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State Dependent Auditory Evoked Hemodynamic Responses Recorded Optically with Indwelling Photodiodes

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Abstract

Implantable optical technologies provide measurements of cerebral hemodynamic activity from freely behaving animals without movement constraint or anesthesia. In order to study state-dependent neural evoked responses and the consequential hemodynamic response, we simultaneously measured EEG and scattered light changes in chronically implanted rats. Recordings took place under freely behaving conditions, allowing us to compare the evoked responses across wake, sleep, and anesthetized states. The largest evoked electrical and optical responses occurred during quiet sleep compared to wake and REM sleep while isoflurane anesthesia showed a large, late burst of electrical activity synchronized to the stimulus, but an earlier optical response.

1. Introduction

Optical imaging of cerebral hemodynamic activity provides an index of neuronal activation and can be used as an indirect probe of neural processing and function. Several investigators have utilized optical brain imaging techniques [1-9], although animals typically remain anesthetized or restrained throughout the recordings. Alterations in conscious states, however, have profound effects on global cerebral perfusion [10], evoked electrical responses [11,12], and evoked hemodynamic responses [2,5]. How these different states affect neurovascular coupling is still not well understood. While some investigators have studied changes in the hemodynamic response between wake and anesthetized conditions [2,5], the wake-like conditions are confined to restrained animals. In order to probe state-dependent neurovascular coupling, we measured changes in local cerebral perfusion across different states of consciousness under freely behaving conditions.

Sleep research has long postulated that sleep leads to restoration of neural tissue [13]; however, direct causal evidence for this theory remains elusive. Previous sleep studies have shown a decrease in metabolic activity during quiet sleep (QS) compared to wake and an increase in metabolic activity during rapid eye movement (REM) sleep compared to QS [10, for review see 14,15]. Additionally, evoked electrical response potentials (ERPs) are larger during QS than wake [11,12] and evoked optical signals are larger in amplitude under wake conditions than anesthetized conditions [2,5]. Other optical studies in humans show slow (on the order of

minutes) net increases in oxygenated hemoglobin and deoxygenated hemoglobin during wakeful stimulation and net decreases during sleep stimulation [16].

In order to systematically investigate evoked cerebral hemodynamic responses during different conscious states, we measured the evoked electrical response along with the evoked optical response of 660 nm light reflected from the rat auditory cortex following auditory stimulation during wake and sleep of freely behaving animals and during isoflurane anesthesia.

2. Methods

Hardware

We measured reflected light changes from the rat auditory cortex using an implanted 1 mm silicon photodiode (Figure 1A, PC1-6, Pacific Silicon Sensors, Westlake Village, CA, USA) and a 660 nm light emitting diode (LED, Figure 1B, 1.6 mW, B5b-436-30, Roithner Lasertechnik, GmbH, Vienna, Austria). We chose 660 nm light because LEDs at this wavelength provided the brightest illumination, which is necessary in order to collect a sufficient number of photons to extract the hemodynamic response with sufficient signal-to-noise. To reduce the LED size while maintaining high intensity output, we thinned the plastic casing of the LED to a width of 1 mm. A small drop of epoxy on the end of the LED acted as a lens to focus the light onto the cortex. The photodiode collected multiply scattered light from the cortex. To maximize light collection efficiency, we coupled the photodiode to a 1 mm thick plastic optical fiber encased in stainless steel hypodermic tubing which was in direct contact with the tissue (Figure 1A). Other methods form an optical window over the cortex, and use lens coupled imaging devices. By placing the optical fiber in contact with the tissue, we reduced photon loss within the free space between the dura and photodiode. The fiber was cut to 8 mm in length and polished prior to implantation.

