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Neurovascular Saturation Thresholds Under High Intensity Auditory Stimulation During Wake

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Abstract

Coupling between neural activity and hemodynamic responses is important in understanding brain function, interpreting brain imaging signals, and assessing pathological conditions. Tissue state is a major factor in neurovascular coupling and may alter the relationship between neural and hemodynamic activity. However, most neurovascular coupling studies are performed under anesthetized or sedated states which may have severe consequences on coupling mechanisms. Our previous studies showed that following prolonged periods of sleep deprivation, evoked hemodynamic responses were muted despite consistent electrical responses, suggesting that sustained neural activity may decrease vascular compliance and limit blood perfusion. To investigate potential perfusion limitations during natural waking conditions, we simultaneously measured evoked response potentials (ERPs) and evoked hemodynamic responses using optical imaging techniques to increasing intensity auditory stimulation. The relationship between evoked hemodynamic responses and integrated ERPs followed a sigmoid relationship where the hemodynamic response approached saturation at lower stimulus intensities than the ERP. If limits in blood perfusion are caused by stretching of the vessel wall, then these results suggest there may be decreased vascular compliance due to sustained neural activity during wake, which could limit vascular responsiveness and local blood perfusion. Conditions that stress cerebral vasculature, such as sleep deprivation and some pathologies (e.g., epilepsy), may further decrease vascular compliance, limit metabolic delivery, and cause tissue trauma. While ERPs and evoked hemodynamic responses provide an indication of the correlated neural activity and metabolic demand, the relationship between these two responses is complex and the different measurement techniques are not directly correlated. Future studies are required to verify these findings and further explore neurovascular coupling during wake by assessing local field potentials, vascular expansion, hemodynamic response localization.

Keywords

ERP; neurovascular coupling; NIRS

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1. Introduction

Neural activation increases regional blood flow and volume to deliver metabolites and remove waste products from activated tissue (Sokoloff, 1981; Attwell and Laughlin, 2001). The coupling between neural activity and blood supply is important for understanding brain function and physiological control. Additionally, brain imaging technologies, such as diffuse optical imaging, functional magnetic resonance imaging (fMRI), and positron emission tomography (PET), rely on metabolic measurements as indicators of neural activity. These measured signals are consequences of the coupling between neural activation and hemodynamic responses. However, neurovascular coupling can be influenced by a number of factors such as stimulus parameters (Blood et al., 1995; Nemoto et al., 2004), oxygen supply (Sicard and Duong, 2005), and vascular responsiveness (Piilgaard and Lauritzen, 2009), each of which is influenced by behavioral state. In order to properly interpret hemodynamic measurements evoked by neural activation, a better understanding of neurovascular coupling is required, especially under waking conditions.

Neural activation increases synaptic activity in the surrounding tissue region, which releases glutamate and triggers astrocytic activation. Neurons and astrocytes respond by releasing vasoactive agents that trigger blood vessel dilation, increasing regional blood flow (Iadecola and Nedergaard, 2007; Jakovcevic and Harder, 2007). While both neurons and astrocytes participate in the hemodynamic response, we measured electrical signals from neural activation. Following neural activation, initial increases in hemodynamic activity are localized to cortical column regions and later response components spread across the tissue (Malonek and Grinvald, 1996; Sheth et al., 2004a; Woolsey et al., 1996). Initial studies of neurovascular coupling showed a greater increase in oxygen supply than consumption following neural activity, describing the phenomenon as “watering the entire garden for the sake of one thirsty flower” (Malonek and Grinvald, 1996) implying that much more blood is delivered than is required. However, these studies were performed under anesthesia, a state in which basal neural activity and metabolic demand is relatively low compared to wake, and is therefore likely to influence the neurovascular response. When compared to the anesthetized state, increased basal neural activity during waking results in increased metabolite demand which causes vascular stretching to supply that demand, thereby limiting subsequent perfusion increases during evoked activity (Schei et al., 2009).

