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# Inflow Versus Deoxyhemoglobin Effects in BOLD Functional MRI Using Gradient Echoes at 1.5 T

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Modified gradient-echo MR techniques were applied to study the effects of inflow on functional brain imaging studies using visual and motor cortex stimulation. The results demonstrate that the large signal changes, seen in previously reported gradient-echo studies at 1.5–2.0 T, are dominated by direct inflow effects, in particular when using a large flip angle and a thin slice. The findings suggest that inflow-based functional imaging, along with Blood Oxygenation Level Dependent (BOLD) functional MRI, may play an important role in future research towards the functional organization of the human brain.

## INTRODUCTION

Recently, the use of  $T_2^*$ -weighted MRI in brain activation studies has resulted in functional images of the human brain.<sup>1–9</sup> During stimulation of the visual or motor cortex the thin layer of cortical gray matter, presumably involved with a particular task performance, was delineated with an unprecedented spatial resolution. The use of  $T_2^*$ -weighted MRI to detect functional involvement of brain regions was first suggested by Ogawa *et al.*<sup>10,11</sup> The underlying rationale is that increased local oxygen extraction during activation is accompanied by an incommensurate increase in local blood flow. The resulting increased blood oxygenation level leads to an increased  $T_2^*$  value, and thus to a signal increase in  $T_2^*$ -weighted MRI (hence also called Blood Oxygenation Level Dependent or 'BOLD' MRI). The influence of blood oxygenation on MR signal intensity was confirmed recently.<sup>3</sup> Functional images are usually generated from gradient echo or echo planar imaging (EPI) studies by subtracting images recorded during the non-activated state from images recorded during the activated state, and show high signal intensity in the 'activated' regions. In spite of the large progress in this field, there are a number of controversies and pitfalls related to functional BOLD MRI.<sup>12–21</sup> Researchers have questioned the ability of functional MRI to accurately localize brain activation in the parenchyma, due to the interfering signals from the larger draining vessels overlaying the cortical structures.<sup>19,20</sup> Others have pointed to motion related subtraction artifacts which may be misinterpreted as local brain activations.<sup>15</sup> Also, combined angiographic techniques and functional imaging studies showed striking similarities between the activated regions and the corresponding

larger vessel structure in the same anatomical region.<sup>20</sup> In addition, a large discrepancy exists in the reported intensity increases in activated areas between studies performed with EPI<sup>1,3–5</sup> and gradient-echo techniques.<sup>6,7</sup> As local increase in  $T_2^*$  related to the BOLD effect is expected to scale at least linearly with the applied static magnetic field strength, functional imaging results obtained at 0.15 T strongly suggest that effects other than BOLD are responsible for the contrast in such functional images.<sup>13</sup>

