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Functional MRI as a tool to assess vision in dogs: the optimal anesthetic

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Abstract

Functional magnetic resonance imaging (fMRI) is a recent advance in neuroimaging that provides a picture of brain activity with excellent spatial resolution.
Current methods used to evaluate canine vision are poorly standardized and vulnerable to bias. Functional MRI may represent a valuable method of testing vision in dogs if the impacts of anesthesia on fMRI are understood. Six dogs were scanned during visual stimulation, each under three different anesthetic protocols (isoflurane, propofol, fentanyl/midazolam) to address the questions: (1) Can visually evoked fMR signals be reliably recorded in anesthetized dogs? and (2) Which anesthetic agent permits the least suppression of visually induced fMR signal in dogs? This study confirms that visual stimuli reliably elicit neural activity and fMR signal change in anesthetized dogs.
No significant differences in images acquired under the three anesthetics were found, and there was no significant relationship between anesthetic dose and brain activity, within the range of doses used in this study. Images obtained during isoflurane anesthesia were more consistent between dogs than those obtained with the other two agents. This reduced variation may reflect the fact that inhalant anesthesia is more easily controlled than intravenous anesthesia under conditions associated with high field fMRI.

Key Words: anesthesia, dogs, fMRI, vision

INTRODUCTION

Evaluating visual function presents the veterinary clinician with a range of challenges. The most obvious of these involves the inability of animals to communicate their perceptions verbally. The Snellen chart, effective for human visual testing, cannot be used. Instead, potentially biased and poorly standardised visual tests are employed. Several methods are currently in use to evaluate canine visual function and all have inherent limitations. Behavioral techniques, like the menace response, evaluate an animal's reactions to stimuli but are open to influence from distraction in the testing environment.[1] Electroretinography (ERG) records retinal activity during visual stimulation,[2,3] and therefore evaluates receptor function, but fails to record visual activity in the brain where perception actually occurs. The visual evoked potential (VEP) of the electroencephalogram (EEG) has the advantage that it does record brain activity, presumably from the thalamus and from the primary visual cortex (V1).[4,5] As there are few descriptions of canine cerebral functional anatomy in the literature, the feline cortex is used as a reference for electrode placement when recording VEPs in dogs, leading to poor standardization of the technique and unreliable results.[6] Due to the limitations associated with all of these methods, not only is assessment of visually normal animals difficult, but objective evaluations of surgical and pharmacologic treatments for visual disease are unavailable.

Blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) is a relatively new technique in medical imaging, which reveals neural activity in the brain with high spatial resolution. BOLD fMRI depends on the principle that increased activity in the cerebral cortex (e.g. visual cortex in response to a visual stimulus) leads to an increase in metabolic activity,[7] and a more pronounced increase in blood flow, localized to the site of neural activity.[8,9] As the increase in blood flow exceeds the metabolic increase and the rate of oxygen extraction from the cerebral microvasculature, an increase in the ratio of oxygenated: deoxygenated hemoglobin results.[7,10,11] Deoxyhemoglobin is paramagnetic[12] and causes attenuation of magnetic resonance signal.[13] The increased oxy: deoxyhemoglobin ratio therefore causes a signal increase, localized to areas of increased neural activity, in properly weighted MR images.[7,14] To detect this BOLD

effect statistically, acquired images of the brain are divided into equal sized discrete volume elements, or voxels. Images obtained during repeated stimulus presentation periods are then compared on a voxel by voxel basis to those obtained during repeated control periods. Voxels in which a significant change in BOLD signal occurs are superimposed on a corresponding anatomic MR image.[9,15]

This new technique has been a highly productive tool for human neuroscientists, particularly with respect to the visual system. One study of particular interest addressed dyslexia in humans.[16] Using fMRI, the authors demonstrated that the medial temporal area (MT) of the brain in dyslexic patients was not activated by movement in the visual field, although there is activation of this region in normal patients presented with moving stimuli. Subtle deficits in cognitive processing of visual motion by dyslexics were also observed during behavioral tests, confirming the fMRI results. Functional MRI has also revealed a great deal about the processing of shape and form in the human visual system,[17] as well as the functional neuroanatomy of visual perception and memory.[18] If several logistic concerns can be addressed BOLD fMRI could be similarly beneficial to veterinary research.

Blood oxygenation level-dependant fMRI is highly sensitive to subject movement[19] so anesthesia will be a necessary limitation imposed on veterinary applications of the technique. Results of a pilot study by our group revealed that functional MR images could be obtained from anesthetized dogs and suggested that anesthesia was the most influential variable affecting image quality.[20] In the present study, the effects of three clinically common anesthetic protocols (isoflurane, propofol and a combination of fentanyl/midazolam) on fMRI were compared during visual stimulation in dogs. Two specific questions were addressed: (1) Can BOLD signal changes be reliably and repeatedly elicited by visual stimuli in anesthetized dogs? (2) Do differences in the quality of fMR images exist when different anesthetic agents are used? To our knowledge this study is the first detailed account of fMRI in dogs.

METHODS

Animals

Six purpose-bred Beagles (one male, five female; mean mass 9.75 ± 0.88 kg (±1 SD)), obtained from the University of Guelph Central Animal Facility, Guelph, Ontario, Canada, were transported to the Animal Care and Veterinary Services facilities at the University of Western Ontario, London, Ontario where they were housed during the experimental period. To establish normality, each dog underwent a thorough ophthalmic examination including slit lamp biomicroscopy, indirect ophthalmoscopy, applanation tonometry, and streak retinoscopy. Dogs were fasted for 12 h prior to each experiment but permitted free access to water. All procedures met with the approval of both the University of Western Ontario Council on Animal Care and the University of Guelph Animal Care Committee.

Imaging

All experiments were performed on a Siemens Varians 4 Tesla Whole Body Magnetic Resonance Scanner housed at the Robarts Research Institute at the University of Western Ontario. The visual stimulus, a 20- by 20-cm vertical grating pattern with 10 black and 10 white bars that alternated position at 5 Hz, was back projected from a digital projector (NEC Corporation model MT800, Tokyo, Japan) located behind the observation window, onto a screen 60 cm from the subject's eyes. The animals' eyelids were held open using nonmagnetic, insulated copper speculae. To prevent corneal dehydration, an elastoviscous, clear corneal shield (Hylashields Nite®, i-med Pharma Inc., Pointe-Claire, Quebec, Canada) was applied to each eye immediately prior to speculum insertion.

