Insights in the rat brain by high resolution BOLD functional MRI

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Functional MRI (fMRI) at a high spatial resolution of 75x75x1000µm³ can be achieved using high field small animal MR scanners and four-element array coils. Fine, detailed sub-structures within the activation area can be detected at unrivaled *in vivo* resolution, thus broadening the understanding of the biophysical principles and origin of BOLD to gain further insight into brain organization and structure. Furthermore, functional brain deficit and recovery after brain diseases like stroke can be investigated in great detail.

Functional MRI (fMRI) is a unique tool to study brain activation in humans and animals. In addition to standard anatomical MRI, fMRI can provide information about function and intactness of different brain regions as well as neuronal pathways and workflow of information between such regions.

The BOLD (blood-oxygenation-level dependent) effect is a quantitative method measuring the change of small local field distortions of oxygenated hemoglobin [1] at high temporal resolution. Therefore, the BOLD effect is mostly recorded with the EPI sequence, a fast sequence able to record several image slices of the rat brain repetitively in 3 seconds or less. Unfortunately, these EPI images often suffer from low spatial resolution and distortion artifacts.

Recent technologies in MRI such as high magnetic field strengths, fast RF-readout electronics and multi-element array coils enable high resolution functional MRI to detect and differentiate small activation volumes at the scale of even sub-structures.

These results provide new insights into the understanding of (i) the BOLD effect, (ii) different aspects such as animal models, anesthesia protocols, stimulation and imaging parameters as well as analysis techniques, (iii) the (changing) structure of the brain, (iv) reorganization and recovery of the brain after diseases like stroke and Alzheimer's disease.

Functional MRI in small animals is achievable in magnets from 4.7 to 11.7 Tesla [2,3]. Most investigations rely on the stimulation paradigm of electrical forepaw stimulation on rats at 7.0 and 9.4 Tesla. As such functional activation studies need to be performed on anesthetized animals, α–chloralose had been identified as combining the requirement of holding the animal still and at the same time allowing stimulus to be processed under such anesthetized condition. However, due to severe side effects of α–chloralose, this protocol is limited to acute experiments. A different, robust approach is used in

our studies with the sedative medetomidine [4], which allows recovery of the animal and is therefore compatible with the requirements for longitudinal experiments [5].

Experimental Protocol

Magnets & Coils: Bruker BioSpec 7.0 Tesla, linear Helmholtz coils, linear surface coils and BioSpec 11.7 Tesla, quadrature resonator, quadrature or four-element surface coils.

Sequence parameters: spin-echo (SE) and gradient-echo (GE) single-shot EPI with field-of-view 16.8 – 25.6 mm, matrix 64 (7.0 Tesla) to 256 (11.7 Tesla, four-element coil), resolution 75–400 µm, up to 16 adjacent slices of 0.5 – 2 mm thickness, echo time 16 ms (GE) and 30 ms (SE), repetition time = 3000 ms, 115 repetitions.

Anesthesia: initiation with 1.5 – 2% halothane or isoflurane, slow exchange against sedative agent.

Sedation: bolus injection of 0.5 ml of sedative mixture (0.3 ml Domitor® in 10 ml saline solution for 300 gram rat) and continuous injection of 1 ml per hour. The sedative agent is the α2 adrenergic agonist medetomidine.

Recovery: intraperitoneal injection of 0.3 ml Antisedan® for 300 gram rat, the α2 adrenergic antagonist atipamezole.

Experimental specialities: special attention during the change from anesthesia to sedation, to avoid noise, movement, and pain. A temperature feedback system is indispensible because the auto-regulation of the animal's body temperature is affected by both anesthetic and sedative. A sanitary pad is put under the animal to absorb the increased urgency from the sedated animal.

Stimulation: electrical forepaw stimulation results in activation of the primary (S1) and secondary (S2) somatosensory cortex, and the thalamus. For this, current pulses (1 – 2 mA of 0.3 ms duration, at 3 or 6 Hz) are used for 15 sec following 45 sec resting period, repeated 5 times. Resultant total scan time is 5 min 45 sec.