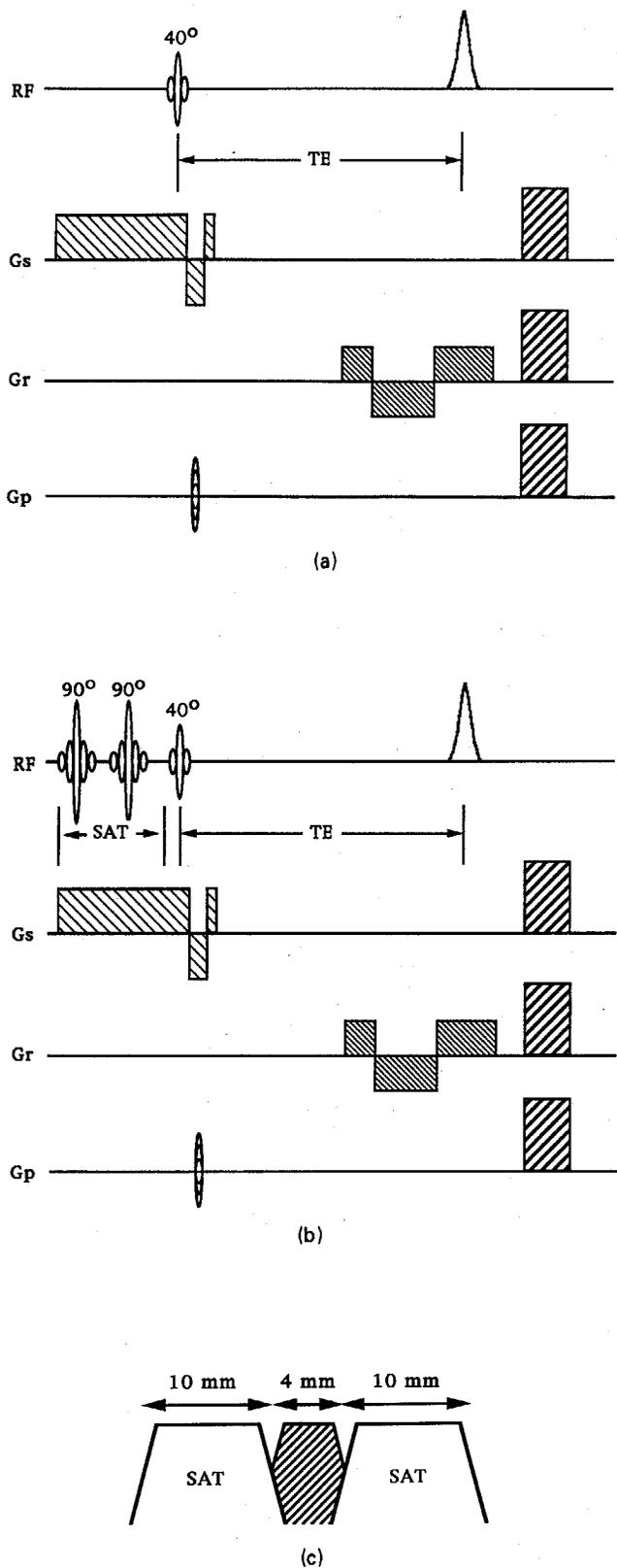
Contrast in gradient-echo images is affected directly by the flow into the observed plane, in particular when using a combination of large flip angle and thin slice. Conditions then closely resemble those of 2D time-of-flight MR angiography methods. Here, we show that inflow effects can indeed dominate the intensity changes in gradient echo functional imaging at 1.5 T. A preliminary account of this work has been presented recently.<sup>14</sup>

## MATERIALS AND METHODS

Three different series of experiments were performed to investigate the effects of inflow on functional images, all of which were based on a conventional gradient-echo method (also called GRASS, FLASH, or FFE), as shown in Fig. 1(a). The intensity increases in activated areas were studied as a function of the variables: (i)  $TE$ , (ii) with enhancement and cancellation of inflow, and (iii) with and without suppression of inflow. All experiments were performed on standard clinical scanners operating at 1.5 T, using circularly polarized RF head coils. The gradient-echo techniques used RF spoiling. Gradient wave forms in slice-select and read-out direction were first-order motion compensated. Gradient crushers were used at the end of each repetition interval to reduce image artifacts. A  $128 \times 128$  image matrix was used with a 20–24 cm field of view.

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Abbreviations used: BOLD, Blood Oxygenation Level Dependent; EPI, echo planar imaging; ROI, regions of interest.



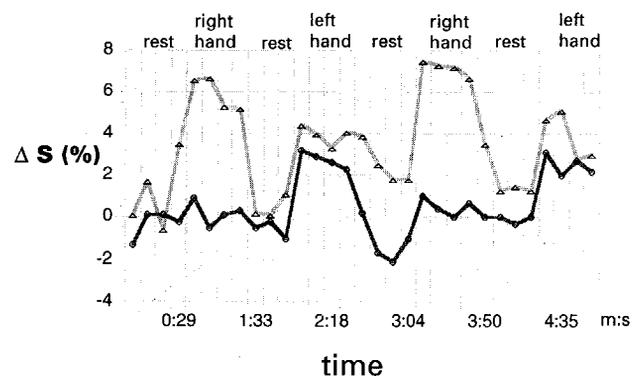
**Figure 1.** Inflow suppression incorporated in a FLASH pulse sequence. (a) Diagram of basic pulse sequence, employing first-order motion compensation in slice select (Gs) and readout (Gr) gradients, and gradient crushers in all directions, including a phase encoding gradient (Gp). The duration of the slice selection gradient lobe was lengthened to allow addition of saturation RF pulses [see (1b)]. (b) Pulse diagram of sequence with inflow suppression. Two 90° RF pulses were added to the basic sequence to saturate (SAT) signal from neighbouring slices. (c) Location of saturation (SAT) slices. The slices are chosen immediately adjacent to the observed slice in order to minimize contributions of in-flowing spins.

