built a model to explain the measured variables by two factors: subject age and the brainstem volume. We validated a fixed-effect linear model with the low number of samples collected. The results follow that the number of voxels activated in RF during the optokinetic stimuli were both significantly correlated with age and brainstem volume. (p = 0.0029, 0.00049 respectively with R-squared 0.801) However, there were no significant correlations for the ASR stimulus.

IV. DISCUSSION

When exposed to vection and acoustic-startle stimuli, we expected significant localized response in both VN and RF. We did not find any significant voxels activated after performing standard analysis via SPM, because the multiple-comparison correction eliminated all effects. We believe this correction is too conservative for use in brainstem at high resolution We therefore report uncorrected statistical analysis to investigate the trend of the response toward the different type of stimulus. We hypothesized that the VN and RF would respond distinctly to optokinetic and acoustic stimuli, respectively. Wildenburg et al (2011) [7] reported localization of a single voxel in the expected vicinity of the lateral vestibular nuclei activated by optokinetic stimuli on 9 healthy subjects. We were unable to replicate these results. We also found that pontine response varied with age, suggesting an additional factor in conducting brainstem imaging research.



(a) (b)

Figure 4. This is an example of a subject (H04) response to both stimuli across the whole brainstem region. (a) Vection stimuli (b) Acoustic stimuli. The phase distribution of the activated voxels is represented in two colors. Red represents response phase less than 3.14 rad, where we would take it is following the stimulus design and blue represents phase over 3.14 rad, anti-correlated to the response. The proximity of voxel correlated and anti-correlated provides potential difficulty in terms of average signal response.

These data provide helpful preliminary evidence in functional differentiation of brainstem nuclei.

Our inability to replicate the Wildenberg result was most likely caused our inclusion of many older individuals, which reduced the strength of response given the correlations of brainstem response and age in Table IV. However, younger subjects gave robust activations that could be clinically useful, but our subject population was very small. Thus, more experiments are needed to validate the ability to routinely localize VN using fMRI.

Variability in the registration step was indeed a serious challenge. Regardless of the high resolution of the fMRI scans, the anatomical boundaries within the brainstem differentiating the nuclei are difficult to discern using most MRI contrasts, particularly at 3T. The boundaries of specific nuclei are not evident from the T1-MPRAGE anatomical scans. We used the MNI-space defined atlas [12] to estimate the positioning of the nuclei, and qualitatively inspected the standard space registration. Generally, registration was imperfect, based on misalignments observed at clear boundaries such as the superficial surfaces of the superior and inferior colliculi. The combination of misalignment and the lack of clear definition of nuclei boundaries weakens to identify the response clearly.

It may be that our across-subjects analysis was foiled because activations and deactivations occur in proximity among the tightly packed nuclei of the brainstem presented in Fig 4. This hypothesis is supported by the bimodal phase distributions observed in the younger subjects. Therefore, when multiple subjects are registered together using available, rather approximate methods, all effects tend to cancel out.

Head movement during the scan is a critical parameter to control for the fMRI experiment. Mean displacement across all scans and subjects was small, <0.5 mm after motion censoring. However, for vection, there was a correlation between age and head motion (r = 0.53, p =0.12), but this correlation was not observed for the ASR. Nevertheless, head motion may have degraded the quality of the fMRI data for the older, naïve subjects, partly explaining the observed trends with age. We also suspect that our motion censoring threshold was too lenient, but the small subject population gave us little latitude in the censoring.

Finally, the experimental design to achieve a reliable response was difficult because the VN and RF fMRI response effect sizes were not known in advance. In order to control the quality of the runs, simultaneous efferent measurement (e.g. eye tracker for visual, muscle activity measures for acoustic stimuli) would provide the quantitative behavior assessment to evaluate the fMRI for quality control. Increasing the number of participants should increase the statistical power, with a greater focus on younger subjects that yield stronger activations. In addition, older and naïve subjects must be more carefully trained with better head restraints to reduce head motion.

V. Conclusion

We investigated the brainstem neural response via fMRI to characterize the effect towards visual/acoustic stimuli. We were unable to replicate results found in an earlier study localizing LVN. The responses in brainstem nuclei were only detectable using uncorrected statistics, suggesting that more sensitive protocols aimed at younger or better trained subjects are required to reliably quantify functional brainstem activity.

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