Each fMRI experiment lasted approximately 7 min and consisted of 10 alternating 9 s periods of stimulus activation followed by corresponding 30 s periods of inactivation. This sequential task activation paradigm permits averaging of image data obtained during the rest and activation state to increase statistical power during voxel by voxel comparison and improve signal-to-noise ratio.[15] For each experiment four shot (i.e. spatial encoding of single slice data was achieved using four identical pulse sequences), echo-planar T2*-weighted whole brain magnetic resonance images depicting BOLD contrast were obtained with a radiofrequency (RF) knee coil (designed for MR imaging of the human knee but ideally sized for a beagle head) for RF transmission and reception. The functional images were obtained in contiguous dorsal sections with echo time (TE) = 15 ms, repetition time (TR) = 750 ms, flip angle = 60°, 64×64 matrix, field of view (FOV) = 12.8 cm (2 mm in-plane resolution), and slice thickness = 2 mm. Immediately following each experiment corresponding whole brain, 3D magnetization-prepared Turbo FLASH (fast low angle shot) T1-weighted anatomical images of each dog were acquired with TE, 6.5 ms; TR, 12.4 ms, flip angle, 11°; and inversion time, 500 ms. These anatomical volumes were acquired in the same FOV as the functional images so that functional data could be superimposed on anatomic images. Head movement was restricted using foam padding within the RF knee coil.

Image processing was performed using the analysis software Stimulate.[21] Voxel by voxel comparison of rest and task state functional images was performed using cross-correlation analysis. This permitted the reference function, to which the time course of BOLD signal was compared, to shift with the acquired signal and accommodate hemodynamic delay between neural activity and the factors underlying the BOLD effect (e.g. blood oxygenation). Statistical significance for the cross-correlation was set initially at $P < 0.10$ and was improved to $P < 0.05$ by restricting significance to clusters with a minimum size of three significant voxels.[22] These voxel clusters were then superimposed onto the T1-weighted anatomic images.

Once image processing was complete, regions that included the lateral geniculate nucleus (LGN) of the thalamus and the

occipital cortex were identified on each image. These areas were used as specific regions of interest (ROI) for further analysis. In all dogs, the LGN region consisted of two bilateral and as-symmetrical-as-possible rectangular volumes transecting five dorsal slices at the level of the thalamus in the diencephalon (see Fig. 1a). Within each slice, boundaries of the two sections of this ROI were placed as equidistant as possible laterally from the third ventricle and medially from the lateral ventricles. The occipital ROI was a larger rectangular volume beginning in the first dorsal slice where the cerebellum could no longer be observed and proceeding dorsally through seven slices (see Fig. 1b). Within each slice, the caudal boundary for this ROI was placed at the occipital pole and the rostral boundary was placed just caudal to the pseudosylvian fissure.

For each anesthetic agent and ROI, the number of significant voxels was determined as a percentage of the total number of voxels within the ROI, to control for slight differences in ROI size. Mean percentage signal change (between the rest and activation states) was also calculated for each anesthetic agent and ROI. These quantitative measures of BOLD activity were used for further analysis. Qualitative assessment of both the images themselves and the time courses of activation with respect to stimulus presentations was also performed.

Anesthesia

Functional MRI experiments were performed on all six dogs anesthetized under each of three different anesthetic protocols: (1) the inhalant, isoflurane (AErrane®, Janssen, North York, Ontario, Canada); (2) the nonopioid intravenous agent, propofol (Rapinovet®, Schering Plough Animal Health, Point-Claire, Quebec, Canada); and (3) a combination of the opioid intravenous agent, fentanyl (Abbott Laboratories Ltd, Toronto, Ontario, Canada), and nonopioid, midazolam (Versed®, Hoffman La Roche Ltd, Mississauga, Ontario, Canada). To avoid data loss resulting from movement artifacts, and to facilitate comparison of results within dogs, each dog was scanned twice under each agent for a total of 36 experimental sessions (six dogs × three anesthetics × two trials). The order of anesthetic agent used for each dog was chosen randomly and the minimum time between consecutive studies on any dog was 72 h.

Prior to each experimental session, mydriasis was achieved using 0.5% tropicamide drops (Diotrope®, Dioptic Laboratories, Markham, Ontario, Canada), 0.01 mg/kg glycopyrolate (Sabex Inc. Boucherville, Quebec, Canada) was given intramuscularly and a cephalic catheter was placed.

Target doses of each agent known to maintain a relatively light, comparable and stable depth of general anesthesia were selected (Table 1).[23] Bolus doses (propofol, 4–6 mg/kg; fentanyl/midazolam, 10–20 μg/kg and 0.5 mg/kg, respectively) were used for induction with the I.V. regimes and, mask induction was used for isoflurane. Once anesthetized, the dogs were intubated, transported a short distance to the MR scanning room, and placed on 100% oxygen, or oxygen and isoflurane. A neuromuscular block was produced using atracurium besylate (Sabex Inc.) at 0.3 mg/kg I.V. This minimized muscle movement and facilitated central fixation of a relaxed eye. Intermittent positive pressure ventilation was employed using a volume-limited ventilator (Airshields® Ventimeter®, Air-Shields Inc., Hatboro, PA, USA) with a respiratory frequency of 10 breaths per minute and a tidal volume of 15 mL/kg.

Maintenance of I.V. anesthesia was achieved using an infusion of propofol (0.4 mg/kg/min), or fentanyl/midazolam (0.80 μg/kg/min and 8.0 μg/kg/min, respectively), administered via a microdrip (Baxter Corporation, Toronto, Ontario) from a preloaded volume limiting system (Buretrol®, Baxter Corporation). Actual infusion doses were calculated by dividing the volume of infusion used during an imaging session by the total time of the session. The intravenous anesthetics were diluted with saline (anesthetic : saline, 1 : 3) to facilitate more accurate infusion and to simultaneously achieve fluid replacement through the same venous access.

Inhalant anesthesia (1.6% end-tidal, expired isoflurane) was maintained using a precision vaporizer (Forane®, Cyprane Ltd, Keighley Yorkshire, England) and a modified Bain breathing system at a fresh gas flow sufficient to prevent rebreathing (150 mL/kg). The Bain system was modified by extending both the inlet and exhaust tubing to 10 m. Dogs were maintained on a 0.9% NaCl infusion (Baxter Corporation) at 5 mL/kg/ h during isoflurane anesthesia. Experiments in which actual induction or infusion doses exceeded the mean of all doses ± two standard deviations were excluded from further analyses.

