Stability of Repeat Measures of CBF in Aged Tg2576 and Wild Type Mice *via* CASL

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Introduction

Cerebral hypoperfusion, which can be measured with arterial spin labeling (ASL), may be an important physiological indicator of neurodegenerative disorders such as Alzheimer's Disease (AD) (1). As such, longitudinal studies probing regional cerebral perfusion changes as a function of disease progression and/or therapeutic intervention may give valuable insights into underlying disease mechanisms. For perfusion measurements to be used in this way, an important first step is to quantify the test/retest stability of the cerebral blood flow (CBF) measurement. In this study, continuous arterial spin labeling (CASL) (2) was performed on five 17-month old App(+)-Tg2576 (TG) mice and five age-matched wild-type (WT) litter mates.

Methods

All animal handling procedures were reviewed and approved by the local IACUC. Ten 17-month old mice (five App(+)-Tg2576 and five App(-) litter mates) were housed on a standard light/dark cycle and provided a high-fat diet (LabDiet 5020) and water *ad libitum*. Mice were anesthetized with 4% v/v isoflurane in a gas mixture of 30% O_2 + 70% N_2. Animals were then placed into a head holder and positioning tray (Bruker, Entlingen). Animal core temperatures were maintained at 37 +/- 1 °C by circulating warm water through the tray and a blanket over the animal, and the free-breathing respiration rate was maintained at 100 +/- 20 breaths per minute by varying isoflurane dose (~1.5% v/v). Temperature and respiration rate were monitored continuously *via* a rectal probe and a pneumatic pillow (SA Instruments, Stony Brook). Scanning was performed on a Bruker 4.7T/40, using a 7-cm actively-decoupled linear transmit volume coil, and a 1.5-cm actively-decoupled quadrature receive coil. Multi-slice transaxial, coronal, and sagittal images where acquired in order to consistently locate the orientation and position of the brain slice for CBF measurement. Inversion recovery data were acquired at ten inversion times using a non-selective inversion pulse, followed by a fast spin echo image readout (TE=8 ms, ETL=16, centric encoding, MTX=128x128, FOV=1.6 cm^2). CBF measurements were acquired using the CASL paradigm. The labeling was accomplished by irradiating the mouse with a 5.9 uT radiofrequency of continuous amplitude for 2.0 seconds at a position 1.5 cm caudal (tag) and rostral (control) to the imaging slice. The imaging was accomplished with a RARE sequence (TR = 2.5 s, TE = 8.0 ms, ETL = 16, centrally encoded, MTX = 128x128, FOV = 1.6 cm^2, THK = 1.0 mm, post-labeling delay=370 ms). Tag and control images were acquired in 6 blocks of four images each for a period of 16 minutes ((4 tag + 4 control) *6 = 48 images total), enabling generation of a CBF map every 80 seconds. This manner of data collection minimizes the errors due to drift in the data and gives a more accurate measure of average CBF over the course of the acquisition. Each mouse was scanned three times in three different imaging sessions.

Analysis

CBF values were fit according to the equation (3):

$$CBF = \frac{\lambda}{T_1}\frac{M_{control} - M_{tag}}{\left[M_{tag} + \left(2\alpha * \exp\left(-PLD/T_{1b}\right) - 1\right)M_{control}\right]}$$

where λ is the blood/brain partition coefficient for water (which is held fixed at the commonly-accepted literature value of 0.9 ml/g), α is the efficiency of the labeling scheme (calibrated with a flow phantom to be 0.9 or 90 % efficient), PLD is the post-labeling delay, T_{1b} is the T_1 of the blood (taken to be 1.7 s), and $M_{control/tag}$ is the pixel-by-pixel intensity from the control and tag images, respectively. Images were registered to a 3D segmented atlas using an internally-developed optimization package.

T_1 values were determined by fitting the inversion recovery data to the 3-parameter expression $M_z(ti) = M_0 - \beta * M_0 * \exp(-ti/T_1)$.

Results

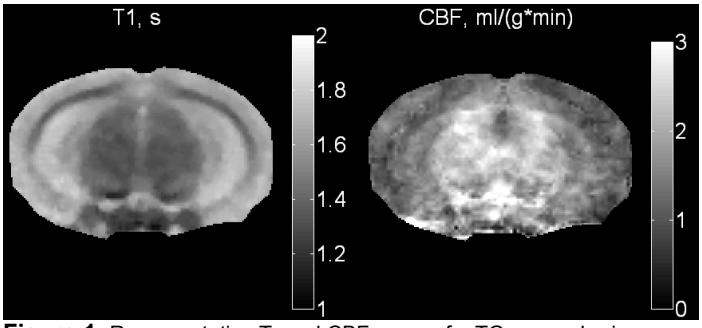

Figure 1. Representative T_1 and CBF maps of a TG mouse brain.

		mean	inter-animal std dev	inter-animal CV	intra-animal average std dev	intra-animal average CV
WT	cortex	1.58*	0.14	0.088	0.08	0.052
	hippocampus	2.02	0.13	0.065	0.08	0.037
	thalamus	2.44	0.19	0.079	0.11	0.048
TG	cortex	1.28*	0.14	0.108	0.13	0.103
	hippocampus	1.88	0.11	0.059	0.12	0.064
	thalamus	2.18	0.18	0.083	0.16	0.074

Table 1. Mean, standard deviation (std dev), and coefficient of variation (CV) of CBF values within designated regions. Values represent inter- and intra- animal standard deviation and CV. *p<0.02.

Discussion

Measurement of cerebral blood flow is subject to considerable instrumental and physiological noise. Use of this parameter in longitudinal studies as a means of assessing either disease progression or therapeutic intervention requires a reliable estimate of the stability of the measurement in disease model populations. As shown in table 1, the coefficient of variation (CV, or relative standard deviation) of measured CBF *across* animals in each group is similar, while repeat CBF measurements *within* TG animals appears to be about twice as variable as those *within* the age-matched WT. Since measurements from these two groups were interspersed, it is reasonable to assume that increased variability in TG mice represents increased physiological noise. This may be due to impaired cerebrovascular autoregulation in the TG mice (4), which results in a diminished ability to regulate CBF in the face of normal blood pressure fluctuations. These results also indicate that the dominant noise contribution is likely physiological rather than instrumental.

With this group size and measurement protocol, CBF was lower in cortex, hippocampus, and thalamus of the TG mice, though the difference was only found to be significant in the cortex.

References

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