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Effect of anesthesia on renal R_2^* measured by blood oxygen level-dependent MRI

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Abstract

Blood oxygen level-dependent (BOLD) MRI is increasingly used to assess renal tissue oxygenation during disease based on the transverse relaxation rate (R_2^*). In preclinical small animal models, the requisite use of anesthesia during imaging may lead to functional changes which influence R_2^* and confound results. The purpose of this study is to evaluate the effects of four common anesthetic compounds on renal R_2^* in healthy mice. Five female ICR mice were imaged with BOLD MRI approximately 25 minutes after induction with isoflurane (Iso; 1% or 1.5%, delivered in 100% O₂), ketamine/xylazine (KX), sodium pentobarbital (PB) or 2,2,2-tribromoethanol (TBE). A significant effect of anesthetic agent on R_2^* was observed in all tissue layers of the kidney, including the cortex, outer stripe of the outer medulla (OSOM), inner stripe of the outer medulla (ISOM), and inner medulla (IM). Pairwise significant differences in R_2^* between specific agents were found in the cortex, OSOM, and ISOM, with the largest difference observed in the ISOM between 1.5% Iso ($26.6 \pm 1.7 \text{ s}^{-1}$) and KX ($66.0 \pm 7.1 \text{ s}^{-1}$). The difference between 1% Iso and KX in the ISOM was not abolished when KX was administered with supplemental 100% O₂ or when 1% Iso was delivered in 21% O₂, indicating that the fraction of inspired oxygen did not account for observed differences. Our results indicate that the choice of anesthesia has a large influence on the observed R_2^* in mouse kidney, and anesthetic effects must be considered in the design and interpretation of renal BOLD MRI studies.

Graphical abstract

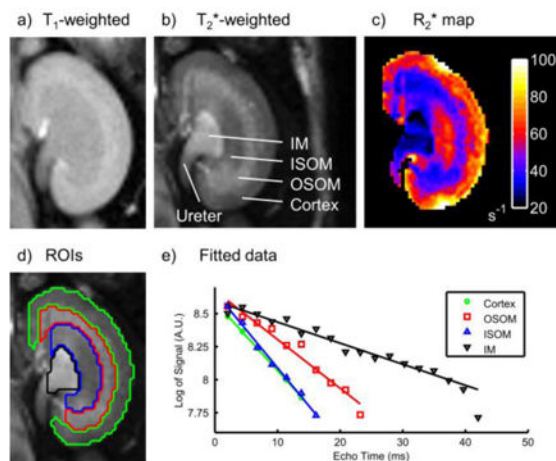
We evaluated the influence four commonly used anesthetics on renal R_2^* in mice. The choice of anesthetic agent significantly affected R_2^* in all tissue layers of the kidney, and observed differences were not attributed to differences in inhaled oxygen between volatile and injectable agents. The results indicate that anesthetic effects must be considered for preclinical renal BOLD MRI.

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Introduction

Renal oxygenation is determined by the balance of oxygen (O_2) delivery and consumption (1). Although renal perfusion accounts for $\sim 25\%$ of the cardiac output, the kidneys are vulnerable to hypoxic injury, particularly within the medulla which normally functions at a low partial pressure of oxygen (pO_2). This is due to a combination of factors, including low medullary perfusion relative to that of the cortex, arterial-to-venous shunting of O_2 through diffusion between countercurrent vessels, and the high aerobic demand associated with reabsorption of sodium. Accumulating evidence has implicated hypoxia in the pathogenesis of acute kidney injury as well as chronic diseases including end-stage renal disease (2,3), diabetic nephropathy (4,5), and hypertension (6). Therefore, there is interest in developing non-invasive clinical methods for evaluating renal oxygenation.

Blood oxygen level-dependent (BOLD) MRI provides a method for non-invasive assessment of renal oxygenation based on the apparent transverse relaxation rate (R_2^*) of renal tissue (7). Because deoxyhemoglobin is paramagnetic (8), an increase in intravascular deoxyhemoglobin concentration leads to an increased R_2^* . Assuming the intravascular and parenchymal pO_2 are in equilibrium, R_2^* therefore serves as a surrogate for tissue oxygenation. BOLD MRI has been applied to a variety of renal diseases both clinically and in preclinical small animal models (for reviews see Li et al. (9) and Zhang et al. (10)).

