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A Low Cost Color Visual Stimulator for fMRI

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Abstract

This low cost visual stimulator was developed for use in small animal imaging. The stimulator uses a single tri-color LED for each eye and can output red, green, or blue light or any combination of the three. When all three LED colors are illuminated at the same time achromatic light is output. The stimulator is almost entirely implemented in software with only minimal electronics. The LEDs are controlled via the parallel port of a desktop computer. Flicker frequency, wavelength, intensity and waveform shape are under software control. The LEDs are coupled to fiber optic cables which run into the MRI scanner room leaving the LEDs and the power source in the control room. Calibration with a radiometer shows the light output to be very linear from zero to full intensity. The stimulator was used in fMRI visual stimulation studies performed on Sprague Dawley rats with an 11.7 Tesla magnet. As the stimulator is software driven, modifications to accommodate other protocols and extensions for new functionality can be readily incorporated. With this in mind, the visual stimulator circuit diagram and software including source code are available upon request.

Keywords

Visual stimulation; magnetic resonance imaging; biomedical measurements

Introduction

Visual stimulation via a flashing light is a valuable tool for examining visual response. The magnitude of the fMRI response varies with both flash frequency and stimulation wavelength (Van Camp et al., 2006). Various light sources have been employed for the

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measurement of flicker response including stroboscopic lights (Van Camp et al., 2006) (Tsunoda et al., 2004), computer displays, (Kim et al., 2000), (Jehle et al., 2009) rotating disks (Garhofer et al., 2004) and LEDs (Manning et al., 2007).

Retinal neurovascular coupling has been widely studied using flickering light stimulation. Accumulative findings suggested that visual stimulation modulates retinal and optic nerve blood flow (Riva et al., 2005). Significant linear relationship has been found between retinal blood flow and the Electroretinography signal, visual evoked potential, and ganglion cell activity (Vo Van and Riva, 1995). Exploring the coupling between light-evoked retinal activity in retinal and choroidal circulation without depth limitation can deepen our understanding of the pathophysiology of ocular diseases and has potential to early detect retinal dysfunction.

Spectral response is generally measured using interference filters or a monochromator, a device that uses grating or a prism to separate a narrow wavelength band from a continuous spectrum light source (Birch and Jacobs, 1975), (Bi et al., 2006). LEDs have also been employed in visual stimulation though only at a single frequency (Manning et al., 2007).

The intensity of light from LEDs is linear with input voltage only over a very limited range. While the relationship between light output and voltage can be obtained by calibration (Hogg, 1991), alternative techniques may be used to obtain more linear control over intensity. One approach is using pulse width modulation (PWM) where the output is changing the average current to the LED (Narra and Zinger, 2004).

Visual stimulation in the MRI environment is challenging. Ferrometallic components are unsuitable for use in the magnetic environment. Other non-ferrometallic parts can produce image artifacts. Electronic components can suffer from induced currents from gradient fields and in turn can interfere with image acquisition. The confined space of the imager makes it difficult to position light sources, especially in small animal imagers.

In this study, the use of fiber optic cables eliminates most of the problems encountered with providing visual stimulation in the confined environment of small animal imager. The fiber optic cables being MRI compatible do not affect the quality of the imaging. A desktop computer in the control room operates all aspects of stimulus delivery including wavelength, intensity, and flicker frequency making for very repeatable experiments. We demonstrate the utility of this device by using functional MRI at 11.7 Tesla to image visual evoked responses in the rat retina.

Materials and Methods

Hardware

The stimulator uses a common anode tri-color LED for each eye (Super Bright LEDs). The peak emission wavelengths are 630nm for red, 525nm for green, and 472nm for blue. The LEDs have a common anode with separate cathodes for each color. The cathodes for each LED color are switched to ground by a channel of an ULN2003 Darlington transistor array. Dropping resistors were chosen to provide a 20ma current for each color matching the manufacturer's specification for nominal output. The ULN2003 switches each LED color from full on to full off. The circuit consists of two tri-color LEDs, one ULN2003 integrated circuit, seven resistors and an additional green LED as a power indicator.