A gas monitor (Criticare Poet® IQ, Criticare Systems Inc., Waukesha, WI, USA) was used to monitor inspired and end-tidal expired CO_2 and O_2 levels during all experiments, as well as inspired and end-tidal expired isoflurane during isoflurane experiments. These parameters were recorded for the 10 min immediately prior to a scanning session, as well as immediately following a session, but they could not be monitored throughout scanning sessions. The powerful magnetic field surrounding the scanner prohibited any metal-containing equipment from being placed within a 10-m radius, a distance which exceeded the sampling capabilities of the gas monitor. Heart rate and oxygen saturation were monitored throughout each session using a pulse oximeter (Nonin Medical Inc. model 8600FO, Minneapolis, MN, USA) and fiber-optic cable. Each animal was covered with a blanket while anesthetized to maintain body temperature and prevent hypothermia.

Statistical analysis

Comparisons of percentage BOLD signal change, and the number of significant voxels per ROI as a percentage of ROI size were performed based on the randomized complete blocks design (RCBD). The SAS systems general linear models procedure (Proc GLM)[24] was used to detect trial by anesthetic treatment interactions for both measures of image quality within each ROI. If no significant trial by treatment

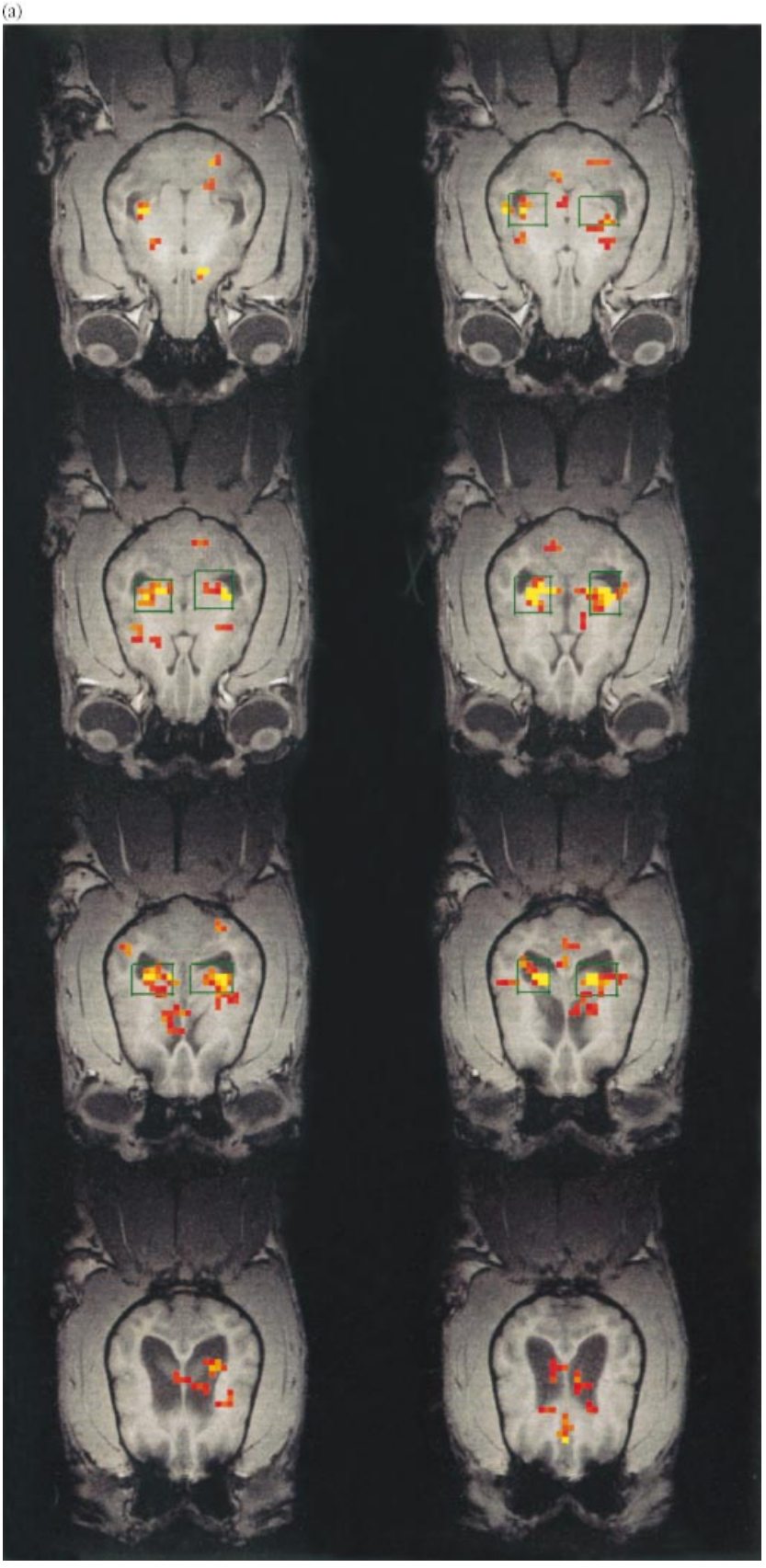
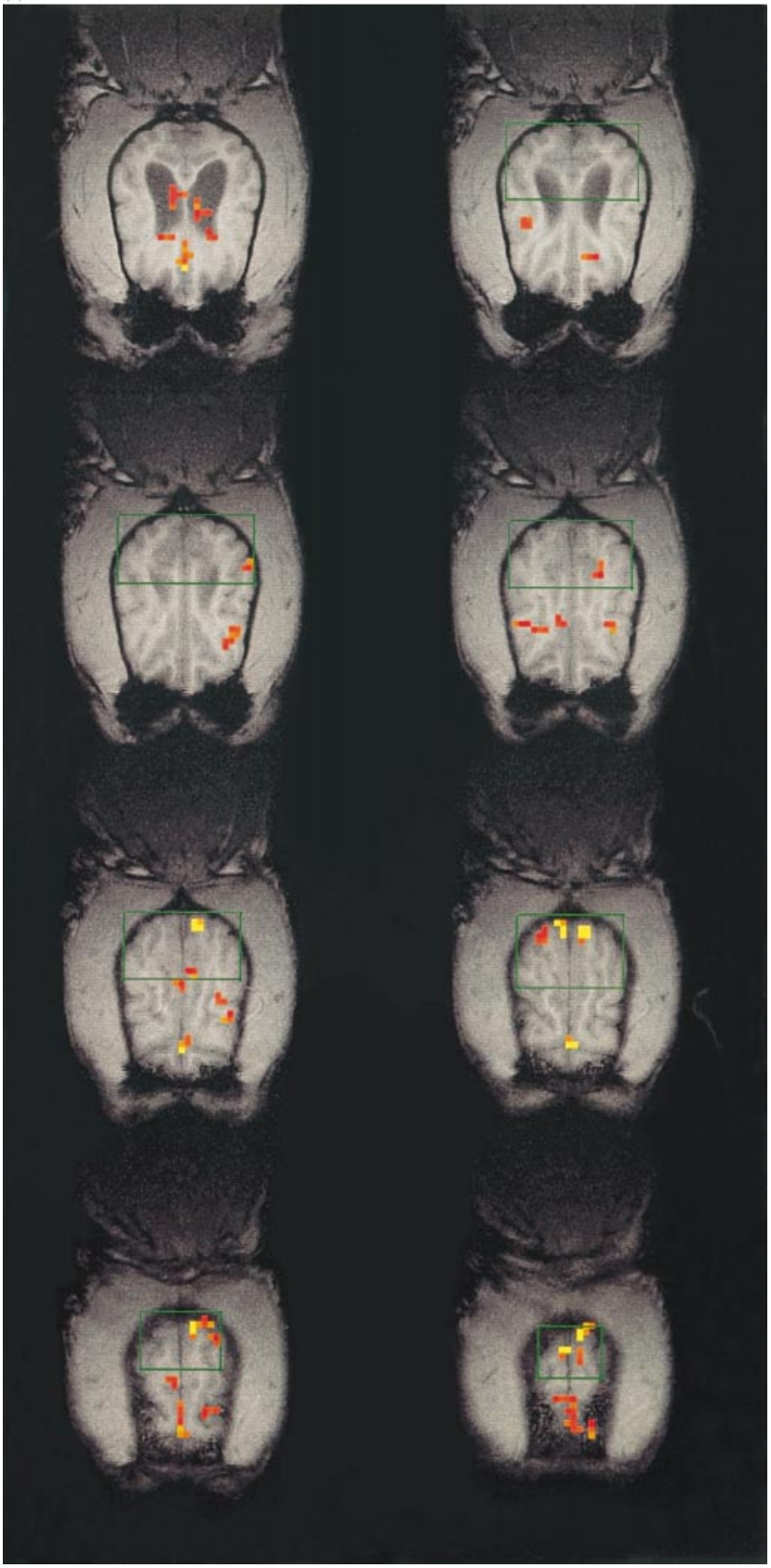