In order to investigate the relationship between neural activity and hemodynamic responses, graded increases in stimulation are needed. Previous studies using varying stimulation frequency have shown linear coupling between neural activity and cerebral blood flow (CBF) (Gerrits et al., 1998; Martindale et al., 2003; Matsuura and Kanno, 2001; Ngai et al., 1999; Ureshi et al., 2004) and cerebral blood volume (CBV) (Martindale et al., 2003; Sheth et al., 2003) in rodents. Other studies varying stimulation intensity have shown nonlinear coupling between evoked field potentials and CBF (Hewson-Stoate et al., 2005; Jones et al., 2004; Ureshi et al., 2005; Nielsen and Lauritzen, 2001) and hemoglobin oxygenation (Devor et al., 2003; Sheth et al., 2004b). To explore the potential for saturation of blood perfusion during wake, we measured evoked responses from the auditory cortex in rats to different stimulation intensities. This paradigm alleviates potential habituation effects that appear with high frequency stimulation.

Tissue state has profound effects on vascular compliance resulting in state-dependent evoked responses and neurovascular coupling (Jones et al., 2008; Schei et al., 2009). Previous studies under sleep deprivation conditions have suggested that there may be limits to evoked vascular responses when the tissue has experienced prolonged periods of wake (Schei and Rector, 2011). While many studies have investigated neurovascular coupling under a variety of anesthetics, few have studied these responses during natural waking

activity. Therefore, the evaluation of neurovascular coupling under different tissue states is of critical importance in order to better understand brain function, thereby better interpret brain imaging signals and evaluate pathological conditions. We investigated the relationship between evoked neural and hemodynamic responses in awake animals to assess potential limitations in vascular responsiveness following high intensity stimulation. We chronically implanted animals with electrodes and optodes to simultaneously measure evoked response potentials (ERPs) and regional changes in hemoglobin oxygenation from the auditory cortex in rodents following varying intensity auditory stimulation. We hypothesize that limits in vascular delivery during the wake state will mute evoked hemodynamic responses while increasing neural responses to high intensity stimulation.

2. Experimental Procedures

We used adult, female Sprague Dawley rats (250-300 g, Simonsen Laboratories, Gilroy, CA, USA, $n = 15$) to simultaneously measure evoked electrical and hemodynamic responses. Prior to implantation, we trained animals to remain still under restrained conditions, using a custom made wrap, for a one hour period (Topchiy et al., 2009). Once animals exhibited consistent good behavior: no movement, chattering, or vocalizations throughout the restraint period, we proceeded with implantation, which has been previously described (Schei et al., 2009). Briefly, animals were anesthetized with Isoflurane anesthesia and implanted with four blunted stainless steel screw electrodes, two over the frontal lobe, one over the right parietal lobe, and one over the left parietal lobe, to measure electric potential differences from a ground reference placed in the occipital lobe (Figure 1, closed circles, modified from (Schei et al., 2009)). Additional screws were used to secure the headstage to the skull (open circles). Two light emitting diodes (LEDs, 1.6 mW, B5b-436-30, Roithner Lasertechnik, GmbH, Vienna, Austria; ELC-880-17, chip 880 nm, 10 mW, 20 mA) emitting 660 nm and 880 nm light were positioned 1 mm rostral to lambda, over the temporal ridge. A photodiode (PC1-6, Pacific Silicon Sensors, Westlake Village, California, USA) was positioned 3 mm rostral to the LED to collect back-scattered light. Since specific regions of the primary sensory cortex are responsible for processing the middle latency and late latency response components to particular sensory stimulation (Penfield and Jasper, 1954; Phillips et al., 2011) Each average was triggered by the stimulus; therefore, the recording electrode and optodes measured those responses involved with the auditory stimulus. We measured electrocardiography (ECG) by placing an insulated stainless steel wire (New England Wire, Lisbon, NH, 212-50F-357-0), with 1 mm end exposed, subcutaneously down the thoracic cavity. The recording wires were connected to a miniature plug and held in place with dental cement. All procedures were approved by the Washington State University Animal Care and Use Committee (IACUC). Animals were given two weeks recovery before recording.