An important consideration for BOLD MRI in animals is the effect of anesthesia on functional measures such as R_2^* . General anesthesia is needed for preclinical imaging but is known to produce several reversible physiologic changes in the kidney, including changes in blood flow, glomerular filtration, and sodium excretion (11). These effects may alter O_2 delivery and/or demand, leading to a change in pO_2 and, consequently, R_2^* . Importantly, the magnitude of the perturbation of R_2^* may depend on the specific anesthetic agent used, as each has different biochemical targets. However, comparative studies on the effects of different anesthetics on renal R_2^* are lacking, and there is currently no recommended standard of anesthesia for BOLD MRI studies.

The purpose of this study is to evaluate the influence of several commonly used anesthetic agents on renal R_2^* in healthy mice. Because inhaled agents are typically delivered with 100% O_2 while only room air is provided for injected agents, we also investigated whether the fraction of inspired oxygen (FiO_2) could explain differences in R_2^* between these agents. Our results show that the choice of anesthetic has a significant effect on R_2^* in all parts of the kidney and highlight the importance of consideration of anesthetic effects in the design and analysis of renal BOLD MRI studies.

Experimental

Animal handling

All experiments complied with the policies of our institution's Animal Care and Use Committee and were conducted using approved animal use protocols. This study included five healthy female ICR mice (mean 28.6 g; 12 weeks of age). The mice had free access to food and water and were housed under standard laboratory conditions of 22°C room temperature, 43% humidity, and a 12:12 hour light/dark cycle. Mouse temperature was continuously monitored during imaging and maintained at 36-37°C using a warm air blower (Small Animal Instruments, Inc., Stony Brook, NY). Respiratory motion was monitored using a pneumatic pillow (Small Animal Instruments, Inc.). Sequential MR exams were performed at five-day intervals to minimize hormonal variation within the estrus cycle.

Anesthesia

Two experiments were performed to separately evaluate the effect of anesthesia and FiO_2 on renal R_2^* . For all exams, each mouse received one type of anesthesia, with BOLD MRI performed approximately 25 minutes post-induction.

In Experiment 1, each animal was administered one of the following anesthetics during each of five separate exams: Isoflurane (Iso) at dosages of 1.5% or 1%, ketamine/xylazine [KX; 100/4 mg/kg intraperitoneal (ip)], sodium pentobarbital (PB; 60 mg/kg ip), and 2,2,2-tribromoethanol (TBE; 500 mg/kg ip). The order of anesthetics for each animal was determined according to a randomized Latin square design to control for variation across individuals and exam number (Table 1). Isoflurane was continuously delivered in 100% O_2 . Dosages for injected agents were chosen to produce approximately 45 minutes of anesthesia, as this duration is realistic for imaging studies.

Experiment 2 was conducted to test whether the difference in FiO_2 between inhaled and injected agents in Experiment 1 was a confounding factor. Experiment 2 included three of the five mice. These animals were administered either 1% Iso delivered in 21% O_2 (versus 100% O_2 in Experiment 1) or KX with supplemental 100% O_2 (versus room air in Experiment 1).

MR Imaging

Imaging of the kidneys was performed using a 4.7 T small animal MR scanner (Agilent Technologies, Santa Clara, CA) and a 35-mm-inner-diameter quadrature volume coil (Agilent Technologies). A single slice through the center of the kidney was imaged in the

coronal plane with 0.23 mm in-plane resolution, 1 mm slice thickness, and 128×128 matrix size. The ureter was used as an anatomic landmark to ensure reproducible slice placement across exam days. T₁-weighted images were acquired using a spoiled gradient-recalled echo sequence with parameters as follows: repetition time, 30 ms; echo time, 3.1 ms; flip angle, 20°; number of averages, 16; acquisition time, 1:01 (min:s). BOLD images were acquired using a multi-gradient-recalled echo sequence with parameters as follows: repetition time, 350 ms; echo time, 2.0 ms; echo spacing, 2.4 ms; number of echoes, 64; flip angle, 30°; number of averages, 4; bandwidth, 100 kHz. Spectral-based fat saturation and end-expiration respiratory triggering were used for the BOLD acquisitions. The acquisition time therefore depended on the average respiratory rate and ranged from 3:39 for TBE to 8:57 for 1% Iso.

Image Analysis

All image analysis was performed in MATLAB (The Mathworks, Natick, MA).

T₂*-weighted images with high signal-to-noise and contrast-to-noise ratios were generated by summation of the BOLD echo images (12). In each kidney, a single region of interest (ROI) was produced for each layer of renal tissue by manual segmentation of the T₂*- and T₁-weighted images (Fig. 1a,b,d). The segmented layers included the cortex, outer stripe of the outer medulla (OSOM), inner stripe of the outer medulla (ISOM), and inner medulla (IM).

