Contribution of Sinerem® Used as Blood-Pool Contrast Agent: Detection of Cerebral Blood Volume Changes during Apnea in the Rabbit

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The authors suggest that ultra-small paramagnetic iron oxide (USPIO) particles used as blood pool contrast agents may increase the sensitivity of midfield MRI (i.e., less than 1.5 Tesla) to physiological variations in cerebral blood volume. This hypothesis was tested on a rabbit model of apnea which increases pCO_2 and cerebral blood volume. Using Sinerem® as the USPIO at a blood concentration of 60 μmol iron/kg body weight, an 8% T_2*-weighted signal decrease could be observed at 1.0 T with 25–33% increase in pCO_2. Comparatively, in the absence of USPIO, T_2*-weighted signal dropped only 4% during apnea and after mild hyperoxygenation beforehand, due to increased deoxyhemoglobin content. These preliminary data suggest that USPIOs could play an important role in functional MRI at midfield strength, by sensitizing the signal to cerebral blood volume changes.

Key words: functional magnetic resonance imaging; blood pool contrast agent; cerebral blood volume; animal model.

INTRODUCTION

Changes in local cerebral hemodynamics and oxygenation resulting from brain function can be detected with MRI (1, 2). Using blood oxygen level dependent (BOLD) contrast (3), activated brain areas, which overcompensate for local increases in oxygen consumption, show increased cerebral blood flow. This overcompensation locally reduces the concentration of deoxyhemoglobin, resulting in an increase in T_2* and a rise in signal in gradient echo MRI. However, the T_2* effects produced by physiological variations of the deoxyhemoglobin content remain small, and the method suffers from a lack of sensitivity, especially at midfield strength (4). Paramagnetic contrast agents, such as Gd-DTPA, have also been used to monitor variations in cerebral blood volume during activation (5). With this approach, the effect on the MR signal is more pronounced. However, because of the recirculation and the plasma clearance of the agent, one must monitor the first passage of a bolus injection for each activated or resting condition, thus limiting the number of studies for any given subject (6). In these conditions, it would be useful to have a blood pool contrast agent that could remain at a sufficiently high, stable concentration in the blood stream during an entire study. The modulation of the agent concentration after variations of the cerebral blood volume could result in MR signal changes more pronounced in amplitude than those observed with the BOLD approach, especially at midfield strength.

This paper describes the use of the long plasmatic clearance of an ultra-small superparamagnetic iron oxide (USPIO) to monitor changes in blood volume in an animal model of apnea. When used as a blood pool contrast agent, it has previously been shown that USPIO is present at a sufficiently stable concentration to allow equilibrium imaging to be conducted (7). The sensitivity to physiological variations in cerebral blood volume at midfield strength (1.0 T) was assessed. Apnea-induced hypercapnia was used to produce cerebral vasodilatation (8) and comparison with the BOLD effect was made.

METHODS

Animals

Sixteen healthy New Zealand white rabbits housed in an approved facility were fasted for at least 12 h, anesthetized with 50 mg/kg intramuscular administration of tiletamin hydrochloride and zolazepam hydrochloride (Zoletil®, Laboratoires Reading, L'Hay-les-Roses, France), tracheotomized, paralyzed with 0.2 mg/kg/0.5h intravenous vecuronium bromide (Norcuron®, Organon Teknika, Fresnes, France), and mechanically ventilated. A Servo-ventilator 900® (Siemens-Elema, Schönander, Sweden) equipped with an oxygen monitor (Oxycheck 2000®, Critikon Inc., Tampa, FL) was connected to a pediatric spirometric unit (SE 302, Siemens-Elema) to be adjusted for small tidal volumes (14 ml/min obtained with a respiratory frequency of 40/min). The protocol consisted of three phases: 3.5 min of equilibration, 2.5 min apnea (ventilator turned off), and 15 min of recovery. A high level of oxygenation before apnea was intended to avoid hemoglobin desaturation during apnea and to isolate the contribution of vasodilation on the signal. On the other hand, moderate hyperoxygenation before apnea should allow comparative evaluation of the BOLD effect during apnea. The oxygen-inspired fraction (IFO_2) was set at 50% (moderate hyperoxygenation) in the first part of the experiment ($n = 9$) and at 70–85% (marked hyperoxygenation) in the following two parts: without ($n = 13$) and with ($n = 14$) contrast agent. Femoral arterial blood gases: (arterial O_2 partial pressure (pO_2), arterial CO_2 partial

