Increased sensitivity to the BOLD-fMRI signal response during electrical forepaw stimulation in mice using a cryogenic RF probe

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INTRODUCTION Advantages of cryogenic radio frequency (RF) probes compared to room-temperature (RT) coils for small animal MR imaging have been recently investigated [1-3]. As in small animal MRI sample noise and thermal receiver noise are of comparable magnitude, cooling of RF coil and preamplifier leads to a significant increase in sensitivity. For structural MR imaging of the mouse brain at 400MHz an increase in sensitivity of up to a factor of 2.5 was found when using cryogenic detection [3]. However, it has not been investigated whether this increased sensitivity can be exploited in functional MRI (fMRI) experiments based on the blood oxygenation level dependent (BOLD) contrast. fMRI involves serial data acquisition with corresponding demands on physiological stability in particular temperature, blood pressure and blood gases; therefore the relative contribution of sample noise in BOLD-fMRI might compromise the sensitivity gain provided by the cryogenic detection. In this study we evaluated the potential sensitivity gain provided by a cryogenic RF probe on the BOLD signal in the somatosensory cortex of mice elicited by electrical stimulation of the forepaws by comparing BOLD responses recorded using a cryogenic RF transceive coil and a RT receive only surface coil, respectively.

METHODS Instrumentation: All experiments were carried out on a Bruker BioSpec 94/30 (Bruker BioSpin MRI, Ettlingen, Germany) small animal MR system. Two RF coil setups were used for the MRI experiments: a) A rectangular RT (293K) quadrature receive only surface coil in combination with a linear polarized bird-cage resonator for transmission (RT, Bruker BioSpin MRI, Ettlingen, Germany). b) A transceive cryogenic quadrature RF surface probe (CryoProbe) operating below 30K with an integrated cooled preamplifier (at 77K) (Bruker BioSpin AG, Fällanden, Switzerland). The CryoProbe has a similar geometry as the RT surface coil, but is additionally equipped with a temperature-controlled, user-adjustable thermal shield to ensure insulation of the animal head from the cold RF coil.

fMRI: In vivo experiments were carried out in strict adherence with the Swiss law for animal protection. Female C57Bl/6 mice were anesthetized using 1.5% Isoflurane in an oxygen/air (20%/80%) mixture. Animals were intubated, artificially ventilated and paralyzed using the neuromuscular blocking agent Pancuronium bromide (1-1.5mg/kg dose). To ensure reproducible positioning, the animals were stereotactically fixated on the same animal support for both RT coil and CryoProbe measurements. Physiologic parameters were monitored by a rectal temperature probe (36±0.5°C) and a transcutaneous electrode on the hind limb measuring pCO_2 (40±10 mmHg). BOLD fMRI experiments were performed using a gradient echo-echo planar sequence (GE-EPI) with the following parameters: 5 slices of 0.5mm thickness (THK) with 0.7mm interslice distance (ISD); spatial resolution (RES): 200x200µm²; TE/TR: 8.5/2500ms; 3 averages; temporal resolution: 7.5s; 112 repetitions; total scan time: 14min. The stimulation paradigm consisted of sequential forepaw stimulation with subcutaneous electrodes following a block design (amplitude: 1.5mA, frequency: 3Hz). One stimulation cycle consisted of a 120s off-period used as baseline and of 4 repetitions of 60s on- and 120s off-periods. Experiments were performed using the RT coil setup (N=13) and the CryoProbe at different thermal shield temperatures of T_{Shield}=20°C (N=6), 27°C (N=6), 30°C (N=13), and 38°C (N=5) to investigate the temperature effect on the BOLD signal response. In addition GE-EPI acquisitions using identical parameter settings were acquired without electrical stimulation.

The relative cerebral blood volume (CBV) was assessed as measure for baseline perfusion using a RARE sequence: 5 slices (THK: 0.5mm; ISD: 0.7mm), RES: 264x214µm², TE/TE_{eff}/TR: 10/40/3000ms; RARE factor: 8; temporal resolution: 21s. Identical positions of CBV and BOLD-fMRI measurements were adjusted. The native signal S_{pre} was estimated prior to the administration of the iron-oxide based contrast agent Endorem (Guerbet, France, dose: 33mg/kg body weight). After an initial wait time of 10min to reach steady-state the post contrast signal S_{post} was acquired. Accordingly, CBV was calculated as: $-100.0/TE*\ln(S_{post}/S_{pre})$.

Data Analysis: Brain activity parametric maps were calculated according to the general linear model (GLM) using Biomap (M. Rausch, Novartis, Basel, Switzerland). For statistical maps, a threshold of p=0.001 and activation cluster size ≥15 voxels were applied on a slice at Bregma -0.10mm. Image SNR (SNR_0) and temporal SNR (tSNR) were calculated according to [4]. Changes in BOLD signal intensity were estimated for a region-of-interest (ROI) drawn in the S1 somatosensory cortical area contralateral to the stimulation side. The maximum BOLD signal (ΔS) was estimated as difference between the baseline and the maximum signal of the first stimulation period. The noise level in fMRI scans was estimated as the standard deviation (σ) of the baseline signal fluctuations for the same ROI from a separate acquisition without stimulation. Statistical analysis of variance (ANOVA) was performed in Origin (OriginLab, MA, USA).