Figure 1. Blood oxygenation level-dependent (BOLD) functional MR, whole brain images in dorsal sections (a, b) with corresponding BOLD activity time courses (c, d) from a dog anesthetized under fentanyl/midazolarn and presented with a vertical grating visual stimulus. All functional data were obtained using four shot echo-planar imaging weighted for T2*. Images were divided into discrete volume elements (voxels) in a 64 × 64 matrix with in plane resolution, 2 mm and slice thickness, 2 mm. Within each voxel, cross correlation was used to detect increases in BOLD signal linked to repeated periods of stimulus presentation. Voxels in which a significant increase in BOLD signal was detected were then superimposed on 3D magnetization prepared Turbo FLASH (fast low angle shot) anatomic images as colored pixels. Bright yellow voxels are those with the greatest signal change between control and activation. Two regions of interest (ROIs) were selected based on regions where activation was reliably observed and where visual activity has been reported in other animals. Their boundaries appear as thin lines on (a) and (b). The first ROI (a) included the lateral geniculate nucleus (LGN) of the thalamus and the second (b) included much of the occipital lobes of both cerebral hemispheres. The number of significant voxels as a percentage of total ROI size was used as one quantitative measure of image quality for further analysis and the mean percentage signal change was used as another. ROIs were always selected in the same slices, based on the same anatomical landmarks between dogs and anesthetics. Percentage signal changes were calculated by comparing baseline and activation BOLD signals, based on the time courses of stimulus presentation (bottom traces in (c) and (d)) and BOLD signal in the LGN region (c) and occipital region (d) over the course of each experiment.

(b)



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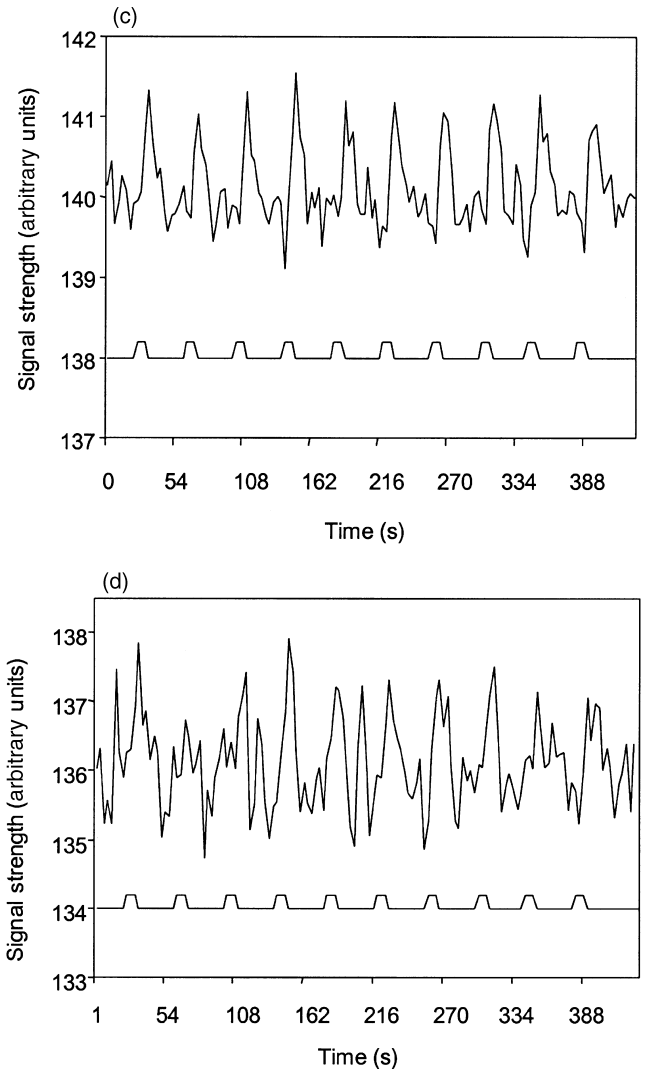

Figure 1. *continued.*

effects were detected, Proc GLM[25] was used to test for drug treatment effects by averaging results from trial 1 and trial 2 for each dog and comparing between anesthetics using a method analogous to repeated measures ANOVA. If a significant trial by treatment effect did occur, SAS Proc Mixed[25] was used to detect simple treatment effects within trial 1 and trial 2 separately. Linear regression analysis for anesthetic dose by both fMRI variables was performed using Statistix (Version 1.0, Analytical Software) by averaging data from trial 1 and trial 2 for each dog, when no significant trial by treatment effects were detected. When a trial by treatment interaction was detected, linear regression was performed by plotting data from the first trial for each dog against anesthetic dose. Significance for all analyses was assessed at $P < 0.05$.

