We studied the effect of stimulus quality on the basic physiological response characteristics of oxygenation-sensitive MRI signals. Paradigms comprised a contrast-reversing checkerboard vs darkness or vs gray light as well as gray light vs darkness in a 2 s/52 s protocol (nine subjects). MRI was performed at 2.0 T using single-shot gradient-echo EPI (TR/TE = 500/54 ms, flip angle 30°). All paradigms elicited almost identical signal intensity time courses comprising a latency period (1–2 s), an activation-induced signal increase (4–4.5% at about 6–7 s after stimulus onset) and a post-stimulus signal undershoot (~1%) that slowly recovered to baseline (about 50 s). Thus, in contrast to findings for sustained stimulation, brief presentations of distinct visual stimuli exhibit similar physiological response characteristics that support the use of a uniform response profile for the evaluation of event related paradigms.

Introduction

The mainstream of magnetic resonance functional neuroimaging techniques focuses on the detection of activation-induced changes in the absolute concentration of deoxygenated paramagnetic hemoglobin in the venous compartments of the cerebral vasculature. Whereas the physical nature of the blood oxygenation level dependent (BOLD) MRI signal has been identified as reflecting the microscopic magnetic field homogeneity within an image voxel, the underlying physiological mechanisms that modulate the net cerebral blood oxygenation are less clearly understood. Possible contributions that accompany a change in neuronal activity originate from changes in cerebral blood flow, blood volume and oxidative metabolism. Their complex interplay and its effect on the spatiotemporal properties of oxygenation-sensitized MRI signals is the subject of ongoing methodological investigations. For example, we previously addressed the temporal response characteristics to visual activation for sustained [1], brief [2] and subsecond [3] stimulus presentations. In these cases, a contrast-reversing checkerboard, a movie, or even stationary diffuse light elicited both a positive BOLD MRI response and a post-stimulus signal undershoot when controlled by darkness.

Materials and Methods

Subjects and functional neuroimaging: A total of nine healthy subjects participated in the study (age...
22–29 years, informed written consent before all examinations). All studies were carried out at 2.0 T (Siemens Vision, Erlangen, Germany) using a standard imaging head coil and oxygenation-sensitive MRI based on single-shot blipped gradient-echo EPI (mean TE = 54 ms, symmetrical coverage of k-space). T2* sensitivity without T1 weighting was achieved by means of a repetition time TR = 500 ms and a flip angle of 30°. Three oblique sections (4 mm thickness, 2 mm gap) parallel to the calcarine fissure covered major parts of the primary and extrastriate visual cortex. The in-plane resolution was adjusted to 2 × 2 mm² (128 × 128 matrix, 256 × 256 mm² field of view).

**Paradigms:** Projection of visual stimuli reached 40 × 30° of the subjects’ visual field (Schafter and Kirchhoff, Hamburg, Germany) [1]. The checkerboard stimulus consisted of a circular pattern of black and white wedges reversing color ten times per second, corresponding to a frequency of 5 Hz. The gray light stimulus was a stationary presentation of diffuse gray light at a luminance that matched the mean luminance of the checkerboard. In all cases the subjects were instructed to fixate a central red cross and maintain attention throughout all experimental runs.

The actual paradigms were either checkerboard vs darkness, checkerboard vs gray light, a modified checkerboard at reduced luminance contrast (20% on a corresponding gray scale) vs gray light, or gray light vs darkness. The main protocol comprised six cycles of 2 s activation and 52 s of control. The stimulation cycles were preceeded by a leading 40 s period of the control stimulus to establish steady state conditions as well as to obtain a true pre-stimulation baseline MRI signal intensity. In addition, we used the same paradigms in an 18 s/36 s protocol (six cycles) to ensure consistency with previous studies.

**Data evaluation:** Stimulus-correlated changes in BOLD MRI signal intensity were analysed by cross-correlation analysis [6]. The reference function was a 6 s wide box-car shifted by 3.5 s with respect to onset of stimulation. These parameters account for hemodynamic latencies as well as rise and fall times in response to a change in visual stimulation. The raw data were subject to temporal filtering using a linear window of 2.5 s width. No spatial filtering was applied.

Quantitative maps of correlation coefficients were obtained by statistical analysis. Based on the individual noise distribution the strategy identifies activated centers by utilizing a high threshold (p < 0.0001) and integrates neighbouring pixels for area delineation [7]. The signal intensity time courses shown here represent mean values from all activated pixels admitted by the aforementioned analysis. Normalization was with respect to pre-stimulation baseline. In addition, the time courses were averaged across all six cycles and three sections using weighting factors that reflect the differences in the number of activated pixels per section. Finally, the data were averaged across subjects.

### Results and Discussion

**Spatial extent of activation:** Figure 1 demonstrates the spatial extent of activation in the occipital brain of a single subject for 2 s/52 s presentations of checkerboard vs darkness (Fig. 1A), checkerboard vs gray light (Fig. 1B) and gray light vs darkness (Fig. 1C). Although the activated areas largely overlap, a more detailed quantitative analysis reveals mean numbers of activated pixels per section of 198 ± 75 for checkerboard vs darkness, 144 ± 60 for gray light vs darkness and 108 ± 38 pixels for checkerboard vs gray light. Thus, the mean areas of activation apparently reflect the basic luminance contrast between conditions. Assuming darkness as control, activation by a 2 s contrast-reversing checkerboard involves more spatially extended neuronal activity than stationary gray light, while processing of a checkerboard vs gray light further reduces the area of activation. This interpretation is in line with other oxygenation-sensitive MRI studies of the human visual system [8–11] suggesting a high correlation between the response strength and the local (spatial and temporal) average of neuronal activity.