The typical RF pulse angle used was 40°, as used in most of the gradient-echo functional imaging studies published.<sup>6,7</sup>

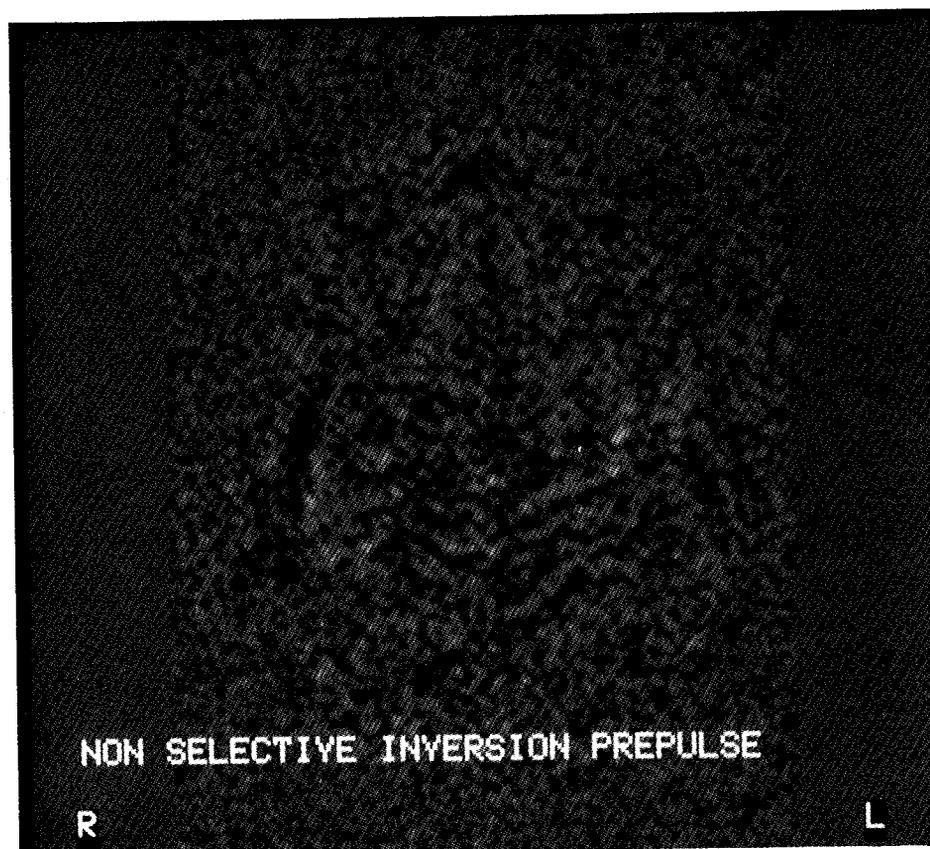
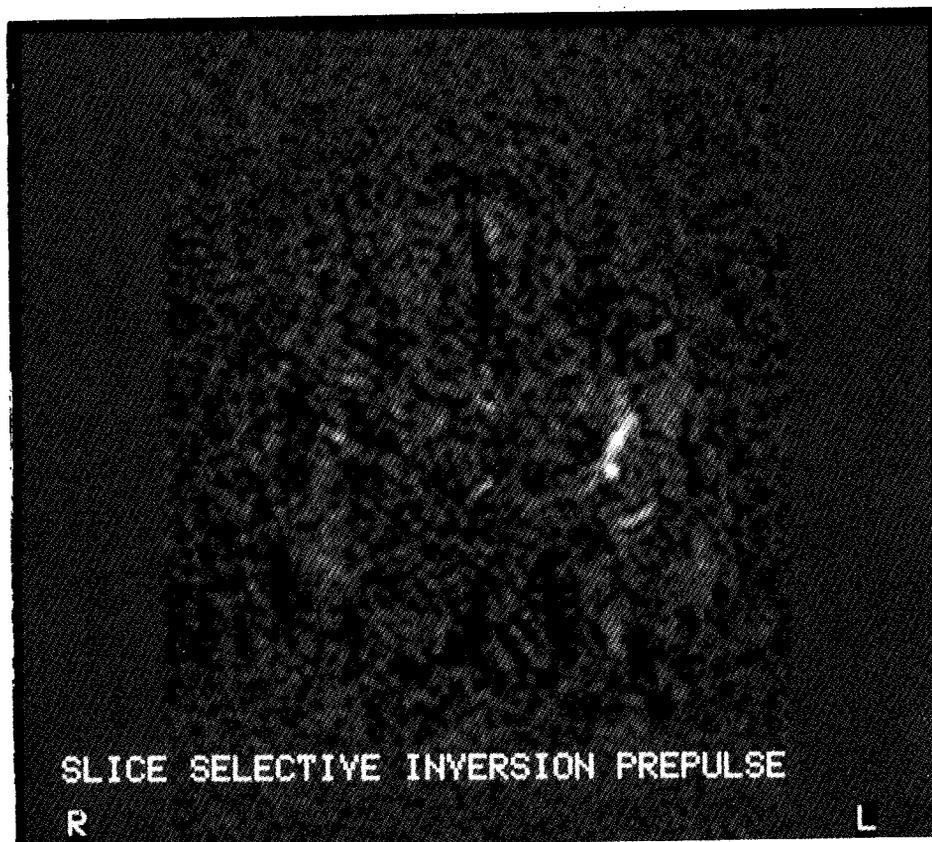
The first two experiments employed a finger tapping paradigm to study motor cortex stimulation and were performed on a 1.5 T Philips Gyroscan ACS-II scanner. A series of 32 dynamic scans was recorded, in which four scans at rest were alternated with four scans during which finger tapping was performed using the right or the left hand. Sagittal localizer images were recorded to position the 10 mm thick oblique transverse slice in the motor strip. Signal changes were studied in both dominant and non-dominant hemisphere. First,  $TE$  was varied between 15 ms, 30 ms and 60 ms while maintaining a  $TR$  of 85 ms. Second, sensitivity to inflow was varied by inserting an inversion prepulse followed by a delay to minimize signal due to stationary spins. In the case of inverting only the observed slice, the inflow sensitivity is enhanced. On the other hand, when using a non-selective inversion, inflow sensitivity is strongly reduced. For this particular purpose, the basic gradient-echo sequence was divided into 10 segments, and an effective inversion delay of 800 ms was selected which suppressed predominantly cerebral spinal fluid and other long  $T_1$  components under the given experimental conditions.

