

Subanesthetic Isoflurane Affects Task-induced Brain Activation in a Highly Specific Manner

A Functional Magnetic Resonance Imaging Study

Wolfgang Heinke, M.D.,* Christian Schwarzbauer, Ph.D.†

Background: Functional magnetic resonance imaging of blood oxygenation level-dependent signal changes offers a very promising approach to investigate activated neural networks during anesthesia.

Methods: Sixteen healthy male volunteers, assigned into two groups of eight subjects (isoflurane group, control group), were investigated by functional magnetic resonance imaging during different experimental conditions. The isoflurane group successively breathed air (baseline condition), isoflurane in air (0.42 vol% inspiratory; isoflurane condition) and air again (recovery condition) while performing a visual search task, whereas the control group breathed air during all experimental conditions. Functional magnetic resonance images were acquired during the entire experimental session. In addition, reaction times and error rates were recorded.

Results: A significant isoflurane-related decrease ($z > 3.1$ corresponding to $P < 0.001$) in task-induced brain activation was found in three distinct cortical regions: the right anterior-superior insula (Talairach coordinates: $x = 32$, $y = 22$, $z = 8$) and the banks of the left and right intraparietal sulcus (Talairach coordinates: $x = -34$, $y = -36$, $z = 32$; $x = 22$, $y = -60$, $z = 41$, respectively). Subcortical structures (lateral geniculate nucleus) and the primary cortices (motor cortex, visual cortex) were not affected. All measured parameters indicated a nearly complete recovery of the affected networks within 5 min.

Conclusions: Our findings indicate that subanesthetic isoflurane affected task-induced activation in specific neural networks rather than causing a global decrease in functional activation.

FUNCTIONAL imaging modalities such as positron emission tomography or single photon emission computed tomography have revealed many details concerning the effect of common anesthetic agents (e.g., isoflurane) on cerebral blood flow and metabolism.¹⁻⁸ The effect of anesthesia on task-induced brain activation, however, is less well established. Functional magnetic resonance imaging (fMRI) offers a very promising approach to investigate this phenomenon.

In comparison to positron emission tomography or single photon emission computed tomography, fMRI

provides higher intrinsic spatial and temporal resolution, and it does not require the injection of exogenous tracers.^{9,10} Therefore, a subject can perform a variety of different tasks during various experimental conditions (e.g., breathing subanesthetic concentrations of a volatile anesthetic agent *vs.* breathing air) within the same imaging session. Most fMRI studies are based on measuring changes in the blood oxygenation level-dependent (BOLD) signal that arise from a complex interplay between cerebral hemodynamics and oxidative metabolism.^{10,11}

Investigation of sedative effects produced by anesthetics in conscious volunteers offers the possibility to study the interaction of these drugs with cognitive processes. It should therefore provide further evidence for a better understanding of the general mechanism of anesthetic action. In the present study, we investigated the effect of subanesthetic isoflurane on functional brain activation induced by a visual search task.

Materials and Methods

Subjects

Sixteen male volunteers between the ages of 20 and 35 yr with no history of trauma or neurologic disease were investigated. All subjects were right-handed. Females were excluded from the study because of the possibility of sex differences in task-induced activation of the brain.¹² The study was approved by the local ethics committee of the Medical Faculty, University of Leipzig. Written informed consent was obtained from each subject before the study. Subjects were instructed not to eat or drink 6 h before the experiment and to refrain from consuming alcohol or using any medication on the day of the experiment. All volunteers took part in a familiarization session before the day of the study in which all tasks and proceedings were practiced.

Experimental Design and Procedure

The volunteers were positioned supine on the gantry of the scanner. A polyethylene mouthpiece with 4 m of flexible tubing was used to connect the subjects to an MR-compatible ventilator. We used a semi-open circuit with a fresh gas flow of 10 l/min. Subjects breathed spontaneously throughout the experiment. To facilitate inspiration, a pressure support of 200 Pa was applied. Nasal breathing was precluded by a nose clamp. Foam padding and a "stiff neck" were used to limit head movements. Heart rate, arterial oxygen saturation, end-

* Research Associate, Department of Anesthesiology and Intensive Care Therapy, University of Leipzig. † Research Associate, Max-Planck-Institute of Cognitive Neuroscience.

Received from the Department of Anesthesiology and Intensive Care Therapy, University of Leipzig, Leipzig, Germany, and Max-Planck-Institute of Cognitive Neuroscience, Leipzig, Germany. Submitted for publication January 24, 2000. Accepted for publication January 3, 2001. Supported by Verein zur Förderung der Forschung und Fortbildung in der Anästhesiologie, Intensiv-, Schmerz- und Notfalltherapie e.V., Leipzig, Germany. Presented in part at the German Congress of Anesthesiology, Wiesbaden, Hessen, Germany, May 5-8, 1999.

Address reprint requests to Dr. Heinke: Klinik und Poliklinik für Anästhesiologie und Intensivtherapie der Universität Leipzig, Liebigstr. 20a, 04103 Leipzig, Germany. Address electronic mail to: heiw@medizin.uni-leipzig.de. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

tidal partial carbon dioxide pressure, and isoflurane concentrations (inspiratory and expiratory) were continuously monitored during the entire session using an MR-compatible monitoring system.

A visual search task was used for stimulation. The subjects were first given a cue comprising a red "L" shape of a given orientation (0° , 90° , 180° , 270°). After a delay of 1.2 s, a 10×10 array of randomly rotating white "L"s was presented on the black background for a period of 2.2 s. Then four of these shapes became stationary and changed their color from white to red, while the remainder continued to rotate. The possible orientations of the stationary "L" shapes were 0° , 90° , 180° , 270° . The stationary shapes always occurred at the same position. The distance from the position of the stationary shapes to the center of the screen was the same, as was the distances between the stationary shapes. The subjects had to determine whether the orientation of any one of the stationary shapes matched that of the cue and had to give a yes-no response using a two-button key pad. Subjects used the index finger (yes response) and middle finger (no response) of the right hand. To introduce a further level of complexity, the coding of the key depended on the orientation of the cue "L" shape. If it was rotated by 90° or 270° , the first key corresponded to "yes" and the second to "no." If it was rotated by 0° or 180° , the coding of the keys was reversed. Reaction times and error rates were recorded. The reaction limit was 2 s. Answers exceeding this limit were discarded. The stimulation protocol consisted of five blocks of control interleaved with five blocks of visual stimulation. During the control task, the subject had to fixate on a gray dot positioned at the center of a black screen. The duration of each block was 30 s, and the entire stimulation experiment lasted 5 min. The stimulation program was written in ERTS (BeriSoft Cooperation, Frankfurt am Main, Germany). Using a liquid crystal display projector, the display was projected onto a translucent screen positioned inside the magnet bore behind the subject's head. The screen was viewed using mirror glasses that were equipped with correction lenses as necessary.