Implantation

During initial surgical procedures, female Sprague-Dawley rats (250-300 g, Simonsen Laboratories, Gilroy, CA, USA, $n = 4$) were anesthetized using isoflurane gas mixed with pure oxygen. We delivered 5.0% isoflurane for the initial induction and then reduced the concentration (2.3% - 2.9%) for the remaining surgical procedure. The anesthesia depth was monitored via the toe pinch reflex, electrocardiogram (EKG), and respiration. All procedures were approved by the Washington State University Animal Care and Use Committee (IACUC). The body temperature was maintained at 37 °C (± 0.5 °C) using a heating pad and rectal thermometer probe and the heart rate and respiration rate were continuously monitored using subcutaneous pin electrodes. After shaving the rat's scalp and fixing the head in a stereotaxic frame, we made an incision down the midline exposing the skull. We retracted the skin, removed the fascia, and detached the temporalis muscle from the skull in order to expose the area over the auditory cortex. Eight 0.9 mm diameter holes were drilled around the skull using a dental drill and blunted stainless steel screws were inset making contact with the cortex without applying pressure or puncturing the dura. One frontal screw and one parietal screw measured EEG, one occipital screw served as a ground reference (Figure 1C, solid circles), and the remaining screws secured the headstage in place (Figure 1C, open circles). A 1.4 mm hole was drilled approximately 3 mm caudal to bregma, over the thinned temporal ridge, for insertion of the photodiode and another 1.4 mm hole was drilled approximately 3 mm caudal to the photodiode for insertion of the LED. Two insulated stainless steel wires with 1 mm exposed wire ends were placed in the thoracic cavity and neck muscles to measure the heart rate (EKG) and muscle activity (EMG), respectively. A miniature plug connected wires from the LED, photodiode, and recording electrodes and all components were secured using dental cement. Flunixin (1.1 mg/kg) was delivered subcutaneously and a liberal amount of antibiotic

ointment was applied to the base of the headstage each day for 3 days following the recovery. Rats were allowed a 2 week recovery period before recording.

Recordings

For freely moving conditions, rats were placed in a 26.5 cm × 26.5 cm × 34 cm acrylic chamber and tethered using a 55 cm long cable and swivel commutator. Signals were amplified, (AC photodiode x200, DC photodiode x1, EEG x1000, EKG x1000, EMG x1000), filtered from 0.1 Hz to 3.2 kHz and digitized at 10 kHz using custom built hardware [17]. To reduce noise in the recordings, the frontal EEG screw electrode was grounded.

In order to generate a substantial hemodynamic response, we stimulated the cortex through a 10 Hz burst of 5 speaker clicks (~64 dB) delivered at random intervals between 2 and 13 seconds throughout the recording. Although we recorded from a large area of cortex, we provided a relatively specific stimulus and therefore assume that the evoked electrical and optical responses were primarily due to auditory stimulation, despite volume conduction [18]. Rats were recorded under freely moving conditions, during wake and sleep states, with food and water available *ad libitum*. In order to achieve adequate sleep, we recorded rats for 24 consecutive hours. Wake/sleep states were sorted into 2 second epochs and scored based on EEG delta power (0 – 3 Hz) and EMG activity. Wake characteristics included high frequency, low amplitude EEG signals along with large amplitude, variable EMG signals. QS states showed low frequency, high amplitude EEG signals along with a constant moderate amplitude EMG from sustained muscle tone. REM sleep exhibited high frequency, low amplitude EEG, similar to wake, with a nearly flat EMG due to muscle atonia [19]. Two 24 hour recordings were averaged together in order to improve signal-to-noise in the optical signal. An anesthetized state was achieved using a mask to deliver isoflurane gas mixed with oxygen at high concentrations (5.0%) for initial induction. The concentration of isoflurane was then reduced to ~2.0% before experimentation to maintain a deep anesthetic plane and was monitored by muscle relaxation, respiratory rate, evoked electrical responses, and burst suppression ratio (BSR). The BSR, defined as the sum of the silent periods over the total epoch time, was 0.74 ± 0.05 . We averaged together 500 stimuli and then allowed the rat one week to recover before the next recording.

All data were analyzed using Octave, an open source data analysis package (www.octave.org). The evoked electrical and optical responses were sorted by state and averaged together across stimuli. The evoked electrical response was recorded from the parietal screw electrode, closest to the auditory cortex. The amplitude of the evoked electrical response potential (ERP) was reported as the amplitude difference between the first peak (P1) and first trough (N1) components of the first ERP.