R₂* maps were calculated from BOLD magnitude images using robust linear regression of the natural logarithm of signal vs. echo time on a voxel-wise basis with a maximum likelihood estimator (13) (Fig. 1c,e). This approach was preferred to nonlinear exponential fitting of the raw data in order to avoid errors due to the nonzero noise floor of magnitude data (14). Furthermore, robust regression was preferred over ordinary least squares due to its relative insensitivity to outliers and noise distribution. Data with raw signal below the Rose criterion (five times the background standard deviation) were excluded from fitting in order to avoid noise amplification at later echo times by the natural logarithm operation. The specific number of data above this threshold varied across tissues due to their different T₁ and R₂* values (Table 2). Following R₂* calculation, each tissue layer ROI was eroded by one voxel to exclude partial volume or segmentation errors, and the average R₂* within the ROI was calculated.

Statistical Analysis

Statistical analysis was performed in Microsoft Excel 2010 (Redmond, WA) with significant differences defined as $p < 0.05$. Data from Experiment 1 were analyzed using an analysis of variance (ANOVA) for three factors (mouse, exam number, and anesthesia) followed by pairwise multiple comparisons using the Holm-Šidák method (15). Data from Experiment 2 were analyzed using a one-tailed paired student t-test. Values are reported in the text as mean ± standard error of the mean (standard deviation divided by the square root of the sample size), unless otherwise indicated.

Results

All mice tolerated all anesthetic doses well and remained in a stable unconscious state throughout imaging. A high goodness of fit was achieved for all voxels (Table 2). The coefficient of determination, R^2 , adjusted for the number of fitted points was at least 0.92 ± 0.12 (mean \pm standard deviation) for all tissues, and the standard error of the estimated R_2^* varied from 5.4 ± 2.9 % (OSOM) to 7.7 ± 6.6 % (cortex; mean \pm standard deviation).

Experiment 1 – Effects of Anesthetic Agents

We found a significant effect of the choice of anesthesia on respiration rate ($p < 0.0001$, ANOVA), indicating that the degree of respiratory depression varied depending on the anesthetic agent (Fig. 2). The lowest respiration rate was observed for 1.5% Iso (57 ± 11 breaths/min), whereas TBE produced the highest rate (140 ± 8 breaths/min). Qualitative differences in the shape of the respiratory waveform were also observed (data not shown). For example, under both 1% and 1.5% Iso we observed short periods of respiratory motion separated by relatively long quiescent intervals at end-expiration, while respiratory motion for other agents was more continuous and sinusoidal.

We found a significant effect of the choice of anesthesia on R_2^* in all four renal tissue layers investigated ($p < 0.0005$, ANOVA; Fig. 3). Post-hoc pairwise comparisons found several significant differences in R_2^* between specific agents within the cortex, OSOM, and ISOM. The largest differences in these tissues were between 1.5% Iso and KX (respectively: 49.7 ± 5.2 s⁻¹ versus 73.6 ± 7.3 s⁻¹ in cortex; 31.6 ± 3.3 s⁻¹ versus 59.8 ± 3.3 s⁻¹ in OSOM; 26.6 ± 1.7 s⁻¹ versus 66.0 ± 7.1 s⁻¹ in ISOM). In general, the R_2^* for injected agents trended toward higher values compared to Iso in all tissue layers.

Experiment 2 – Effect of Inspired Oxygen

R_2^* did not differ in any region between 1% Iso delivered with 21% O₂ and 1% Iso delivered with 100% O₂ (Fig. 4). In the ISOM, KX combined with 100% O₂ resulted in a significantly higher R_2^* than did 1% Iso delivered with 21% O₂, suggesting that a difference in FiO₂ does not fully explain differences between Iso and KX observed in Experiment 1 for this tissue. No statistically significant differences between these combinations of anesthesia and FiO₂ were observed in other renal tissues, possibly due to the low number of animals this experiment.

Discussion

Preclinical imaging, including BOLD MRI, is a valuable tool for investigating the etiology of disease and for evaluating treatments. This study demonstrates that anesthetic agents have significantly different effects on the R_2^* measured in all tissue layers of the kidney in mice, with the most pronounced differences occurring within the outer medulla. The apparent sensitivity of R_2^* in the outer medulla to the choice of anesthesia may be due to its relatively hypoxic state in general. The medulla is also the most common site of R_2^* differences observed with disease or diuretic challenge (16–20). The large range of R_2^* values observed in this study is not negligible compared to the magnitude of these

differences, so controlling for the influence of anesthesia is essential to avoid confounding results.

