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Light Stimulus Frequency Dependence of Activity in the Rat Visual System as Studied With High-Resolution BOLD fMRI

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Van Camp, Nadja, Marleen Verhoye, Chris I. De Zeeuw, and Annemie Van der Linden. Light stimulus frequency dependence of activity in the rat visual system as studied with high-resolution BOLD fMRI. *J Neurophysiol* 95: 3164–3170, 2006. First published January 4, 2006; doi:10.1152/jn.00400.2005. The neurophysiology of the rodent visual system has mainly been investigated by invasive and ex-vivo techniques providing fragmented data. This area of research has been deprived of functional MRI studies based on blood oxygenation level dependent (BOLD) contrast, which allows a whole brain approach with a high spatial and temporal resolution. In the present study, we looked at the neurovascular response properties of the visual system of the pigmented rat, focusing on the visual cortex (VC), the superior colliculus (SC) and the flocculus-paraflocculus of the cerebellum (FL-PFL), using BOLD fMRI under domitor anesthesia. Visual stimulation was performed monocularly or binocularly while flashing light from a strobe unit was presented. For each structure, we assessed the flashing frequency that evoked the optimal BOLD response: Neither the VC nor the FL-PFL displayed frequency dependence during monocular visual stimulation, but were most sensitive to low frequencies (1–5 Hz) when flashing light was provided binocularly. The SC responded optimally to high flashing rates (8–12 Hz) during both monocular and binocular stimulation. The signal intensity changes in the VC and FL-PFL were locked to the stimulation period, whereas the BOLD response in the SC showed a similar onset but a very slow recovery at offset. The VC and FL-PFL, but not the SC, showed signs of binocular competition. The observed correlation between frequency-dependent responses of different visual areas during binocular visual presentation suggests a functional relationship between the VC and FL-PFL rather than between the SC and FL-PFL.

INTRODUCTION

The CNS of all vertebrates has three main visual pathways. These include the *thalamofugal pathway*, which is formed by the retinal projections to the primary visual cortex via the lateral geniculate nucleus of the thalamus and is involved in visual distinction of form and color as well as perception of visual motion (Hubel and Wiesel 1998); the *tectofugal pathway*, which is formed by a direct projection from retinal cells to the superior colliculus and is primarily involved in visual orientation and spatial attention (Wurtz and Goldberg 1972); and finally, the *accessory optic system*, which relays retinal slip signals for self-motion and gaze stabilization, either directly from the retina or indirectly via the visual cortex to the nuclei of the optic tract and visual tegmental relay zone, which in turn project to the inferior olive at which the climbing fiber input to the Purkinje cells in the cerebellum originate (Simpson et al. 1996).

Current knowledge of the functional organization of the rodent visual system is fragmented and has been acquired exclusively through invasive or ex vivo techniques. Several studies have shown that all optic nuclei in the rodent brain respond to changes in light intensity, particularly to rapid ON-OFF stimuli (Cooper and Allen 1995; Cooper and Thurlow 1991; Cooper et al. 1991; Toga et al. 1995) The superior colliculus displayed the most pronounced response, while the lateral geniculate nucleus and second-order sites of the visual cortex were less responsive. It has been suggested that the subcortical components of the visual system rather than the primary visual cortex play an important role in the processing of intensity information (Cooper and Allen 1995; Cooper and Thurlow 1991). Stimulation frequency-dependent studies with the use of patterned and flashing light have suggested that the superior colliculus is more sensitive than the lateral geniculate nucleus (Toga et al. 1995) and primary visual cortex (Lu et al. 2004; Montero and Jian 1995). Unfortunately, all techniques used so far to study the rodent visual brain system have poor temporal (autoradiographic, c-fos studies) or spatial (electrophysiology and optical imaging) resolution. However, functional MRI (fMRI) based on blood oxygen level dependent (BOLD) contrast provides the opportunity to visualize individual responses of different brain structures at a high spatial resolution and follow them longitudinally over time, while assessing the entire brain. To date, such studies have not yet been performed on the visual system in rodents.