RESULTS

Data from 36 experimental sessions were processed. Five sessions were excluded due to motion artifacts and one fentanyl/midazolam session was excluded because anesthetic infusion dose exceeded the mean ± 2 SD. Despite these exclusions, at least 1 of 2 trials for each dog under each anesthetic was included in all analyses.

Target and actual infusion doses of each anesthetic agent are provided in Table 1. In all dogs, and under all three anesthetic agents, significant BOLD signal change of between 0.3 and 1.1% was elicited by visual stimulation. Representative images and the corresponding time courses of activity from one dog under fentanyl/midazolam anesthesia are shown in Fig. 1. Voxels in which BOLD signal change correlated with the time course of stimulus presentation appear as colored pixels on the anatomic image, the bright yellow pixels representing the greatest signal change between the control state and activation state.

		Isoflurane (end-tidal %)	Propofol (mg/kg/min)	Fentanyl/Midazolam (ug/kg/min)	(ug/kg/min)
Dog	Target	1.6	0.40	0.80	8.0
Bibi1		N/A	0.20	0.50	5.0
Bibi2		1.4	0.26	0.83	8.3
Pakum1		N/A	0.36	1.30	13.0
Pakum2		1.4	N/A	0.40	4.0
Starr1		1.3	0.27	1.30	13.0
Starr2		1.5	0.36	0.90	9.0
Shadow1		1.3	0.44	0.89	8.9
Shadow2		1.4	0.33	0.88	8.8
Diosa1		1.6	0.33	1.70*	17*
Diosa2		1.3	0.36	0.92	9.2
Broadway1		1.6	0.33	0.90	9.0
Broadway2		1.8	0.36	0.75	7.5
Mean		1.5	0.33	0.94	9.4
S.D.		0.2	0.07	0.35	3.5

N/A, not available.

Table 1 Anesthetic target doses and actual doses for isoflurane, propofol and fentanyl/midazolam used to anesthetize dogs for BOLD fMRI. Intravenous agents were administered using a drip infusion and isoflurane using a vaporiser and Bain circuit. One experiment was excluded (*) from subsequent analysis because the dose exceeded the mean ± 2 standard deviations.

Table 2 Evaluation of trial by anesthetic effects on differences between three anesthetic treatments for two measures of BOLD signal (mean percentage signal change, PSC; and percentage of significant voxels per total number of voxels within ROI, SVX) in two regions of interest (lateral geniculate nucleus, LGN; and occipital cortex, OCC)*

Variable	*F*	*P*
LGN PSC	0.50	0.62
LGN SVX (log transformed)	4.41	0.04†
OCC PSC	0.67	0.54
OCC SVX	0.19	0.83

*Randomized complete blocks design, SAS general linear model (Proc GLM)[25] to detect significant drug by trial effects with 2 degrees of freedom; $n = 30$.
†Significant difference.

No obvious qualitative trends with respect to fMR image quality were observed between the three anesthetic protocols. With all three anesthetics, significant activity in response to visual stimulation was observed consistently, though not exclusively, in a region that included the LGN of the thalamus, and throughout the occipital lobe of cerebral cortex. These regions were included in ROIs for all further analyses.

Results of the randomized complete blocks design (RCBD) general linear models procedure (Proc GLM)[25] to detect trial by treatment effects for both fMR variables in both regions of interest are presented in Table 2. Only the log-transformed percentage of significant voxels within the LGN region was significantly affected by trial treatment interactions (log transform was applied based on the residual plot of these data). This trial-by-treatment effect occurred because there was a significant difference between observations from the first fentanyl/midazolam trial and first isoflurane trial ($F = 2.73$, $P = 0.02$, d.f. = 10), but not between observations from the second trials for these protocols ($F = -0.76$, $P = 0.47$, d.f. = 10). Based on this discrepancy, data from the two trials were considered separately during further analysis and SAS Proc Mixed[26] was employed. All data met the normality assumptions of the general linear model, but equal variance assumptions may have been violated.

End-tidal CO_2 levels for each experiment were also analysed. The mean of end-tidal CO_2 levels recorded at the start and the end of an experiment was used because the magnetic field prohibited continuous use of the gas monitor throughout scanning sessions. No significant trial by treatment interaction effects ($F = 0.06$, $P = 0.94$, d.f. = 2) were observed for recorded end tidal CO_2 values using Proc GLM. A treatment effect for CO_2 was significant ($F = 1.45$, $P = 0.03$, d.f. = 2), however, because CO_2 values recorded under fentanyl/midazolam (47.1 ± 4.9 mmHg) were significantly greater than under propofol (43.4 ± 2.5 mmHg); end tidal CO_2 values under isoflurane (45.6 ± 3.0 mmHg) did not differ significantly from values obtained under either of the other protocols.

Comparisons of mean percentage BOLD signal change and significant voxels/ROI between the three different anesthetics are presented in Fig. 2. No significant differences were observed for any category. Based on their distributions, however, data obtained under isoflurane, especially for significant voxels/ROI in both the LGN and occipital regions seems qualitatively less variable (Fig. 2, Table 3). Unfortunately, a definitive statistical test of equal variance is not available using the RCBD model.