There are several possible mechanisms by which anesthesia may affect deoxyhemoglobin concentration and, consequently, R_2^* in the kidney. For example, changes in systemic arterial blood oxygenation (p_aO_2) may occur due to depression of respiratory rate. In this study, 1.5% Iso produced the most severe respiratory depression (57 ± 11 breaths/min), whereas breath rates for all other agents were at least 82 ± 10 breaths/min on average. If respiratory depression under anesthesia was a major determinant of renal R_2^* , one might expect R_2^* to be higher for 1.5% Iso than for the injected agents; however, the opposite relationship was observed. Furthermore, similar values of R_2^* were observed for 1% Iso and 1.5% Iso in Experiment 1, despite a nearly two-fold difference in respiratory rate (109 ± 11 breaths/min for 1% Iso). These results suggest that p_aO_2 was not limiting renal R_2^* as a result of depression of respiratory rate. Consistent with this, previous reports using invasive probes in rodents have shown at least 99% arterial hemoglobin saturation under Iso delivered with 100% O_2 (21,22) and 97% saturation when delivered with 21% O_2 (21).

It is also possible that differences in FiO_2 during anesthesia may influence p_aO_2 , as inhaled agents are typically delivered in 100% O_2 while injected agents do not involve supplemental oxygen. However, our findings in Experiment 2 suggest against a major role for this effect. The R_2^* within the ISOM was higher for KX with 100% O_2 than for 1% Iso with 21% O_2 , which would not be expected based on FiO_2 alone. In addition, R_2^* values were not different between 1% Iso delivered with 100% O_2 or 21% O_2 . Although Experiment 2 is limited by a low sample size, its results support the hypothesis that the significant differences in Experiment 1 are not simply an artifact of FiO_2 differences, and that pharmacologic effects play a role. This is consistent with previous results in mice which showed similar p_aO_2 values between the conscious state and KX (23) anesthetization without supplemental oxygen, suggesting that FiO_2 under KX is not a limiting factor.

A decrease in blood flow to the kidney may also influence R_2^* by reducing O_2 delivery. Depression of cardiac function and vasodilation are well-documented effects of general anesthesia, and the severity of depression is agent dependent (11). A previous study by Janssen et al. (24), in particular, evaluated the hemodynamic effects of Iso, KX, and PB in mice at dosages similar to those used in the present study (Iso, 1% in 100% O_2 ; KX, 50/10 mg/kg ip; PB, 50 mg/kg ip) and compared these values to the conscious state. Interestingly, the reported reduction of cardiac index by these agents covary with our values of R_2^* (Fig. 5), suggesting that differences in renal R_2^* between anesthetic agents may be associated with reduced delivery of oxygenated blood due to effects on systemic circulation. The importance of consideration of anesthetic effects may therefore extend beyond renal BOLD MRI to include perfusion imaging as well as functional imaging of organs other than the kidney.

Finally, R_2^* may be influenced by changes in metabolic O_2 demand within renal cells. Unlike other organs in which perfusion changes in response to demand for O_2 , the kidney changes its metabolic rate in response to perfusion. This is because the majority of renal O_2 consumption is due to reabsorption of filtered sodium, and a change in blood flow alters the

filtered load of sodium. As an example, increasing renal blood flow by administration of atrial natriuretic peptide has been shown to paradoxically decrease renal pO_2 in rats, while conversely pO_2 has been shown to increase following acute hypoperfusion (25). Due to the complex relationship between renal blood flow and O_2 consumption, it is unclear how large a role O_2 demand plays in our observations of R_2^* in the present study. Direct measurement of O_2 extraction by the kidney, in concert with BOLD MRI, may help identify the relative contributions of O_2 delivery and consumption to R_2^* under anesthesia.

A limitation of the present study is that injected agents were evaluated at only one dose, while the effect of each agent on R_2^* is likely dose dependent. In addition, metabolism of these agents over time may cause the functional response and effect on R_2^* to be time dependent. Time-dependent effects may be more pronounced for agents injected as a single dose than for inhaled or intravenous agents which can be administered continuously and titrated to a desired level of anesthesia. We sought to standardize the anesthetic state by choosing dosages which produced 45 minutes of anesthesia and by imaging at approximately the same time post-induction. Additional factors, such as the strain, gender or age of the mice, may also influence the R_2^* of the kidney under particular anesthetics, as these factors have been shown to affect anesthetic response in general (26,27).