In the present study, we assessed all three visual pathways of the rat simultaneously using BOLD fMRI during stroboscopic light presentation. To obtain unambiguous results, we have restricted the quantification of the BOLD response to those brain structures that are accurately delineable based on clear anatomical boundaries: the visual cortex, superior colliculus, and flocculus-paraflocculus of the vestibulocerebellum. To control for possible auditory stimulation in these structures from the clicking sounds accompanying the flashing of the stroboscope, we designed a stimulation paradigm to perform fMRI of the rat visual system while compensating for auditory evoked BOLD responses. Ultimately this approach allowed us to investigate: *1*) whether the visual cortex (V1 and V2), superior colliculus, and (para)flocculus are activated by stroboscopic light, and which stimulus frequencies do provoke an optimal BOLD response in these areas; *2*) whether the activation patterns in the areas that are sensitive to particular frequencies of stroboscopic stimulation show particular tem-

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poral correlations with the stimulus pattern; *3*) whether and how the BOLD responses of different structures are related to each other; and *4*) to what extent binocular (as compared with monocular) visual stimulation may compromise the neurovascular response.

METHODS

Animal preparation

Eight male black hooded Long Evans rats with pigmented eyes [320 ± 20 (SE) g], were initially anesthetized with a intramuscular injection of Ketamine (75 mg/kg, Ketalar, Parke-Davis, Belgium) and Xylazine (5 mg/kg, Rompun, Bayer, Germany), which was necessary to position the head of the rats in a custom-made plastic stereotactic device consisting of blunt ear plugs and a tooth bar. Thirty minutes after Ketalar/Rompun induction, anesthesia was switched to Medetomidine (Domitor, Pfizer), starting with an initial bolus of 0.08 mg/kg (IM) and continuing after 40 min with an IM infusion of 0.15 $mg.kg^{-1}.h^{-1}$. Rats were spontaneously breathing throughout the entire experiment. Breathing rate and end tidal pCO_2 were measured using a pneumatic pillow and a CapStar-100 End-Tidal CO_2 Analyser (Linton Instruments, UK), respectively. Body temperature was monitored with a rectal probe and maintained at (37.5 ± 0.5°C) with a feedback-controlled heating pad (T/Pad and T/Pump, Gaymar Institute, Kent Scientific Corporation, Litchfield, CT). The eyes of the rats were covered with Vaseline to prevent them from drying out. In four rats, the left eye was sealed for monocular visual stimulation; in four other rats, both eyes were left uncovered to allow for binocular visual stimulation.

MRI

The imaging was performed on a 7T horizontal bore magnet (MRRS) with 8-cm aperture and self-shielded gradients with a strength of 400 mT/m (Oxford Instruments). Radiofrequency (RF) transmission was done with a Helmholtz coil (diam: 50 mm). A circular surface coil (diam: 24 mm) placed above the dorsocaudal part of the brain was used for receiving the RF pulses.

Ten high-resolution coronal gradient echo images (TR/TE: 500/6ms, FOV: 35 mm, slice thickness: 1 mm, acquisition matrix: 256x128) were positioned at IA 8 mm to IA −5 mm using scouting images acquired along three orthogonal directions. Subsequently, fMRI was performed with a T_2^*-weighted multislice gradient echo sequence at the same position as the high-resolution images (TR/TE: 400/14 ms, FOV: 35 mm, acquisition matrix: 128x64, temporal resolution: 25.6 s per experiment).

Stimulus and stimulation paradigm

A strobe unit was placed two meters in front of the magnet. Between the bore of the magnet and the lamp, a hollow pipe with a diameter equal to the magnet bore was placed for optimal light stimulation with the least amount of light scattering.

The stimulation paradigm consisted of a rest period, followed by a period during which the lamp of the strobe unit was covered and the rat was exposed only to the clicking noise of the strobe unit (auditory stimulation in complete darkness), and thereafter by a period during which the rat was exposed to both light and clicks (combined visual and auditory stimulation used as "visual" stimulation). Each period lasted for 2 min and included five fMRI experiments. A sequence of three periods (2 min rest, 2 min clicks, 2 min light and clicks) was repeated three times. Within each fMRI run (consisting of 3 repetitive sequences), the light stimulus frequency was kept constant. Five different frequencies (1Hz, 5Hz, 8Hz, 10Hz, 12Hz) were presented in random order during five separate fMRI runs within the same animal. This experimental setup was performed on eight rats with monocular stimulation or ($n = 4$) or binocular stimulation ($n = 4$).

Data Analysis

The fMRI images were zerofilled to 128x128 and processed off-line with Medx software. Spatial filtering was performed to the data by gaussian smoothing with a kernel of 3x3 pixels, but no temporal filtering was applied.

To investigate different BOLD responses, data analysis was carried out in two different ways. First, periods of auditory stimulation in complete darkness (clicking noise) were statistically compared with periods without any stimulation ("auditory" stimulation vs. rest) using a nonpaired *t*-test. Pixels with a significant change of signal intensity ($P < 0.05$) compared with the rest period were considered to have been activated by auditory stimulation. The frequency spectrum of the extremely short clicks probably contains a lot of high-frequency components, which may evoke auditory responses in the cortex, superior colliculus, or flocculus-paraflocculus.