The fMRI imaging protocol is illustrated in figure 1. The 16 volunteers were divided into two groups, each consisting of eight subjects. The first group took part in the actual isoflurane experiment (isoflurane group), and the second group took part in a control experiment (control group). The isoflurane experiment lasted 30 min and included three 5-min stimulation blocks. During the first stimulation block (baseline condition [BC]) performed at 0 to 5 min, the subjects breathed air. The vaporizer was then adjusted to 0.42 vol% isoflurane so that the participants were breathing isoflurane in air at 5 to 20 min. The second block (isoflurane condition [IC]) was performed at 15 to 20 min. A tactile stimulation was performed on the big toe 30 s before the IC started to ensure that every subject was aware of the task and was

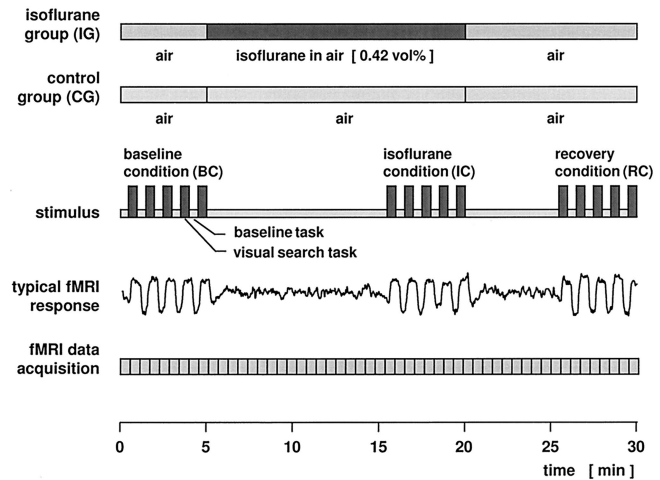


Fig. 1. Schematic representation of the experimental protocol. The stimulus (visual search task *vs.* baseline task) was presented during three epochs corresponding to the following experimental conditions: baseline condition, isoflurane condition, and recovery condition. During each experimental condition, five blocks of the baseline task were presented in an interleaved manner with five blocks of the visual search task. The duration of each block was 30 s. Functional magnetic resonance imaging (fMRI) data were continuously acquired during the entire experiment. As a representative example we show a typical fMRI signal response of a single voxel in the visual cortex. It was obtained by multisubject averaging over the isoflurane group. Note how the fMRI signal follows the switching on and off of the external stimulus.

not asleep during this part of the session. After the IC, the vaporizer was closed and the subjects breathed air again. The third block (recovery condition [RC]) was performed at 25 to 30 min to investigate the recovery from the preceding IC. Functional magnetic resonance images were acquired in a continuous fashion during the entire experimental session with a repetition time of 2 s. The control experiment was identical to the isoflurane experiment, except that the subjects breathed air during the entire experiment.

Functional Magnetic Resonance Imaging

All experiments were performed on a 3 Tesla whole-body scanner equipped with a quadrature birdcage head resonator and an actively shielded whole-body gradient set capable of switching 30 mT/m in 500 μ s. The MPIL software package developed at the Max-Planck-Institute of Cognitive Neuroscience in Leipzig was used to drive the console.

Functional magnetic resonance imaging was performed on the basis of a conventional blipped echoplanar imaging sequence.¹³ Imaging parameters were as follows: matrix size, 64×64 ; field of view, 19.2×19.2 cm; in-plane spatial resolution, 3×3 mm; slice thickness, 5 mm; repetition time, 2 s; echo time, 30 ms. Sixteen axial slices were acquired covering the entire brain.

Before the fMRI experiment, high-resolution anatomic images were obtained from the same slices using a mod-

ified driven equilibrium Fourier transform (MDEFT) imaging sequence¹⁴ with the following imaging parameters: matrix size 256×256 ; field of view, 19.2×19.2 cm; in-plane spatial resolution, 0.75×0.75 mm; slice thickness, 5 mm; inversion time, 0.65 s; recovery time, 0.65 s; echo time, 6.1 ms; total acquisition time, 5.5 min.

In a different imaging session, a three-dimensional (3D) data set was acquired using 3D MDEFT imaging.¹⁵ Imaging parameters were as follows: matrix size, $256 \times 256 \times 128$; field of view, $25.0 \times 25 \times 19.2$ mm; spatial resolution, $0.98 \times 0.98 \times 1.5$ mm; inversion time, 0.65 s; recovery time, 0.65 s; echo time, 6.1 ms; total acquisition time, 19 min.

Image Processing and Statistical Analysis

Functional data sets were acquired with a repetition time of 2 s per volume. Since each volume consisted of 16 slices acquired in a serial manner, the temporal delay between slices was corrected by sinc-interpolation. In addition, data were corrected for bulk motion using a rigid-body realignment algorithm. Image time series were then displayed as a movie to ensure that no substantial through plane motion was present. A spatial Gaussian filter with a full width at half maximum of 5 mm was applied to each image. Finally, a baseline correction of the image series was performed by applying a high-pass filter with a cutoff frequency of 1/120 Hz.

Statistical evaluation was based on a least-squares estimation using the general linear model for serially autocorrelated observations.¹⁶⁻¹⁹ First, for each individual subject, statistical parametric maps were generated. The design matrix was generated with a boxcar (square wave) function and a response delay of 6 s. The model equation, including the observation data, the design matrix, and the error term, were convoluted with a Gaussian kernel with a dispersion of 4 s (full width at half maximum). The model includes an estimate of temporal autocorrelation that is used to estimate the effective degrees of freedom. For each experimental condition (BC, IC, RC), contrasts between visual search and baseline task were calculated using *t* statistics. Subsequently, *t* values were converted to *z* values. The resulting *z* maps were coregistered onto the corresponding 3D anatomic data sets using the two-dimensional high-resolution MDEFT images as a reference, and finally transformed into a stereotactic Talairach coordinate system.²⁰

