RANDOM VS REGULAR STIMULATION PARADIGMS IN SCATTERED LIGHT IMAGING EXPERIMENTS

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Introduction

In contrast to functional imaging based on hemodynamic changes, we have imaged fast intrinsic optical changes *in vivo* that closely track the dynamics of electrical responses such as event related potential (Rector, Rogers et al. 2001a). While differences in illumination methods may partly explain our ability to resolve faster responses, differences in stimulation protocols may also contribute. In scattered light imaging experiments, the predominant signals are absorbance changes associated with slow hemodynamic responses that accompany electrical activation of neural tissue. Many investigators have demonstrated a reasonable spatial correspondence of hemodynamic signals during neural activity, including changes in blood oxygenation, blood flow and blood volume, with responses that can be observed electrically (Frostig, Lieke et al. 1990; Malonek, Dirnagl et al. 1997). Some hemodynamic changes can be measured as early as 100 ms post stimulus, but larger signals evolve over many seconds. Most studies have used regular inter-stimulus intervals or EKG-locked stimuli, with various attempts to correct for cardiac pulsations. It is difficult to remove hemodynamic and vascular artifact with regular stimulus intervals, and the large metabolic effects could mask smaller and faster optical signals which are related to electrical activation. We directly compared random vs regular stimulation to assess the impact of the different paradigms on the temporal progression of the optical signals.

Methods

Seven rats were used for acute imaging experiments. Animals were anesthetized with Ketamine and Xylazine, then head-fixed in a stereotaxic instrument. A section of skull overlying the barrel cortex, just large enough to accommodate the optical instrument, was removed. Respiration, heart rate, and temperature were monitored continuously. Scattered light changes were detected by a CCD imager (TC211, Texas Inst., 192x165 pix, 13.75x16 μm pix size) coupled to an image conduit in direct contact with the tissue (Figure 1). Illumination (660 nm LED) was delivered via optical fibers arranged around the perimeter of the image conduit. Four silver wire electrodes at the interface were used to record evoked electrical potentials. The stimuli consisted of brief (0.1 ms) twitches of a single whisker delivered by a metal tube attached to a small speaker. Stimuli were delivered at either regular or random intervals. Images were acquired at 100 frames per second and electrophysiological signals were collected at 10 KHz. Data were collected and averaged over 250 to 4000 stimuli per trial using custom signal conditioning and acquisition hardware and software.

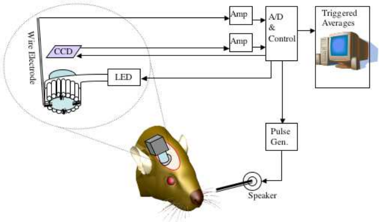

Figure 1. Experimental Set-up

Results

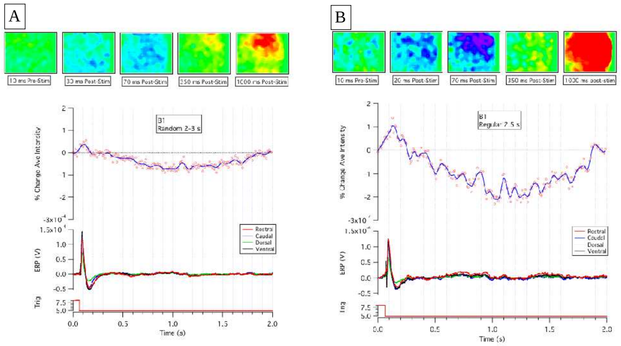

Figure 2. Time-triggered averages when using A) random and B) regular inter-stimulus intervals (ISI). Images are displayed on the same color scale with cool colors representing increases in light and warm colors representing decreases.

A comparison of results for two different stimulation paradigms is shown in Figure 2. The optical response for regular inter-stimulus intervals was larger than that obtained using random inter-stimulus intervals (ISI), but was corrupted by large oscillations that corresponded to cardiac and respiratory rhythms. While the fast optical response at t = 30 ms post-stimulus is clearly seen for random ISI, the slower response predominates in the cardiac-related signal seen for regular ISI. The functional images for random ISI show a fast response more localized than the slower vascular response which may be preferentially distributed over the vasculature.