Second, periods of visual stimulation (including clicking noise) were statistically compared with periods during which the animals were exposed only to the clicking noise of the stroboscopic lamp (visual stimulation versus auditory stimulation), using a nonpaired *t*-test. Pixels with a significant change of signal intensity compared with the auditory stimulation period ($P < 0.05$) were considered to have been activated by visual stimulation only. The results of these analyses are mentioned in the RESULTS section as BOLD response during visual stimulation.

The resulting functional activation maps display the z-score of each voxel. The z-scores of significantly activated pixels ($P < 0.05$) were overlaid on the high-resolution images, with z scores color coded on a predetermined color scale (Fig. 1).

To quantify the BOLD response, we delineated three regions of interest on high-resolution anatomical images and projected them onto the corresponding statistical z-score maps for further analysis.

The cortex, including visual cortex, was delineated from IA 4 mm to IA 1 mm. The superior colliculus was delineated on MRI images at position IA 3 to IA 1 mm. The flocculus-parafloccculus were delineated from IA 2.8 mm to IA −0.8 mm.

Within each region of interest, the relative signal intensity (%SI) change was measured in the 10 pixels demonstrating the highest significance levels (superior colliculus: $P < 0.0005$, visual cortex:

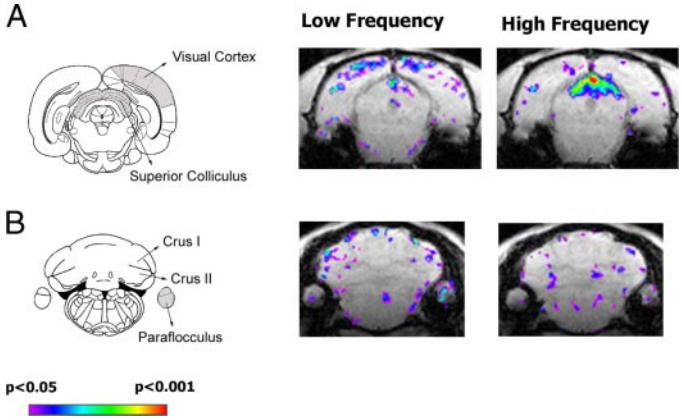

FIG. 1. *A* and *B*: the functional activation maps on binocular visual stimulation overlying high-resolution magnetic resonance images at approximate positions IA 2 mm and −3 mm. On the left, schematic drawings of the corresponding coronal sections, adapted from Paxinos and Watson (Paxinos and Watson 1986), are shown. The columns separate activation maps after low- and high-frequency visual stimulation performed on the same rat. Cerebellar activations were present in all animals, though the variability of cerebellar activations among different animals was very high. The color bar at the bottom left of the figure shows the significance of the activation maps.

$P < 0.01$, parafloccular complex: $P < 0.025$) during visual stimulation. The selected voxels were verified as being grouped in clusters of at least three pixels. In case of single voxels, it was clear that it was always the most significantly activated voxel that was selected, which was surrounded by a cluster of at least three less significantly activated voxels. Single voxels were observed mostly in the cerebellum, where signal changes were small and significance levels are lower as compared with the superior colliculus. Therefore the statistical analysis was repeated for the cerebellum, applying the following criterion: only clusters of more than three voxels occurring at a significance of at least $P < 0.05$ were included in the analyses (data not shown). No differences between the results or interpretation were observed when comparing clusters of at least three pixels at a significance level of $P < 0.05$ with single pixels at a significance of $P < 0.025$ (as applied in the current paper). As the significance levels in the cerebellum were much lower than those observed in the superior colliculus and visual cortex, false activations of single voxels, if present, were likely to be found in this structure. However, redoing the statistics with different criteria showed that false activations of single pixels were not likely to occur with the data analysis of the present paper. Therefore it was concluded that the statistical criterion was adequate for a reliable quantitative analysis of the BOLD contrast in all brain areas investigated.

The signal intensity change was calculated relative to the mean signal intensity of the images during the first period of auditory stimulation. In addition, for the superior colliculus, the signal intensity change to visual stimulation was calculated relative to the first rest period.

Each time point of the stimulation periods was included in the statistical analysis of the difference in BOLD response to varying frequencies. This was calculated in the three structures of interest at all stimulation frequencies and for mono- or binocular stimulation.