The hemodynamic response was measured in terms of changes in 660 nm light. Light entered the cortex and was multiply scattered before being detected by the photodiode. Due to the relatively high number of photons absorbed by hemoglobin at this wavelength, we assume that our signal is predominantly affected by changes in absorption rather than scattering [20]. Additionally, according to the hemoglobin absorption curve, deoxygenated hemoglobin absorbs ten times more 660 nm light than oxygenated hemoglobin. However, changes in oxyhemoglobin and deoxyhemoglobin concentrations are coupled with changes in blood volume and flow. Figure 2 shows the sensitivity of the optical signal to changes in tissue oxygen saturation (top) and to changes in blood volume (bottom) for varying initial oxygen saturation conditions. As the blood becomes more oxygenated, the optical response is more sensitive to changes in deoxygenated hemoglobin and less sensitive to changes in blood volume. A detailed analysis is given in the Appendix. Bulk brain tissue oxygen saturation is lower than arterial oxygen saturation; thus, we expect that our optical signal will be affected by a combination of changes in deoxyhemoglobin concentration and blood volume. Since we are limited to one

wavelength, the deoxyhemoglobin and blood volume components cannot be separated and additional studies are needed using at least two wavelengths.

To assess evoked changes in the hemodynamics due to neural activation, the AC optical signal was divided by the DC optical signal reporting fractional changes (dI/I) and then inverted so that an increase in signal corresponded to an increase in blood volume/deoxyhemoglobin. The signal peak was defined as the first local maximum following stimulation and the trough was defined as the local minimum following the peak. The peak and trough amplitude were measured in referenced to baseline activity. In order to average across multiple recordings, the optical responses during wake, QS, and REM were normalized to the QS peak. We chose QS because responses were most consistent in this state. Since the isoflurane recordings took place at a different time, these data were not normalized. Evoked electrical and optical response amplitudes and times were measured for 4 animals and the mean and standard error were calculated. Statistical significance was calculated using a Mann-Whitney test. In order to test the state-dependence of the neurovascular response, we plotted the ERP amplitude (P-N1) versus the optical response amplitude (peak-trough) for each individual recording.

3. Results

Figure 3 shows a sample of the ERPs across state from one rat. Under all conditions, the ERP was largest for the first stimulus and subsequent ERPs corresponding to subsequent stimuli were smaller. As previously reported, ERPs during QS were significantly larger in amplitude compared to wake. REM sleep ERPs were similar in size and shape to the ERPs during wakefulness [12]. Under isoflurane anesthesia, a small evoked response appeared after the first stimulus followed by a large, elongated, and late peak due to averaged burst activity synchronized to the stimulus. The average of the bursts forms a response that appears similar to a large and late evoked response, but the underlying mechanisms that generate the burst is different [11]. An example of synchronized individual bursts and the burst average are shown in the lower inset of Figure 3.

Typical evoked optical responses from one rat across all states are shown in Figure 4. While some initial fast optical components may have been present, we did not collect a sufficient number of trials to resolve the fast signal [21]. This study focused on two components from the slower hemodynamic response. During wake, the optical signal showed an initial peak that occurred around 1.8 s followed by a successive trough at 3.3 s. During QS, there was a large increase in dI/I at 2.3 s and a decrease at 4.1 s. During this state, the signal was phase shifted where the peak and trough appeared later compared to the other states. REM sleep exhibited a peak at 1.5 s followed by a trough at 3.3 s. Under isoflurane anesthesia, the evoked optical signal had an initial increase at 1.6 s and a subsequent decrease at 3.2 s.

Evoked optical responses across states for 4 animals are shown in Figure 5. The wake/sleep amplitudes were normalized to the QS peak for each rat. Note that the optical amplitude scaling was twice as large during QS (Figure 5B) than during wake (Figure 5A) and REM (Figure 5C). Since the isoflurane recordings took place on a different day than the wake/sleep recordings, the trace amplitudes were presented as changes from baseline conditions and the amplitudes are based on a different scale. The peaks and troughs in the responses were marked with arrows. The traces show similar trends in peak and trough amplitudes and times during each state along with the variability between animals.