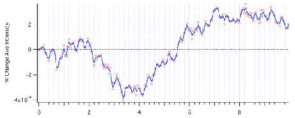

Figure 3. Optical response during longer regular ISI.

For short ISIs (2.5 s), we often do not achieve baseline conditions before the start of the next stimulus. When longer regular ISI are used, a slow oscillatory component becomes apparent. This component is likely due to the 0.1 Hz oscillations present in vasomotion (Mayhew, Askew et al. 1996).

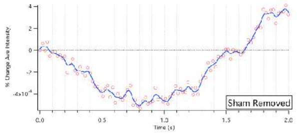

Figure 4. Optical response for regular ISI with interleaved sham control data subtracted.

One strategy for removing these vascular artifacts might be to subtract control images which use interleaved sham stimuli. However, even after subtracting control images, we found that the vascular responses dominated the optical signal (Figure 4) suggesting that periodic vascular components also related to the stimulus become synchronous with the regular stimuli.

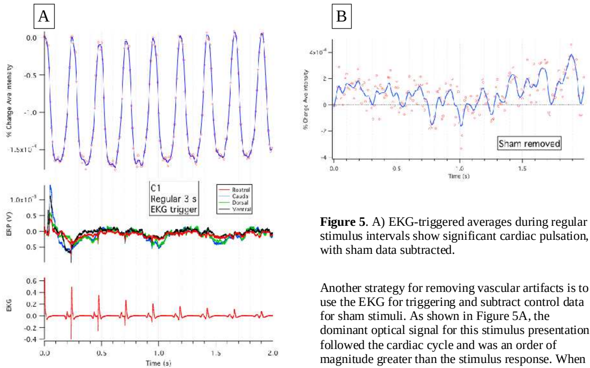

Figure 5. A) EKG-triggered averages during regular inter-stimulus intervals show significant cardiac pulsation, and B) with sham data subtracted.

Another strategy for removing vascular artifacts is to use the EKG for triggering and subtract control data for sham stimuli. As shown in Figure 5A, the dominant optical signal for this stimulus presentation followed the cardiac cycle and was an order of magnitude greater than the stimulus response. When control data was subtracted, the faster responses were masked by insufficient removal of the cardiac pulsations.

Conclusions

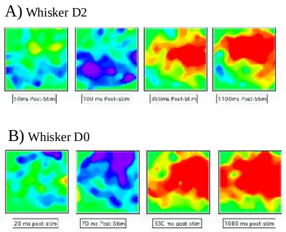

Figure 6. Optical responses for different whiskers stimulated using random inter-stimulus intervals. The fast response had different spatial patterns for the two cases, and was also located in different areas than the later and more diffuse hemodynamic responses which may be preferentially distributed over the vasculature.

We found that use of regular inter-stimulus intervals produced average images that contained cardiac, respiratory, and 0.1 Hz oscillatory components that appear in an average. Each of these processes produced significant vasculature artifact in the functional images. Subtraction of control data did not reduce vascular artifacts in optical measurements when using regular inter-stimulus intervals suggesting periodic vascular components that are not in random phase with the stimulus. While this strategy does remove background level vascular signals, it does not remove the enhanced vascular response to neural tissue activation. This activation-dependent vascular and 0.1 Hz response dominated the optical signal, obscuring the fast intrinsic optical signals. Preliminary evidence suggests that cardiac phase can synchronize to cortical stimulation, indicating that heart/brain networks are tightly coupled (Sandman, Vigor-Zierk, et al., 1992). In contrast, when random inter-stimulus intervals were used, the cardiac, respiratory, and 0.1 Hz oscillatory components were in random phase with the stimulus. Although hemodynamic signals were still present, they were not dominant, allowing imaging of fast optical signals, which track evoked electrical responses closely and are confined spatially (Figure 6). With improvements in signal-to-noise, imaging of fast intrinsic optical signals could be useful for future study of neural activation dynamics in the somatosensory cortex, as well as other systems.

References

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