To minimize movement artifact, animals were restrained during recordings. A speaker was placed approximately 7 cm above the animal's head and delivered a train of five auditory clicks (0.3 ms pulse width, 5 Hz) at random intervals between 8 and 22 seconds. This stimulation frequency generated the largest hemodynamic responses in awake animals (Martin et al., 2006). Additionally, since the hemodynamic impulse response begins approximately 1 second after stimulation, the duration of stimulation did not affect the hemodynamic response characteristics. We varied the intensity of the stimulation between 50 dBA to 90 dBA, in 5 dBA intervals. Each recording day consisted of a 65 dBA stimulus intensity recording as a control and either 1 or 2 testing stimulus intensities. Animals were recorded daily and the stimulus intensity recording paradigm was randomly alternated across a period of approximately 2 months. A continuous stream of data was amplified (photodiode $\times 1$, EEG $\times 1000$, ECG $\times 1000$), filtered (0.1 Hz - 3.2 kHz), and digitized (20 kHz, 24 bits) using custom built hardware (Rector and George, 2001).

The light entered the tissue and scattered; photons collected by the detector followed a banana-shaped mean trajectory from the source to the detector. Hemodynamic information primarily arose from the superficial layers of the tissue between the light source and detector (Okada et al., 1995). The recording electrode was positioned between the light sources and detector, but slightly lateral due to physical size constraints. Volume conduction and signal averaging assisted in reducing non-specific neural activity and recording auditory ERPs. Thus, electrical and optical signals indicate evoked neural and hemodynamic activity from the same region of the auditory cortex.

Based on the different absorption properties of oxygenated and deoxygenated hemoglobin (HbO₂ and Hb, respectively), we calculated relative changes in concentration using the modified Beer-Lambert law (Boas et al., 2001; Huppert et al., 2009). Deoxygenated hemoglobin was calculated by

$$Hb = \frac{\frac{\Delta A_{\lambda_1}}{x} \epsilon_{Hb\lambda_2} - \frac{\Delta A_{\lambda_2}}{x} \epsilon_{Hb\lambda_1}}{\epsilon_{Hb\lambda_1} \epsilon_{HbO_2\lambda_2} - \epsilon_{Hb\lambda_2} \epsilon_{HbO_2\lambda_1}}, \quad (1)$$

optical where ΔA is the change in absorbance for a specified wavelength, x is the pathlength, ϵ is the extinction coefficient for either HbO₂ or Hb at a specified wavelength (Prahl, 1999). Oxygenated hemoglobin was calculated by

$$HbO_2 = \frac{\frac{\Delta A_{\lambda_2}}{x} \epsilon_{Hb\lambda_1} - \frac{\Delta A_{\lambda_1}}{x} \epsilon_{Hb\lambda_2}}{\epsilon_{Hb\lambda_1} \epsilon_{HbO_2\lambda_2} - \epsilon_{Hb\lambda_2} \epsilon_{HbO_2\lambda_1}}. \quad (2)$$

A pathlength correction factor was not used since the two wavelengths were sufficiently separated to minimize cross-talk (Boas et al., 2004). Total hemoglobin (HbT) was calculated as the sum of HbO₂ and Hb,

$$HbT = HbO_2 + Hb. \quad (3)$$

All data was analyzed using octave (www.octave.org), an open source numerical computation package. Stimuli from a single recording were averaged together and sorted by stimulus intensity into 5 dB bins. To assess electrical activity, we measured the P1 and N1 components of the first ERP response in the train of five (Knight et al., 1985) and the rectified response integrated for 1 second following the first stimulus in the series. The ERP P1-N1 amplitude provided information on synchronous cortical activity while the integrated ERP provided information about the total neural activity. Statistical significance was calculated using a Mann-Whitney U-test. To compare responses across animals, we normalized the response to the P1-N1 amplitude or integrated ERP from the 65 dBA condition, respectively.

To assess the hemodynamic responses, we measured the HbO₂ peak amplitude and Hb trough amplitude. In addition, we rectified HbO₂ and Hb responses and integrated for 5 seconds starting at 1 second following the first stimulus in the series. Statistical significance was calculated using a Mann-Whitney U-test. The evoked hemodynamic responses were normalized to the HbO₂ peak amplitude or integrated response following the 65 dBA intensity condition, respectively.

In order to measure saturation thresholds of the ERP and hemodynamic responses, we plotted the ERP versus evoked hemodynamic responses to different intensity stimuli. Data were first fit to a linear regression model,

$$y=mx+b, \quad (4)$$

where y is the hemodynamic response, x is the electrical response which produces the hemodynamic response, m is the change in the hemodynamic response over the change in the electrical response to different intensity stimulation, and b is the intercept. We expect that a null electrical response would not generate a hemodynamic response; therefore, we set $b = 0$ in the linear regression.