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pressure (pCO_2), and percentage of hemoglobin saturation with O_2 (HbSat)) were analyzed at time 0 (control), after apnea, and after recovery. Femoral arterial blood pressure was controlled throughout the experiment (monitor Minimon 7131®, Kontron, Basel, Switzerland).

Contrast Agent

The USPIO (AMI-227, Sinerem®, Guerbet, Aulnay-sous-Bois, France) was injected intravenously in the third part of the experiment at a dose of 60 μmol iron/kg body weight when IFO_2 before apnea was 70–85%. These nanoparticles (30 nm in diameter as measured by photon correlation spectroscopy) consist of an iron oxide crystalline core (4–6 nm diameter as measured by electron microscopy) coated with a dextran surfactant. The T_2 relaxivity of the Sinerem® is 0.83×10^5(mol/liter)$^{-1}$ s^{-1} at 37°C and 20 MHz in plasma. Under the same conditions T_1 relaxivity is 0.27×10^5(mol/liter)$^{-1}$ s^{-1}. Since Sinerem® has a long plasmatic half life, the blood concentration changes only minimally during image acquisition (21 min). In the rat, the plasmatic half life is 118 min with a mean rate time of 170 min fitted by a decreasing monoexponential curve (7). In the rabbit, this was shown to be longer than 240 min at the dose used in this study. This agent is not toxic for human use and is currently undergoing clinical trials.

MRI

The animals were positioned prone in the head coil of a 1.0 T whole-body imager (Magnetom Impact®, Siemens, Erlangen, Germany). Three-plane images were first obtained to localize a coronal slice bisecting the hemispheres at the level of the basal ganglia. Magnetic field homogeneity was adjusted on this slice. Three series of images were acquired: one during each part of the experiment, with IFO_2 50% without Sinerem®, with IFO_2 70–85% without Sinerem®, with IFO_2 70–85% and Sinerem®. Each series included 128 images obtained every 10 s to cover the three phases of each part: 22 images during baseline, 15 during apnea and 91 during recovery. The imaging sequence is a T_2*-weighted FLASH sequence (fast low angle shot (9)) (FOV = 150 mm, TR = 54 ms, TE = 40 ms, flip angle = 10°, matrix size = 128 × 128, slice thickness = 5 mm, acquisition time = 9 s). The transmitter frequency and gain as well as receiver gain were kept constant throughout the experiment for each rabbit. In addition, a reference tube of gadolinium-DOTA 25 mM (Dotarem®, Laboratoires Guerbet, Aulnay-sous-Bois, France) was positioned near the rabbit head for normalization of the signal intensities.

Data Analyses

Images were transferred to a Sun Sparc 10® workstation (Sun Microsystems Inc., Mountain View, CA). They were processed with Analyse® software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN). Circular 0.7 cm^2 regions of interest were chosen in the cerebral gray matter and in the reference tube (Fig. 1). Statistical tests were performed for each animal: baseline and apnea signal intensities were compared after normalization to the signal of the reference tube for each animal between the three parts of the experiment using a two-sided paired t test for each condition (FIO_2 and Sinerem®). Additionally, the average percentage of signal enhancement (100 × {baseline-apnea}/baseline) for the group was