In the third experiment, visual stimulation paradigms were performed using flashing lights and varying checkerboard patterns using a 7.8 Hz frequency. Sagittal localizer images were used to position a 4 mm thick oblique slice through the calcarine cortex. A series of 36 dynamic scans was recorded, in which six scans at rest were alternated with six scans during which visual stimulation was performed. The experiments were performed on a 1.5 T GE/SIGNA scanner. Inflow suppression was realized by inserting two slice selective prepulses [Fig. 1(b)], suppressing two 10 mm thick slices adjacent to the observed slice [Fig. 1(c)]. A more rectangular profile of these slices was generated by using double-side lobed sinc RF wave forms. For comparison, the experiment was repeated without these saturation RF prepulses. Activation images were created by subtracting the sum of images obtained without stimulation from the sum of images recorded during stimulation.

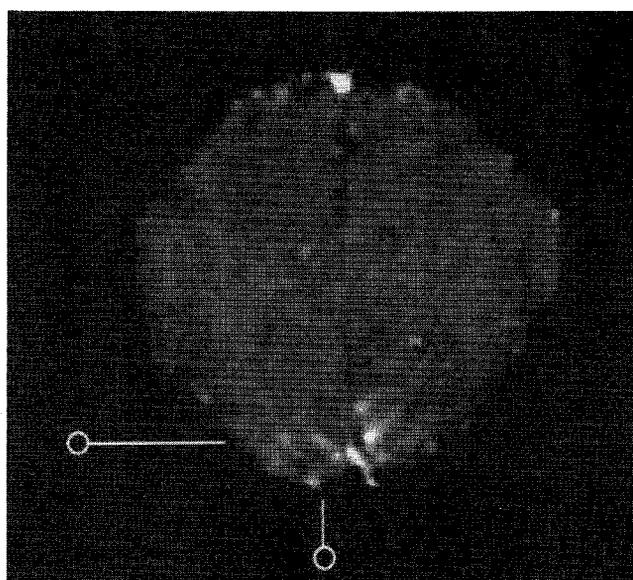
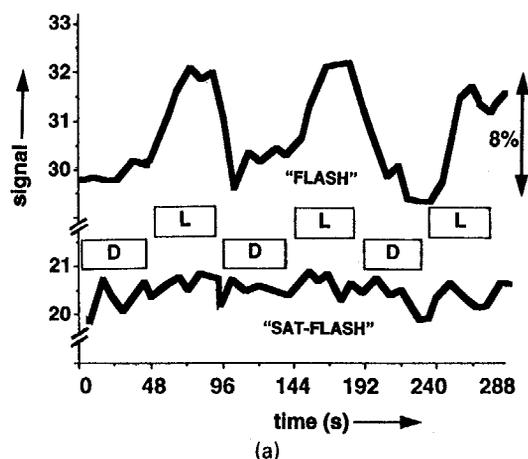
Consent was obtained from each volunteer. The optical stimulation studies were approved by NIH IRB.



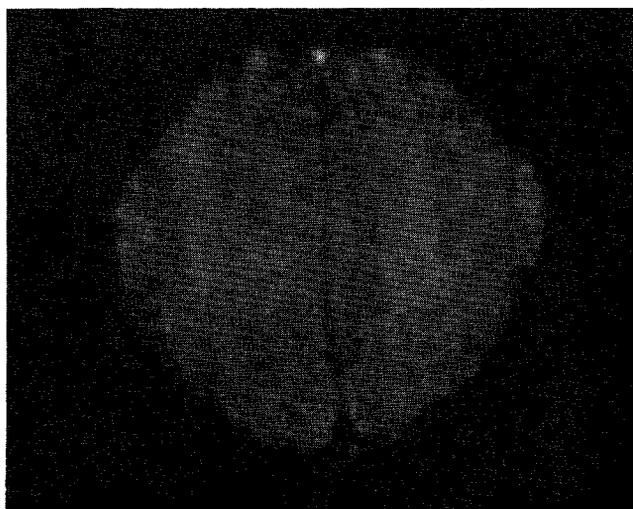
**Figure 2.** Time intensity diagram of the relative signal change in the precentral gyrus of the left and right hemispheres during a finger tapping series. The response shows the asymmetry between the dominant ( $\Delta$ ) and non-dominant ( $\circ$ ) hemispheres of this right-handed volunteer.



**Figure 3.** Subtraction images during motor cortex activation. Each image represents the combination of four images obtained during rest combined with four images obtained during right-handed finger tapping. (a) shows the result using a slice-selective inversion prepulse to enhance inflow effects, whereas (b) shows the effect using the same protocol with a non-selective-inversion prepulse to suppress inflow effects.



(b)



(c)

**Figure 4.** Example of the effect of inflow suppression in a checkerboard visual stimulation study. Signal changes upon stimulation are shown for an oblique slice through the visual cortex. (a) Time course of signal from a small region indicated in (b) within the visual cortex in FLASH (top) and SAT-FLASH (bottom) experiments. 'D' and 'L' indicate dark and light stages of the stimulation paradigm, respectively. (b) FLASH activation image. Increased signal intensity is seen in the posterior part of the visual cortex area (bottom of image). (c) SAT-FLASH activation image. Compared to the FLASH image (in b), a strong reduction of areas with increased signal intensity is observed, especially within the visual cortex.

**Table 1.** Comparison of visual stimulation measurements ( $n = 9$ ) performed without (FLASH) and with inflow saturation (SAT-FLASH). The average percentage signal increase upon activation is given for regions of interest (ROI) in the visual cortex area encompassing the brightest intensity areas in the difference images

ROI size	Average signal increase for FLASH (%) <sup>a</sup>	Average signal increase for SAT-FLASH (%) <sup>b,c</sup>
20–40 $\mu$ L	10.2	3.3
40–80 $\mu$ L	6.3	2.3
2–3 $\mu$ L	3.3	1.2

<sup>a</sup> For each subject, five to seven ROI were studied.