It is important to emphasize, however, that luminance differences regulate the spatial extent of activation only for very brief stimulus presentations that hamper the perception of detailed features, i.e. the full stimulus quality. Complementary observations for slightly longer activation periods suggest differences in spatiotemporal complexity of the stimuli to become the dominant factor with regard to spatial extent. In line with this more intuitive expectation, the use of an 18 s/36 s protocol (same group of subjects, maps not shown) results in a larger number of admitted pixels for presentations of checkerboard vs darkness (371 ± 118) or checkerboard vs gray light (316 ± 69) than for gray light vs darkness (298 ± 115). The fact that the total areas are almost twice as large as those for the 2 s/52 s protocol may be explained by the stronger BOLD MRI signal increase and enhanced undershoot obtained for longer stimulus presentations: see [2] for a comparison of 1.6 s (4–5% MRI signal increase) and 10 s checkerboard stimuli (5–6% MRI signal in-
crease) in protocols using 90 s of darkness as control.

In general, the observation that rather distinct stimuli such as a contrast-reversing checkerboard and a stationary gray screen result in substantial overlap of activated areas may be surprising at first glance. However, we should keep in mind that the columnar organization of the visual cortex (and also of other cortices) occurs on a spatial scale not resolved by the applied MRI technique. Thus, selective and condition-specific processing of distinct stimulus feature [12] takes place beyond the resolution of the present maps which blur respective representations within functional areas.

Nevertheless, the quantitative data seem to provide an indirect measure of the degree of neuronal activation, i.e. the visual system’s workload required to process either luminance contrast or differences in stimulus complexity. For the basic stimuli and conditions investigated here, the underlying changes in neuronal activity most likely reflect the recruitment or release of specialized subsets of neuronal populations rather than variations in the degree of neuronal firing (or its synchronization) within a given population.

**Temporal response characteristics:** The mean BOLD MRI signal time courses for the paradigms tested are summarized in Fig. 2. They correspond to checkerboard vs darkness (Fig. 2A), checkerboard vs gray light (Fig. 2B), checkerboard at 20% luminance contrast vs gray light (Fig. 2C) and gray light vs darkness (Fig. 2D). Qualitatively, all paradigms yield an initial hemodynamic latency as well as an activation-induced BOLD MRI signal increase and a post-activation signal undershoot. Because the 52 s recovery period turned out to be sufficient for a return to pre-stimulation baseline, these temporal profiles may be considered to represent the true activation response to physiologically decoupled 2 s events.

A more detailed analysis reveals minor though consistent differences between paradigms. For this purpose the full width at half height of the maximum MRI signal strength may be taken as a measure for the temporal response width of the positive BOLD response. It turns out that this width is about 7.0 s for paradigms with darkness as control (Fig. 2A,D), but only about 5.5 s for those using gray light as control (Fig. 2B,C).

Together with the fact that the 4.5% positive BOLD MRI response strength for a contrast-reversing black and white checkerboard differs only slightly from the 4.0% for a checkerboard at reduced luminance or even gray light, our observations suggest that the initial response to a functional challenge is dominated by a gross, rapid and rather non-specific
delivery of blood into activated brain areas that leads to an almost uniform hemodynamic behavior within the first few seconds after stimulation onset. Depending on the paradigm there may be differences in the spatial distribution of activations, but within an activated region the physiologic adjustment yields almost the same degree of venous hyperoxygenation as detectable by oxygenation-sensitive MRI. In fact, marked differences in stimulus quality are required to induce at least small differences in response strength. Moreover, the flow effect seems to occur at maximum possible speed. This is mainly based on previous findings for a 10 s contrast-reversing checkerboard where BOLD MRI signal increases of up to 6% required > 10 s after stimulation onset [2].

The strength of the undershoot signal is about −1.0% in all cases in good agreement with previous findings for physiologically decoupled brief stimuli [2,3]. However, even stronger signal decreases are observed after sustained activation [1] or after multiple cycles of repetitive activation [2,3,5]. Although the physical effect must be ascribed to an increase of the absolute concentration of deoxyhemoglobin in the affected image voxels, the underlying physiologic mechanism still remains unclear with slow alterations in oxidative metabolism [13] or hemodynamics [14] representing the most likely explanation.

**Conclusion**

A key result of the present work is that even marked differences in stimulus quality have little or no influence on the temporal characteristics of BOLD MRI responses to brief visual activation. The observation of a latency period, an activation induced positive BOLD response, and a post-activation undershoot, independent of the use of darkness or gray light as control condition, confirms and extends the validity of previous findings for brief stimuli [2,3] and event related protocols [5]. The findings suggest that the physiological response strength during the first few seconds after the onset of a functional challenge is dominated by a non-specific blood flow adjustment not reflecting detailed stimulus features.

The observation of a uniform hemodynamic response function provides experimental support for evaluation strategies that attempt to analyze BOLD MRI responses to event related paradigms by assuming a single reference profile [15,16]. Although the present work does not solve the problem of linear or non-linear overlap of responses, it demonstrates a standardized response for physiologically decoupled single trials.

In addition, the small paradigm-specific differences in the temporal response width are beneficial when used in event related studies. Because pertinent para-
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Digms tend to map only subtle differences between functional states, i.e., conditions with almost identical degrees of neuronal activity, they may result in sharper response functions with slightly faster rise and fall times. This effect may help to reduce interstimulus intervals in repetitive event-related protocols. However, further work is necessary to establish more detailed relationships between the degree of synaptic activity or, more likely, the extent of involvement of different neuronal populations and the observables accessible by oxygenation-sensitive MRI.

References


Received 3 February 1999; accepted 22 February 1999