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Cranioplastic Surgery and Acclimation Training for Awake Mouse fMRI

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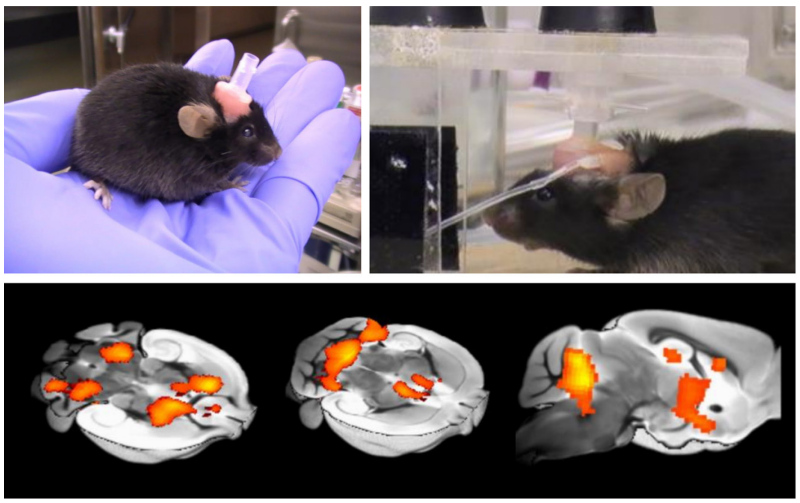
[Abstract] MRI is a promising tool for translational research to link brain function and structure in animal models of disease to patients with neuropsychiatric disorders. However, given that mouse functional MRI (fMRI) typically relies on anesthetics to suppress head motion and physiological noise, it has been difficult to directly compare brain fMRI in anesthetized mice with that in conscious patients. Here, we developed a new system to acquire fMRI in awake mice, which includes a head positioner and dedicated radio frequency coil. The system was used to investigate functional brain networks in conscious mice, with the goal of enabling future studies to bridge fMRI of disease model animals with human fMRI. Cranioplastic surgery was performed to affix the head mount and the cupped-hand handling method was performed to minimize stress during MRI scanning. Here we describe the new mouse fMRI system, cranioplastic surgery and acclimation protocol.

Graphic abstract:



Awake fMRI system to investigate the neuronal activity in awaked mice.

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Keywords: Awake fMRI, Functional MRI, 15q *dup* mouse, Autism, Diffusion tensor imaging, Functional connectivity, Structural connectivity

[Background] High-field mouse fMRI is an important translational tool to bridge the gap between invasive research in mouse models of neuropsychiatric diseases and clinical research in patients. However, compared to human fMRI, mouse fMRI studies are typically limited due to anesthesia, which is necessary to suppress body motion and stress during fMRI acquisition. Performing mouse fMRI studies under light anesthesia can ameliorate body motion, but the extent to which functional brain networks and connectivity are influenced by light anesthesia remains unclear. In general, small doses of dexmedetomidine, medetomidine, isoflurane or a mixture of these anesthetics are commonly used to achieve light anesthesia to investigate brain function in rodents (Grandjean *et al.*, 2014; Bukhari *et al.*, 2017; Tsurugizawa *et al.*, 2019 and 2020a). However, these anesthetics induce not only suppression of consciousness (Munglani *et al.*, 1993) but also vasoactive modulation of neurovascular coupling (Tsurugizawa *et al.*, 2010 and 2016), which potentially impacts the relationship between neuronal activation and vascular response. In particular, light sedation alters the BOLD response to physiological stimuli and thus affects the validity of task-based fMRI (Tsurugizawa *et al.*, 2013a). Anesthesia clearly depresses consciousness, even if residual brain function is comparable to an awake state. In addition, it is impossible to perform cognitive tasks under anesthesia. In summary, fMRI under anesthesia, even with light anesthesia, is not comparable to fMRI in a conscious state, and thus rodent fMRI performed in an anesthetized state considerably narrows opportunities to comprehensively characterize brain function and connectivity. Hence, we developed an awake fMRI system for mice that does not necessitate anesthesia, thus enabling mouse fMRI experiments that are analogous to human fMRI.