<sup>b</sup> With SAT-FLASH, the number of bright intensity areas was reduced by 75–80%. Data in this column are based on the remaining ROI with significant signal increase.

<sup>c</sup> Signal stability was 4–9% for the smallest ROI and 0.5–1.5% for the largest ROI.

## RESULTS

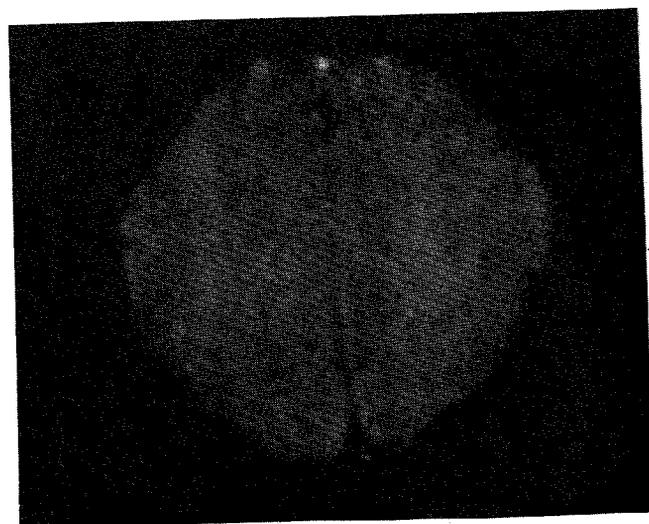
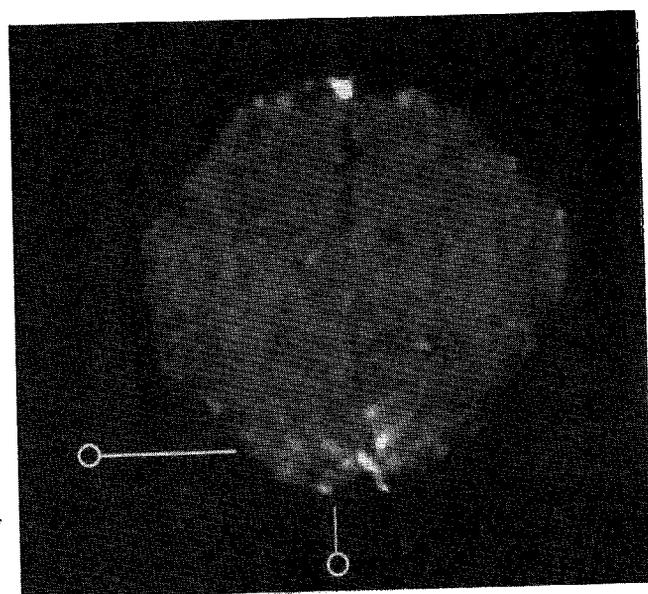
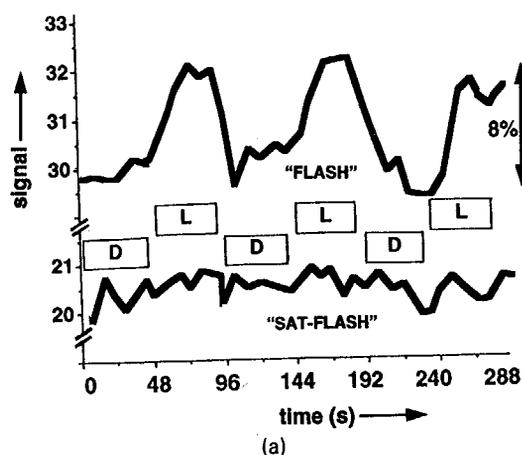
The motor cortex activation studies using the standard gradient-echo sequence as described showed an asymmetric response of a focal area located in the precentral gyrus of the dominant and non-dominant hemisphere (Fig. 2). The averaged ( $n = 5$ ) signal increase in the dominant motor cortex was 4.2% upon finger tapping with the dominant hand, whereas no increase was observed in the non-dominant motor cortex. When finger tapping was performed with the non-dominant hand, signal increases in the dominant and non-dominant hemisphere were 2.6 and 2.4%, respectively. Studies were performed at  $TE = 15, 30$  and  $60$  ms. The observed signal increases measured at these different  $TE$  values (all other experimental parameters were kept constant) corresponded to transverse relaxation rate changes ( $\Delta R_2^*$ ) of 0.93, 0.68 and 0.58 s, respectively.