We compared the ERP amplitude, measured as the difference between P1 and N1 and normalized to QS, across states (Table 1). The ERP amplitude was significantly larger during QS than wake, REM and isoflurane ($p < 0.05$). As expected, the ERP amplitude did not differ between wake and REM.

We compared the optical response peaks and troughs across states by plotting the amplitude and time for each animal (Figure 6A). The mean amplitude and time from the 4 rats were plotted for each state along with the vertical and horizontal error bars, respectively. The peak amplitude was significantly larger ($p < 0.05$) during QS than wake or REM; however, the trough amplitude did not significantly differ (circled data points, mean and standard error values shown in Table 1). Both the optical peak and trough during QS occurred later in time than during isoflurane ($p < 0.05$).

In order to illustrate the relationship between the ERP response and the optical response, we plotted the optical amplitude (peak-trough) versus the ERP amplitude (P1-N1) across each state (Figure 6B). During wake, both the ERP and optical amplitude were small compared to QS. During REM, there was a larger range of ERP and optical amplitudes but, on average, lower response amplitudes compared to QS. The ERP amplitude during QS was similar to the burst amplitude during isoflurane anesthesia. Additionally, the optical responses were similar in amplitude.

4. Discussion

Optical studies under freely behaving conditions allowed us to probe the processes involved in neurovascular coupling under wake, sleep, and anesthetized states. The ERP signals during REM sleep were nearly identical to those during wakefulness. While the averaged evoked optical responses were similar in time and amplitude, the individual optical responses experienced high variability in the signal, as evident by a lower signal-to-noise ratio compared to QS. Under wakefulness, movement artifact may have contributed to the noise in the signal. The large, late trough in rat 4 may be residue of a strong movement artifact. During REM sleep, however, movement artifact does not contribute since animals experience muscle atonia, a characteristic of REM sleep. In rat 3, it was difficult to distinguish between deep QS and REM. As a result, the peak and trough in the REM optical response may have shifted later in time, having a time course similar to QS.

The ERP signal during QS exhibited a significant amplitude increase compared to the wake and REM states. The optical peak amplitude was significantly larger during QS, yet the trough amplitude did not significantly differ. The larger hemodynamic response during QS may be due to a lower initial blood perfusion baseline and a larger fractional change. During wake and REM there may be a larger degree of ongoing neuronal activity and a concomitant larger blood perfusion baseline, resulting in a smaller fractional change. Additionally, since blood flow decreases during quiet sleep [10, for review see 14,15], the larger amplitude hemodynamic response during QS may result from blood vessels initially being in a less dilated state, with greater ability to dilate in response to neural activation, resulting in a larger vessel cross-sectional area change. During wake, however, blood vessels may be in a semi-dilated state because they are continually providing metabolites to waking tissue with higher ongoing activity levels, and may undergo a smaller change in diameter due to saturation effects. Alternatively, larger ERPs during QS may result from increased synchronous neural activity recruiting more cells and exhibit a greater need for metabolites. As a result, there may be a larger recruitment of blood or increased deoxyhemoglobin concentration within the activated area during QS compared to wake and REM. These conclusions require additional experiments with multiple wavelengths to further investigate the nature of these hemodynamic changes.

Anesthetics alter the state of cortical cells through enhanced inhibition, potentially affecting neurovascular coupling. Isoflurane anesthesia showed a small initial evoked response along with a late synchronization of electrical burst activity that was larger on average than the waking response [11], but elicited a significantly earlier evoked hemodynamic response. The hemodynamic response may result from a convolution of the ERP and burst responses which

could not be dissected in the present study. The earlier phase shift in the optical signals seen under isoflurane anesthesia may result from a suppression of neurovascular control, or decreased latency in the overall neural response since the ERP also appeared earlier (see Figure 3). Further studies to relate the electrical activity and the optical response are needed to investigate the effects of different anesthetic agents on neurovascular coupling.