Previous studies, including our own, have developed fMRI systems and protocols to acquire fMRI in awake mice. These have been used to investigate responses to fear conditioned stimulation (Harris *et al.*, 2015) and optogenetics (Desai *et al.*, 2011) and to investigate resting state functional connectivity (Bergmann *et al.*, 2016; Yoshida *et al.*, 2016; Madularu *et al.*, 2017). Key limitations of the protocols used in these studies include the use of restraint tubes, absence of earplugs to reduce scanner noise (Mowery *et al.*, 2019; Kurioka *et al.*, 2020) and unclear animal handling and acclimation methods. We first developed an awake-mouse fMRI system to investigate the intragastric stimulation of capsaicin (Tsurugizawa *et al.*, 2013b). More recently, we enhanced the system with the addition of a head fixation apparatus and acclimation training. The new system was used to investigate brain function in mice with a chromosome duplication (15q *dup*) resulting in abnormal behavior resembling ASD symptoms (Nakatani *et al.*, 2009; Tsurugizawa *et al.*, 2020b).

In this report, we describe our fMRI system and protocol for acquiring fMRI in awake mice, including cranioplastic surgery, acclimation training and fMRI acquisition. The protocol enables routine resting-state and task-fMRI mouse studies, where anesthetization of animals introduces a major confound.

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Materials and Reagents

1. Small gauze pad
2. C57BL6J mice (8-15 weeks)
3. Super-Bond C & B (Sun medical)
4. GC UNIFAST Trad (GC Dental Products Corp., Aichi, Japan)
5. Male (VRSP6) and female (VRF6) Plastic Leur Fitting (Nordson Medical, US)
6. Earplugs for humans, made of Polyurethane (3M Company, Minnesota)

Equipment

1. A 4.7T horizontal MRI Avance IIII system (Bruker, Germany)
2. Mouse head positioner (custom made)
3. Dedicated mouse volume coil (30 mm diameter) to transmit/receive the radio frequency signal, combined with semicircular acrylic transparent plastic pipe for mouse bed (Takashima Seisakujo Co., Ltd)
4. Respiration/heart rate monitor system (Model 1025, SA Instruments, Stony Brook, NY, USA)

Procedure

A. Cranioplastic surgery
   1. Surgery
      a. Anesthetize mice with isoflurane (1.5% with air).
      b. Remove head skin and exposed the cranium.
      c. Carefully polish the surface of the cranium with a small gauze pad containing physiological saline.
      d. Place super-bond (Super-Bond C & B) on the skull.
      e. Mount cranioplastic acrylic cement (GC UNIFAST Trad) on the superbond with a male plastic Luer Fitting (Figure 1).
      f. Mice could recover for more than a week after surgery. Carefully check animal body weight and behavior every day.

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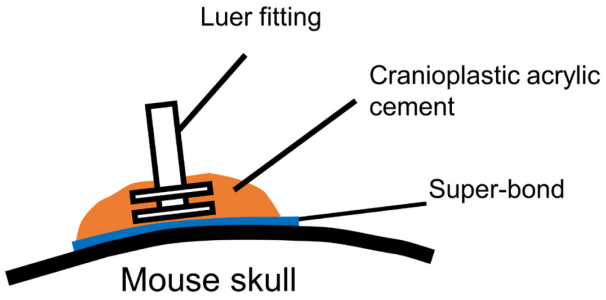

Figure 1. Cranioplastic surgery. A thin layer of super-bond was placed on the skull. The Luer fitting was then rapidly fixed with cranioplastic acrylic cement.

B. Acclimation training

1. Mouse handling during recovery
   a. Continue handling during the recovery for at least 1 week. The handler lift the mice with cupped hands and hold the animal for around 30 s (Figure 2).
   b. We confirm that mice do not urinate and do not jump from the cupped hands during handling.
   c. Once body weight increased compare to pre-surgery weight, train the mice to acclimatize to the awake fMRI conditions before fMRI experiments.