Data were also fit to the Hill equation (Hill, 1910),

$$R=R_{max} * \frac{I^n}{K^n+I^n}, \quad (5)$$

where R is the hemodynamic response, R_{max} is the maximum hemodynamic response, I is the electrical response, K is the electrical response which produces a hemodynamic response at one half R_{max} , and n is the Hill coefficient. In this fit, the electrical activity was the driving factor and the change in hemoglobin concentration was the response. We compared the linear and nonlinear models and found that the nonlinear model best fits the ERP versus hemodynamic response data significantly better than the linear model ($p < 0.01$, F Test). We used Monte Carlo simulations to calculate the 95% confidence intervals for the fits to the Hill equation.

3. Results

Typical ERPs to different intensity stimulation from 2 different animals are shown in Figure 2 illustrate the consistency of responses across multiple recordings as well as different animals. The largest responses follow the first stimulus in the train and responses to the following four stimuli were smaller. A stimulus frequency of 1 Hz does not show habituation in subsequent ERP responses (Franceschini et al., 2008); however, it generates a smaller evoked hemodynamic response and a train of impulses interfere with the hemodynamic response, potentially influencing the characteristics. Increasing the stimulus intensity generated larger amplitude ERPs. The average and standard error ERPs, normalized to the P1-N1 amplitude from the 65 dBA condition, to different intensity stimuli from fifteen animals are shown in Figure 3. With increasing stimulus intensity, the initial ERPs following the first stimulus in the train were larger in amplitude. The lower panel expands the first and second ERPs in the train to illustrate the increased amplitude of the ERP components. We measured the amplitude of the P1 and N1 ERP components marked with the dashed lines.

The P1 and N1 ERP components were larger with increasing stimulation intensity (Figure 4, top panel; $\rho = 0.97$, Spearman correlation). At low intensity stimulation (50 dBA - 70 dBA), the ERP P1-N1 amplitude did not significantly differ from 65 dBA; however, with higher intensity stimulation, the ERP P1-N1 was significantly larger ($p < 0.01$, Mann-Whitney U-test). Similarly, the integrated ERP was significantly larger following higher intensity stimulation compared to the integrated ERP following 65 dBA stimulation (Figure 4, bottom panel; $p < 0.01$, Mann-Whitney U-test; $\rho = 0.98$, Spearman correlation)

Example evoked hemodynamic responses from two animals are shown in Figure 5. We found an increase in HbO2 concentration and a decrease in Hb concentration following stimulation. As with the electrical responses, hemodynamic responses were larger amplitude with higher intensity stimulation. The average and standard error evoked hemodynamic responses, normalized to the HbO2 peak amplitude from the 65 dBA condition, to different intensity stimuli from fifteen animals are shown in Figure 6. Following the auditory

stimulus, there was an increase in HbO₂ concentration (Figure 6 A) with a peak occurring around 2.88 ± 0.02 s. Similarly, following stimulation there was a decrease in Hb concentration (Figure 6 B) with a trough occurring around 3.16 ± 0.06 s. Changes in HbT concentration are shown in Figure 6 C. The HbT waveform illustrates the temporal differences between the HbO₂ and Hb responses.

Both the HbO₂ peak amplitude and the Hb trough amplitude increased with larger intensity stimulation (Figure 7; HbO₂ $\rho = 1$, Hb $\rho = 0.98$, Spearman correlation). At low intensity stimulation, the HbO₂ peak and Hb trough did not significantly differ from the 65 dBA intensity stimulation; however, at high intensity stimulation, the HbO₂ peak and Hb trough were significantly larger ($p < 0.01$, Mann-Whitney U-test). Similarly, the integrated HbO₂ and Hb responses increased with higher intensity stimulation (HbO₂ $\rho = 0.85$, Hb $\rho = 0.85$, Spearman correlation). At low intensity stimulation the HbO₂ and Hb integrated responses did not significantly differ, but at high intensity stimulation the integrated responses were significantly. The combined HbO₂ and Hb amplitudes ($\rho = 0.98$, Spearman correlation) and integrated responses ($\rho = 0.85$, Spearman correlation) were significantly larger at higher intensity stimulation.