In conclusion, this study demonstrates that the choice of anesthesia has a significant effect on the observed values of R_2^* in healthy mouse kidney. The design of BOLD MRI studies must consider this effect, and we recommend standardization of anesthetic protocols to minimize variation in R_2^* .

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Abbreviations

BOLD	Blood oxygen level-dependent
FiO₂	Fraction of inspired oxygen
IM	Inner medulla
ip	Intraperitoneal
Iso	Isoflurane
ISOM	Inner stripe of the outer medulla
KX	Ketamine/xylazine
OSOM	Outer stripe of the outer medulla
p_aO₂	Arterial partial pressure of oxygen
pO₂	Partial pressure of oxygen
PB	Sodium pentobarbital
R₂*	Transverse relaxation rate
ROI	Region of interest
TBE	Tribromoethanol

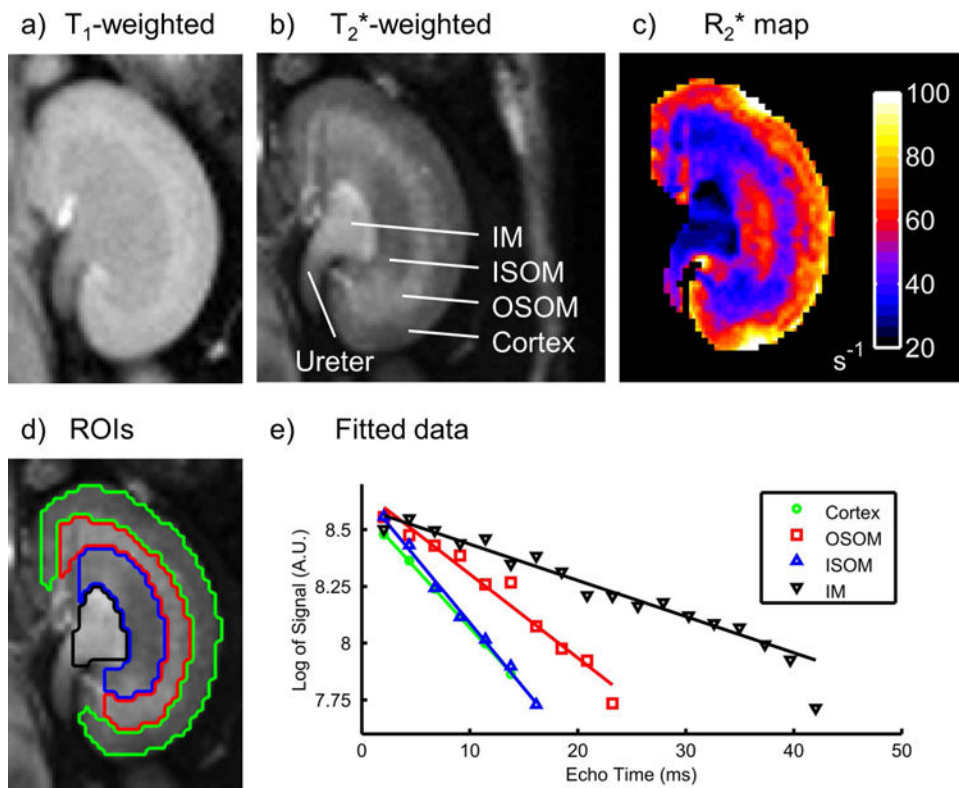


Figure 1. Typical T₁-weighted (a) and T₂*-weighted (b) kidney images with the corresponding R₂* map (c). Four distinct tissue layers are clearly visible in the T₂*-weighted image: cortex, outer stripe of the outer medulla (OSOM), inner stripe of the outer medulla (ISOM) and inner medulla (IM). Regions of interest were produced by manual segmentation of the T₁- and T₂*-weighted images (d). Example R₂* fits from each region of interest are shown (e).