A similar hemodynamic response was reported by Chen-Bee et al. where a peak in the inverted optical response occurred around 1 s and a trough occurred around 4 s while the animal was anesthetized with Nembutal and atropine [7]. These studies, however, were conducted using a whisker twitching stimulus paradigm and the optical measurements used 635 nm light. Regardless of these different recording protocols, the similarities between the optical responses reported here under isoflurane anesthesia are striking. However, our sleep and waking responses show a significantly different amplitude and timing that demonstrate behavioral state modulation of neurovascular coupling.

Since most optical studies are performed under a variety of different anesthetics and since alterations in the conscious state drastically affect the optical recordings, hemodynamic response models must address the behavioral state of the tissue under investigation. Different anesthetics may alter the neurovascular coupling as evident by the data presented here and elsewhere [2,5,7]. If the changes in blood perfusion were directly proportional to electrical activity, we would expect a linear increase in the hemodynamic response under QS. However, since the increase in the hemodynamic response under QS was not only larger amplitude, but also phase shifted later in time, a non-linear effect on neurovascular coupling is evident. Furthermore, the relationship between the two responses appears to differ depending on the state of the tissue. Studies with different stimulus intensities to investigate the coupling changes between the electrical and optical response are required. Investigation of the optical response during state transitions can further probe changes in state-dependent coupling.

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Appendix

5. Appendix

Neural activation elicits changes in local oxygenated hemoglobin concentrations and blood volume. These changes can be measured using optical techniques which rely on the absorption properties of blood [22]. For short distances we can assume that scattering is negligible and attenuation of light through a medium can be described by the Beer-Lambert Law

$$I = I_0 10^{-\mu_\lambda d} \quad (1)$$

where μ_λ is the absorption coefficient and d is the path length of light. Considering only the oxyhemoglobin and deoxyhemoglobin components, the absorption coefficient can be written as a linear combination of the oxy- and deoxyhemoglobin absorption extinction coefficients, ϵ_λ , and the total hemoglobin concentration, c .

$$\mu_\lambda = \epsilon_{\lambda_{HbO_2}} c + \epsilon_{\lambda_{Hb}} c \quad (2)$$

Changes in absorption are described as

$$A = \log\left(\frac{I_0}{I}\right) \quad (3)$$

$$A = S \epsilon_{\lambda_{HbO_2}} c d + (1 - S) \epsilon_{\lambda_{Hb}} c d \quad (4)$$

where S is the fractional oxygenated hemoglobin saturation. The sensitivity of absorption changes to oxygenated hemoglobin saturation can be calculated as

$$\frac{dA_{\lambda}}{dS} = \epsilon_{\lambda_{HbO_2}} c d - \epsilon_{\lambda_{Hb}} c d \quad (5)$$

We solved for the total hemoglobin concentration and path length using Equation 4.

$$c d = \frac{A}{\left(S \epsilon_{\lambda_{HbO_2}} + (1 - S) \epsilon_{\lambda_{Hb}}\right)} \quad (6)$$

The sensitivity of absorption to changes in oxygenated hemoglobin saturation as a function of wavelength is

$$\frac{dA_{\lambda}}{dS} = \frac{A \left(\epsilon_{\lambda_{HbO_2}} - \epsilon_{\lambda_{Hb}} \right)}{\left(S \epsilon_{\lambda_{HbO_2}} + (1 - S) \epsilon_{\lambda_{Hb}} \right)} \quad (7)$$

where A = 0.7404 for the example shown in Figure 4. This sensitivity curve is shown in top trace of Figure 2 for varying the initial oxygenated hemoglobin saturation conditions. At 660 nm wavelength, the optical signal becomes more sensitive to changes in deoxygenated hemoglobin at larger fractional oxygenated hemoglobin saturation.