To reduce the number of variables and limit the data set, we grouped the stimulation frequencies in two different frequency ranges: low frequencies, including 1 and 5 Hz, and high stimulation frequencies, including 8, 10, and 12 Hz (Correa-Lacarcel et al. 2000).

Statistical analyses were performed using student *t*-test applying SPSS12.0 for Windows. All data are means ± SE.

RESULTS

Despite the background scanning noise and the earplugs in the animal restrainer, small but significant ($P < 0.05$) BOLD changes during auditory stimulation could be discerned in all structures of interest (see METHODS section). Therefore BOLD responses obtained during periods of visual stimulation were statistically compared with those obtained during auditory stimulation. The BOLD amplitudes and the temporal changes were quantified and analyzed in the visual cortex, the superior colliculus, and the flocculus-paraflocculus of the cerebellum for each flashing frequency during mono- or binocular visual presentation (Fig. 1). Different activation characteristics were observed depending on the stimulus frequency and presentation: monocular or binocular.

Binocular visual stimulation

The presentation of flashing light evoked significant BOLD responses in all structures of interest, with the most pronounced response in the superior colliculus and substantially lower responses in the visual cortex and the flocculus-paraflocculus. In the superior colliculus and visual cortex, the BOLD activations were clearly bilateral (Fig. 1). The BOLD contrast in both the visual cortex (Fig. 2*A*, black bars) and flocculus-paraflocculus (Fig. 2*C*, black bars) decreased by more than 30% during increasing flashing rates ($P_{\text{visual cortex}} = 0.0001$ and $P_{\text{flocculus-paraflocculus}} = 0.0001$), while in the superior colliculus, an increase of ≤60% was observed as compared with low-frequency flashing light ($P_{\text{superior colliculus}} = 0.0001$; Fig. 2*B*, black bars).

Temporal characteristics of the BOLD response

Signal intensity traces demonstrated that the BOLD response in all regions of interest remained elevated during the entire stimulation period, lasting for two minutes (Fig. 3*A,B* and Fig. 4*A*). The time traces in these structures (Fig. 3*A* and 3B) illustrate the persistent smaller response to high-frequency stimulation (light gray trace) as compared with low-frequency stimulation (black trace) already pointed out in Fig. 2*C*.

While the BOLD response in the cortex and cerebellar structures remained restricted to the period of stroboscopic light presentation, the temporal response pattern in the superior colliculus showed a similar onset but exceeded the stimulation period and decreased on consecutive stimulation periods (Fig. 4, *A* and *B*). This change was most clear at the highest flashing rate (light gray trace in Fig. 4*A*), for which the maximum

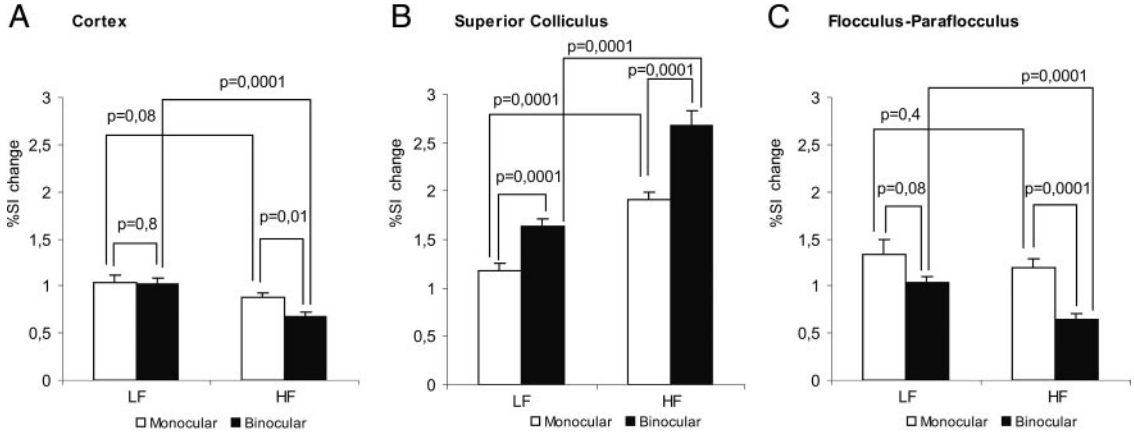

FIG. 2. The column charts display the mean ± SE ($n_{\text{monocular}} = 4$; $n_{\text{binocular}} = 4$) of the percent signal intensity changes during the period of visual stimulation relative to the mean of the signal intensity of the first period of auditory stimulation. White bars indicate BOLD contrast during monocular visual stimulation, black bars during binocular visual stimulation. *A–C*: the responses in the visual cortex, the superior colliculus, and the flocculus-paraflocculus, respectively. LF, low frequency; HF, high frequency.