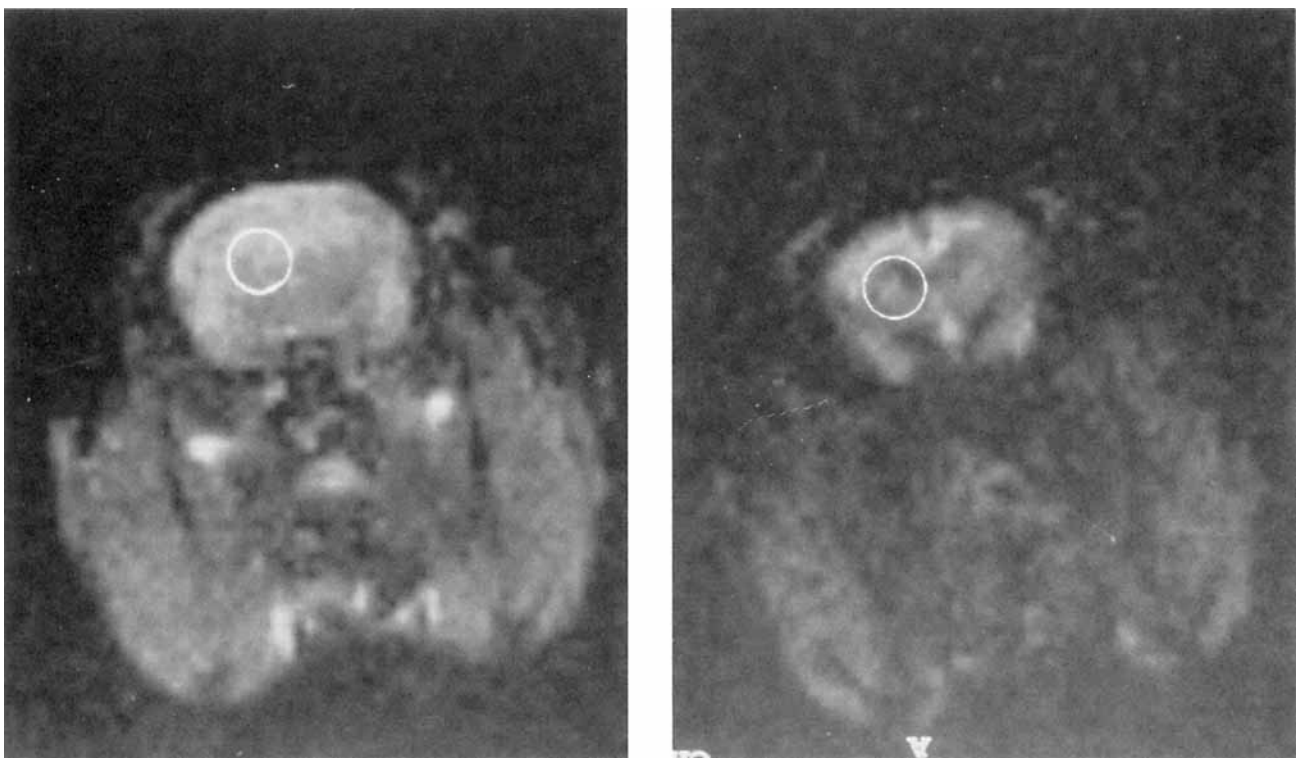

FIG. 1. T_2*-weighted coronal images of rabbit head before and after Sinerem®.