In order to account for changes in blood volume, the absorption coefficient can be written as

$$\mu_{\lambda} = (1 - V) \mu_{tissue} + V \left(S \epsilon_{\lambda_{HbO_2}} c + (1 - S) \epsilon_{\lambda_{Hb}} c \right) \quad (8)$$

where V is the fractional blood volume to total volume and μ_{tissue} is the absorption coefficient of the tissue. The sensitivity of absorption to changes in blood volume can be written as

$$\frac{dA_{\lambda}}{dV} = -\mu_{tissue} d + S \epsilon_{\lambda_{HbO_2}} c d + (1 - S) \epsilon_{\lambda_{Hb}} c d \quad (9)$$

and the path length is

$$d = A \left[\frac{1}{(1 - V) \mu_{tissue} + V S \epsilon_{\lambda_{HbO_2}} c + V (1 - S) \epsilon_{\lambda_{Hb}} c} \right] \quad (10)$$

The sensitivity of absorption to changes in blood volume becomes

$$\frac{dA_\lambda}{dV} = A \frac{\left(-\mu_{tissue} + S \epsilon_{\lambda_{HbO_2}} c + (1 - S) \epsilon_{\lambda_{Hb}} c \right)}{(1 - V) \mu_{tissue} + V S \epsilon_{\lambda_{HbO_2}} c + V (1 - S) \epsilon_{\lambda_{Hb}} c} \quad (11)$$

where $A = 0.7404$ for the example rat in Figure 4, $\mu_{tissue} = 2.63 \text{ cm}^{-1}$ [23], $V = 5.2\%$ [24], and $c = 5.36\text{E-}3$ moles/liter [25]. We assumed that we are predominantly collecting photons traveling through gray matter, that the optical properties of gray matter are similar between human tissue and rat tissue, and that the hemoglobin concentration is similar to whole blood. A plot of the absorption sensitivity to changes in blood volume as a function of wavelength is shown in the bottom trace of Figure 2, varying the initial oxygenated hemoglobin saturation. At 660 nm, the higher the fractional oxygenated hemoglobin saturation, the less sensitive the signal is to changes in blood volume.

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Figure 1.

A 1 mm diameter photodiode (A) was coupled to a 1 mm optical fiber encased in hypodermic tubing and covered with black epoxy. The distal end of the optical fiber was cut to 8 mm length, polished, and placed directly in contact with the cortical tissue to collect light from the cortex. To illuminate the tissue with 660 nm light, an LED (B) was cut down to a 1 mm width and covered with black epoxy. Rats (C) were implanted with an LED and photodiode pair which were placed over the right temporal ridge (T) and 3 mm caudal to Bregma (B) with 3 mm between the LED and photodiode. To assess behavioral state, EEG screw electrodes were implanted in the frontal and parietal lobes and a ground reference screw was placed in the

occipital lobe (solid circles). Five additional screws served as anchors for the headstage (open circles). Neck EMG and EKG wires recorded muscle activity and heart rate.

Figure 2.

The top trace shows the sensitivity of the optical signal to oxyhemoglobin changes as a function of wavelength, described by Equation 7 in the Appendix, for varying initial oxygenated hemoglobin saturation conditions. At 660 nm, the optical signal is more sensitive to changes in deoxyhemoglobin with higher oxyhemoglobin saturation. The lower trace shows the sensitivity of the optical signal to changes in blood volume for varying initial oxygenated hemoglobin saturation conditions. At 660 nm, the optical signal is less sensitive to changes in blood volume with higher oxyhemoglobin saturation, described by Equation 11. An inset of the hemoglobin absorption curve is shown with a line drawn at 660 nm.

Figure 3.

Sample evoked electrical responses from one animal for wake, sleep, and anesthetized states were averaged across stimuli and plotted across time. The vertical lines represent each stimulus in the burst. The ERP amplitude, measured from the first peak (P1) to the first trough (N1), was significantly larger during QS than during wake. During REM sleep, the ERP was similar in amplitude to the ERP during wake. Under isoflurane anesthesia, a small amplitude ERP appeared after the first stimulus followed by a late burst of activity synchronized to the stimulus. The lower inset panel demonstrates electrical recordings from a different rat using single click stimuli. The top trace shows several burst events that synchronize to the stimulus and are stacked on top of each other. The bottom trace shows the corresponding average of the bursts, which appears very similar to a late ERP, but is unrelated to mechanisms that underlie the ERP [11]. The dotted line indicates the time of the stimulus.