All control of the stimulator is via the computer parallel port. Each of the three colors from the two LEDs requires an output data bit from the parallel port. These six control signals are used to toggle channels of the ULN2003. The stimulator can input synchronization pulses

from the scanner or output synchronization pulses to the scanner via two BNC connectors. Output synchronization pulses use a seventh parallel port output data bit. Synchronization pulses from the scanner are monitored using a parallel port input status line.

The LEDs are coupled to 2.5mm glass bundle fiber optic cables 8m in length (Fiberoptic Components). The fiber optic cables are passed through a wave guide to the MRI room leaving all electronics in the control room.

Software

The C++ based software is designed to deliver stimulation for a block protocol. A simple graphical user interface (GUI) controls the setup of stimulus presentation timing. The rest time, stimulation time, and number of blocks, are set via the GUI. The flicker frequency and intensity of stimulation are also software controlled. The flicker frequency can be varied from 0.1 to 100 Hz. The output can either be a square wave or a sine wave.

Stimulation can be delivered to the left eye, right eye or both eyes. Any combination of LED colors can be selected as well. All three colors are combined for achromatic light.

The intensity is controlled by pulse width modulation (PWM) where the output is modulated by changing the ratio of power on time to power off time. The LED duty cycle varies from continuously on for maximum intensity to completely off. Each 5ms time slice is divided into on and off times with the minimum on or off time $1/1000^{\text{th}}$ of the time slice. The 5ms time slice produces a 200Hz flash rate that is well above the critical fusion frequency for rats (Wells et al., 2001). The Windows high performance timer which has microsecond or better accuracy is used for timing.

Calibration

The stimulator output was calibrated using an International Light Technologies IL-1700 Research Radiometer (International Light Technologies). Irradiance was measured for each LED color over a range from full intensity (1.0) to the minimum selectable value (0.001). The detector was positioned 27mm from the end of the fiber optic cable. Intensity was measured using a Pritchard 1980A photometer (Photo Research).

fMRI

Two fMRI studies using Sprague Dawley rats were performed using a Bruker Biospec 11.7T scanner with institutional approval and in accordance with the Statement for the Use of Animals in Ophthalmic and Vision Research. The first used blood oxygenation level-dependent functional MRI (BOLD fMRI) to examine the hemodynamic response of the rat retina (De La Garza et al., 2011). The second study examined the change in retinal blood volume using an intravascular monocrystalline iron oxide nanoparticle (MION) contrast agent (Shih et al., 2011).

For the BOLD fMRI study 10 normal adult male Sprague Dawley rats (250–350 g) were initially anesthetized with approximately 2% isoflurane in air, intubated and mechanically ventilated. Afterwards, the animals were secured in an MRI-compatible rat stereotaxic headset with ear and tooth bars, and isoflurane was reduced to 0.9–1.0% after animals had been paralyzed with pancuronium bromide (3 mg/kg first dose, 1.5 mg/kg/h, intraperitoneal injection). Animals were studied with (n=5) and without (n=5) the application of atropine, phenylephrine and proparacaine eye drops. To apply the eye drops the animal was slid out of the scanner, eye drops were applied then the animal was slid back in without re-shimming.

A custom made circular surface coil was placed over the left eye of the rat. The distance between the eye and the end of the fiber optic cable was 6mm. A single mid-sagittal imaging slice bisecting the center of the eye is used for imaging in order to minimize partial-volume effect (PVE) due to retinal curvature. BOLD fMRI was acquired using single-shot gradient-echo, echo-planar imaging with inversion recovery. The visual paradigm consisted of five epochs of (60 s OFF and 30 s ON), followed by 180 s of OFF. The stimulation frequency was 8Hz.

For the blood volume study the MION contrast agent (blood half-life >3 hrs) was administered intravenously (30 mg Fe/kg, i.v). The rats were orally intubated, ventilated, paralyzed, and anesthetized with α -chloralose (60 mg/kg first dose, followed by 30 mg/kg/hr, i.v. infusion). A custom made circular surface coil was placed over the left eye of the rat. Each fMRI experiment consisted of five periods of 96 s of stimulation OFF and 96 s of stimulation ON. fMRI blood volume changes were measured using conventional gradient-echo sequence with spectral width = 14 kHz, TR = 150 ms, TE = 5 ms, FOV = 7.7×7.7 mm, slice thickness = 1 mm, acquisition matrix = 128×64, yielding a nominal resolution of 60×60×1000 μ m with a temporal resolution of 9.6 s.