The primary statistical analysis described above yielded 48 *z* maps ($Z_{c,s,g}$) corresponding to the three conditions (*c* = BC, IC, RC), the 8 subjects per group (*s* = 1, 2...8), and the 2 groups (*g* = isoflurane group, control group). It is reasonable to assume that differences between experimental conditions in the isoflurane group are not solely caused by anesthesia but may also

reflect carryover effects (*e.g.*, habituation, fatigue, learning). However, using an appropriate statistical design, separation of isoflurane from carryover effects can be achieved by comparison with a suitable control group. For this purpose, we used the concept of the “split-plot” factorial design,²¹ using planned contrasts to increase statistical sensitivity. We expected a specific interaction between group and condition: isoflurane effects should be dominant during the IC and may be still present during the RC of the isoflurane group, whereas carryover effects in both groups should be the same. The latter assumption is reasonable because both groups underwent the same experimental procedure, except that the control group was breathing air during the entire experiment. To separate isoflurane from carryover effects, we calculated the following planned contrasts between the experimental conditions (*c* = BC, IC, RC) from the individual *z* maps for each of the 16 subjects:

$$C = Z_{BC}/2 - Z_{IC} + Z_{RC}/2 \quad (1)$$

These contrasts are quantitative measures that describe the difference between the IC in comparison to the mean of the BC and RC. In the isoflurane group, these differences may be caused by both isoflurane and carryover effects, whereas in the control group, differences may only arise as a result of carryover effects. The effect of isoflurane can therefore be separated from carryover effects by comparing the contrasts obtained in both groups. For this purpose, we applied a *t* test for independent samples. The resulting *t* map was finally converted into a *z* map. Hereafter we refer to this *z* map as “interaction map” because it displays the isoflurane-induced interaction between the two groups.

However, we were not only interested in the isoflurane-induced changes in the task-induced activation pattern, but also in the activation pattern itself. We therefore calculated six activation maps ($A_{c,g}$) corresponding to the three experimental conditions (*c* = BC, IC, RC) and the two groups (*g* = isoflurane group, control group) by multisubject analysis of the individual *z* maps²²:

$$A_{c,g} = \sum_{s=1}^8 Z_{c,s,g} \sqrt{8} \quad (2)$$

Multisubject analysis was used for two reasons: to increase statistical sensitivity and to reduce the amount data.

Finally, the interaction map showing isoflurane-related changes in task-induced activation was superimposed onto an activation map ($A_{c=BC,g=IG}$) that indicates the entire task-induced activation pattern during the BC. Significant task-induced activation ($z > 5.5$, uncorrected for multiple comparisons) was color-coded in uniform blue, whereas a red-yellow color scale was used to display the level of significance in isoflurane-related

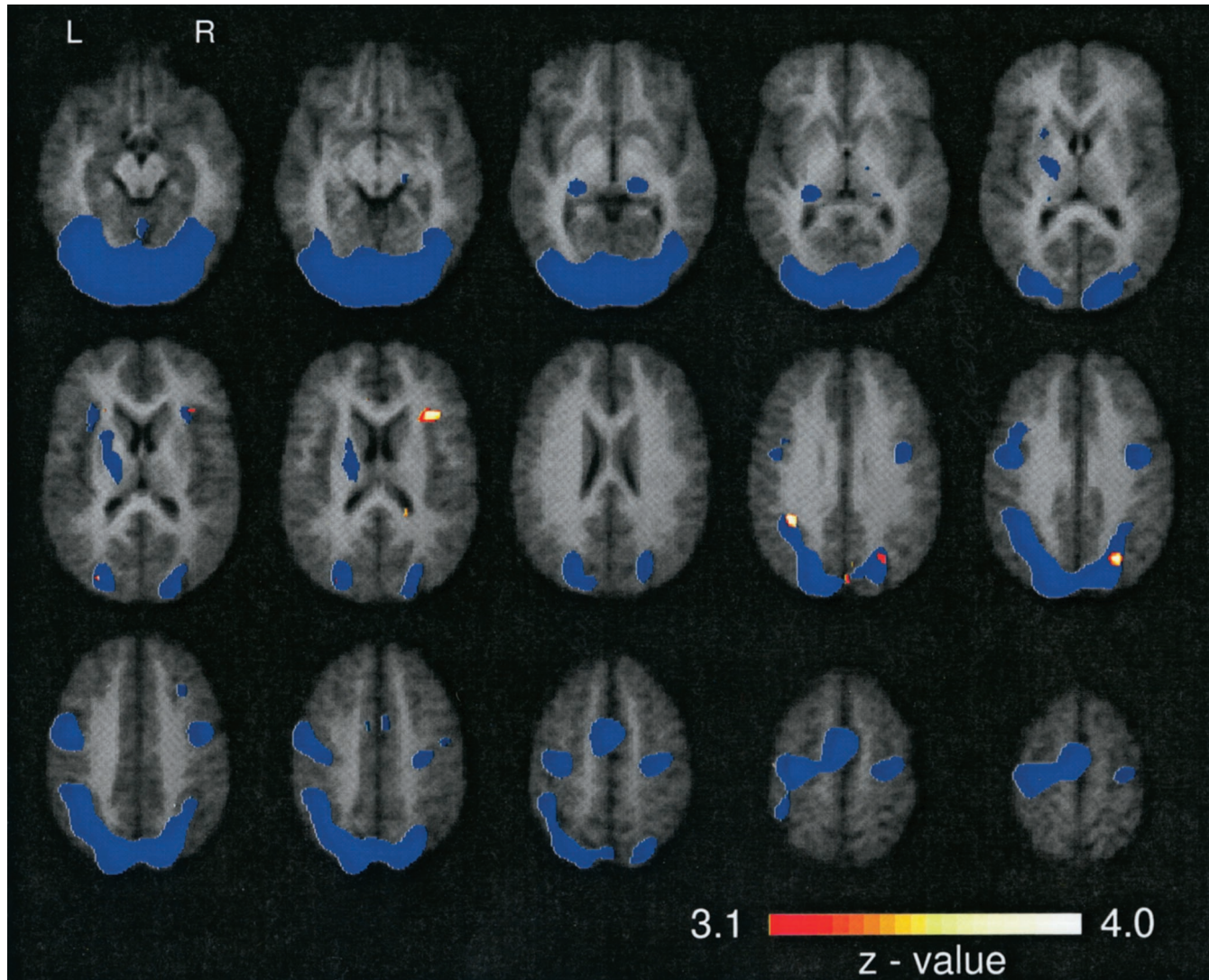


Fig. 2. Functional interaction map showing isoflurane-related changes in task-induced activation. Significant decrease in activation ($z > 3.1$ corresponding to $P < 0.001$) is visible in three distinct cortical regions: the right antero-superior insula, the left intraparietal sulcus (ascending–horizontal branch), and right intraparietal sulcus (horizontal branch); a three-dimensional representation of these regions is given in figure 3. Voxels with a significant increase in task-induced activation were not found at this level of significance. Regions of significant task-induced activation that were not affected by isoflurane are shown in uniform blue ($z > 5.5$ corresponding to $P < 10^{-7}$, uncorrected for multiple comparisons). L = left; R = right.

changes. Correction for multiple comparison was omitted because of this reasonable high threshold. Color-coded regions were then overlaid onto a 3D anatomic data set that was obtained by multisubject averaging over all individual 3D anatomic data sets ($n = 16$) in the Talairach coordinate system.