Figure 4.

Inverted evoked optical responses were averaged across wake, sleep, and anesthetized states and plotted across time in this example from one rat. The vertical lines represent each stimulus in the burst. To compare sleep state related responses, the wake, QS, and REM traces were normalized to the QS peak while the isoflurane trace was reported as fractional change from baseline, pre-stimulus conditions. An increase in the inverted signal corresponded to an increase in 660 nm light absorption (a decrease in reflected light) and an increase in deoxyhemoglobin/blood volume. The peak amplitude was larger during QS than wake and REM, but the trough amplitude did not significantly differ. Both the peak and the trough were shifted later in time during QS than wake, REM, and isoflurane.

Figure 5.

Optical response traces from 4 animals are shown for wake (A), QS (B), REM (C), and isoflurane (D). The peaks and troughs, marked by arrows, show the amplitude and timing trends. The variability between rats may be caused by different amounts of movement artifact and/or placement differences. During wake, rat 4 showed a large, late trough that may have been influenced by movement artifact. Rat 3 showed thicker traces in QS and REM, as well as rat 4 during isoflurane due to decreased signal-to-noise in these recordings.

Figure 6.

The peak amplitude versus time is plotted for all 4 animals across states (A). The wake/sleep responses were normalized to the QS peak while the isoflurane response was reported as changes from baseline. The mean peak amplitudes and times are plotted with vertical and horizontal standard error bars, respectively. During QS, the peak amplitude was significantly larger compared to wake and REM. The QS peak was shifted significantly later in time compared to isoflurane. The trough amplitude versus time is plotted for all 4 animals across states along with the mean and standard error (circled region). Unlike the peak, the QS trough amplitude did not significantly differ between wake and REM, but it occurred significantly later in time compared to isoflurane. A plot of the optical amplitude (peak-trough) versus the ERP amplitude (P1-N1) for individual recordings is shown in B. Wake states exhibited lower amplitude ERP and optical responses while QS states exhibited larger ERP and optical amplitudes. Evoked responses during REM were similar in amplitude to wake responses, but experienced larger variations between animals. During isoflurane, the optical response was similar in amplitude to QS, and the synchronized burst amplitudes were similar to QS ERP amplitudes.

The mean and standard error values of the electrical and optical responses from 4 animals are shown. The ERP amplitude (P1 – N1), normalized to QS, was smaller during wake, REM, and isoflurane ($p < 0.05$) compared to QS. The isoflurane burst amplitude did not significantly differ from the QS ERP. As previously reported, the ERP amplitude during REM was not statistically different than wake. The optical response amplitudes during wake and sleep were normalized to QS while the optical response amplitude of the isoflurane response was reported as changes from baseline. During QS, the optical response peak amplitude was larger than wake and REM ($p < 0.05$). The QS trough, however, was not significantly larger than wake or REM. Both the QS peak and trough occurred significantly later in time than isoflurane ($p < 0.05$).

Table 1

States	Electrical		Optical	
	ERP Amplitude	Peak Amplitude	Peak Time	Trough Amplitude
Wake	$0.51 \pm 0.085^*$	$0.11 \pm 0.02^*$	1.66 ± 0.12	-0.61 ± 0.2
QS	1.00	1.00	2.38 ± 0.22	-0.36 ± 0.05
REM	$0.486 \pm 0.088^*$	$0.16 \pm 0.06^*$	2.50 ± 0.60	-0.33 ± 0.1
Isoflurane	$0.16 \pm 0.029^*$	$6.3e-5 \pm 2e-5$	$1.63 \pm 0.027^*$	$-4.5e-5 \pm 2e-5$
Iso burst	1.44 ± 0.40			$3.34 \pm 0.16^*$

*
p < 0.05