Comparing the evoked neural response to the evoked hemodynamic response showed a sigmoidal relationship (Figure 8). At high stimulus intensities, the ERP continued to increase while the hemodynamic response approached saturation. Data was fit to Equation 5 and the parameters are shown in Table 1. At low stimulation levels (50 - 65 dBA), the ERP P1-N1 amplitude, integrated ERP, HbO₂ peak, and Hb trough were similar. As the stimulus intensity increased, the ERP P1-N1 amplitude, integrated ERP, HbO₂ peak, and Hb trough increased. At high stimulation levels, the ERP P1-N1 amplitude and integrated ERP increased while the HbO₂ peak and Hb trough leveled off. The individual response components to varying stimulation intensities were calculated from the average evoked responses shown in Figures 3 and 6.

4. Discussion

ERP and evoked hemodynamic responses following varying intensity auditory stimulation showed a nonlinear relationship during the wake state. As the stimulus intensity increased, both the ERP and evoked hemodynamic response increased. At high intensity stimulation, however, the evoked HbO₂ and Hb amplitude and integrated response plateaued while the ERP P1-N1 amplitude and integrated response continued to increase. Under high intensity stimulation, the vasculature may saturate, approaching limitations in blood perfusion due to limited vessel compliance.

We found that the evoked changes in HbO₂ and Hb were large relative to the evoked changes in HbT. Our signals may be dominated by changes in oxygenated hemoglobin concentration in the venules (Hillman et al., 2007). As a result, there may be large changes in blood flow, which elicits large changes in oxygenated hemoglobin concentration while changes in blood volume were not as prevalent. Others have also shown that changes in neural activity are highly correlated with changes in cerebral blood flow (Masamoto et al., 2008).

While the evoked neural and hemodynamic responses correlated to increasing intensity stimulation, the coupling between these responses remains complex. Electrical and optical measurement techniques provide an indirect assessment of the neurovascular relationship, making interpretation difficult. Due to volume conduction of electrical signals and widespread synaptic activity during sensory processing as well as diffuse vascular responses, both local and global, it is difficult to determine that the same tissue region is

responsible for the electrophysiological and optical responses. Both the electrophysiological and the optical responses are related to activity integrated from a relatively extended area, in which individual neuronal and vascular activities could add up according to different rules. Time-triggered averages to specific stimuli help to narrow the responses to activation of the auditory cortex; however, limitations with the present methods preclude a direct causal relationship.

To further investigate neurovascular coupling and the potential effects of vascular expansion limits in more localized regions, additional studies are required. Single- or multi-unit spiking activity can reflect activity levels from specific cells generating action potentials, but do not accurately report activity from a small group of cells which may be served by a micro-capillary bed. Local field potentials may correspond better to population dynamics of neural activity from a small group of cells and changes in regional hemodynamics correlate more strongly to local field potentials than spiking activity (Lauritzen, 2001; Logothetis et al., 2001; Logothetis, 2002). Additionally, better indications of vascular expansion and blood flow could be obtained through direct microscopic recordings of vascular changes using implantable video techniques (Rector and George, 2001).

Since our animals were fully awake and restrained during the recordings, there is a possibility that blood pressure changes due to stress could influence the results. Several precautions were taken to minimize stress during recordings and decouple stress and evoked responses. Animals were trained to remain still while wrapped and conditioned to the recording paradigm; stimulus intensities were randomized across recording days and within recording sessions; and behavioral monitoring showed little or no chattering, movement, and vocalizations. Additionally, we did not observe significant heart rate increases with higher intensity stimulation ($p < 0.01$, Mann-Whitney U-test). However, it is difficult to completely eliminate stress from recording procedures and it may have contributed to increased blood flow during high intensity stimulation. Future studies could incorporate blood pressure measurements to allow for correlation between stress, high intensity stimulation, and evoked responses.

Previous studies investigating neurovascular coupling have shown a power law relationship between ERP and hemodynamic responses to increasing intensity stimuli where the electrical response approached saturation at lower stimulus intensities than the hemodynamic response (Devor et al., 2003; Hewson-Stoate et al., 2005; Sheth et al., 2004b). However, these studies employed anesthetized preparations that may affect neurovascular coupling. Anesthetized conditions may allow for vessels to relax and be more compliant so that the hemodynamic response may approach saturation at higher stimulus intensities than during waking conditions. Furthermore, the coupling between neural activity and hemodynamic responses has been shown to be dependent on the stimulus paradigm (Nemoto et al., 2004). The duration of stimulation may also effect the coupling. Long duration stimulation generates robust hemodynamic responses, but may also increase basal blood flow.