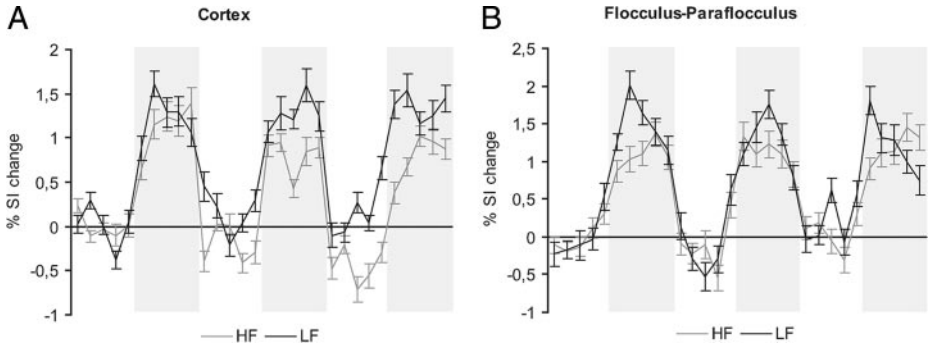

FIG. 3. Line charts demonstrating signal intensity changes (in percentage ± SE) in the visual cortex (*A*) and flocculus-paraflocculus (*B*) during binocular visual stimulation (shaded boxes) at LF stimulation (black line) and HF stimulation (light gray line). Signal intensity changes are averaged over 4 animals and calculated relative to the first auditory stimulation period.

response was reached at nearly the end of each stimulation period. After the first visual presentation, it recovered very slowly and did not reach baseline again until five experiments later (i.e., after approximately 4 min) at the start of the second stimulation period. During this slow recovery, a second maximum was observed. After the second stimulation period, the BOLD response already returned to baseline after two experiments (i.e., 2 min), and the second maximum was less pronounced (arrows Fig. 4*A*).

Correlation of the BOLD responses to varying frequencies in different parts of the visual system

Correlation analyses of the response patterns during binocular visual stimulation with increasing frequencies revealed a significant positive correlation ($r = 0.2$, $P < 0.01$) between the neurovascular response in the flocculus-paraflocculus and the cortex, and a significant inverse correlation between the flocculus-paraflocculus and superior colliculus ($r = -0.2$, $P < 0.01$).

Monocular visual stimulation

While the BOLD activations spread bilaterally during binocular visual presentation, monocular visual stimulation (left eye sealed) yielded mainly activations in the contralateral hemisphere. Increasing flashing frequencies with monocular stimulation did not produce significantly decreased responses in the visual cortex ($P_{monocular} = 0.08$; Fig. 2*A*, white bars) or the flocculus-paraflocculus ($P = 0.4$; Fig. 2*C*, white bars), while an increased response was still observed in the superior colliculus ($P = 0.0001$; Fig. 2*B*, white bars).

Temporal characteristics of the BOLD response

Monocular temporal data are not shown, as they were identical to the observations after binocular visual stimulation, except that no amplitude differences were discerned in the signal traces of the visual cortex and the flocculus-paraflocculus in response to high or low flashing rates. This is in contrast to the superior colliculus, where the response to high frequen-

