# How to image CBF in the mouse brain

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## Introduction: how can we measure CBF in the rodent brain?

Assessment of tissue perfusion in the brain has been the focus of many clinical and research studies, and a variety of MRI techniques for measuring capillary blood flow have been proposed for human and animal studies.

*Dynamic susceptibility contrast (DSC) in the rodent brain?*
Although DSC is sensitive enough to provide reliable relative measurements of blood flow and blood volume even in low-flow conditions in humans, absolute quantification is still difficult. In small rodents, depending on the anesthetic used and the brain region, capillary blood flow can be 2–10 times higher than in humans. A dynamic first-pass measurement of the contrast agent through the organ therefore would require extremely short bolus injections and imaging times. Also, repetition of a dynamic susceptibility contrast MRI measurement is only possible after complete washout of the contrast agent, precluding serial measurements in most cases.

*Arterial Spin Labeling*
Arterial spin labeling (ASL) MRI techniques have been widely developed and used in clinical research studies, but were originally developed in animals. In ASL, high capillary blood flow is advantageous because the signal is directly proportional to capillary flow. Owing to the increase in T1 with field strength, the high B0 fields commonly used in small-animal MRI are also beneficial, although T2 effects partly counterbalance this advantage for certain readout techniques. Moreover, ASL permits comparatively easy and reliable quantitative measurements of cerebral blood flow (CBF) which can be repeated during the experiment. Its repeatability along with its ability to provide quantitative measurements also eases follow-up studies and enables functional brain studies. ASL MRI is therefore the method of choice for mapping capillary blood flow in small rodents.

## Available ASL Methods

*Pseudo-continuous, continuous and dynamic ASL*
With these techniques, magnetic labeling is achieved distal from the slice(s) of observation. Continuous techniques require dedicated labeling coils and drivers whereas pseudo-continuous techniques mimic a continuous label with a standard transmit coil and minimize magnetization transfer effects by a special pulse scheme. All of these techniques were shown in theory and experimentally to provide significantly better sensitivity than their pulsed ASL counterparts, however a quantitative assessment of blood flow appears more difficult in absolute terms. Dynamic ASL techniques use a particular time-dependent function for continuous labeling to add important information to the CBF measurement, such as arterial transit times and tissue T1.

*Pulsed ASL – FAIR*
In animal studies, Flow-sensitive Alternating Inversion Recovery (FAIR) is the most widespread labeling scheme for pulsed ASL. The readout of the magnetization difference produced by inflowing blood into the capillary system is less efficient than in continuous ASL due to long recovery periods, but the labeling zone is placed symmetrically and close to the observation slice leading to better robustness with respect to arterial transit times and to an easier modeling. FAIR, however, has some limitations when multi-slice experiments are to be performed. Look-Locker inversion-recovery techniques were first proposed for rodent cardiac applications, but also had their entry into brain studies. They were shown to provide slightly better sensitivity than classical FAIR techniques and to open new ways to the exploration of the magnetization dynamics after labeling including assessment of the shape of the “magnetic bolus”. Look-Locker techniques also provide an inherent and simultaneous measurement of T1.

## Current challenges in mouse brain ASL

*Anesthesia and animal position*
Anesthetics have a known impact on either cardiac function, brain function or microvascular hemodynamics, and the combination of all these effects will lead to modifications of CBF measured. In particular, isoflurane, the most commonly used anesthetic, has a known vasodilatory effect. Besides interpretation of absolute baseline CBF measurements, this can also impact the results obtained on animal models, since it will also modify the response of the vascular system to challenges as found in pathology. Preconditioning in stroke by isoflurane is an example, in which the anesthetic directly interferes with vascular function in a beneficial way. Gaseous anethetics in general tend to increase CBF, whereas barbiturates or a ketamine/xylazine combination will diminish CBF.
Vertical MR systems generally have higher field strenghts and are therefore advantageous for ASL assessment of CBF. The animal position, however, modifies baseline CBF, and horizontal systems should be preferred. In pulsed ASL, due to very rapid circulation of blood in rodents, it is necessary to provide large labeling zones including a significant portion of the body. Larger volume excitation coils in horizontal systems are therefore an advantage.

*Transit times, influence of larger vessels and venous outflow*
Not only CBF, but also blood flow in larger arteries and veins is high in small rodents. Recent approaches take into account that the exchange time between capillary blood and tissue is not negligibly small, and that a significant portion of labeled blood will not contribute to the ASL signal. Arterial bulk flow can produce overestimation of the measured CBF. Compared with human studies, however, arterial transit times (for blood traveling from the labeling zone to the imaging slice) are very small and can, in general, be neglected.