In one study comparing neurovascular coupling between wake and anesthetized conditions, there was a linear coupling during wake which was not present in the anesthetized rat when changing stimulus frequency (Martin et al., 2006). Higher frequency stimulation may initiate inhibition effects, reducing metabolic demand as well as the hemodynamic response. By varying the stimulus intensity, rather than frequency, we observed the hemodynamic response approach saturation at lower stimulus intensities than the electrical response. Taken together, these studies illustrate fundamental differences in neurovascular coupling between wake states and anesthetized states.

Our results suggest that there are limits to perfusion during high intensity auditory stimulation under waking conditions. Most hemodynamic studies use anesthetized or sedated conditions, which could reduce basal neural activity and thereby decrease metabolic demand. Waking activity may be a highly metabolically active state due to energetic demands associated with many spontaneous action potentials and sodium-potassium pumps constantly working to restore membrane potential gradients. Basal blood delivery may be higher during this state, as evident by small changes in HbT but large changes in HbO₂ and Hb, compared to anesthetized or quiet sleep states where metabolism may be lower (Alkire et al., 1995, 1999; Nakao et al., 2001; Braun et al., 1997). Under high intensity stimulation, the vasculature has shown limits in responsiveness. These results suggest that compliance issues may be more important during wake than during anesthesia. Situations which continually stress the vasculature, such as sleep deprivation or pathologies (e.g., epilepsy), may decrease vascular compliance, approach saturation limits in metabolic delivery, and lead to tissue trauma.

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Figure 1.

Rats were implanted with 2 light emitting diodes (LEDs, 660 and 880 nm) placed over the right parietal lobe and a photodiode placed 3 cm rostral to the LEDs. Bregma (B), lambda (L), the midline (M), and the temporal ridge (T) were used for positioning reference. Blunted stainless steel screw electrodes were placed in the frontal and parietal lobes (closed circles), measuring potential differences from the ground electrode placed in the occipital lobe. Additional screws were placed in the skull to support the implant (open circles). An electrocardiography (ECG) wire was placed next to the thoracic cavity. This figure was modified from (Schei et al., 2009).

Figure 2.

Average (black traces) and standard error (gray traces) evoked response potentials (ERPs) to different intensity stimuli illustrate typical recordings from two different animals. The vertical lines represent the train of 5 speaker clicks (5 Hz, 8-22 s ISI). The largest responses followed the first speaker click and habituation effects muted the subsequent responses to the following four speaker clicks. For increasing stimulus intensity, we observe increased ERP amplitudes following the first click.

Figure 3.

Average (black traces) and standard error (gray traces) evoked response potentials (ERPs) to different intensity stimuli were normalized to the P1-N1 amplitude at 65 dBA and averaged across 15 animals. The solid vertical lines represent the train of 5 speaker clicks (5 Hz, 8-22 s ISI). The dashed vertical lines indicate the P1 and N1 components of the ERP following the first click. The region in the dotted box is expanded in the bottom panel to illustrate ERPs from the first and second speaker clicks. The vertical scale bar is twice the normalized ERP P1-N1 amplitude.

Figure 4.

A) The evoked response potential (ERP) P1-N1 amplitude to different intensity stimuli, normalized to the ERP P1-N1 from 65 dBA, show that responses are larger with increased intensity stimuli ($\rho = 0.97$, Spearman correlation). Higher intensity stimuli (75 dBA) elicited ERPs with P1-N1 amplitudes significantly larger than the 65 dBA intensity stimulus (* $p < 0.01$, Mann-Whitney U-test). B) The ERP integrated across the train of stimuli for different intensity stimuli, normalized to the integrated ERP from 65 dBA, increased with higher intensity stimuli ($\rho = 0.98$, Spearman correlation). Responses to higher intensity stimuli (80 dBA) that were larger than responses to the 65 dBA intensity stimulus (* $p < 0.01$, Mann-Whitney U-test).

Figure 5.