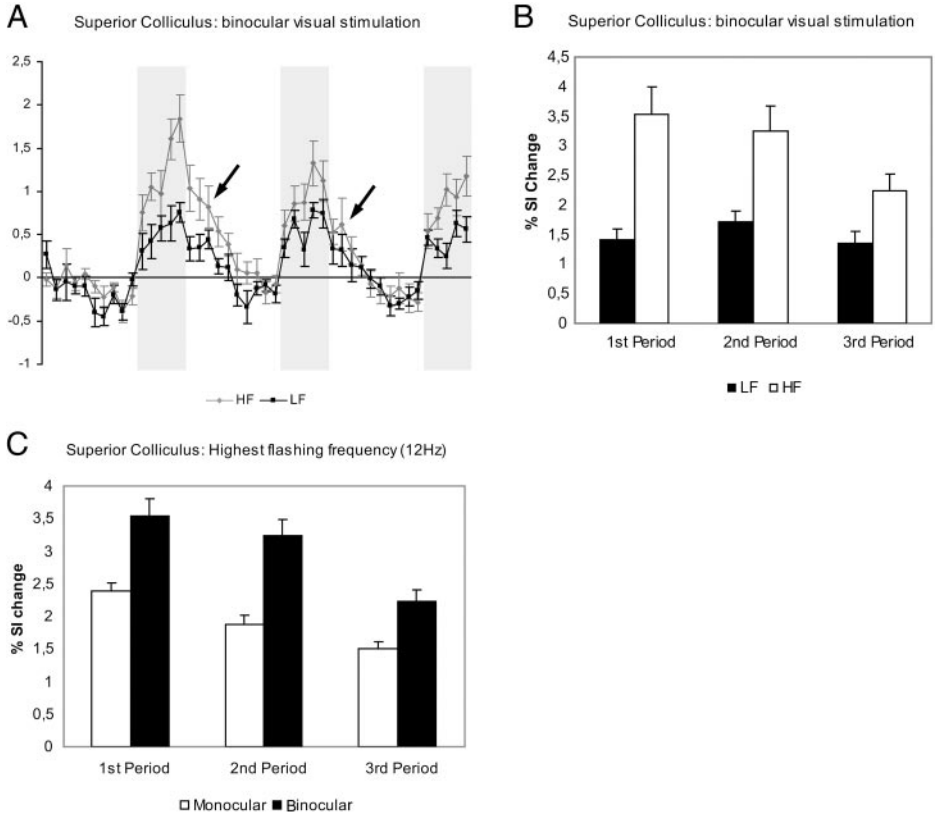

FIG. 4. The temporal responses observed in the superior colliculus during binocular visual stimulation (shaded boxes in *A*). Traces (*A*) and mean of the signal intensity (%SI) changes ± SE (*B*, *C*) are averaged over 4 animals. *A*: signal intensity traces during visual stimulation at high flashing frequencies (HF) (light gray line) and low flashing frequencies (LF) (black line). Arrows indicate a secondary response maximum. Signal intensity traces are calculated relative to the first rest period. *B*: average BOLD signal intensity changes (±SE) during each visual stimulation period at high flashing frequencies (white, HF) and low flashing frequencies (black, LF). Habituation is observed at the third stimulation period ($P = 0.001$), at high stimulation frequencies. *C*: comparison of average BOLD changes (±SE) during each visual stimulation period during monocular (white) or binocular (black) visual stimulation at the highest flashing rate (12 Hz). The amplitude declined more during binocular visual stimulation than during monocular visual stimulation.

cies remained larger than the response to low frequencies. The slow recovery of the response after the first visual stimulation period still existed during monocular visual presentation.

Correlation of the BOLD responses to varying frequencies in the different parts of the visual system

In contrast to its effect on the temporal BOLD response in the superior colliculus, changing the flashing rate of the visual stimulus had little or no effect on the temporal BOLD response in the visual cortex and flocculus-paraflocculus and none of the BOLD response patterns could be related with each other.

Binocular versus monocular visual stimulation

The BOLD response in the visual cortex and flocculus-paraflocculus displayed no differences between monocular or binocular low-frequency visual stimulation ($P_{\text{monocular versus binocular}} = 0.8$ for cortex and $P_{\text{monocular versus binocular}} = 0.08$ for the cerebellum; Fig. 2*A*). However, at high flashing rates, the response decreased by ≤20% in the cortex and by 40% in the flocculus-paraflocculus during binocular visual stimulation ($p_{\text{monocular versus binocular}} = 0.01$ for the cortex, Fig. 2*A*; $P_{\text{monocular versus binocular}} = 0.0001$ for the cerebellum, Fig. 2*C*). The signal in the superior colliculus increased by almost 40% on binocular stimulation with both low and high flashing rates ($P_{\text{monocular versus binocular}} < 0.0001$ for low frequency and high frequency; Fig. 2*B*).

Temporal characteristics of the BOLD response

In neither the paraflocculus nor the visual cortex was a different temporal response pattern observed during binocular as compared with monocular flashing light presentation. However, in the superior colliculus, binocular stimulation at the highest flashing rate (12 Hz) yielded a steeper decline in response during consecutive stimulation periods compared with the same frequencies presented monocularly (Fig. 4*C*).