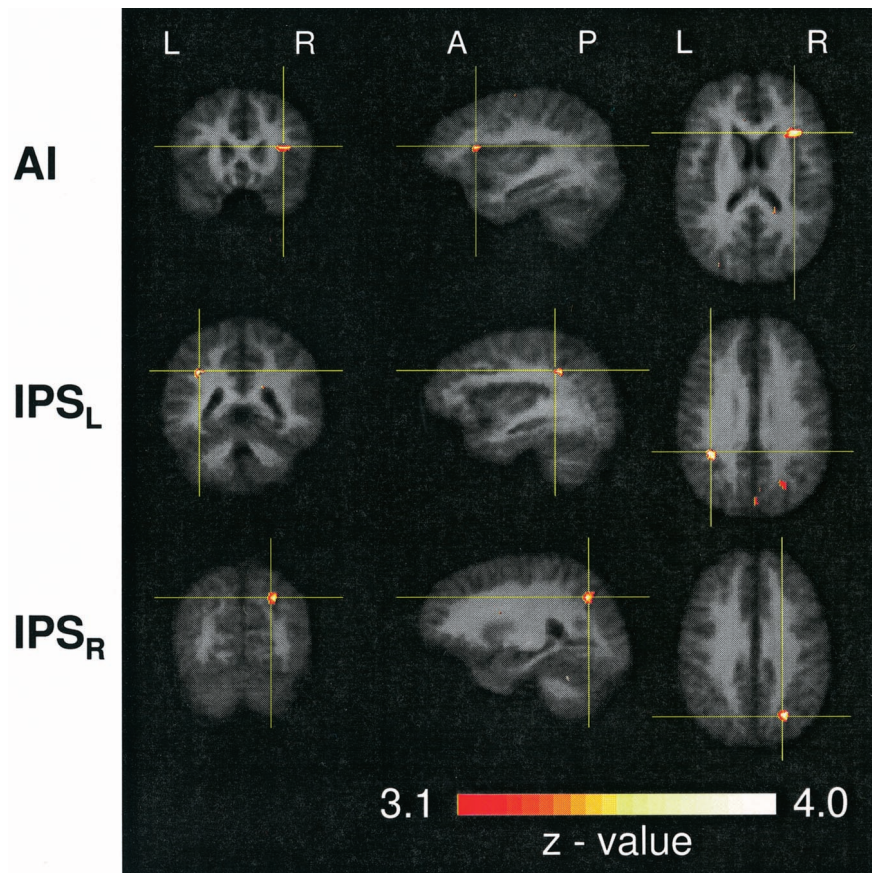
Image processing and statistical analysis were performed on the basis of the LIPSIA software package developed at the Max-Planck-Institute of Cognitive Neuroscience in Leipzig.²³ The routine for generating the interaction map was written in IDL (Research Systems, Boulder, CO).

Physiological data (heart rate, breathing rate, arterial oxygen saturation, end-tidal carbon dioxide values) of the isoflurane group were compared between experimental conditions (BC, IC, RC). A one-way repeated-

measures analysis of variance was used to compare normally distributed data (heart rate, breathing rate, arterial oxygen saturation), whereas a repeated-measures analysis of variance on ranks (Friedman test) was applied to analyze data that were not normally distributed (end-tidal carbon dioxide).

Average changes in reaction times were calculated for each group (isoflurane group, control group) comparing IC *versus* BC and RC *versus* BC. The t statistic was used to analyze the statistical significance of the results. In addition, the error rates (number of actual errors/number of possible errors) were calculated for each group and condition. For a statistical analysis of the results, we applied a one-way analysis of variance on ranks (Kruskal-Wallis test), because data were not normally distributed.

Fig. 3. Three-dimensional representation of the functional interaction map showing regions with a significant isoflurane-related decrease ($z > 3.1$ corresponding to $P < 0.001$) in task-induced activation. AI = antero-superior insula; IPS_L = left intraparietal sulcus; IPS_R = right intraparietal sulcus. The Talairach coordinate of the selected voxels are: $x = 32, y = 22, z = 8$ (AI); $x = -34, y = -36, z = 32$ (IPS_L); $x = 22, y = -60, z = 41$ (IPS_R). From left to right: coronal, sagittal, and axial view. L = left; R = right; A = anterior; P = posterior.



Results

Functional Magnetic Resonance Imaging

To separate isoflurane-related effects from carryover effects (*e.g.*, habituation, fatigue, learning), we performed a statistical comparison between the isoflurane group and the control group (see Methods). The resulting interaction map is depicted in figure 2. The red-to-yellow color scale displays the level of significance. The threshold was set to $z = 3.1$, which corresponds to a probability of $P = 0.001$. Significant decrease in activation is visible in three distinct cortical regions: the right antero-superior insula, the left intraparietal sulcus (ascending-horizontal branch), and the right intraparietal sulcus (horizontal branch); a 3D representation of these regions is shown in figure 3. Voxels with a significant increase in task-induced activation were not found at this level of significance. Regions of significant task-induced activation ($z > 5.5$) that were not affected by isoflurane are shown in uniform blue. These regions include the lateral geniculate nucleus, the striate and extrastriate visual cortex, the motor cortex, the supplementary motor area, and the left putamen, as well as large parts of the anterior insula and the banks of the intraparietal sulcus.

A quantitative analysis of isoflurane-related changes is shown in figure 4. Representative voxels were selected

in the three regions affected by isoflurane (antero-superior insula, left intraparietal sulcus, right intraparietal sulcus) as well as in three regions not affected by isoflurane (lateral geniculate nucleus, visual cortex, motor cortex). For each group (isoflurane, control), the group-specific z values calculated according to equation 2 are plotted *versus* the experimental condition (BC, IC, RC). In the antero-superior insula, left intraparietal sulcus, and right intraparietal sulcus, the isoflurane group shows a clear decrease in functional activation during the IC in comparison to the control group. During the RC, functional activation almost reached BC. A statistical comparison of this “decrease-recovery” behavior between both groups using a split-plot factorial design (see Methods) yielded significant differences ($z > 3.1$). However, no significant isoflurane-related effects were detectable in visual cortex, motor cortex, and lateral geniculate nucleus. The plot corresponding to motor cortex nicely demonstrates that a control group is indispensable for a correct interpretation of isoflurane-related effects. The isoflurane group shows a distinct decrease-recovery behavior that could be misinterpreted as an isoflurane-related effect. However, the control group shows a comparable time course. The observed decrease-recovery behavior is therefore a result of carryover effects (*e.g.*, habituation, fatigue, learning) and not related to isoflurane.