Average (black trace) and standard error (gray trace) evoked hemodynamic responses to different intensity stimuli from two typical animals illustrate that the increase in oxygenated hemoglobin concentration (HbO2) and decrease in deoxygenated hemoglobin concentration (Hb) was larger with increasing intensity stimuli. The vertical lines represent the train of 5 speaker clicks (5 Hz, 8-22 s ISI).

Figure 6.

Average (black trace) and standard error (gray trace) evoked hemodynamic responses to different intensity stimuli were normalized to the oxyhemoglobin (HbO₂) peak amplitude following 65 dB stimulation and averaged across 15 animals. The vertical lines represent the train of 5 speaker clicks (5 Hz, 8-22 s ISI). The HbO₂ peak and deoxyhemoglobin (Hb) trough were larger in amplitude with higher intensity stimulation. The total hemoglobin (HbT) response was smaller in amplitude than the HbO₂ and Hb responses (note that the scale is half of the HbO₂ and Hb scales).

Figure 7.

A) Evoked oxyhemoglobin (HbO2) response peak amplitudes and deoxyhemoglobin (Hb) response trough amplitudes to different intensity stimuli, normalized to the HbO2 peak from 65 dBA stimulation, are shown for 15 animals. Responses increased with higher intensity stimuli (HbO2 $\rho = 1$, Hb $\rho = 0.98$, Spearman correlation). B) Rectified HbO2 and Hb evoked response amplitudes were combined for each stimulus intensity ($\rho = 0.98$). C) Evoked HbO2 and Hb integrated responses are shown for different intensity stimuli (HbO2 $\rho = 0.85$, Hb $\rho = 0.85$). D) Rectified integrated HbO2 and Hb evoked responses were combined for each stimulus intensity ($\rho = 0.85$). Higher intensity stimuli elicited significantly larger HbO2 and Hb response amplitudes and integrated responses compared to the 65 dBA intensity stimulus (* $p < 0.01$, Mann-Whitney U-test).

Figure 8.

Hemodynamic responses versus evoked response potentials (ERPs) to different intensity stimuli fit to the Hill equation (black trace, Equation 5) along with 95% confidence intervals calculated from Monte Carlo simulations (gray traces). The top two rows show the hemodynamic responses versus the integrated ERP. The hemodynamic response approached saturation at a lower stimulus intensity compared to the integrated ERP. The bottom two rows show the hemodynamic response versus the ERP P1-N1 amplitude. The hill equation fit shows that the hemodynamic response approached saturation at a lower stimulus intensity than the ERP P1-N1 amplitude; however, the confidence intervals were larger compared the fits to the integrated ERP.

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Table 1

Fit parameters for hemodynamic responses versus integrated ERP and P1-N1 amplitudes fit shown in Figure 8. The K , n , and R_{max} parameters were found using a least squares fit to the Hill equation (Equation 5). Goodness of fit is reported by R^2 . To illustrate saturation effects, we reported the integrated ERP and P1-N1 amplitude at 90% of R_{max} . The hemodynamic response amplitudes and integrated responses approached saturation at a lower stimulus intensity than the integrated ERP and P1-N1 amplitude. The integrated ERP approached saturation at a lower stimulus intensity than the ERP P1-N1 amplitude.

Σ ERP						
HbO2 Amplitude	Hb Amplitude	HbO2 + Hb Amplitude	Σ HbO2	Σ Hb	Σ (HbO2 + Hb)	
K	1.56	1.62	1.59	1.66	1.68	1.68
n	4.60	5.10	4.79	4.91	6.73	5.80
R_{max}	8.30	8.18	16.5	25.8	26.2	52.0
90%	2.51	2.50	2.52	2.60	2.34	2.45
R^2	0.99	0.99	0.99	0.99	0.99	0.99

ERP Amplitude						
HbO2 Amplitude	Hb Amplitude	HbO2 + Hb Amplitude	Σ HbO2	Σ Hb	Σ (HbO2 + Hb)	
K	1.65	1.83	1.74	2.03	2.00	1.99
n	3.59	3.47	3.50	3.20	3.87	3.56
R_{max}	8.53	8.91	17.4	30.3	29.9	59.4
90%	3.05	3.45	3.26	4.0%	3.53	3.69
R^2	0.99	0.99	0.99	0.98	0.99	0.99