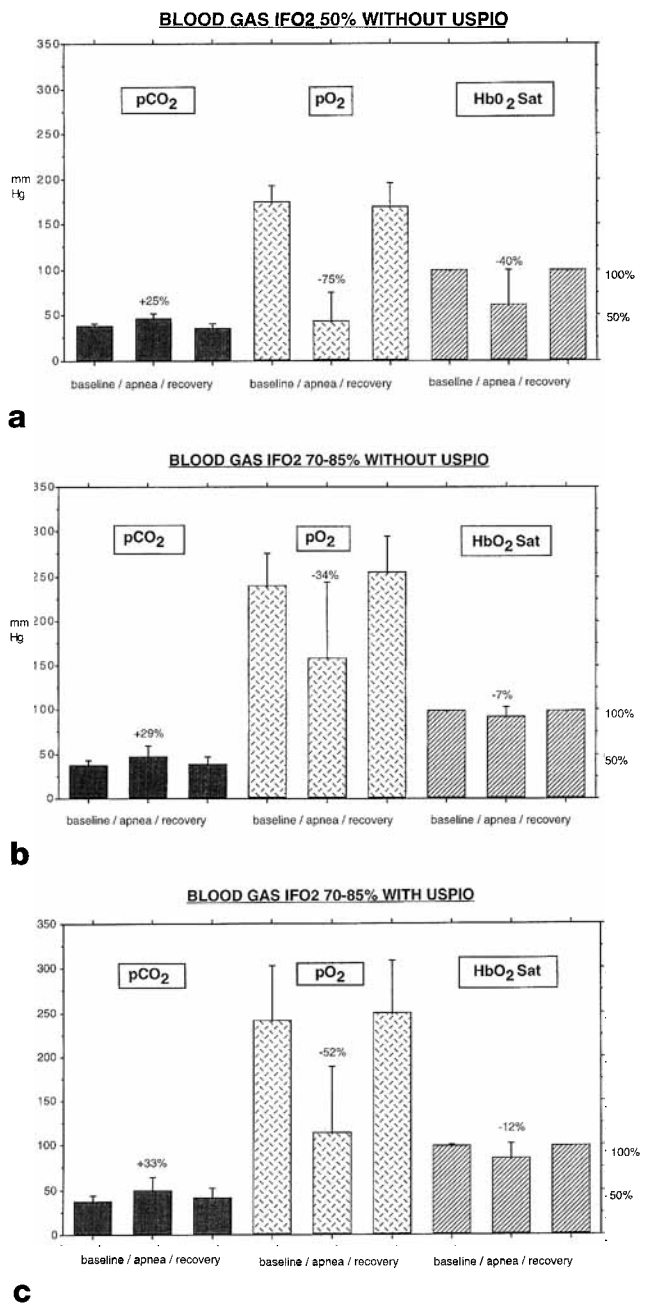

FIG. 2. Blood gas analysis.

tested against the null hypothesis using a two-sided *t* test.

RESULTS

Blood Gas Analysis

The three main physiological parameters monitored during baseline, apnea, and recovery periods are summarized in Fig. 2. In all cases pCO_2 increased reproducibly by 25–33% during apnea in the three series (IFO_2 50% without Sinerem® and IFO_2 70–85% without and with Sinerem®). Moreover, pO_2 and HbSat during apnea were strongly linked to IFO_2 before apnea. After a pre-oxygenation of IFO_2 50%, pO_2 decreased by 75% (from 175–45 mmHg) and hemoglobin desaturated by 40% (from 100–60%). After IFO_2 70–85%, pO_2 decreased by 35–50% (from 240–140 mmHg) and hemoglobin saturation was not significantly changed (7–12%).

MRI

The report of the dynamic study of signal intensity is summarized in Table 1 and Fig. 3. When pre-apnea IFO_2 was only 50%, hemoglobin desaturation during apnea was detectable as a signal decrease (-4% $P < 0.007$) in gray matter. Immediate return to baseline occurred at the end of apnea followed by a signal overshoot.

With 70–85% IFO_2 before apnea, no direct effect on MR signal could be detected during apnea. After injection of 60 μmol iron/kg body weight Sinerem®, MR signal intensity decreased by 45% (31–59%) as compared with precontrast images (Fig. 1). A marked signal decrease (-8% $P < 0.001$) occurred during apnea only when Sinerem® was present in the blood stream. This lower signal persisted after the end of the apnea episode before returning slowly to baseline.

