Development of an MRI method for awake mouse imaging using soft immobilization and a fast MR acquisition procedure

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Introduction: In MRI research on animals, mouse MRI studies greatly facilitate the elucidation of the pathogenic mechanisms underlying various diseases and the appropriate medical treatments, due to the remarkable advances in mouse genetic technology. It is difficult to acquire accurate MR images when an imaging target moves in the scanner, and thus treatment with anesthesia and fixing apparatuses are usually required. However, the physiological conditions are reportedly different between anesthetized and awake animals [1]. Moreover, some MRI studies for awake animals reported that the brain activation profiles were affected by anesthesia [2], and that stimulus-dependent BOLD signal enhancement was much larger in awake animals than in anesthetized animals [3]. Therefore, methods for awake MRI are attracting increased attention, to exclude the unfavorable effects of anesthesia. In the current awake MRI methods, fixing apparatuses implanted in the brain by surgery [4] and acclimation procedures by training [5] have mainly been applied to suppress head movement in the scanner, and thus these methods require technical proficiency and days for recovery from surgery or for training.

The aim of this study is to develop an easy-to-operate method for awake MRI, without surgery and training. Here we evaluated an awake MRI method for mouse brain imaging, which uses softer immobilization with clothes for mice and a fast MR acquisition procedure.

Methods: MRI experiments were performed with a 7.0 Tesla Bruker Biospec 70/20 scanner and a mouse brain 4-channel phased array surface coil (Bruker BioSpin). Four types of mouse clothes were designed to fit the mouse body (Fig. 1) and used for the MRI measurements. Home-made apparatuses to fix the clothes were designed and attached to an MRI cradle during the measurements. The clothes in Fig. 1 with additional home-made attachment apparatuses are referred to as design 1. The clothes attached to the apparatuses in a different manner from that of design 1 are referred to as design 2. The clothes with two types of modified hood covers to fit a mouse head in addition to design 2 are referred to as designs 3 and 4, respectively. The experiments were performed on adult male C57BL/6 mice (22–25 g). Mice were randomly assigned to one out of four groups containing three animals per group for each clothing design. For the first experience of wearing the clothes, the mice were anesthetized with 3% isoflurane, put in the clothes, and fixed to the MRI cradle. At 60 minutes after cessation of the anesthesia, FISP images (FOV=18 x 18 mm, matrix =90 x 90, resolution=0.2 x 0.2 mm, slice thickness=1.5 mm, number of slices=5, TR/TE=2.3/1.15 ms, FA=60°, NEX=1, and scan repetition time=1 s) were acquired for 30 minutes. Standard deviations (SDs) of the movements of mouse brains in the three directions of Left-Right, Superior-Inferior, and Anterior-Posterior were calculated using SPM8, based on the acquired FISP images. The target SD value of the movements was set to 0.2 mm, corresponding to the spatial resolution of the FISP images [6]. Throughout the experiments, the mice were kept at 37±1°C by a heating pad, and respiration and heart rates were monitored.

Fig. 1 A mouse wearing the designed clothes. The designed clothes have four holes for legs and Velcro tapes on the back and head, to fit the entire body. Based on this basic design, four types of mouse clothes were developed for the MRI measurements.

Results: The movement analyses revealed that the design 2 clothes were the best at suppressing mouse brain movement, among all of the tested clothes during the fast MR acquisition (Fig. 2). The SDs of the movements of mice wearing the design 2 clothes were less than the target value of 0.2 mm in the three directions of Left-Right (L–R), Superior–Inferior (S–I), and Anterior–Posterior (A–P), among which the Anterior-Posterior movement provided the biggest value. The respiration and heart rates remained within the range normally observed in awake mice. As a result, mouse brain MR images with suppressed motion artifacts were obtained with our awake MRI method, using the design 2 clothes without surgery and training (Fig. 3).

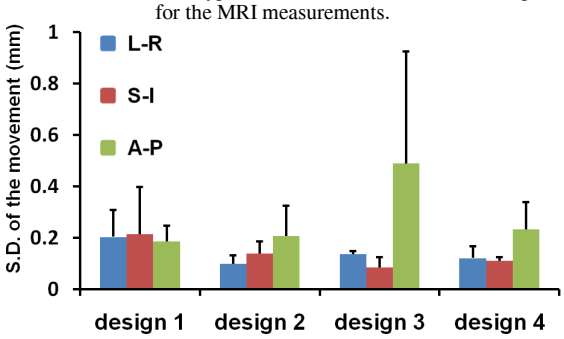

Fig. 2 Evaluation of the mouse brain movements during the MRI measurements.
The standard deviations (SDs) of the movements in the three directions of Left–Right (L–R), Superior–Inferior (S–I), and Anterior–Posterior (A–P) were calculated for the four types of mouse clothes, based on the acquired FISP images.

Discussion: The reasons why our mouse clothes suppressed the brain movements are considered to be due to the softer clothes used for immobilization, rather than harder materials such as an acrylic cylinder, and the wrapping of the entire body in the clothes, instead of few fixation points to lower the pressure at the sites contacted by the fixing apparatuses. By improving the clothes to suppress the Anterior-Posterior movement, a much higher SNR of images is expected. Our method does not require technical proficiency for the immobilization, and thus appears to support the elucidation of physiological differences between anesthetized and awake animals by overcoming the weak points in the conventional awake MRI methods.

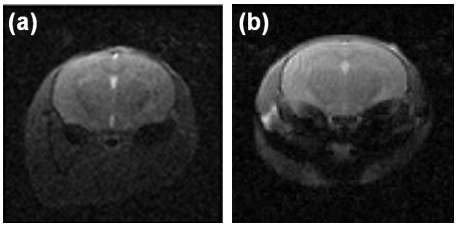

Fig. 3 Comparison of the brain MR images between awake and anesthetized mice.
Identical axial slices of the brain FISP images of an awake (a) and a 3% isoflurane-anesthetized (b) mouse are presented.

Conclusions: We successfully developed a concise method for awake MRI analyses of mice without surgery and training, using softer immobilization with mouse clothes and a fast MR acquisition procedure. Our method will greatly contribute to brain function studies by combined use with fMRI methods, such as BOLD experiments.

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