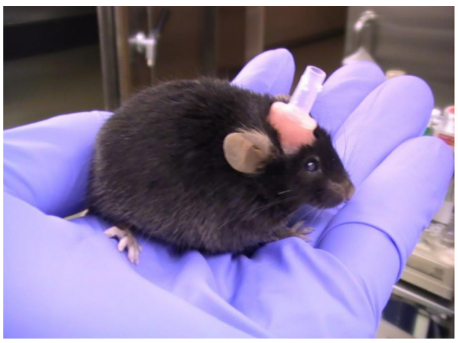

Figure 2. Handling with cupped hands. Photo shows the handling of a representative mouse with implantation of the Luer fitting to the skull.

2. Acclimation training
   a. Train mice for 4 days to acclimatize to the awake fMRI conditions before fMRI experiments. Train them at the same time each day (10:00-18:00) to minimize the effects of circadian rhythm variations.
   b. During the first 2 days, use a pseudo-MRI system consisting of a non-magnet bore and a head positioner (Figure 3 and Figure 4). The acrylic bar is tightly fitted with an elastic tube. Then train the mice in the MRI bore for the next 2 days.

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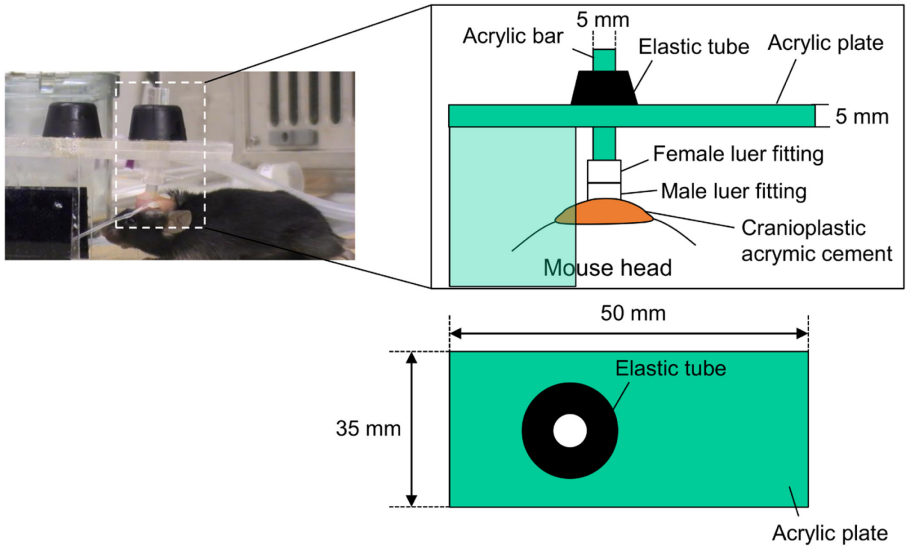

Figure 3. Fixation of the head with the custom-made head positioner. Photo of the fixed head (left) and schematic figure of the head fixation system. The head fixation system was custom made.