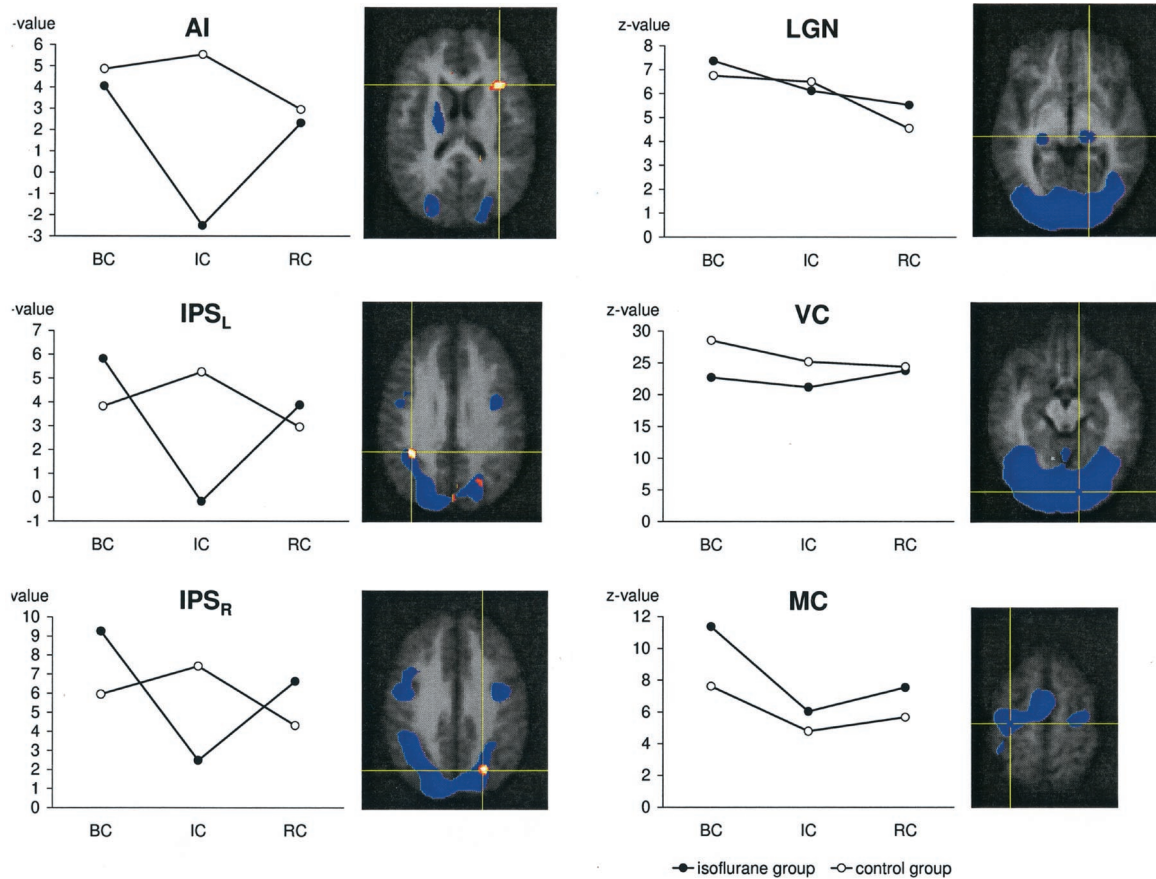


Fig. 4. Quantitative analysis of isoflurane-related changes in task-induced brain activation. Representative voxels were selected in six different regions: AI = anterio-superior insula; IPS_L = left intraparietal sulcus; IPS_R = right intraparietal sulcus; VC = visual cortex; LGN = lateral geniculate nucleus; MC = motor cortex. The plots show the group-specific z values calculated according to equation 2 for each group (isoflurane, control) and condition (BC = baseline condition; IC = isoflurane condition; RC = recovery condition). Comparing the corresponding time courses of the isoflurane and control group reveals a significant isoflurane-related decrease ($z > 3.1$ corresponding to $P < 0.001$) in AI, IPS_L , and IPS_R . No such effect, however, is visible in VC, LGN, and MC.

Reaction Times and Error Rates

Figure 5 shows the average change in reaction times comparing IC *versus* BC and RC *versus* BC. The isoflurane group showed a significant increase of $26.5 \pm 10.6\%$ during the IC ($P < 0.0002$). During the RC, reaction time was still prolonged: $4.9 \pm 3.7\%$ ($P < 0.01$). No significant changes in the reaction times were found for the control group. The corresponding changes in reaction time of the control group are not significant.

The average error rates under BC, IC, and RC were 5.7 ± 8.6 , 25.1 ± 23.1 , and $14.9 \pm 18.0\%$, respectively, for the isoflurane group and 6.5 ± 3.7 , 10.0 ± 7.1 , and $8.5 \pm 6.6\%$, respectively, for the control group. Differences between error rates are not significant as proven by a statistical comparison of both groups.

Behavioral State of the Subjects

No subject fell asleep during the IC, as proved by reaction times. Most of the subjects compared their behavioral state during the isoflurane experiment with a slightly intoxicated state. During an interview conducted

after the experimental session, they reported that details of the search task became blurred.

Physiologic Data

Physiologic data (heart rate, oxygen saturation, and breathing rate) of the isoflurane group did not show any

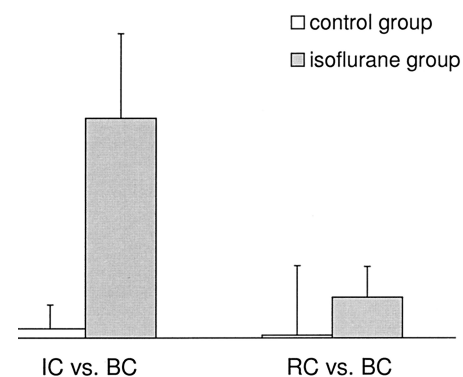


Fig. 5. Average change in the reaction times comparing isoflurane *versus* baseline condition (IC *vs.* BC) and recovery *versus* baseline condition (RC *vs.* BC).

Table 1. Physiologic Data (Isoflurane Group) under Baseline, Isoflurane, and Recovery Conditions

	BC	IC	RC
HR	71.5 ± 5.5	70.9 ± 6.6	68.8 ± 7.2
Sao ₂	94.0 ± 2.5	93.7 ± 2.9	93.6 ± 2.4
BR	12.2 ± 2.9	10.5 ± 4.2	10.4 ± 2.9

Mean values and standard deviations were calculated from the individual results of all subjects (n = 8). No significant differences were found between different conditions.