Three sets of blood volume experiments were performed varying stimulus wavelength, luminance, and flicker frequency. For these experiments, the distance between the eye and the end of the stimulator fiber optic cable was 6mm. The order of the experiments was randomized. The first experiment used a fixed luminance (374 cd/m²) with flicker frequencies of 1, 10, 20, and 60Hz using achromatic light. The second experiment individually stimulated at the frequencies of the three LED components (630nm, 525nm, and 472nm) with equal quanta of 2.39×10^{13} quanta/s/cm² at a 10Hz flicker frequency. The third experiment varied the luminance of the stimulation using a 10Hz achromatic flicker with a duty cycle of 50%. The luminance was set at 81, 234 and 374 cd/m².

Results

Calibration with the IL-1700 showed that the irradiance output was linear with the software selected intensity value (fig 1). A linear regression fit of the data resulted in a minimum R² value of 0.9998 for all three frequencies.

For the BOLD fMRI study (fig 2A), visual stimuli from the 8 Hz diffuse achromatic light increased BOLD responses in the retina $5.0 \pm 0.8\%$ from activated pixels and $3.1 \pm 1.1\%$ from the whole-retina region of interest compared with baseline. After eye drop application, activated pixels in the ciliary muscles were largely eliminated.

For the blood volume fMRI study, the MION contrast agent and the high field scanner provided sufficient resolution to detect visually-evoked retinal and choroidal responses. With varying flicker frequencies of achromatic light, the retinal layer region of interest showed a peak response compared to baseline at 10Hz (fig 2B) while the choroidal layer showed no statistically meaningful variation in response to flicker frequency. The responses to red (630nm), green (525nm), and blue (472nm) light at a flicker frequency of 10Hz with equal light quanta exposure (2.39×10^{13} quanta/s/cm²) varied between the retinal and choroidal layers. When the luminance levels were varied at a fixed flicker frequency of 10Hz, the retinal layer responses increased with increasing luminance at a greater rate than the choroidal layer.

Discussion

The irradiance calibration showed the output of the visual stimulator to be extremely linear. This is relatively easy to achieve with a PWM controlled LED system. Trying to vary the

intensity of an LED by varying the current is problematic as the current/intensity curve is not linear and is different for each color LED. The LED rise and fall times are under a microsecond so when on, the LED is essentially always at full intensity. The intensity output of the PWM controlled LED is a linear function of the LED duty cycle. Using this device and functional MRI we detected visual evoked responses in the rat retinas.

For the BOLD study, the retinal activation was in step with the light stimulus. Moreover, time-series of anatomical and functional MRI images showed that the pupils and ciliary muscles moved in correlation with the stimulus onset before eye drops. These movements were markedly reduced or eliminated after the application of eye drops.

The blood volume fMRI study showed that the retinal vessels respond to changing frequency, wavelength and luminance to a greater degree than the choroidal vessels. The retinal results compare favorably with previous work. The rat retina has peak sensitivity to light of a wavelength of about 510nm (Neitz and Jacobs, 1986). The highest response in this study was to the 525nm LED. The peak flicker frequency was at 10Hz with a drop off at 30Hz. (Jehle et al., 2009) showed a peak in control rats at 19 Hz with a drop off at 38Hz. It is difficult to make a direct comparison due to the differences in measurement intervals.

This visual stimulation system works well in an MRI environment. The MRI compatible fiber optic cables are the only components in the MRI room eliminating the possibility of interference with scanning. The flexible software control allows for tight synchronization with the MRI scanner protocol and accurate control of the stimulus presentation. The materials cost of the stimulator is low (~\$400) with the fiber optic cables comprising the bulk of the cost. The cost of the remaining components including an external power supply is around \$40. One limitation of the system is that it requires a computer with a parallel port. Many new computers come without parallel ports though PCI and PCIe parallel port expansion cards are readily available. Due the tight timing requirements the software works best with at least a dual core processor as the timing loop can monopolize a single processor.

One great advantage of this software driven system is the ability extend its capabilities without having to build a new device. While only sine and square wave output is available currently, adding additional waveforms is only a programming task. By providing the source code for the stimulator other investigators will have the opportunity to modify and extend the software to fit their particular protocol.

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Figure 1.

Figure 2.