Analysis: "Stimulate" [6], "FSL" [7] or Bruker "FUN" program. Statistical t-test over activated periods versus resting periods. BOLD activation is expected to be 2 – 3 mm^2 (SE) and 7 mm^2 (GE) with 1 – 3% BOLD contrast for the S1 cortex in rats.

Results and Discussion

BOLD fMRI using electrical forepaw stimulation is a very stable technique for neuronal activation experiments in rodents. It can be combined with other imaging techniques like T_2 imaging for lesion depiction, diffusion tensor imaging and perfusion imaging. Depending on the equipment, different resolutions can be achieved, showing the activation from a small but unresolved cluster down to fine sub-structured areas.

At 7.0 Tesla, using linear surface coils and older

Figure 1

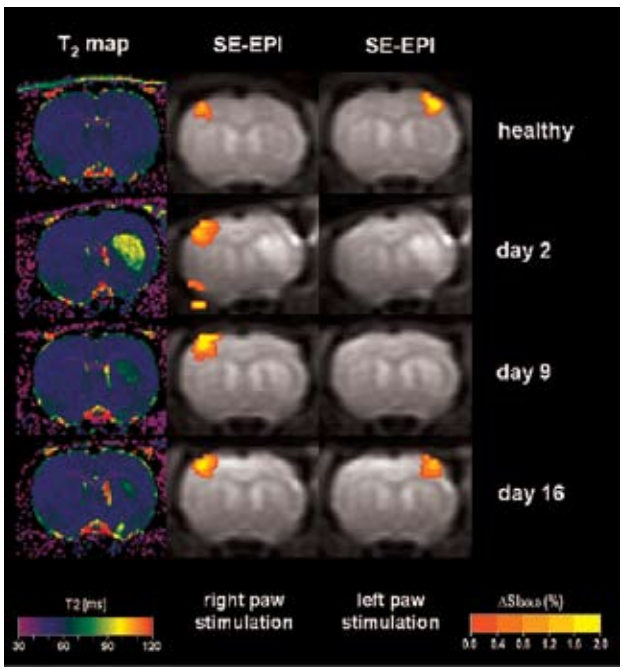


Fig. 1: BOLD activation at 7.0 Tesla on animals after stroke, with a resolution of 400x400x2000 $µm^3$. T_2-maps (left column) indicate the lesion on the right hemisphere. BOLD activation on the left healthy hemisphere (center column) and of the ischemic right hemisphere (right column) indicates transient loss of activation during the first two weeks following stroke, followed by full recovery at day 16.

DBX electronics, BOLD contrast with a resolution of 400x400x2000 µm³ can be achieved. In combination with T_2-maps, stroke lesion and loss of functional activity and its spontaneous recovery (Fig. 1) was characterized [4,5]. Higher field strength of 11.7 Tesla, quadrature surface coils and faster AVANCE II readout electronics increased SNR and resolution to less than 200x200x2000 µm³, fine enough to detect small structures along the pathway of neuronal activity like the secondary somatosensory cortex S2 and thalamus (Fig. 2) with BOLD contrast. With a four-element array coil, matrices up to 256x256 were achieved, which led to a resolution of 75x75x1000 µm³. At this high resolution the activation becomes more resolved enabling the detection of shape and sub-structures in S1 area, S2 and thalamus (Fig. 3).

In general, BOLD contrast is increasing with field strength due to increasing susceptibility sensitivity. Increased spatial resolution further enhances the BOLD sensitivity due to reduced partial volume effects. BOLD contrast was found to increase from 1.5% at a resolution of 400x400x1000 µm³ to 4% at 150x150x1000 µm³ at 11.7 Tesla using the quadrature coil. At even higher resolutions, where sub-structures become detectable, BOLD contrast dropped due to decreased SNR. A direct increase of BOLD contrast with field strength, as observed up to 7.0 Tesla, could not be observed in these studies (Fig. 4), due to decreasing extravascular contributions from venules and arterioles, and due to decreasing intravascular contributions. These changes are explained by very short T_2 values of blood at such high field [3]. However, increasing field strength provides higher S/N which, when invested into higher spatial resolution, provides not only access to finer details of sub-structures but also increases the BOLD amplitude (because of less "dilution" by partial volume effect).