BC = baseline condition; IC = isoflurane condition; RC = recovery condition; HR = heart rate in beats per minute; Sao₂ = oxygen saturation (%); BR = breathing rate in cycles per minute.

significant difference between the three experimental conditions (table 1). Breathing of isoflurane resulted in an inspiratory concentration of 0.42 vol% at the beginning and 0.44 vol% at the end of the IC. The corresponding expiratory concentrations were 0.29 and 0.31 vol%. The time courses of the inspiratory and expiratory concentrations of isoflurane during the entire session are shown in figure 6. The end-tidal carbon dioxide values were slightly increased (1.5 mmHg) during the IC ($P < 0.05$; fig. 7).

Discussion

In most brain regions, our study reveals no isoflurane-related changes in task-induced activation, including all areas associated with primary information processing (e.g., lateral geniculate nucleus, primary visual cortex, motor cortex; fig. 2). However, in three focal cortical regions (anterio-superior insula, left intraparietal sulcus, and right intraparietal sulcus), isoflurane significantly decreased task-induced activation (fig. 4). This finding indicates that subanesthetic isoflurane affected task-induced activation in specific neural networks rather than causing a global decrease in functional activation.

The parietal association areas, *i.e.*, the intraparietal sulci, are known to be involved in visuo-spatial attention.^{24,25} Recently, the intraparietal sulcal cortex has

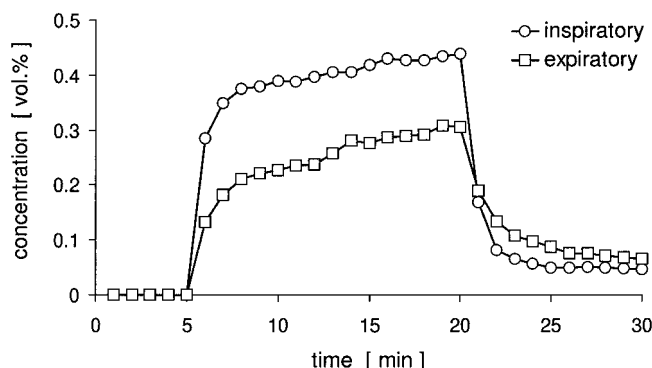


Fig. 6. Time courses of inspiratory and expiratory isoflurane concentrations during the entire experimental session obtained by averaging the individual results of all subjects of the isoflurane group (n = 8).

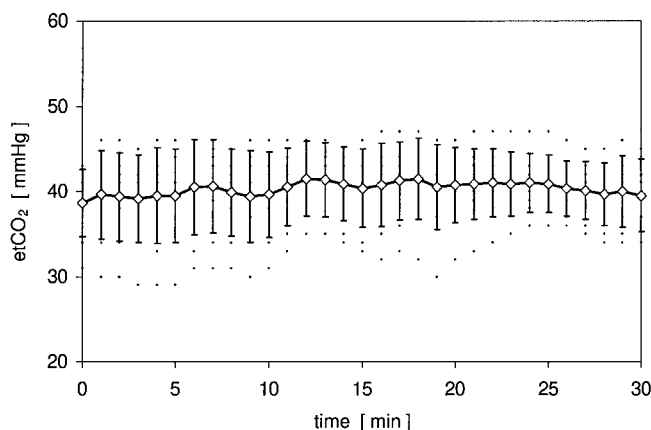


Fig. 7. Time courses of end-tidal carbon dioxide (ETCO₂) obtained by averaging the individual results of all subjects of the isoflurane group (n = 8).

been suggested to reflect a common neural substrate underlying multiple modes of visual selective processing and attention. Furthermore, there is evidence that the parietal cortex, particularly the banks along the intraparietal sulci, represent an area responsible for suppressing irrelevant distractors to maintain visual attention.²⁵ The anterior insula plays an important role in visual working memory as shown by Courtney *et al.*²⁶ Dove *et al.*²⁷ found bilateral activation of the anterior insula related to a task-switching paradigm. Pollmann and von Cramon²⁸ reported activation of the antero-superior insula (especially in the right hemisphere) associated with the difficulty of a visual search task.

The quantitative analysis of the affected regions (fig. 4) shows an isoflurane-related decrease in task-induced activation that is accompanied by a significant increase in the average reaction time (fig. 5). This increase in reaction time clearly demonstrates an impairment of cognitive performance. Despite this impairment, all subjects were conscious and able to respond to the stimuli. Considering the isoflurane-related decrease in task-induced activation in the parietal association cortex and the insula (see antero-superior insula, left intraparietal sulcus, and right intraparietal sulcus regions in fig. 4), we interpret the observed deterioration of cognitive performance by a selective impairment of attention and working memory processes in the visual domain. However, the preserved functional activation at the thalamic level in the early visual cortices and motor cortices during isoflurane suggests a regular perception and processing of the stimuli in these regions (see lateral geniculate nucleus, visual cortex, and motor cortex regions in fig. 4).

With regard to the difference in spatial extent and threshold used for displaying the activation and interaction maps (figs. 2-4), the following two factors should be considered. First, the activation maps were derived using a fixed-effect model, whereas for the interaction maps a second-level analysis was required (see Methods). Second-level analysis usually shows lower statisti-

cal power, mainly because error terms are estimated on the basis of interindividual rather than intraindividual variance. Second, the activation maps depict the difference between an experimental task and a resting baseline, whereas the interaction maps depict the interaction between identical tasks. For the interaction maps, the conditions compared are more similar than they are in the case of the activation maps.

The measured changes in reaction times and task-induced activation almost reached baseline within 5 min after exposure to 0.42 vol% isoflurane (figs. 4 and 5), which suggests a nearly complete recovery of cognitive functions. Recently published investigations based on psychomotor tests have demonstrated a similar rapid recovery after inhalation of subanesthetic isoflurane in healthy young volunteers.^{29,30}

For the interpretation of our results, a discussion of the underlying biophysical mechanisms³¹⁻³³ and the possible interactions with isoflurane seems useful. Increased neural activity is accompanied by a regional increase in oxidative metabolism, cerebral blood flow, and cerebral blood volume. All of these parameters affect the regional deoxyhemoglobin concentration. Because of its paramagnetic properties, deoxyhemoglobin causes distortions of the local magnetic field that can be visualized by BOLD-based fMRI.