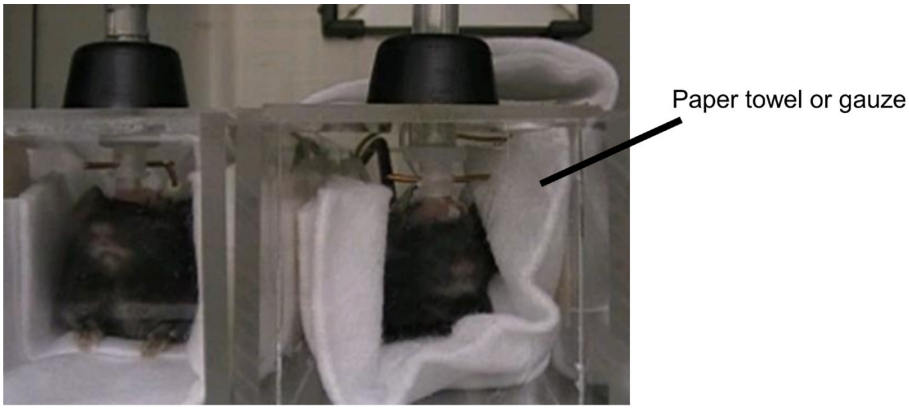

Figure 4. Acclimation training with pseudo-MRI head positioner

c. Anesthetize mice in anesthesia chamber using 2% isoflurane with air.
d. Once mice are anesthetize, stop isoflurane and rapidly and tightly fix their head with the non-MRI head fixation system (Figure 3).
e. The earplug is cut down to fit to the mouse ears and put into the mouse ear canal using tweezers (Figure 5D). The size of dedicated earplugs should be bigger than ear canal so as not to make a gap with the ear canal.
f. Gently wrap the body with paper towel or gauze so that the mice can move their limbs within the paper towel (or gauze) cover (Figure 4).
g. In general mice awoke within 10 min following cessation of isoflurane delivery. This is confirmed using electroencephalography (Tsurugizawa *et al.*, 2020b).
h. Mice remain in the pseudo-MRI apparatus for 30 min on the first day and 90 min on the second day.

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i. Monitor the respiratory rate and heart rate during the training. A small pneumatic pillow, by which respiration is measured, is attached under the animal's abdomen with surgical tape. The electrodes for electrocardiogram (ECG) are inserted under the skin.

3. Awake fMRI
   a. Following 2 days training outside of the magnet, start acclimation training in the magnetic bore.
   b. Anesthetize mice in anesthesia chamber with 2% isoflurane with air.
   c. Continue isoflurane anesthesia via the mask (1.5% with air) during the setup.
   d. Fix the animal's head in the dedicated volume coil with head positioner and the animal's body is positioned on the bed (Figure 5A). Use the same head positioner as Figure 3. The size of dedicated earplugs should be bigger than ear canal so as not to make a gap with the ear canal.
   e. Insert dedicated earplug (cut down to a 5 mm length) into the mouse ear canal (Figure 5D).

