Detecting responses to single light flashes in the rodent brain using laser doppler and fMRI at 9.4T

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Purpose: Two important features of functional activation models are the application of meaningful physiological stimuli and anesthetic conditions that neither reduce nor inhibit the cortical neural or hyperemic responses. Visual stimulation is a non-invasive physiological stimulus widely used in human brain imaging. In the albino rat, flash and flicker stimulation has been extensively used to examine the neural response in the visual cortex [1], but there have been few investigations of visual stimulus-induced cerebral blood flow changes [2, 3, 4]. Ergo, in order to establish a model for functional hyperemia in the rat visual cortex due to both single flash and continuous flicker, the hyperemic responses to a range of stimulation frequencies flash and flicker stimulations were measured with fMRI and laser Doppler flow (LDF) technique under medetomidine (Domitor), a non-narcotic sedative and analgesic that is gaining wider acceptance in fMRI animal studies.

Methods: 6 Sprague Dawley rats (300 –380g) were used in the fMRI (4 rats) and LDF (2 rats) studies. The right femoral artery and vein were cannulated and used for invasive blood pressure monitoring and for continuous IV drug administration. A tracheotomy allowed for mechanical ventilation with 30% O2-70% N2. Surgery was performed under isoflurane (1.4%) vaporized into the ventilatory gas. After surgery, isoflurane was gradually reduced to zero as a continuous infusion of Domitor (0.1mg/kg/hr) and pancurium bromide (2mg/kg/hr) was started. A blue, remotely-controlled, LED was placed ~4cm in front of each eye. The visual stimulation protocol consisted of randomly varying the LED flickering at the frequencies of 0.2, 1, 5, 10 Hz. The stimuli were presented to both eyes and then to each eye separately. The physiologic parameters - mean arterial blood pressure, arterial blood gases, pulse oximetry, pulse, temperature, respiratory rate, inspired / expired O2 and CO2 - were maintained under normal physiologic ranges throughout all experiments. **fMRI study**: Each stimulation sequence began with an OFF period of 40 seconds followed by three repetitions of ON for 20 seconds and OFF for 40 seconds (total scan time 3minutes 40seconds). Gradient echo scans (Single shot EPI, TE = 18.39 ms, TR = 2 ms, MTX 96 x 96, FOV = 4 cm, Number of repetitions = 110, 10 scans 1mm apart, acquisition time = 3 minutes 40 seconds) were acquired on a 9.4T Bruker MRI scanner. **LDF study:** Under isoflurane anesthesia, prior to Domitor/pancuronium bromide infusion, two 3mm x 3mm sections of skull were thinned to translucency over both primary visual cortices (V1) (-7mm caudal, ±3.0mm lateral from bregma). The LDF probes were shielded from the LEDs. the cortical functional hyperemic response to seven stimulation trials (10s ON, 50s OFF) was bilaterally assessed through the thinned skull with the laser Doppler flow (LDF) probes suspended above both V1s. At each frequency, the LDF signal was normalized to 10s of the baseline preceding each stimulus block and averaged.

Results and Discussion: Strong fMRI and LDF functional responses were observed in both V1s following both bilateral and single-sided stimulation. The 5% LDF response to the 0.2Hz flash stimulation was significant, although lower in magnitude than the 12% response observed at the highest flicker rate of 10 Hz flicker. The fMRI signals from the lateral geniculate nucleus (LGN) and superior colliculus (SC) peaked at 10Hz and was present at all stimulation frequencies. BOLD activation was observed in V1 at 0.2Hz, 1Hz, and 5Hz and was greatest at 1Hz. We establish for the first time, a robust fMRI response to bilateral and single-sided, discrete flash stimuli in V1 of medetomidine-anesthetized albino rats. Additionally, we demonstrate significant fMRI responses to bilateral and single-sided flicker at frequencies of 1, 5, and 10 Hz in the V1, LG, and SC. These flash and flicker responses were also observed in V1 using LDF.

Figure 1.)

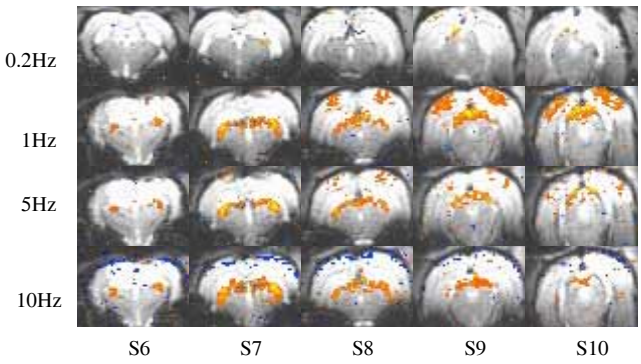

Figure 2.)

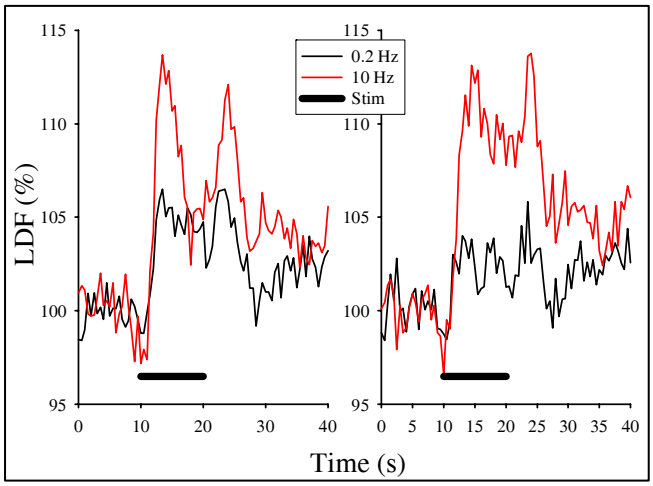

Figure 1.) Shown are frequency dependent BOLD responses in 1mm contiguous slices in the posterior rodent brain at various stimulation frequencies.

Figure 2.) The effects of 0.2Hz flash and 10Hz flicker stimulation on the LDF% change from baseline in the left and right V1.

References: 1. Imas, O.A., et al. Anesthesiology, 102(5):937-37, 2005. 2. Schulte, M.L., et al, Neurosci Lett. 394(1):63-8, 2006. 3. Van Camp, N, et al. J. Neurophysiol., 95(5), 3164-70, 2006. 4. Huang, W., et al., Proc.Natl. Acad. Sci. U.S.A., 93:6037-6042, 1996.