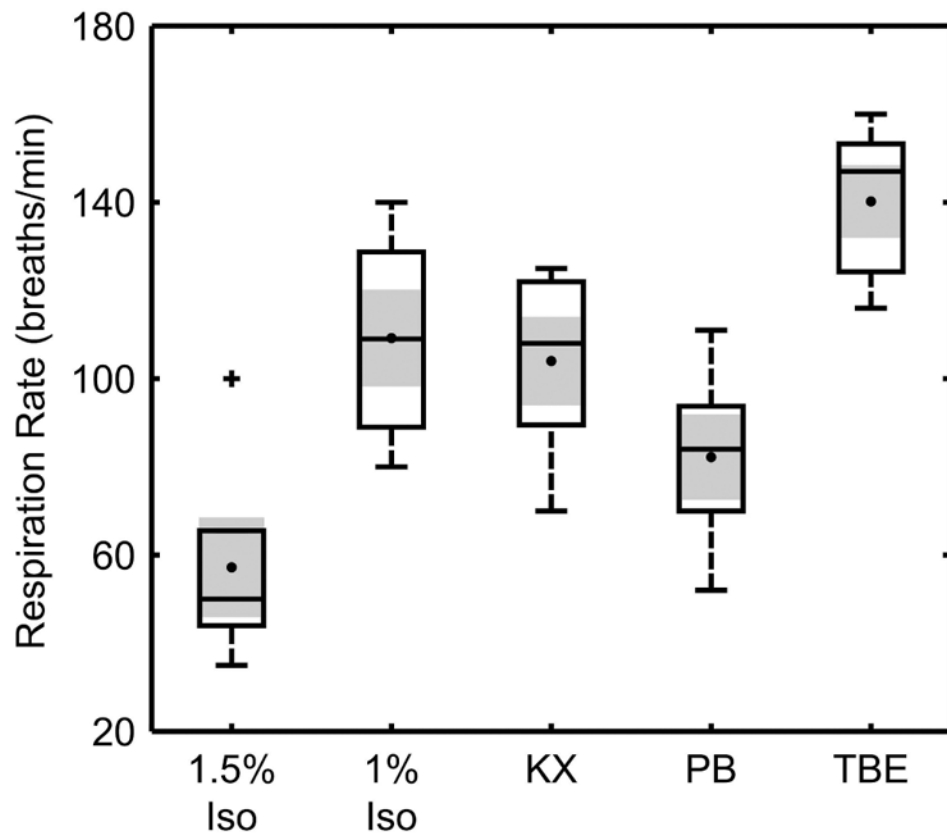


Figure 2. Box-and-whisker plots of respiration rates during BOLD imaging under different anesthetic agents ($n = 5$) with the mean (\bullet) and standard error (shading) overlaid. A significant effect of anesthesia on respiratory rate was observed ($p < 0.0001$, ANOVA). Iso = isoflurane; KX = ketamine/xylazine; PB = sodium pentobarbital; TBE = tribromoethanol.