Slice-selective and non-slice-selective inversion prepulses were used to enhance and attenuate inflow effects. A slice-selective inversion prepulse actually increased the observed signal changes considerably. Signal changes up to 20% were found under these conditions. With non-slice-selective inversion, signal changes in the motor cortex during activation dropped below the noise level (Fig. 3).

Nine out of 12 visual activation studies showed significant signal increases in the calcarine cortex upon stimulation. Signal increases of up to 30% were registered, depending on volunteer and location. The signal changes observed in the calcarine cortex during visual stimulation dropped below noise level when applying saturation prepulses (third series of experiments). Figure 4 shows an example of the effect of the application of saturation prepulses in a checkerboard visual stimulation experiment. Table 1 gives a summary of visual stimulation studies.

## DISCUSSION

The hemispheric asymmetry observed in the motor cortex studies shows that the signal changes do correspond with focal brain activation. Similar results have



**Figure 4.** Example of the effect of inflow suppression in a checkerboard visual stimulation study. Signal changes upon stimulation are shown for an oblique slice through the visual cortex. (a) Time course of signal from a small region indicated in (b) within the visual cortex in FLASH (top) and SAT-FLASH (bottom) experiments. 'D' and 'L' indicate dark and light stages of the stimulation paradigm, respectively. (b) FLASH activation image. Increased signal intensity is seen in the posterior part of the visual cortex area (bottom of image). (c) SAT-FLASH activation image. Compared to the FLASH image (in b), a strong reduction of areas with increased signal intensity is observed, especially within the visual cortex.

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## DISCUSSION

The hemispheric asymmetry observed in the motor cortex studies shows that the signal changes do correspond with focal brain activation. Similar results have

been reported at higher field strengths.<sup>8</sup> The possibility to determine brain dominance by this method illustrates that irrespective of the exact mechanism, functional imaging using gradient echoes at 1.5 T may give valuable information. The results, obtained with visual stimulation confirmed previously reported findings<sup>6,7</sup> and also demonstrate the ability to map physiology changes in brain locations globally corresponding with the known functional organization of the brain. However, assuming a susceptibility dominated contrast mechanism, the change in  $R_2^*$  is determined by the change in oxygenation level during stimulation and should be independent of  $TE$ . The observed variation of  $\Delta R_2^*$  with  $TE$  contradicts this assumption and therefore suggests that other contrast mechanisms may play an important role. As physiological studies have pointed out that local blood flow may increase by 10–30% during brain activation, the most likely other mechanism which accounts for the MR observations is direct measurement of increased (slow) inflow during stimulation. This idea is substantiated by the results of motor and visual stimulation. The correspondence between inflow sensitivity and signal increases in activated regions is evident in motor, as well as visual stimulation. This indicates that inflow effects contribute substantially to the contrast mechanism in BOLD type studies using gradient echoes. Since not all effects could be explained by inflow effects (Table 1), we suspect that a deoxygenation contrast mechanism is still present, but signal changes related to this mechanism are

much smaller than previously assumed. This is confirmed by recent 3D BOLD functional imaging studies with very high intrinsic S/N ratio.<sup>22</sup>

## CONCLUSIONS

The results demonstrate that the large signal changes seen in previously reported functional imaging studies using gradient-echo techniques are mainly caused by direct inflow effects. Our findings do not exclude the use of gradient-echo techniques for measurement of susceptibility changes, but indicate that inflow effects have to be accounted for carefully. Exploiting the inflow effect may even increase the potential of MR to study functional processes by tailoring pulse sequences to exactly this effect.<sup>16,17</sup> Therefore, both BOLD and inflow contrast mechanisms can play an important role in functional MRI. However, since inflow effects take place over larger distances, the use of a technique based on these effects may not lead to accurate localization of activated regions.

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