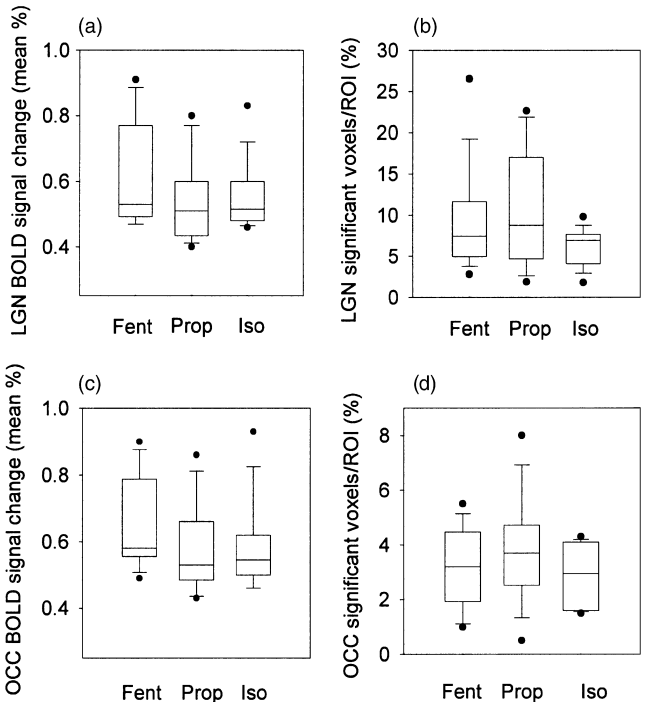

Figure 2. Boxplots of two quantitative measures of blood oxygenation level-dependent (BOLD) MR images in two regions of interest (ROIs) obtained from six anesthetized dogs. The bottom and top lines of each box represent the 25th and 75th percentiles, respectively, and the middle line represents the median. Horizontal lines below and above boxes represent the 10th and 90th percentiles, respectively, and black dots represent data points outside this range. Each dog was anesthetized under three anesthetic agents: fentanyl/midazolam (Fent), propofol (Prop), and isoflurane (Iso). There were no significant differences in the lateral geniculate nucleus (LGN) ROI for mean percentage BOLD signal changes (a): $F = 1.97$, $P = 0.19$, d.f. = 2, or for percentage of significant voxels per ROI (b): $F = 1.57$, $P = 0.26$, d.f. = 2 under the three different anesthetic agents, nor were differences significant in the occipital ROI between mean percentage signal changes (c): $F = 3.0$, $P = 0.10$, d.f. = 2 or percentage of significant voxels per ROI (d): $F = 1.4$, $P = 0.56$, d.f. = 2 under the three different anesthetic agents. The randomized complete blocks design general linear model procedure,[25] analogous to repeated measures ANOVA, was used to test three of the four categories, because it permitted averaging of data from repeated trials. This procedure was not valid for comparison of number of significant voxels/ROI as a percentage of voxel size in the LGN region because a significant trial by treatment effect had previously been detected (Table 2). These data were compared using SAS Proc Mixed,[26] which considers trials individually. Although not testable statistically using the model employed, the variance of all four categories under isoflurane anesthesia is less than under the other anesthetic agents (Table 3), particularly in (b) and (d).

Some variation in anesthetic dose between experiments was unavoidable, especially for the intravenous agents. To

Table 3 Standard deviations of BOLD fMRI data obtained from six dogs for two quantitative measures of image quality (mean percentage signal change, PSC; and number of significant voxels/ROI as a percentage of ROI size, SVX) in two regions of interest (lateral geniculate nucleus, LGN; and occipital lobe, OCC). A statistical test for equal variance is unavailable using the statistical model employed. Qualitatively, standard deviations for isoflurane data are smaller than those for propofol and fentanyl/midazolam data, especially for SVX in both ROIs

Variable	Fentanyl/Midazolam	Propofol	Isoflurane
LGN PSC	0.17	0.13	0.11
LGN SVX	6.70	7.25	2.37
OCC PSC	0.15	0.15	0.14
OCC SVX	1.60	2.06	1.09

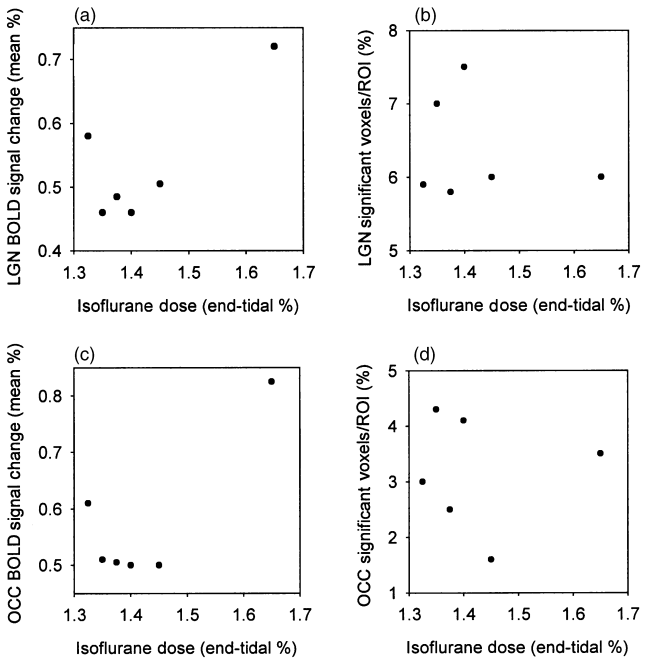

Figure 3. Scatter plots of two quantitative measures of BOLD fMR image quality in two brain regions of interest (ROIs) against isoflurane dose, obtained during visual stimulation in isoflurane-anesthetized dogs. Linear regression analyses were performed. There was no significant relationship between mean percentage BOLD signal change in the lateral geniculate nucleus (LGN) ROI (a): $r^2 = 0.14$, $F = 0.49$, $P = 0.53$, d.f. = 4 and no significant relationship between the number of significant voxels/ROI as a percentage of voxel size and dose in the LGN region (b): $r^2 = 0.05$, $F = 0.21$, $P = 0.67$, d.f. = 5. There was no significant relationship between mean percentage BOLD signal change and dose in the occipital (OCC) RO I (c): $r^2 = 0.48$, $F = 2.8$, $P = 0.19$, d.f. = 4 or between dose and number of significant voxels/ROI as a percentage of ROI size in the OCC region (d): $r^2 = 0.001$, $F = 0.94$, $P = 0.94$, d.f. = 5. Each data point represents the mean of two experimental sessions for a single dog if data from both sessions were available.

determine if this variation had an effect on BOLD activity, linear regression analyses were performed for anesthetic dose against both percentage signal change and significant voxels per ROI (Figs 3 and 4). No significant relationship between anesthetic dose and either measure of BOLD signal was observed in either ROI.