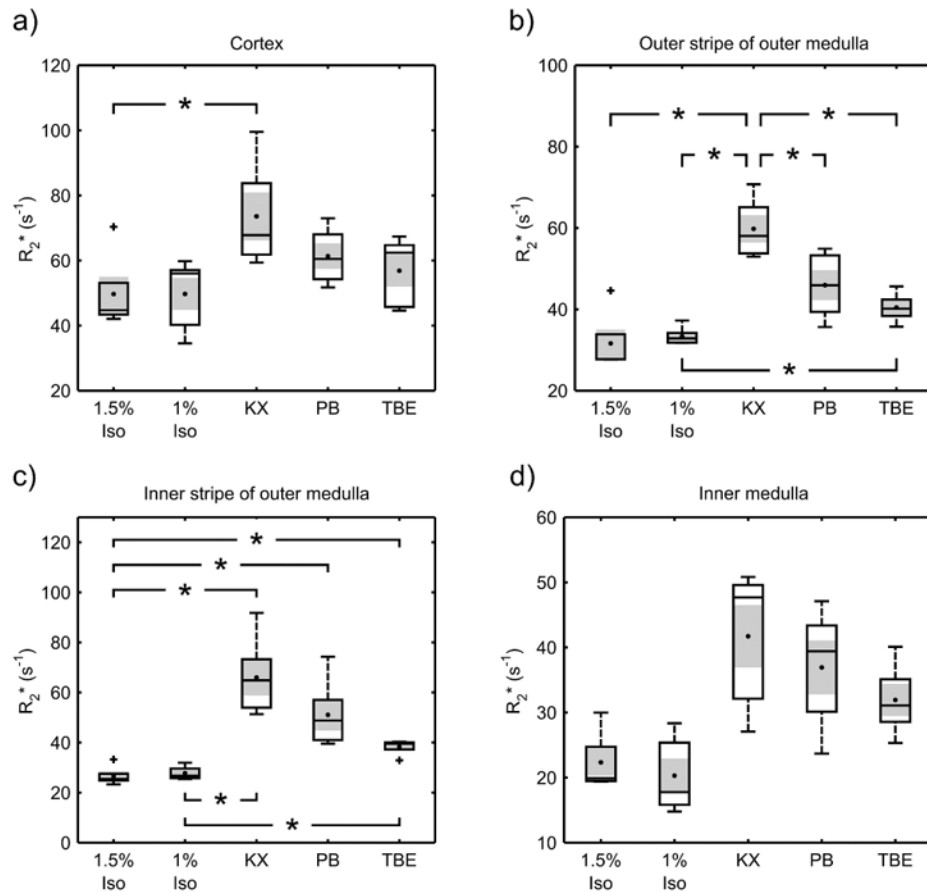
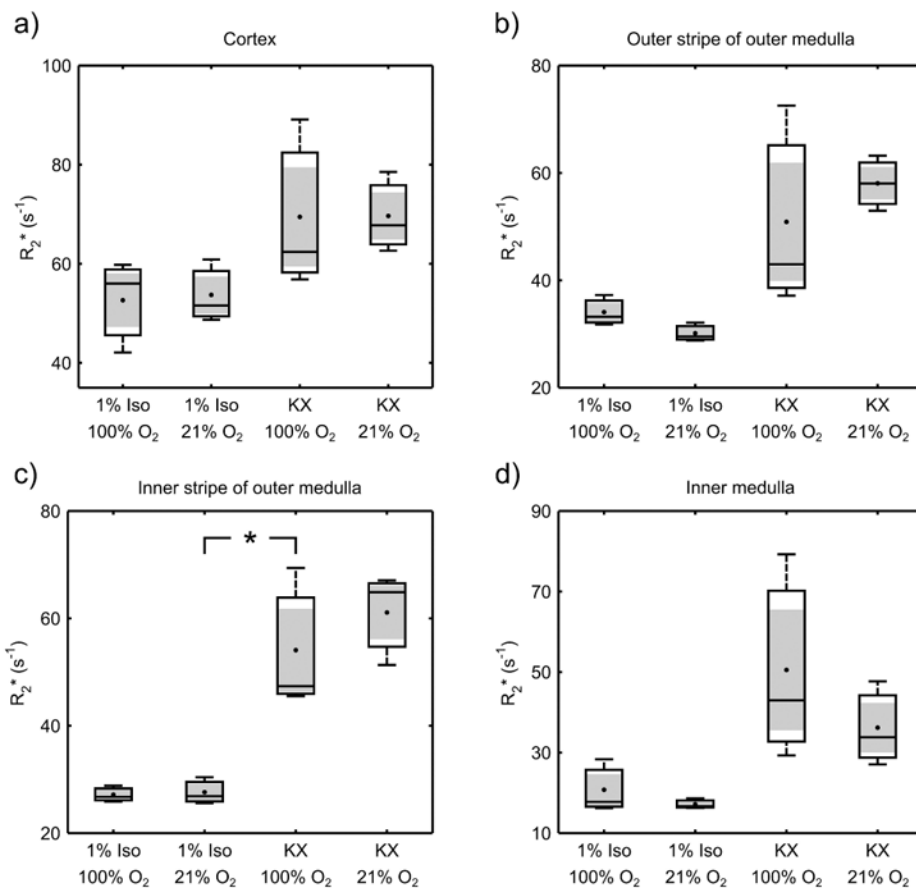


Figure 3. Box-and-whisker plots of the average R_2^* in the cortex (a), outer stripe of the outer medulla (b), inner stripe of the outer medulla (c), and inner medulla (d) for different anesthetic agents ($n = 5$) with the mean (\bullet) and standard error (shading) overlaid. A significant effect of anesthetic agent was observed for all four tissues ($p < 0.0005$, ANOVA). Multiple comparisons found several significant differences between specific agents in the cortex and inner and outer stripes of the outer medulla ($p < 0.05$, Holm-Šidák method; significant differences indicated by *). Iso = isoflurane; KX = ketamine/xylazine; PB = sodium pentobarbital; TBE = tribromoethanol.

**Figure 4.**

Box-and-whisker plots of the average R_2^* in the cortex (a), outer stripe of the outer medulla (b), inner stripe of the outer medulla (c), and inner medulla (d) for 1% isoflurane and ketamine/xylazine while breathing either 100% or 21% O₂ (n = 3), with the mean (•) and standard error (shading) overlaid. R_2^* within the inner stripe of the outer medulla was significantly higher for ketamine/xylazine with 100% O₂ compared to isoflurane with 21% O₂ (p < 0.05, one-tailed student t-test; significant difference indicated by *). Iso = isoflurane; KX = ketamine/xylazine.