DISCUSSION

Functional MR imaging widely relies on the BOLD effect, which depends on the changes in deoxyhemoglobin content (3). We were able to reproduce the BOLD effect at 1.0 T in the rabbit, as well as to study the vasodilation induced by increased pCO_2. In the experimental setting of apnea, it was possible to dissociate the decrease in hemoglobin saturation and the increase in pCO_2 (blood volume). Blood pool contrast agents may be used to increase the sensitivity of MRI to physiological changes in cerebral blood volume. The purpose of this study was to verify the capability of Sinerem® to significantly improve the detection of vasodilation. The fact that increases in pCO_2 could be maintained at similar levels (25–33%) during apnea suggests that vasodilation was equivalent in the three series (IFO_2 50%, IFO_2 70–85% with and without Sinerem®) and that our experimental model is stable and reproducible.

Hemoglobin desaturation during apnea and anoxia results in increased deoxyhemoglobin content responsible for the BOLD effect of MR signal decrease at high field strength (10). The BOLD effect could also be seen in our experiment performed at 1.0 T. The signal decreased by 4% during apnea when IFO_2 was 50% beforehand and when pO_2 fell from 175–45 mmHg after 2.5 min of apnea. This reduction is not in contradiction with the observation made at 2.0 T using the echo-planar technique in the cat by Turner *et al.* (11), who did not observe any change in signal during the apnea part of their experiment. In their experiment, apnea lasted only 1 min, which resulted in a pO_2 fall from 180 to 73 mmHg. Furthermore Kennan *et al.* (12) suggest that a change of 10 mmHg in pO_2 may produce a signal drop of 2–10% depending on the ambiant pO_2. The most drastic effect is expected below 40 mmHg although direct comparisons of MR signal changes may not be valid because, in addition to physiological parameters, MR signal intensity also depends in detail on the pulse sequence type and acquisition parameters.

Table 1
Mean Normalized Intensity During Baseline[a] and Apnea[b]

Animal	IFO_2 50% Without Sinerem®		IFO_2 70–85% Without Sinerem®		IFO_2 70–85% With Sinerem®	
	baseline	apnea	baseline	apnea	baseline	apnea
1	0.43 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	0.43 ± 0.01	0.24 ± 0.00	0.24 ± 0.01
2			0.51 ± 0.01	0.51 ± 0.01	0.30 ± 0.01	0.27 ± 0.01
3			0.92 ± 0.01	0.92 ± 0.01	0.66 ± 0.01	0.60 ± 0.01
4	0.43 ± 0.01	0.42 ± 0.01	0.41 ± 0.01	0.42 ± 0.01	0.32 ± 0.04	0.30 ± 0.01
5			0.45 ± 0.01	0.46 ± 0.02	0.21 ± 0.01	0.20 ± 0.01
6			0.59 ± 0.02	0.59 ± 0.02	0.22 ± 0.01	0.19 ± 0.01
7			0.58 ± 0.02	0.58 ± 0.02	0.20 ± 0.01	0.19 ± 0.02
8	0.56 ± 0.02	0.53 ± 0.03				
9			0.33 ± 0.01	0.33 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
10	0.40 ± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.40 ± 0.01	0.20 ± 0.00	0.19 ± 0.01
11	0.49 ± 0.01	0.47 ± 0.01	0.51 ± 0.01	0.52 ± 0.01	0.25 ± 0.01	0.24 ± 0.01
12	1.05 ± 0.01	0.95 ± 0.07			0.37 ± 0.01	0.34 ± 0.01
13	0.41 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.28 ± 0.03	0.24 ± 0.01
14			0.41 ± 0.01	0.41 ± 0.01	0.30 ± 0.01	0.24 ± 0.02
15	0.55 ± 0.01	0.53 ± 0.03	0.55 ± 0.01	0.55 ± 0.02	0.27 ± 0.01	0.26 ± 0.00
16	0.58 ± 0.01	0.56 ± 0.02				
(%) Mean variation	−4% ($P < 0.007$)[c]		No		−8% ($P < 0.001$)[c]	

[a] Mean value on baseline.
[b] Mean value on the last 10 images of apnea.
[c] Two-sided *t* test.