DISCUSSION

Flashing light of varying stimulation frequencies evoked significant BOLD responses in the visual cortex, superior colliculus, and flocculus-parafloccular complex in the rat. When binocular stimulation was used, both the visual cortex and flocculus-paraflocculus seemed most sensitive to low frequencies, while the response in the superior colliculus was strongest at the highest flashing rates. However, when monocular instead of binocular visual stimulation was provided, this general frequency-dependent response pattern changed in the visual cortex and flocculus-parafloccular complex, but not in the superior colliculus. In addition, we demonstrated that the temporal pattern of the obtained BOLD response was different in the superior colliculus compared with the other two visual areas.

Below, we discuss the spatial and temporal aspects of the response characteristics in the visual cortex, floccular complex, and superior colliculus, as well as the binocular inhibition.

Functional MRI on flashing light stimulation: Spatial aspects

VISUAL CORTEX. The current study demonstrated for the first time a prominent BOLD response in the visual cortex of the rat visual system. Our findings agree with findings of BOLD responses observed in the visual cortex and lateral geniculate nucleus of the awake rabbit (Wyrwicz et al. 2000) after flashing light stimulation at 8 Hz. Moreover, our findings are also compatible with an fMRI study done on the visual system of the mouse in that we also appear to have stimulated the M pathway of the visual cortex by rapidly changing light intensity (Huang et al. 1996). Although no BOLD responses have been reported in structures outside the visual cortex for the mouse visual system (Huang et al. 1996), our observation that the BOLD responses were smaller in the visual cortex than in the superior colliculus is in line with several other studies in rat. First, electrophysiological studies have revealed that only 5% of the neurons in the visual cortex respond to stationary stimuli flashing ON-OFF in their receptive field (Burne et al. 1984), while this percentage can reach a level of 70% in the superior colliculus (Gonzalez et al. 1992); and second, autoradiographic studies have also shown a low metabolic response to light flashes in the visual cortex compared with other visual structures. Thus we conclude that BOLD responses to stroboscopic light stimulation in rat are moderately but significantly present in the visual cortex.

FLOCCULAR COMPLEX. To the best of our knowledge, the present study is the first to show BOLD responses in the floccular complex after flashing light stimulation. These responses may relate to activities in the cerebellar cortex evoked by climbing fiber inputs and/or inputs from the mossy fiber–parallel fiber system (Kistler and De Zeeuw 2003). Electrophysiological studies in rabbits demonstrated that flickering light stimuli at a frequency of 1 Hz causes synchronized bursting of olivary neurons and high local field potential (LFP) activities in the flocculus (Maekawa and Simpson 1973). However, higher stimulation frequencies appear to induce more complex spike activities, but less simple spike activities and smaller floccular LFPs raise the possibility that the mossy fiber–parallel fiber system at least contributes to the LFPs (Buchtel et al. 1972; Maekawa and Kimura 1974; Maekawa and Simpson 1973). Thus since our visually evoked fMRI responses showed that the flocculus-paraflocculus display a decreased response on increasing stimulation frequencies, the BOLD responses may correlate best to activities in the mossy fiber– parallel fiber pathway.

SUPERIOR COLLICULUS. In the present study, the BOLD response in the superior colliculus was so large that the entire structure was lit up on the functional BOLD maps. However, increasing the significance level in the presentation of the activation maps restricted the BOLD response to the superficial layers, in which it is known that they receive visual input, as outlined in the introduction (Correa-Lacarcel et al. 2000; Montero and Jian 1995; Toga et al. 1995). The superior colliculus is known to be highly sensitive to moving stimuli and to be involved in saccadic eye movements (Paxinos 1995; Wurtz and Goldberg 1972). We have demonstrated that the superior colliculus displays a higher sensitivity to high than to low flashing frequencies. This is in contrast to the findings of Correa-Lacarcel, who demonstrated the opposite using red light as a visual stimulus and c-fos labeling as a spatial indicator of related activities (Correa-Lacarcel et al. 2000). This difference may be explained by the different experimental settings used by Correa-Lacarcel and colleagues, in which rats

were adapted to complete darkness and visual stimulation was provided by a laser beam producing monochromatic red light.

Functional MRI on flashing light stimulation: temporal aspects

VISUAL CORTEX. The temporal BOLD response pattern observed in the visual cortex followed the on and off switching of the visual stimulus rather accurately. In the cat visual cortex, neurons can be temporally entrained on flashing light, which evokes a persistent oscillatory response during the entire stimulation period (Rager and Singer 1998). During this process, the retinocortical system exhibits preferences for frequencies in the θ (4–8 Hz) or slow α range (8 to $\leq$13 Hz) (Rager and Singer 1998). Our findings diverge from these results in that we observed a larger BOLD response at lower (1–5 Hz) as compared with higher (8–12 Hz) flashing frequencies during binocular visual stimulation. Possibly this difference is related to species differences, as Girman(Girman et al. 1999) performed electrophysiological recordings of the rat primary cortex during monocular visual stimulation and obtained maximal neuronal responses to stimuli of low range frequencies varying from 0.43 Hz to 6.88 Hz.