## What can we learn from CBF mapping in mouse brain?

ASL has provided useful, sometimes essential information in mouse model studies of various human pathologies. Findings, limitations and challenges will be discussed on the following models.

tumors
stroke (MCAo model)
infectious disease
Alzheimer's disease

ASL has also contributed to a better understanding of brain activation fMRI. Especially the combination of BOLD and blood volume information along with CBF measurements permit to obtain valued information on vascular function.

## Conclusions

The use of ASL as a quantitative CBF measurement and mapping tool is unique because it permits both longitudinal studies and the assessment of diffuse modifications of absolute CBF, which are not related to a specific region.
CBF measurements are generally done along with other MRI modalities and strengthen the observations made.

References

1. Hendrich KS, Kochanek PM, Melick JA, Schiding JK, Statler KD, Williams DS, Marion DW, Ho C. Cerebral perfusion during anesthesia with fentanyl, isoflurane, or pentobarbital in normal rats studied by arterial spin-

labeled MRI. *Magn Reson Med* 2001;46(1):202-206.
2. Detre JA, Leigh JS, Williams DS, Koretsky AP. Perfusion imaging. *Magn Reson Med* 1992;23(1):37-45.
3. Williams DS, Detre JA, Leigh JS, Koretsky AP. Magnetic resonance imaging of perfusion using spin inversion of arterial water. *Proc Natl Acad Sci U S A* 1992;89(1):212-216.
4. St Lawrence KS, Wang J. Effects of the apparent transverse relaxation time on cerebral blood flow measurements obtained by arterial spin labeling. *Magn Reson Med* 2005;53(2):425-433.
5. Pell GS, Thomas DL, Lythgoe MF, Calamante F, Howseman AM, Gadian DG, Ordidge RJ. Implementation of quantitative FAIR perfusion imaging with a short repetition time in time-course studies. *Magn Reson Med* 1999;41(4):829-840.
6. Kim SG. Quantification of relative cerebral blood flow change by flow-sensitive alternating inversion recovery (FAIR) technique: application to functional mapping. *Magn Reson Med.* 1995 Sep;34(3):293-301.
7. Calamante F, Thomas DL, Pell GS, Wiersma J, Turner R. Measuring cerebral blood flow using magnetic resonance imaging techniques. *J Cereb Blood Flow Metab* 1999;19(7):701-735.
8. Thomas DL, Lythgoe MF, Pell GS, Calamante F, Ordidge RJ. The measurement of diffusion and perfusion in biological systems using magnetic resonance imaging. *Phys Med Biol* 2000;45(8):R97-138.
9. Silva AC, Kim SG, Garwood M. Imaging blood flow in brain tumors using arterial spin labeling. *Magn Reson Med* 2000;44(2):169-173.
10. Pell GS, Lythgoe MF, Thomas DL, Calamante F, King MD, Gadian DG, Ordidge RJ. Reperfusion in a gerbil model of forebrain ischemia using serial magnetic resonance FAIR perfusion imaging. *Stroke* 1999;30(6):1263-1270.
11. Prass K, Royl G, Lindauer U, Freyer D, Megow D, Dirnagl U, Stockler-Ipsiroglu G, Wallimann T, Priller J. Improved reperfusion and neuroprotection by creatine in a mouse model of stroke. *J Cereb Blood Flow Metab* 2007;27(6):1290.
12. Stefanovic B, Schwindt W, Hoehn M, Silva AC. Functional uncoupling of hemodynamic from neuronal response by inhibition of neuronal nitric oxide synthase. *J Cereb Blood Flow Metab* 2007;27(4):741-754.
13. Gunther M, Bock M, Schad LR. Arterial spin labeling in combination with a look-locker sampling strategy: inflow turbo-sampling EPI-FAIR (ITS-FAIR). *Magn Reson Med* 2001;46(5):974-984.
14. Ordidge RJ, Wylezinska M, Hugg JW, Butterworth E, Franconi F. Frequency offset corrected inversion (FOCI) pulses for use in localized spectroscopy. *Magn Reson Med* 1996;36(4):562-566.
15. Kober F, Iltis I, Izquierdo M, Desrois M, Ibarrola D, Cozzone PJ, Bernard M. High-resolution myocardial perfusion mapping in small animals in vivo by spin-labeling gradient-echo imaging. *Magn Reson Med* 2004;51(1):62-67.
16. Thomas DL, Lythgoe MF, Calamante F, Gadian DG, Ordidge RJ. Simultaneous noninvasive measurement of CBF and CBV using double-echo FAIR (DEFAIR). *Magn Reson Med* 2001;45(5):853-863.
17. Herscovitch P, Raichle ME. What is the correct value for the brain--blood partition coefficient for water? *J Cereb Blood Flow Metab* 1985;5(1):65-69.
18. Kober F, Iltis I, Cozzone PJ, Bernard M. Myocardial blood flow mapping in mice using high-resolution spin labeling magnetic resonance imaging: influence of ketamine/xylazine and isoflurane anesthesia. *Magn Reson Med* 2005;53(3):601-606.
19. Thomas DL, Lythgoe MF, Gadian DG, Ordidge RJ. In vivo measurement of the longitudinal relaxation time of arterial blood (T1a) in the mouse using a pulsed arterial spin labeling approach. *Magn Reson Med* 2006;55(4):943-947.
20. Meyer ME, Yu O, Eclancher B, Grucker D, Chambron J. NMR relaxation rates and blood oxygenation level. *Magn Reson Med* 1995;34(2):234-241.
21. Scremin OU, Holschneider DP, Chen K, Li MG, Shih JC. Cerebral cortical blood flow maps are reorganized in MAOB-deficient mice. *Brain Res* 1999;824(1):36-44.
22. Okamoto H, Meng W, Ma J, Ayata C, Roman RJ, Bosnjak ZJ, Kampine JP, Huang PL, Moskowitz MA, Hudetz AG. Isoflurane-induced cerebral hyperemia in neuronal nitric oxide synthase gene deficient mice. *Anesthesiology* 1997;86(4):875-884.
23. Sicard K, Shen Q, Brevard ME, Sullivan R, Ferris CF, King JA, Duong TQ. Regional cerebral blood flow and BOLD responses in conscious and anesthetized rats under basal and hypercapnic conditions: implications for functional MRI studies. *J Cereb Blood Flow Metab* 2003;23(4):472-481.
24. Penet MF, Viola A, Confort-Gouny S, Le Fur Y, Duhamel G, Kober F, Ibarrola D, Izquierdo M, Coltel N, Gharib B, Grau GE, Cozzone PJ. Imaging experimental cerebral malaria in vivo: significant role of ischemic brain edema. *J Neurosci* 2005;25(32):7352-7358.
25. Tsekos NV, Zhang F, Merkle H, Nagayama M, Iadecola C, Kim SG. Quantitative measurements of cerebral blood flow in rats using the FAIR technique: correlation with previous iodoantipyrine autoradiographic studies. *Magn Reson Med.* 1998 Apr;39(4):564-73.
26. Kober F, Duhamel G, Cozzone PJ. Experimental comparison of four FAIR arterial spin labeling techniques for quantification of mouse cerebral blood flow at 4.7 T. *NMR Biomed*. 2008;21(8):781-92.