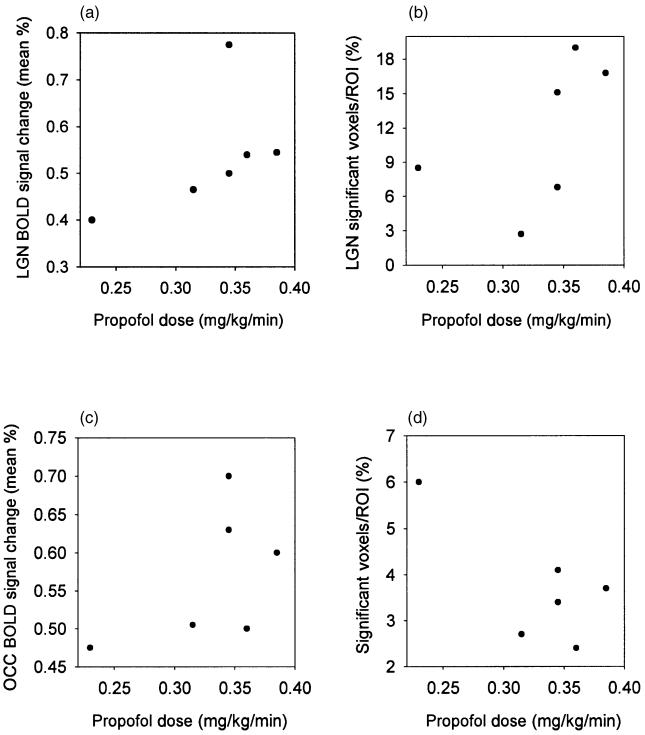

Figure 4. Scatter plots of two quantitative measures of blood oxygenation level-dependent (BOLD) fMR image quality in two brain regions of interest (ROIs) against propofol dose, obtained during visual stimulation in propofol-anesthetized dogs. Linear regression analyses were performed. There was no significant relationship in the lateral geniculate nucleus (LGN) ROI between dose and mean percentage BOLD signal change (a): $r^2 = 0.28$, $F = 1.56$, $P = 0.28$, d.f. = 5) or number of significant voxels/ROI as a percentage of ROI size (b): $r^2 = 0.29$, $F = 1.63$, $P = 0.27$, d.f. = 5, nor was there a significant difference in the occipital (OCC) ROI between dose and mean percentage BOLD signal change (c): $r^2 = 0.28$, $F = 1.61$, $P = 0.27$, d.f. 5 or number of significant voxels/ROI as a percentage of ROI size (d): $r^2 = 0.58$, F 4.2, $P = 0.10$, d.f. = 5. Each data point represents the mean of two experimental sessions for a single dog if data from both sessions were available.

DISCUSSION

A pilot study by our group demonstrated, for the first time, that neural activity can be recorded from the brains of anesthetized dogs using BOLD fMRI.[21] The current study confirms the results of the pilot study and is the first to compare effects of different anesthetic protocols on fMRI activity in any animal. Our results are consistent with fMRI data recorded from visual cortex in cats anesthetized with isoflurane.[26,27] Mean percentage signal changes (0.3–1.1% for the present study) are considerably less than those observed with a repeated visual stimulus presentation paradigm in awake humans. This is not surprising given the fact that general anesthesia depresses central nervous system activity by definition and also reduces basal cerebral blood flow.[28] Lahti *et al.* compared somatosensory fMR responses of rats, awake and restrained, as well as during propofol anesthesia, and noted anesthetic inhibition of BOLD activity.[29] The use of 100% oxygen for ventilation in the present study may have

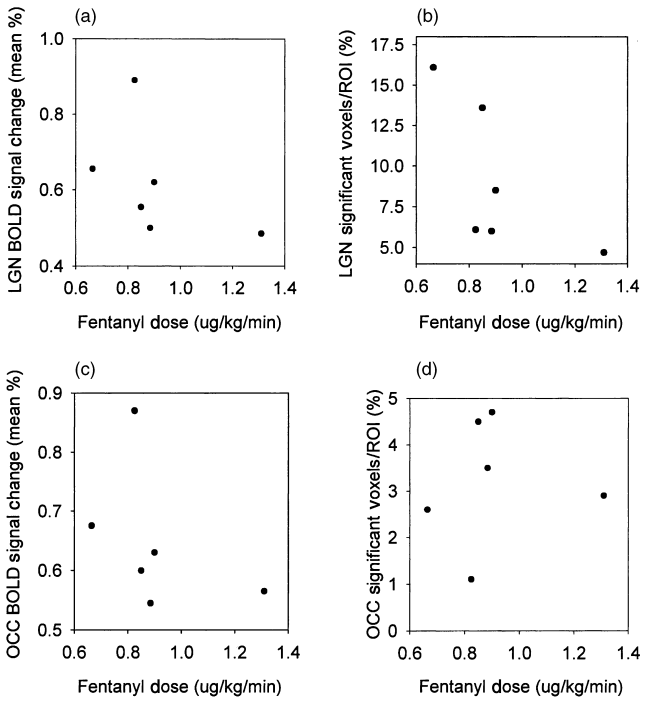

Figure 5. Scatter plots of two quantitative measures of blood oxygenation level-dependent (BOLD) fMR image quality in two brain regions of interest (ROIs) against fentanyl dose, obtained during visual stimulation in fentanyl/midazolam anesthetized dogs. Linear regression analyses were performed. There was no significant relationship in the lateral geniculate nucleus (LGN) ROI between dose and mean percentage BOLD signal change (a): $r^2 = 0.24$, $F = 1.27$, $P = 0.32$, d.f. = 5 or number of significant voxels/ROI as a percentage of ROI size (b): $r^2 = 0.47$, $F = 3.49$, $P = 0.14$, d.f. = 5, nor was there a significant difference in the occipital (OCC) ROI between dose and mean percentage BOLD signal change (c): $r^2 = 0.17$, $F = 0.84$, $P = 0.41$, d.f. = 5 or number of significant voxels/ROI as a percentage of ROI size (d): $r^2 = 0.005$, $F = 0.02$, $P = 0.89$, d.f. = 5. Each data point represents the mean of two experimental sessions for a single dog if data from both sessions were available.

further compounded signal attenuation. Changes in the ratio of deoxy: oxyhemoblobin between the control state and the stimulus presentation state, the basis of the BOLD effect, may have been reduced because of high levels of dissolved oxygen in the blood. The use of N_2O and O_2, in combination, may be more appropriate for future canine fMRI studies to limit this potential source of signal loss. The slightly stronger signals (approx. 1.6%) obtained by Kim *et al.* from isoflurane anesthetized cats ventilated with a 70 : 30 N_2O:O_2 mixture supports this suggestion.[27] Despite reduced signal strength, BOLD activity has been reproducible in all fMRI studies of anesthetized animals to date, including the present study.