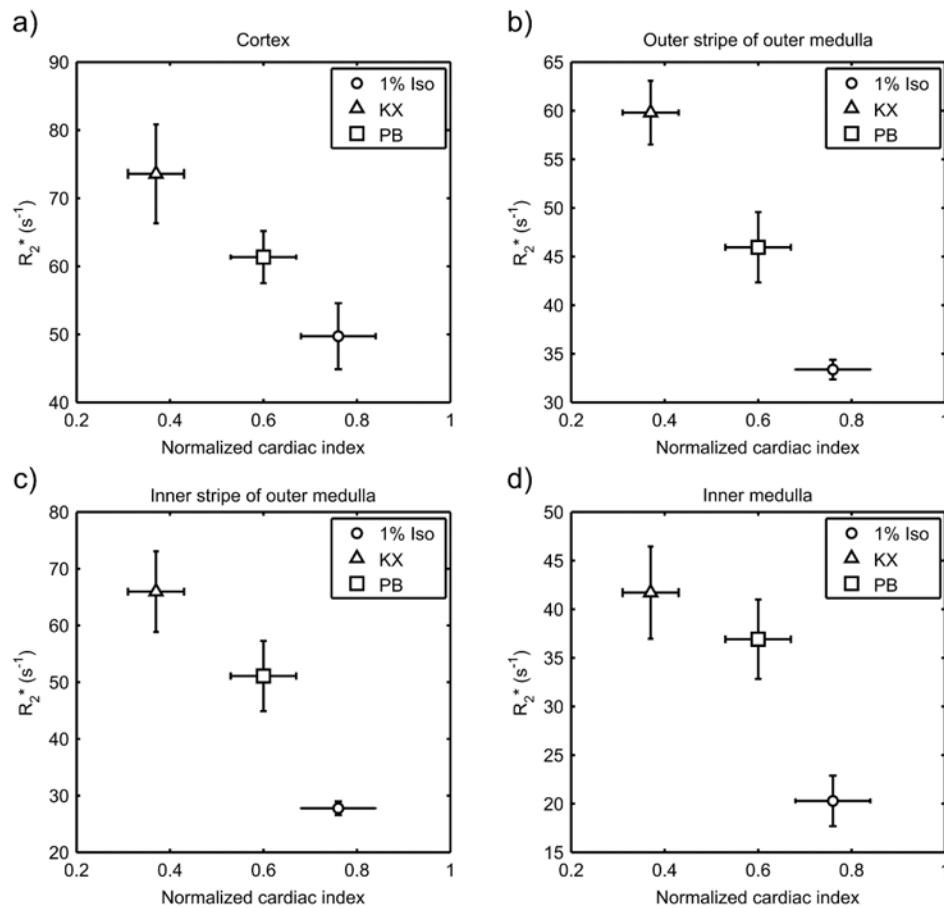


Figure 5.

R_2^* results from Experiment 1 of the present study ($n = 5$) compared to cardiac index (cardiac output per unit body mass) normalized to the awake state, as reported by Janssen et al. (24). Depression of cardiac index correlated with higher values of R_2^* in the cortex (a), outer stripe (b) and inner stripe (c) of the outer medulla, and inner medulla (d). Error bars reflect standard error of the mean. Sample sizes for cardiac index, as reported by Janssen et al., were 10, 9 and 8 for 1% Iso, KX, and PB, respectively. Iso = isoflurane; KX = ketamine/xylazine; PB = sodium pentobarbital.

Table 1

Order of anesthetic agents for Experiment 1. Iso = isoflurane; KX = ketamine/xylazine; PB = sodium pentobarbital; TBE = tribromoethanol.

Mouse	Exam Number				
	1	2	3	4	5
1	1.5% Iso	KX	TBE	PB	1% Iso
2	KX	TBE	1% Iso	1.5% Iso	PB
3	PB	1.5% Iso	KX	1% Iso	TBE
4	1% Iso	PB	1.5% Iso	TBE	KX
5	TBE	1% Iso	PB	KX	1.5% Iso

Table 2

Goodness of fit parameters for R_2^* estimation. Data are expressed as mean \pm standard deviation across all voxels evaluated in the study. OSOM = outer stripe of outer medulla; ISOM = inner stripe of outer medulla; IM = inner medulla.

Tissue	Fitted points	Adjusted R^2 of fit	Standard error of R_2^* (%)
Cortex	10.0 \pm 3.4	0.93 \pm 0.13	7.7 \pm 6.6
OSOM	15.1 \pm 5.0	0.95 \pm 0.05	5.4 \pm 2.9
ISOM	16.1 \pm 6.4	0.95 \pm 0.06	5.7 \pm 3.0
IM	21.3 \pm 8.8	0.92 \pm 0.12	6.3 \pm 6.3

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