FLOCCULAR COMPLEX. During binocular stimulation, the frequency response pattern observed in the flocculus-paraflocculus correlated with that observed in the visual cortex, whereas the superior colliculus displayed an inverse neurovascular activity. Although both cortex and superior colliculus are known to project to the flocculus-paraflocculus, another pathway exists from the superior colliculus to other areas in the cerebellum, such as the vermal lobules VI and VII, which are involved in eye-head movements during saccades (Akaike 1985; Burne and Woodward 1984). Crispino (Crispino and Bullock 1984) demonstrated that responses recorded at the surface of the superior colliculus evoked by bilateral flashes were augmented by cerebellar stimulation of the dorsal vermis (lobules V–VII). In this respect, observed correlations could confirm this closer relationship between the floccular complex and the visual cortex rather than between either of these structures and the superior colliculus.

SUPERIOR COLLICULUS. The superior colliculus is differentiated from the cortex and paraflocculus by its unique response pattern to visual stimulation. At its most sensitive frequencies, the BOLD contrast remained elevated and reached a second climax even after stimulation had ceased. Molotchnikoff (Molotchnikoff et al. 1987) suggested that collicular cells could "memorize" for several seconds various features present in the environment. In a recent study, he demonstrated that the oscillatory activity of the collicular neurons during OFF responses on flash offset is mediated by nitrogen oxide (NO), a vasoactive mediator that increases cerebral blood flow (Yang et al. 2000). These findings may be related to each other, but it would be interesting to investigate these responses at a much higher temporal resolution. In addition, binocular visual stimulation at high flashing rates resulted in a decreased BOLD response at increasing stimulation periods. This finding might reflect neuronal habituation (adaptation) of neuronal activity and hence a smaller BOLD response. Habituation is a common feature of cells from the deep collicular layers (Goldberg and Wurtz 1972; Paxinos 1995). Thus the BOLD trace appears to reflect the temporal response properties of the collicular neuronal population, though this needs to be confirmed in future studies.

Does BOLD fMRI reveal binocular inhibition?

During binocular visual stimulation, the amplitude of the BOLD contrast (i.e., the amplitude of the entire temporal signal intensity trace) in the visual cortex and the flocculus-paraflocculus decreased significantly at increasing frequencies, while it remained the same during monocular stimulation. This reduced response to increasing frequencies when both eyes were stimulated might suggest intrahemispheric binocular competition (Olk and Hartje 2001), as hypothized for humans (Eglin 1987; McKeever 1971; Moscovitch 1986). It is assumed that homologous bilateral stimulation results in interhemispheric exchange of information and that the information processing takes place in the hemisphere that is dominant for that particular task. In all mammals, ocular dominance columns, i.e., the grouping of cells with similar eye preference into columns, develop in the visual cortex, particularly during the critical period, during which the columnar architecture is highly susceptible to alterations in visual input (Katz and Crowley 2002). Thurlow (Thurlow and Cooper 1988) demonstrated the existence of ocular dominance patches in the visual cortex of the adult rat. The present data suggest an interhemisperic interaction (inhibition) in the visual cortex during the binocular presentation of flashing light at high frequencies. Chen and colleagues (Chen et al. 2005) recently showed that elevated endogenous GABA levels are correlated with decreased BOLD fMRI signals during electrical paw stimulation in the rat brain. Shmuel (Shmuel et al. 2002) related the negative BOLD response in humans during visual stimulation to a depression in neuronal activity. Therefore a decrease of the BOLD response might be interpreted in the context of binocular inhibition.

In conclusion, we have demonstrated for the first time that BOLD fMRI during visual stimulation with varying frequencies of light is a sensitive technique that is able characterize the temporal aspects and flashing frequency dependence of the entire visual system of the rat. Our observations largely correspond with literature findings on electrophysiological, autoradiographical, and c-fos studies of the rat visual system, and confirm the sensitivity of the superior colliculus to flashing light at high frequency, whereas cortex and flocculus seem more sensitive to low frequencies. The data also suggest a closer functional relationship between visual cortex and cerebellar structures compared with superior colliculus and cerebellar structures. In addition, we have shown a unique response pattern in the superior colliculus after repetitive visual stimulation. This will be elaborated in future studies.

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