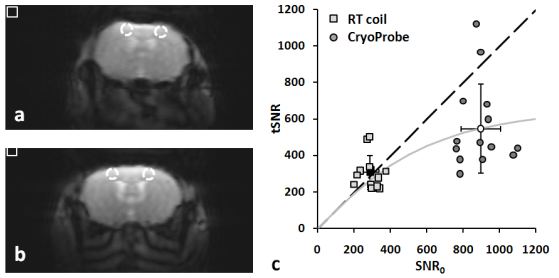

Figure 1: Representative GE-EPI images acquired using RT coil **(a)** and CryoProbe **(b)**, respectively. Dashed circles indicate left and right somatosensory areas (S1), while squares were selected for raw image noise estimation. **(c)** Temporal SNR (tSNR) plotted versus image SNR (SNR_0). Dashed line represents line of equity, while gray line indicates the result of linear least-squares fitting according to [4] (λ=0.00144, R^2=0.94).

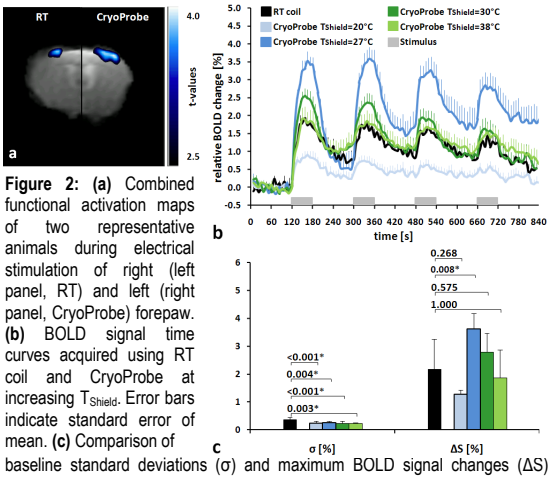

Figure 2: (a) Combined functional activation maps of two representative animals during electrical stimulation of right (left panel, RT) and left (right panel, CryoProbe) forepaw. **(b)** BOLD signal time curves acquired using RT coil and CryoProbe at increasing T_{Shield}. Error bars indicate standard error of mean. **(c)** Comparison of baseline standard deviations (σ) and maximum BOLD signal changes (ΔS) between RT coil and CryoProbe at increasing T_{Shield}. Error bars indicate standard deviations (SD) over different fMRI scans. Asterisks indicate statistical differences of CryoProbe values in comparison to the RT coil (p<0.05).

RESULTS For all acquisitions good image quality with only minor spatial distortions was achieved using both coil setups (Fig. 1a, 1b). The comparison of CryoProbe versus RT coil yielded an increase in SNR_0 and in tSNR of a factor of 3.10±0.66 and of 1.77±0.96, respectively (Fig. 1c). BOLD signal responses during forepaw stimulation were found in all experiments and for both coil setups, however, resulting maximum BOLD signal changes (ΔS) largely varied using the CryoProbe at different T_{Shield} (Fig. 2b, 2c). In contrast, analysis of the baseline fluctuations (σ) revealed significant reductions for the CryoProbe of a factor of 1.6±0.6 (T_{Shield}=20°C), 1.5±0.4 (T_{Shield}=27°C), 1.7±0.6 (T_{Shield}=30°C), and 1.6±0.3 (T_{Shield}=38°C) compared to the RT coil (Fig. 2c). Further experiments estimating CBV showed also a dependency of the baseline perfusion from T_{Shield}. An inverse proportional relation of ΔS and CBV (Fig. 3b) was found when excluding the CryoProbe value at T_{Shield}=20°C (considered as outside of the physiological range).

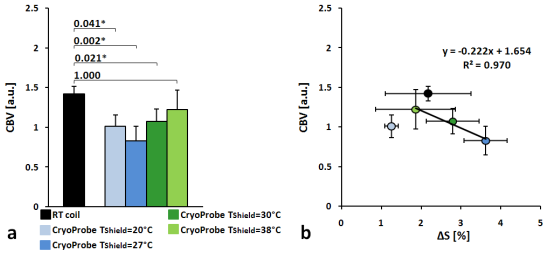

Figure 3: (a) CBV measurements using the RT coil (N=4) and the CryoProbe at T_{Shield}=20°C (N=3), 27°C (N=3), 30°C (N=10), and 38°C (N=3). **(b)** Correlation between CBV and maximum BOLD signal response. Solid line represents linear regression of the CryoProbe values for T_{Shield}=27 - 38°C.

DISCUSSION Comparison of a cryogenic transceive quadrature RF probe with a RT quadrature receive only coil revealed a significant increase in image SNR, while the temporal SNR gain was slightly compromised as expected according to [4]. The cryogenic cooling resulted in a reduction of baseline noise in BOLD experiments of a factor of 1.59 on average. The effect of the CryoProbe shield temperature on the BOLD amplitude is mediated through variation of baseline CBV and can be considered as further optimization parameter. Care has to be taken to stay within the physiological range for CBV. Thus, beside the increased image quality provided by the Cryoprobe allowing for better discrimination of the anatomical structures its thermal shield enables defined thermal conditions at the animal head in contrast to conventional RT measurements. The use of a cryogenic RF probe leads to reduced BOLD signal variations, increasing the statistical power and thus the reproducibility of fMRI experiments.

REFERENCES [1] Doty FD. et al. NMR Biomed 2007 (20):304-25; **[2]** Ratering D. et al. Proc. ISMRM 2007, 383; **[3]** Baltes C. Et al., NMR Biomed 2009, 22(8):834-42; **[4]** Kruger G. et al. MRM2001 (46):631-37

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