Changes in the regional deoxyhemoglobin concentration arise from a complex interplay between cerebral hemodynamics and oxidative metabolism.^{10,11} An increase in oxygen consumption or venous blood volume results in an increase in deoxyhemoglobin and in a decrease of the BOLD signal, whereas an increase in blood flow produces a decrease in the regional deoxyhemoglobin concentration, resulting in an increase in the BOLD signal. Typically, the BOLD signal is found to be increased during periods of neural stimulation. This implies that changes in the deoxyhemoglobin concentration caused by blood flow exceed changes caused by oxidative metabolism and cerebral blood volume. Therefore, regional cerebral blood flow is the dominant parameter in BOLD-based fMRI.

The measurable BOLD signal change depends on two different factors. First, the change in neural activation induced by the stimulus, and second, the coupling between oxidative metabolism and cerebral hemodynamics. At this point the question arises whether the measured isoflurane-related decrease in functional activation solely reflects a decrease in neuronal activation or was partly caused by a change in the coupling between oxidative metabolism and cerebral hemodynamics. As demonstrated in rats, however, the coupling between cerebral blood flow and metabolism is intact during isoflurane anesthesia.³⁴ This suggests that the measured changes in BOLD contrast during isoflurane reflect regional changes in neural activity rather than alterations

in the coupling between cerebral hemodynamics and oxidative metabolism.

For the isoflurane group, end-tidal carbon dioxide was slightly increased (1.5 mmHg) during the IC (fig. 7). We therefore cannot exclude a moderate increase in baseline cerebral blood flow (*i.e.*, the cerebral blood flow value during the baseline task). An additional moderate increase in baseline cerebral blood flow could result from the vasodilatory properties of the anesthetic agent.³⁵ However, the magnitude of absolute cerebral blood flow changes induced by visual or motor stimulation was demonstrated to be independent of the baseline values.³⁶⁻³⁸ During hypercapnia induced by breathing of 6% CO₂, Maximilian *et al.*³⁶ found a fairly constant task-induced cerebral blood flow response among highly variable baseline values in the visual cortex. Similar results were obtained by Li *et al.*³⁷ in the motor cortex. Shimosegawa *et al.*³⁸ reported a significant effect of hypocapnia on task-induced cerebral blood flow response in the visual cortex but found no differences between normocapnia and hypercapnia. An additional argument is that even if global hemodynamic changes had a significant effect on the measured BOLD signal changes, this would be global in nature and therefore could not explain the observed regional changes in task-induced activation.

The effect of isoflurane on task-induced activation is dependent on the dose of the anesthetic agent. At 0.3% (expiratory), Logothetis *et al.*³⁹ found no significant isoflurane-related changes in the monkey brain during visual stimulation. At a comparable expiratory concentration (fig. 6), we observed a significant decrease in task-induced activation in three distinct cortical regions of the human brain; however, most brain regions were not affected by isoflurane (fig. 2). No activation was found at 1.3% in the human somatosensory cortex during sensory stimulation⁴⁰ and at 2.0% in the monkey cortex during visual stimulation.³⁹ Combination of these results supports the hypothesis of a dose-dependent gradual decrease of task-induced activation. The question of how anesthetic drugs affect brain activation during the transition from the conscious to the unconscious state opens a fascinating field for future research.

In conclusion, subanesthetic isoflurane affected information processing during a visual search task by an impairment of distinct parietal cortical structures and of the right insular cortex, resulting in a reduced cognitive performance. The information transfer and processing in subcortical structures at the thalamic level (lateral geniculate nucleus) as well as in the primary cortices (primary visual cortex, motor cortex) was not impaired. These results suggest that neural networks with highly differentiated functions in the association cortices are more sensitive to sedative concentrations of isoflurane than cortical areas with a lesser degree in functional specialization. Functional data as well as reaction times demon-

strate a nearly complete recovery of the brain from subanesthetic isoflurane within 5 min.

The authors thank Derk Olthoff, M.D., Ph.D. (Department of Anesthesiology and Intensive Care Therapy, University of Leipzig, Leipzig, Germany), D. Yves von Cramon, M.D., Ph.D. (Max-Planck-Institute of Cognitive Neuroscience, Leipzig, Germany), David G. Norris, Ph.D. (Max-Planck-Institute of Cognitive Neuroscience), Stefan Pollmann, Ph.D. (Max-Planck-Institute of Cognitive Neuroscience), and Volker Bosch, Ph.D. (Max-Planck-Institute of Cognitive Neuroscience) for valuable comments; and Grit Wenge (Max-Planck-Institute of Cognitive Neuroscience), Katrin Wiesner (Max-Planck-Institute of Cognitive Neuroscience), Sabine Busker-Rössig (Max-Planck-Institute of Cognitive Neuroscience), and Pjotr S. Zbgyev (Max-Planck-Institute of Cognitive Neuroscience) for technical support.

