Investigation of the BOLD response in the rat's visual system using a visual flash stimulus

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Introduction:

The ability to process temporally varying stimuli is important for the perception of moving targets. One method of investigating the visual system's temporal distinguishing capacity is to examine the response to a flickering light stimulus[1]. In a previous study we performed fMRI on the rat's visual system by presenting stroboscopic flashes at different frequencies. We quantified the response in several cerebellar and midbrain regions and demonstrated that, the superior colliculus response was most reproducible. We now have extended these experiments focusing on: 1. the response in the visual cortex which is in generally most sensitive to moving stimuli, but also to stationary flashing light; 2. the comparison of the response characteristics of the superior colliculus with those of the cortex; 3. the comparison between stimulation of two or only one eye.

Materials & Methods:

Animal model: Male black hooded Long Evans rats (n=6, 250-300g) were anesthetized with an intramuscular injection of ketamine (75mg/kg, Ketalar, Parke-Davis, Belgium) and xylasine (5mg/kg Rompun, Bayer, Germany) for animal handling prior to the experiment. During the entire experiment the rats breathed spontaneously and breathing rate, expired pCO_2 and temperature were permanently monitored. Before inserting the rat in the magnet, anesthesia was switched to a continuous infusion of domitor (0.15mg/kg/hr, IM). Above the dorsal brain a surface receiving coil (diameter: 24mm) and helmholtz transmitting coil (diameter 50mm) were placed. *MRI:* The imaging was performed on a 7T horizontal bore magnet (MRRS) with 8 cm aperture and self-shielded gradients with a strength of 0.1 T/m (Oxford Instruments). Ten coronal, high resolution, gradient echo images from IA 5mm to IA -5mm were positioned on the scouting images (TR/TE: 500/6msec, FOV: 35mm, slice thickness: 1mm, acquisition matrix: 256x128). Functional MRI was performed with a T_2*-weighted multislice gradient echo sequence at the position of the high resolution images: TR/TE: 400/14msec, FOV 35mm, acquisition matrix: 128x64. *Stimulus and stimulation paradigm:* A strobe unit was placed 2 meters in front of the magnet. The paradigm consisted of a rest period, followed by a period during which the rat was only exposed to the clicks of the strobe unit and a period during which the rat was exposed to both light and clicks. Each period consisted of five experiments and the epoch of three periods was repeated three times. Within each fMRI experiment the light stimulus frequency was kept constant. Seven different frequencies were tested: 1Hz, 5Hz, 8Hz, 10Hz, 12Hz, 20Hz (n=3) and 50Hz (n=1). In a preliminary experiment the right eye of two rats was closed and shielded. *Data Analysis*: The data were zerofilled to 128x128 and processed with Medx software. A gaussian smoothing with a kernel of 3x3 pixels was applied to the data set. Regional activation following the light stimulation was calculated from an unpaired t-test between the data set of the light/click stimulated images and click stimulated images. On the high resolution images, the visual cortex, superior colliculus and optic pretectum were delineated as regions of interest. The signal intensity trace of the most activated pixels (Z >2; p <0.005) was calculated and analyses were done on percentage signal intensity change during stimulation. ANOVA with repeated measures and Tukey Honest post-hoc test were applied to test the different response to various frequencies and stimulation periods.

Results:

Only the visual cortex and superior colliculus responded significantly different to various frequencies of flash stimulation ($p<0.0001$, figure 1). In the visual cortex, a maximal response was observed at a stimulation frequencies of 5 and 1Hz, whereas the response at 5Hz was significantly larger than the one at 12Hz ($p=0.002$), 20-50Hz ($p=0.0002$) and 8Hz ($p=0.03$). The response in the superior colliculus was most prominent at 8 and 12Hz, and at both significantly larger as 1Hz ($p<0.01$), 20-50Hz ($p<<0.0001$), and 5Hz ($p<0.05$) (figure2). Analyzing the BOLD response in the visual cortex and superior colliculus revealed that: **1)** There was no significant correlation between the BOLD responses in visual cortex and superior colliculus ($R^2=0.029$); **2)** The intensity of the activation in the cortex was significantly lower than the SC for all frequencies ($p<0.01$); **3)** The BOLD response in the superior colliculus showed habituation upon repeated stimulation periods and at a stimulation frequency of 12 Hz, this habituation became significant from the third epoch onwards ($p<0.01$). The cortex did not display a similar habituation to any of the tested frequencies; **4)** If the BOLD response at various frequencies was normalized to the response at 1Hz, we observed that even at 20Hz the response in the superior colliculus was still significantly larger than in the cortex. Only at 50Hz there was no difference between the response in superior colliculus and cortex, both having a smaller response relative to 1Hz (figure 2). After stimulation of only one eye (n=2), the BOLD response in the superior colliculus decreased ($p=0.006$) but still peaked around the same frequencies as compared to stimulation of both eyes (8-12 Hz). In the cortex the BOLD response did not decrease after one eye stimulation but the maximal response frequency shifted from 1Hz ($p<0.001$) and 5Hz to 5Hz and 8Hz ($p<0.017$) (figure 2).

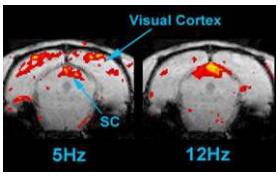

Fig 1: High resolution image at IA 1.5mm, with overlying activation map at flash stimulation frequencies 5 and 12Hz.

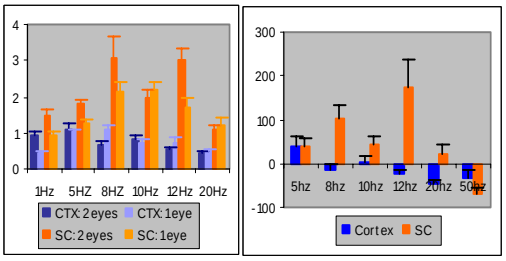

Fig 2: Left graph: Percent SI change in cortex and SC after stimulation of one or both eyes at different frequencies. Right graph: SI change relative to 1Hz in cortex and SC after stimulation of both eyes.

Discussion:

The superior colliculus is known to be highly sensitive to temporally varying stimuli, as we confirm here and have demonstrated before[2]. Electrophysiological studies have demonstrated that only 5% of the neurons in the visual cortex respond to stationary stimuli flashing on-off in their receptive field[3]. This may explain the lower BOLD contrast discerned in the visual cortex. Human fMRI studies during flash stimulation, demonstrated that different visual cortex regions displayed different frequency sensitivity with 7Hz being the optimal frequency of the striate cortex (cfr. primary visual cortex of the rat). We could demonstrate that 1, 5 and 8Hz are also important frequencies for the rat's visual cortex. More experiments will be necessary to confirm the shift in optimal response frequency when stimulating only one eye. The mammalian visual system can be seen as a set of pathways working largely in parallel[4]. Our data demonstrate that the functioning of the cortex is not correlated to the functioning of the superior colliculus or visa versa. This may explain why the cortex and superior colliculus have different optimal response frequencies. We have also demonstrated habituation in the superior colliculus, which is a common feature of cells from the deep collicular layers[4] and a higher flicker fusion threshold in the superior colliculus as compared to the visual cortex.

References

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