27. Foley LM, Hitchens TK, Kochanek PM, Melick JA, Jackson EK, Ho C. Murine orthostatic response during prolonged vertical studies: effect on cerebral blood flow measured by arterial spin-labeled MRI. *Magn Reson Med* 2005;54(4):798-806.
28. Kennan RP, Suzuka SM, Nagel RL, Fabry ME. Decreased cerebral perfusion correlates with increased BOLD hyperoxia response in transgenic mouse models of sickle cell disease. *Magn Reson Med* 2004;51(3):525-532.
29. Kim SG, Uğurbil K. Comparison of blood oxygenation and cerebral blood flow effects in fMRI: estimation of relative oxygen consumption change. *Magn Reson Med.* 1997 Jul;38(1):59-65.
30. Frank LR, Lu K, Wong EC. Perfusion tensor imaging. *Magn Reson Med* 2008; 60 (6): 1284-91
31. Calamante F, Lythgoe MF, Pell GS, Thomas DL, King MD, Busza AL, Sotak CH, Williams SR, Ordidge RJ, Gadian DG. Early changes in water diffusion, perfusion, T1, and T2 during focal cerebral ischemia in the rat studied at 8.5 T. *Magn Reson Med* 1999;41(3):479-485.
32. Barbier EL, Silva AC, Kim HJ, Williams DS, Koretzky AP. Perfusion Analysis Using Dynamic Arterial Spin labeling (DASL). *Magn Reson Med* 1999; 41: 299-308.
33. Barrett T, Brechbiel M, Bernardo M, Choyke PL. MRI of Tumor Angiogenesis. *J Magn Reson Imaging* 2007; 26: 235-49
34. Dai W, Garcia D, de Bazelaire C, Alsop DC. Continuous flow-driven inversion for arterial spin labeling using pulsed radio frequency and gradient fields. *Magn Reson Med* 2008; 60 (6): 1488-97