Summary and Outlook

Functional MRI in combination with high magnetic fields and recent coil technology can provide high-resolution images that enable detection of small, highly resolved activation areas and small activation magnitudes on single-shot EPI images of good quality, even at highest field strength. Future studies on animal models and methods will benefit from these results and will contribute to the better understanding

Figure 2

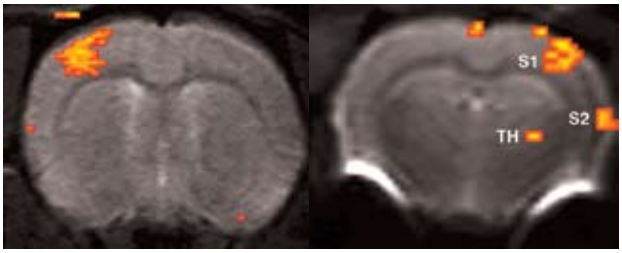


Fig. 2: BOLD activation at 11.7 Tesla using the quadrature surface coil with a resolution of (left) 300x300x 2000 µm³ for high quality and (right) 150x200x2000 µm³ for high sensitivity.

Figure 3

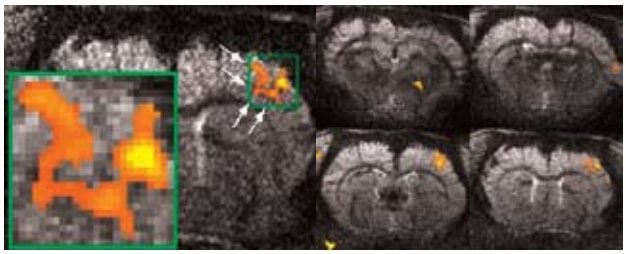


Fig. 3: High resolution BOLD activation at 11.7 Tesla using the four-element array coil with a resolution of 75x75x1000 µm³ for detection of (left) sub-structures within S1, and activation within other regions along the neuronal pathway: S2 and thalamus.

Figure 4

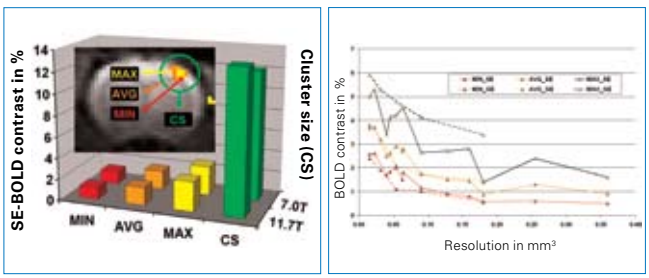
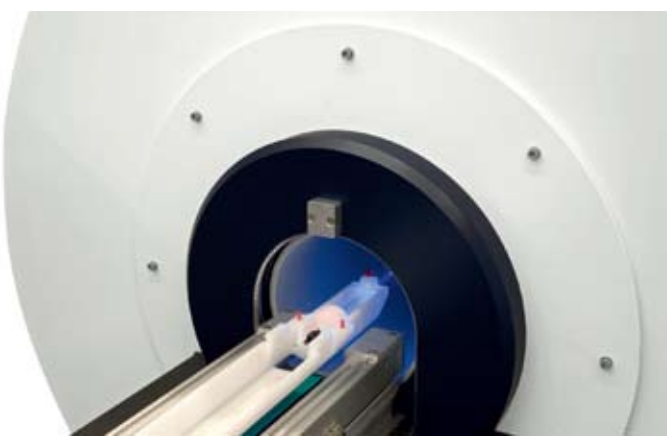


Fig. 4: Comparison of BOLD activation (size and magnitude) for understanding the field dependence of the BOLD effect at 7.0 and 11.7 Tesla with a resolution of 400x400x2000 µm³.



of the structure and, particularly, the function of the brain.

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