References

- Alkire MT, Haier RJ, Barker SJ, Shah NK, Wu JC, Kao YJ: Cerebral metabolism during propofol anesthesia in humans studied with positron emission tomography. *ANESTHESIOLOGY* 1995; 82:393-403
- Alkire MT, Haier RJ, Shah NK, Anderson CT: Positron emission tomography study of regional cerebral metabolism in humans during isoflurane anesthesia. *ANESTHESIOLOGY* 1997; 86:549-57
- Alkire MT, Pomfrett CJD, Haier RJ, Gianzero MV, Chan CM, Jacobson BP, Fallon JH: Functional brain imaging during anesthesia in humans. *ANESTHESIOLOGY* 1999; 90:701-9
- Enlund M, Andersson J, Hartvig P, Valtysson J, Wiklund L: Cerebral normoxia in the rhesus monkey during isoflurane- or propofol-induced hypotension and hypocapnia, despite disparate blood-flow patterns. *Acta Anaesthesiol Scand* 1997; 41:1002-10
- Wessen A, Widman M, Andersson J, Hartvig P, Valind S, Hetta J, Långström B: A positron emission tomography study of cerebral blood flow and oxygen metabolism in healthy male volunteers anesthetized with etlanolone. *Acta Anaesthesiol Scand* 1997; 41:1204-12
- Veselis RA, Reinsel RA, Beattie BJ, Mawlawi OR, Feshchenko VA, DiResta GR, Larson SM, Blasberg RG: Midazolam changes cerebral blood flow in discrete brain regions. *ANESTHESIOLOGY* 1997; 87:1106-17
- Reinstrup P, Ryding E, Algotsson L, Berntrman L, Uski T: Regional cerebral blood flow (SPECT) during anaesthesia with isoflurane and nitrous oxide in humans. *Br J Anaesth* 1997; 78:407-11
- Reinstrup P, Ryding E, Algotsson L, Messeter K, Asgeirsson B, Uski T: Distribution of cerebral blood flow during anesthesia with isoflurane or halothane in humans. *ANESTHESIOLOGY* 1995; 82:359-66
- Bandettini PA, Wong EC: Magnetic resonance imaging of human brain function: Principles, practicalities, and possibilities. *Neurosurg Clin N Am* 1997; 8:345-71
- Forster BB, MacKay AL, Whittall KP, Kiehl KA, Smith AM, Hare RD, Liddle PF: Functional magnetic resonance imaging: The basics of blood-oxygen-level-dependent (BOLD) imaging. *Can Assoc Radiol J* 1998; 49:320-9
- Ogawa S, Lee TM, Kay AR, Tank DW: Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci USA* 1990; 87:9868-72
- Levin JM, Ross MH, Mendelson JH, Mello NK, Cohen BM, Renshaw PF: Sex differences in blood-oxygenation-level-dependent functional MRI with primary visual stimulation. *Am J Psychiatry* 1998; 155:434-6
- Schmitt F, Stehling MK, Turner R: Echo-Planar Imaging: Theory, Technique and Application. Berlin, Springer, 1998
- Norris DG: Reduced power multislice MDEFT imaging. *J Magn Reson Imaging* 2000; 11:445-51
- Lee JH, Garwood M, Menon R, Adriany G, Andersen P, Truwitt CL, Ugurbil K: High contrast and fast three-dimensional magnetic resonance imaging at high fields. *Magn Reson Med* 1995; 34:308-12
- Friston K: Statistical parametric maps in functional imaging: A general linear approach. *Hum Brain Mapp* 1994; 2:189-210
- Worsley KJ, Friston K: Analysis of fMRI time-series revisited—again. *Neuroimage* 1995; 2:359-65
- Zarahn E, Aguirre K, d'Esposito M: Empirical analyses of BOLD fMRI statistics. I. Spatially unsmoothed data collected under null-hypothesis conditions. *Neuroimage* 1997; 5:179-97
- Aguirre K, Zarahn E, d'Esposito M: Empirical analyses of BOLD fMRI statistics. II. Spatially smoothed data collected under null-hypothesis and experimental conditions. *Neuroimage* 1997; 5:199-212
- Talairach P, Tournoux J: A Stereotactic Coplanar Atlas of the Human Brain. Thieme, Stuttgart, 1988
- Kirk RE: Split-plot factorial design: Design with group-treatment confounding. *Experimental Design: Procedures for the Behavioral Sciences*, 3rd Edition. Pacific Grove, Brooks/Cole Publishing Company, 1995, pp 512-20
- Bosch V: Statistical analysis of multi-subject fMRI data: Assessment of focal activations. *J Magn Reson Imaging* 2000; 11:61-4
- Lohmann G, Müller K, Bosch V, von Cramon DY: Lipsia: A software package for the evaluation of functional MRI data. Technical report, Max-Planck-Institute of Cognitive Neuroscience, Leipzig, Germany, 2000
- Krause BJ, Schmidt D, Mottaghy FM, Taylor J, Halsband U, Herzog H, Tellmann L, Müller-Gärtner HW: Episodic retrieval activates the precuneus irrespective of the imagery content of word pair associates: A PET study. *Brain* 1999; 122:255-63
- Wojciulik E, Kanwisher N: The generality of parietal involvement in visual attention. *Neuron* 1999; 23:747-64
- Courtney SM, Ungerleider LG, Keil K, Haxby JV: Transient and sustained activity in a distributed neural system for human working memory. *Nature* 1997; 386:608-11
- Dove A, Pollmann S, Schubert T, Wiggins CJ, von Cramon DY: Prefrontal cortex activation in task switching: An event-related fMRI study. *Cogn Brain Res* 2000; 9:103-9
- Pollmann S, von Cramon DY: Object working memory and visuospatial processing: Functional neuroanatomy analyzed by event-related fMRI. *Exp Brain Res* 2000; 133:12-22
- Zacny JP, Yajnik S, Lichtor JL, Klafta JM, Young CJ, Thapar P, Rupani G, Coalson DW, Apfelbaum JL: The acute and residual effects of subanesthetic concentrations of isoflurane/nitrous oxide combinations on cognitive and psychomotor performance in healthy volunteers. *Anesth Analg* 1996; 82:153-7
- Zacny JP, Sparacino G, Hoffmann PM, Martin R, Lichtor JL: The subjective, behavioral and cognitive effects of subanesthetic concentrations of isoflurane and nitrous oxide in healthy volunteers. *Psychopharmacology* 1994; 114:409-16
- Raichle ME: Behind the scenes of functional brain imaging: A historical and physiological perspective. *Proc Natl Acad Sci USA* 1998; 95:765-72
- Malonek D, Grinvald A: Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: Implications for functional brain mapping. *Science* 1996; 272:551-4
- Malonek D, Dirnagl U, Lindauer U, Yamada K, Kanno I, Grinvald A: Vascular imprints of neuronal activity: Relationships between the dynamics of cortical blood flow, oxygenation, and volume changes following sensory stimulation. *Proc Natl Acad Sci USA* 1997; 94:14826-31
- Hansen TD, Warner DS, Todd MM, Vust LJ: The role of cerebral metabolism in determining the local cerebral blood flow effects of volatile anesthetics: Evidence for persistent flow-metabolism coupling. *J Cereb Blood Flow Metab* 1989; 9:323-8
- Black S, Michenfelder JD: Cerebral blood flow and metabolism, *Clinical Neuroanesthesia*, 2nd Edition. Edited by Cucchiara RF, Black S, Michenfelder JD. New York: Churchill Livingstone Inc., 1998, pp 1-40
- Maximilian VA, Prohovnik I, Risberg J: Cerebral hemodynamic response to mental activation in normo- and hypercapnia. *Stroke* 1980; 11:342-7
- Li TQ, Moseley ME, Glover G: A FAIR study of motor cortex activation under normo- and hypercapnia induced by breath challenge. *Neuroimage* 1999; 10:562-9
- Shimosegawa E, Kanno I, Hatazawa J, Fujita H, Iida H, Miura S, Murakami M, Inugami A, Ogawa T, Itoh H, Okudera T, Uemura K: Photic stimulation study of changing the arterial partial pressure level of carbon dioxide. *J Cereb Blood Flow Metab* 1995; 15:111-4
- Logothetis NK, Guggenberger H, Peled S, Pauls J: Functional imaging of the monkey brain. *Nat Neurosci* 1999; 2:555-62
- Antognini JF, Buonocore MH, Disbrow EA, Carstens E: Isoflurane anesthesia blunts cerebral responses to noxious and innocuous stimuli: A fMRI Study. *Life Sci* 1997; 61:349-54