A methodologic problem reported by Jezzard *et al.* involved maintaining eye position in anesthetized animals during presentations of visual stimuli.[26] They relied on the use of corrective lenses to fixate an animal's gaze on the stimulus and reported some complications with this method. In the present study, the neuromuscular block provided by atracurium was an effective alternative. It resulted in a centrally fixated, relaxed eye position and helped reduce other muscle movements, to which fMRI is extremely sensitive.

There were no obvious qualitative differences in images obtained under isoflurane, propofol or fentanyl/midazolam anesthesia. Activity was detected in brain areas known to play a role in visual processing in other mammals, with all three anesthetic regimes. In cats, roughly 80% of optic tract fibres terminate and synapse in the lateral geniculate nucleus (LGN) of the thalamus. Lateral geniculate fibres in turn innervate visual cortex in the occipital lobes.[30] This organisation is preserved in nonhuman primates and humans,[31] and almost certainly in dogs, although direct data are not available. Based on this known functional mammalian anatomy, and on the significant BOLD activity observed in these areas in the present study, a LGN region of interest (ROI) and an occipital ROI were selected for further analysis. This excluded the few significant voxels outside of ROIs that could have been associated with visual activity, or could simply have reflected non visual, oscillatory brain activity, which by chance matched the time course of stimulus presentation.

Qualitatively, there appeared to be less variation in signal strength and size of activated region in the LGN than in the occipital cortex, which could reflect site-specific differences in anesthetic activity, but these differences were not statistically significant. Within both ROIs the mean percentage signal change between rest and stimulus presentation, as well as the number of significant voxels as a percentage of ROI size under the three different anesthetic agents, were compared. Although no significant differences were detected, there appeared to be less variation in responses under isoflurane anesthesia than under either of the I.V. protocols. This may reflect logistic complications of intravenous anesthetic infusion when working with a powerful MR scanner. A 4-T magnetic field is many times more powerful than the earth's magnetic field and, as such, interferes with electronic and mechanical equipment. All apparatus for anesthetic monitoring and delivery therefore had to be kept a minimum of 10 m from the MR scanner. This meant that the drip infusion rate for I.V. anesthetic maintenance had to be set manually, prior to the onset of scanning, and could not be adjusted until the completion of the hour-long session. Over an hour, slight variations in drip rate could have caused considerable differences in the total volume of anesthesia administered. In addition, a change in leg position within the RF coil might have increased resistance to the infusion, adding to variation in the I.V. anesthetic dose. In contrast, the isoflurane dose was set as a percentage of inspired gas using a dial on an anesthesia machine and was confirmed using the gas monitor, both of which were located the required 10 m from the magnet. The use of an intravenous infusion pump would have facilitated more precise control of I.V. anesthetic dose.

Despite the observed variation in anesthetic dose, significant dose-dependent effects of anesthesia on fMRI were not detected within the range of doses administered. It remains plausible, however, that the increased variation in percentage

signal change and percentage of significant voxels/ROI with I.V. administered protocols reflects reduced precision of anesthetic administration during imaging. One would expect that with increasing anesthetic dose, and therefore increased anesthetic depth, neural activity and BOLD signal would be reduced. Dose-dependent inhibitory relationships between both isoflurane and propofol and measures of cerebral activity, like the EEG, have been reported.[32,33] We may have failed to detect a significant relationship between anesthetic dose and BOLD signal because the range of doses to which these dogs were exposed was too small to elucidate the correlation. One methodologic objective of the study, in fact, was to maintain anesthetic dose as closely as possible between experiments. Variation probably occurred because of difficulties associated with maintaining a constant anesthetic infusion from a considerable distance. Further study using a wider range of anesthetic doses would help resolve this issue.

Blood carbon dioxide levels may have influenced the results of this study, as well, because of the considerable influence on cerebral blood flow exerted by CO_2.[24] A statistically significant difference in end-tidal CO_2 between the three anesthetic protocols was detected, though the small range of values (43.4 ± 2.5–47.1 ± 4.9 mmHg) may not have been biologically significant. We did attempt to maintain CO_2 levels as closely as possible by ensuring dogs were stabilized with an end-tidal CO_2 level of between 38 and 42 mmHg before being placed in the scanner. With continuous monitoring, changing CO_2 levels could have been detected quickly and addressed by modifying ventilation rate or tidal volume. Variation was also compounded by the fact that dogs were very briefly disconnected from ventilation during removal from the scanning room at the end of each session. This may have caused CO_2 accumulation so that values recorded at the end of the session overestimated end-tidal levels as they actually occurred during imaging.

Despite these potential sources of variation, reproducible BOLD signals in brain regions with known visual functions in other mammals were recorded. The reliability of fMRI results during anesthesia has important clinical and research implications. Restraining animals during fMRI is problematic because neural activity associated with factors such as stress or motor output against the restraints could confound data associated with the stimulus of interest. In addition, eye movements cannot be restrained, so fMRI investigations of visual activity are virtually impossible in awake animals, except perhaps in highly trained nonhuman primates. Also, if fMRI is eventually to have clinical application in veterinary medicine, the stress associated with restraint is unlikely to be accepted by pet owners. Human implications also exist in that, currently, human patients incapable of remaining still for extended periods (i.e. small children, the mentally ill) are not candidates for fMRI. Establishing, then, that fMR signals can be reproducibly recorded under general anesthesia is an important finding.

Our results demonstrate that BOLD signal change, and useful fMR images can be reliably elicited using visual stimulation in anesthetized dogs under three different anesthetic protocols. This is particularly encouraging for both clinical and research application of fMRI in veterinary medicine, especially with respect to vision. We did not find differences in fMR activity between anesthetic agents, or dose-dependent relationships between anesthesia and fMRI. It does seem, however, that isoflurane permitted more consistent BOLD activity than the intravenous anesthetic protocols. This may reflect the fact that inhalant anesthesia is more easily controlled under conditions associated with fMRI.

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