In our experiment the fast recovery of the signal after restoration of oxygenation is in keeping with a rapid reversible biochemical reaction such as that which is presumed to occur in the BOLD effect.

Our experimental setting dissociates the effect of vasodilation and hemoglobin desaturation depending only on FIO_2 before apnea. No significant hemoglobin desaturation (7–12%) occurs during apnea after $IF0_2$ 75–80% when pO_2 remains between 240 and 140 mmHg and BOLD effect cannot occur (12). Indeed no significant change in signal could be seen spontaneously during apnea in the second part of the experiment (i.e., without Sinerem®) because the MR signal was not directly sensitive to variations in cerebral blood volume in the conditions of the experiments.

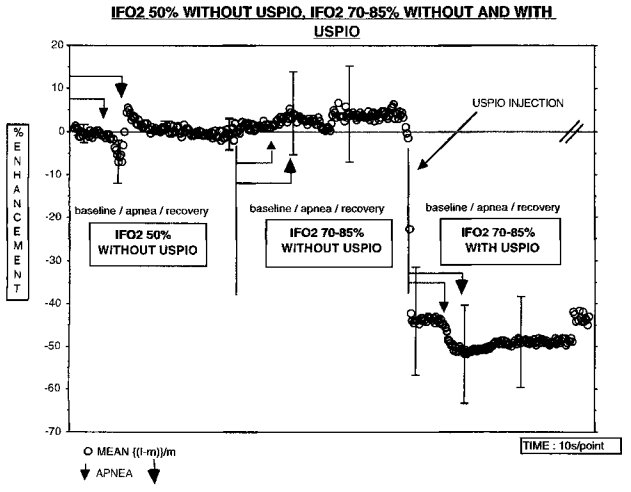

FIG. 3. Signal time evolution curve. Error bars represented standard deviation of the parameter over population at the central time point of baseline and recovery and at the end of the apena.

Sinerem® is a blood pool contrast agent. It is not initially recognized by the macrophage monocyte phagocytic system because it has a small hydrodynamic diameter (30 nm) and a hydrophilic dextran coating. In this study the injection of 60 μmol iron/kg body weight produced a prominent (45% decrease) and prolonged effect lasting at least 30 min on T_2*-weighted signal intensity of the brain parenchyma. The high magnetic moment intravascular circulating agent decreases the signal intensity of the brain as a result of two mechanisms of proton dephasing: T_2 relaxation interactions of the outer-sphere type (13, 14) and magnetic susceptibility effect inducing microscopic field inhomogeneities in which water protons diffuse (15, 16). It affects the signal derived from a larger fraction of protons than that contained in the vascular space. This indirectly renders the small capillary density of 5% significant on the signal intensity. It is therefore able not only to reflect the cerebral blood volume without the contingency of first pass imaging (17, 18) but also to sensitize the signal to variations in cerebral blood volume. When diffusible chelates of gadolinium are used, the total injection time is a significant factor that determines the time to the maximum plasmatic concentration and thus the plasmatic concentration depends on the injection pattern as entry function (6). This problem is avoided using Sinerem® as blood pool contrast agent because the plasmatic concentration rapidly becomes independent from the injection pattern. Additionally it may be considered to be constant throughout the imaging procedure which lasts 21 min since this time scale is short in comparison to the plasmatic half-life of the agent.

In the last part of the experiment, Sinerem® was able to sensitize the signal to variations in cerebral blood volume. The 8% signal decrease observed during apnea after IFO_2 70–85% with Sinerem® is independent of the BOLD effect and related to the increased amount of Sinerem® in voxels brought by vasodilation during hyper-

capnia. The large decrease in the baseline signal caused by the introduction of Sinerem® is a significant drawback of this method, although it could be less detrimental with the use of a smaller dose than the 60 μmol iron/kg body weight of this study.