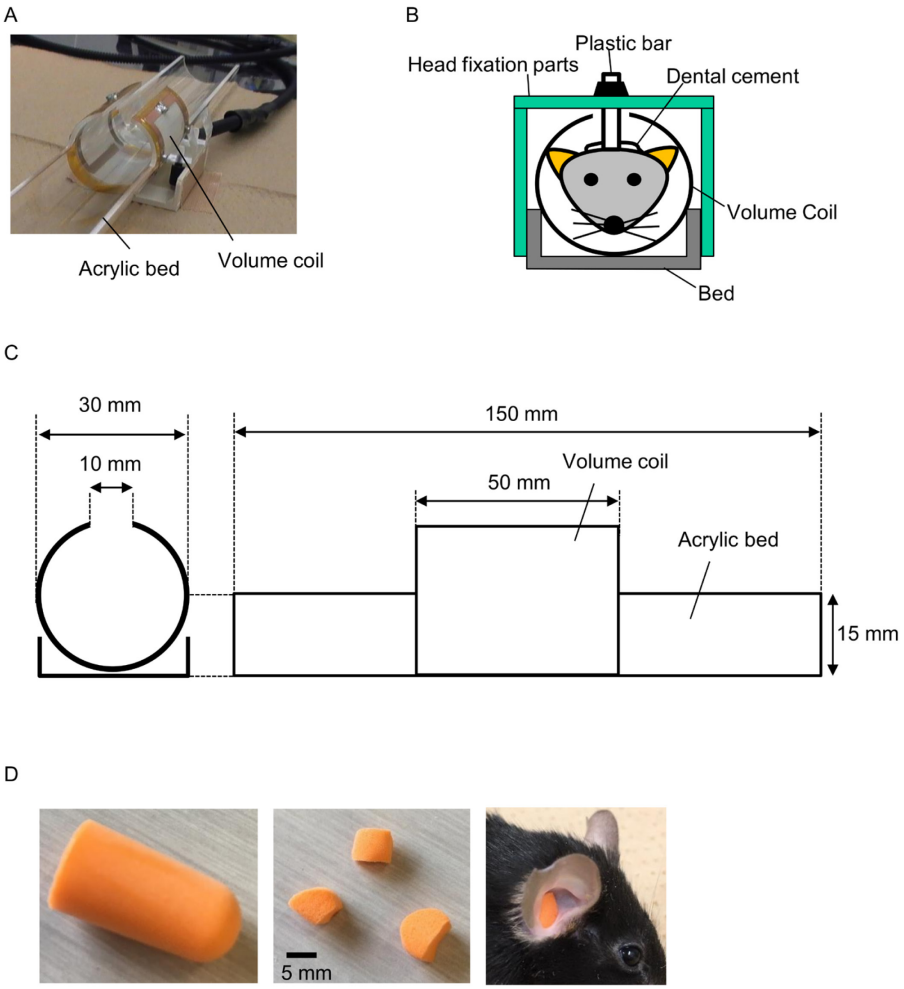

Figure 5. Dedicated volume coil, head fixation system, and eay plugs. A. Dedicated mouse volume coil and bed. B. Schematic figure of the fixation of mouse head. C. Diagram of the volume coil and bed. D. Left: Earplug for human and (D center) dedicated earplug for mouse.

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D. Right: The ear of mouse with dedicated earplug. The picture and schematic figure are from Tsurugizawa *et al.* (2020b).

f. Gently wrap the body with paper towel or gauze so that they can move their limbs within the paper towel (or gauze) cover.
g. Adjust basic frequency, magnetic field homogeneity, reference pulse gain and receiver gain. This adjustment is automatically performed by Bruker system.
h. Acquire an anatomical image with rapid acquisition with relaxation enhancement (RARE) sequence following cessation of isoflurane using the following parameters: time of repetition, 2,500 ms; effective echo time, 60 ms; RARE factor, 8; acquisition matrix, 128 × 128; field of view, 16 mm × 16 mm; slice thickness, 1 mm; 15 slices and four averages.
i. Start to acquire fMRI data 10 min after cessation of anesthesia using the following parameters: time of repetition, 2,000 ms; echo time, 21 ms; acquisition matrix, 80 × 80; field of view, 16 mm × 16 mm; slice thickness, 1 mm; 15 slices and four averages.
j. Monitor the respiratory rate and heart rate during the MRI experiment (Model 1025, SA Instrument).
k. Perform fMRI training (3rd and 4th days) with the same protocol as the fMRI experiment.
l. Complet the acclimation training when the heart rate and respiratory rate are reduced to nominal levels (Figure 6). If they do not decrease, we continue acclimation training.

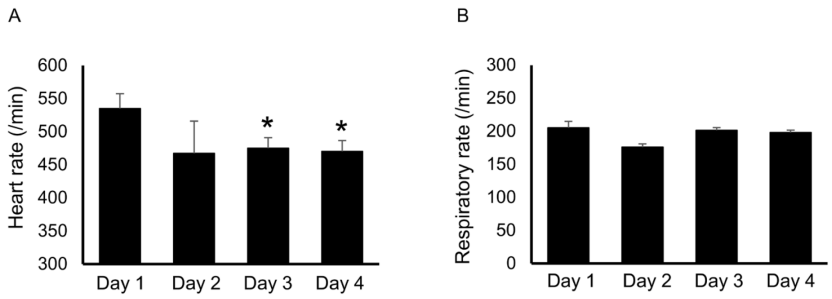

Figure 6. Heart rate and respiratory rate during acclimation training. (A) Heart rate and (B) respiratory rate reduced to nominal levels at the end of the training (n = 15). *$P < 0.05$ by Tukey-Kramer multiple comparison test. Data are from Tsurugizawa *et al.* (2020b).

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Competing interests

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The authors declare no competing interests.

Ethics

All animal experimental procedures in the present study were approved by the institutional review board of animal ethical committee who followed institutional guidelines in Ethical Committee of AIST (2020-0365-A) and the Ethics Committee of RIKEN Brain Science Institute (W2019-2-042).

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