The slow signal recovery after the end of apnea is evocative of persistent vasodilation, which is a long hemodynamic reversible reaction. The longer recovery time from vasodilation could also explain the overshoot of signal after apnea in the first part of the experiment with IFO_2 50% beforehand. Indeed it could correspond to locally increased oxyhemoglobin related to the combination of persistent increased cerebral blood volume and in-flow of reoxygenated blood. This effect is consistent with the signal increase reported by others in the 1st min of recovery from respiratory challenge studied in the cat brain with echo-planar MRI (11). This is also connected to the increased signal observed on reperfusion after transient cat brain ischemic episodes (19).

The dissociation between the BOLD effect and vasodilation in the current apnea experiments allows speculation on the impact of Sinerem® for the detection of hemodynamic change, which may occur during brain activation. This 8% signal decrease observed with Sinerem® is larger than the 4% decrease of the BOLD effect as expected from the simulations comparing oxygenation contrast with hemoglobin and relaxation induced by superparamagnetic iron compounds (12). In brain activation, however, the effects of the two mechanisms would be opposite. Local brain activation would result in an increase in the blood pool agent content (increase in cerebral blood volume) and a decrease in the deoxyhemoglobin content (increase in cerebral blood flow proportional to the increase in blood volume). Nevertheless even taking into account the difference in order of magnitude, the amplitude of the Sinerem® effect could probably compensate for the possible rise in signal produced by BOLD. Indeed the change in relaxation rate of the blood depending linearly on the deoxyhemoglobin concentration is expected to remain small. For example, for the full range of change in the deoxyhemoglobin level from 0–100%, Ogawa *et al.* found only a maximum sensitivity in signal response of 20% at 7.0 T (20). Although various means could also be considered to decrease the BOLD effect, further work would help to better define the respective contribution of the vasodilation and of the BOLD effect in brain activation, and to fully evaluate the potential role of the two mechanisms in functional MRI at various field strengths.

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REFERENCES

1. K. K. Kwong, J. W. Belliveau, D. A. Chesler, I. E. Goldberg, R. M. Weisskoff, B. P. Poncelet, D. N. Kennedy, B. E. Hoppel, M. S. Cohen, R. Turner, H. M. Cheng, T. J. Brady, B. R. Rosen, Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc. Natl. Acad. Sci.* **89,** 5675–5679 (1992).
2. P. A. Bandettini, E. C. Wong, R. S. Hinks, R. S. Tikofsky, J. S. Hyde, Time course EPI of human brain function during task activation. *Magn. Reson. Med.* **25,** 390–397 (1992).
3. S. Ogawa, D. W. Tank, R. Menon, J. M. Ellermann, S-G. Kim, H. Merkle, K. Ugurbil, Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc. Natl. Acad. Sci.* **89,** 5951–5955 (1992).
4. R. Turner, P. Jezzard, H. Wen, K. K. Kwong, D. Le Bihan, T. Zeffiro, R. S. Balaban, Functional mapping of the human visual cortex at 4 and 1.5 Tesla using deoxygenation contrast EPI. *Magn. Reson. Med.* **29,** 277–279 (1993).
5. J. W. Belliveau, D. N. Kennedy, R. C. McKinstry, B. R. Buchbinder, R. M. Weisskoff, M. S. Cohen, J. M. Vevea, T. J. Brady, B. R. Rosen Functional mapping of the human visual cortex by magnetic resonance imaging. *Science* **254,** 716–719 (1991).
6. A. Villringer, B. Rosen, J. W. Belliveau, J. L. Ackerman, R. B. Lauffer, R. B. Buxton, Y-S. Chao, V. J. Wedeen, T. J. Brady, Dynamic imaging with lanthanide chelates in normal brain: contrast due to magnetic susceptibility effects. *Magn. Reson. Med.* **6,** 164–174 (1988).
7. C. Chambon, O. Clément, A. LeBlanche, E. Schouman-Claeys, G. Frija, Superparamagnetic iron oxides as positive MR contrast agents: in vitro and in vivo evidence. *Magn. Reson. Imaging* **11,** 509–519 (1993).
8. M. Wahl, L. Schilling, Regulation of cerebral blood flow - a brief review, *in* "Monitoring of Cerebral Blood Flow and Metabolism in Intensive Care" (A. W. Unterberg, G-H. Schneider, W. R. Lanksch, Eds.), *Acta Neurochir.* [Suppl] vol. 59, pp. 3–10, Springer-Verlag, Wien, 1993.
9. A. Haase, J. Frahm, D. Matthaei, W. Hänicke, K. D. Merboldt, FLASH Imaging. Rapid NMR Imaging using low flip angle pulses. *J. Magn. Reson.* **67,** 258–266 (1986).
10. F. Prielmeier, K-D. Merboldt, W. Hänicke, J. Frahm, Dynamic high-resolution MR Imaging of brain deoxygenation during transient anoxia in the anesthetized rat. *J. Cerebr. Blood Flow Metab.* **13,** 889–894 (1993).
11. R. Turner, D. LeBihan, C. T. W. Moonen, D. Despres, J. Frank, Echo-planar time course MRI of cat brain oxygenation changes. *Magn. Reson. Med.* **22,** 159–166 (1991).
12. R. P. Kennan, J. Zhong, J. C. Gore, Intravascular susceptibility contrast mechanisms in tissues. *Magn. Reson. Med.* **31,** 9–21 (1994).
13. R. N. Muller, P. Gillis, F. Moiny, A. Roch, Transverse relaxivity of particulate MRI contrast media: from theories to experiments. *Magn. Reson. Med.* **22,** 178–182 (1991).
14. P. Gillis, S. H. Koenig, Transverse relaxation of solvent protons induced by magnetized spheres: application to ferritin, erythrocytes, and magnetite. *Magn. Reson. Med.* **5,** 323–345 (1987).
15. D. L. White, K. P. Aicher, A. A. Tzika, J. Kucharczyk, B. L. Engelstad, M. E. Moseley, Iron-dextran as a magnetic susceptibility contrast agent: flow-related contrast effects in the T2-weighted spin-echo MRI of normal rat and cat brain. *Magn. Reson. Med.* **24,** 14–28 (1992).
16. Y. Rozenman, X. Zou, H. L. Kantor, Cardiovascular MR imaging with iron oxide particles: utility of a superparamagnetic contrast agent and the role of diffusion in signal loss. *Radiology* **175,** 655–659 (1990).
17. R. Weissleder, G. Elizondo, J. Wittenberg, C. A. Rabito, H. H. Bengele, L. Josephson, Ultrasmall superparamagnetic iron oxide: characterisation of a new class of contrast agents for MR imaging. *Radiology* **175,** 489–493 (1990).
18. I. Berry, M. Gigaud, C. Chambon, E. Canet, D. Gracia-Meavilla, C. H. Manelfe, Efficacy of ultrasmall superparamagnetic iron oxide in the detection of experimental focal cerebral ischemia. (submitted to *Stroke*)
19. A. J. De Crespigny, M. F. Wendland, N. Derugin, E. Kozniewska, M. E. Moseley, Real-time observation of transient focal ischemia and hyperemia in cat brain. *Magn. Reson. Med.* **27,** 391–397 (1992).
20. S. Ogawa, T. M. Lee, B. Barrere, The sensitivity of magnetic resonance image a rat brain to changes in the cerebral venous oxygenation. *Magn. Reson. Med.* **29,** 205–210 (1993).