HHS Public Access

Author manuscript

*J Neurosci Methods*. Author manuscript; available in PMC 2018 January 31.

Published in final edited form as:

*J Neurosci Methods*. 2017 August 01; 287: 53–57. doi:10.1016/j.jneumeth.2017.06.008.

# A non-invasive restraining system for awake mouse imaging

**Dan Madularu**[a,b,*,1], **Axel P. Mathieu**[b,1], **Chathura Kumaragamage**[b,c], **Lauren M. Reynolds**[d], **Jamie Near**[a,c], **Cecilia Flores**[a], and **M. Natasha Rajah**[a,b,e]

[a]Department of Psychiatry, Faculty of Medicine, McGill University, Montreal, QC, Canada

[b]Brain Imaging Centre, Douglas Mental Health University Institute, McGill University, Montreal, QC, Canada

[c]Department of Biomedical Engineering, McGill University, Montreal, QC, Canada

[d]Integrated Program in Neuroscience, McGill University, Montreal, QC, Canada

[e]Department of Psychology, Faculty of Arts, McGill University, Montreal, QC, Canada

## Abstract

**Background—**Preclinical neuroimaging allows for the assessment of brain anatomy, connectivity and function in laboratory animals, such as mice and rats. Most of these studies are performed under anesthesia to avoid movement during the scanning sessions.

**Method—**Due to the limitations associated with anesthetized imaging, recent efforts have been made to conduct rodent imaging studies in awake animals, habituated to the restraint systems used in these instances. As of now, only one such system is commercially available for mouse scanning (Animal Imaging Research, Boston, MA, USA) integrating the radiofrequency coil electronics with the restraining element, an approach which, although effective in reducing head motion during awake imaging, has some limitations. In the current report, we present a novel mouse restraining system that addresses some of these limitations.

**Results/Comparison to other methods—**The effectiveness of the restraining system was evaluated in terms of three-dimensional linear head movement across two consecutive functional MRI scans (total 20 min) in 33 awake mice. Head movement was minimal, recorded in roughly 12% of the time-series. Respiration rate during the acclimation procedure dropped while the bolus count remained unchanged. Body movement during functional acquisitions did not have a significant effect on magnetic field ($B_0$) homogeneity.

**Conclusion/novelty—**Compared to the commercially available system, the benefit of the current design is two-fold: 1) it is compatible with a range of commercially-available coils, and 2) it allows for the pairing of neuroimaging with other established techniques involving intracranial cannulation (i.e. microinfusion and optogenetics).

[*]Corresponding author at: Brain Imaging Centre, Douglas Mental Health University Institute, McGill University, 6875 Lasalle Blvd Montreal, QC, H4H 1R3, Canada. dan.madularu@gmail.com (D. Madularu).

[1]These authors contributed equally to this work.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jneumeth.2017.06.008.

## 1. Introduction

Preclinical magnetic resonance imaging (MRI) is a rapidly growing field aimed at bridging the translational gap between animal and human research. Advancement of preclinical MRI methods can assist in drug discovery and development, providing insights into the neurobiological determinants of psychiatric and neurological disorders. However, a key issue facing small animal MRI is controlling for head movement during scanning (Ferris et al., 2011). At the moment, the typical solution involves the use of anesthesia and/or surgical intervention to maintain the animal's head in a steady position (Haensel et al., 2015). The use of surgery or intubation during this procedure is invasive and terminal, thus not allowing for repeated scanning sessions. The use of anesthesia has also been shown to be problematic, particularly in pharmacological magnetic resonance imaging studies since anesthetics are neuromodulators and vasodilators, and therefore likely to alter/impact blood-oxygen-level dependent (BOLD) signal (Haensel et al., 2015). An in-depth discussion on the comparison between awake and anesthetized rodent imaging is beyond the scope of this article, as this topic has been covered in detail elsewhere (for reviews, see Ferris et al., 2011; Haensel et al., 2015). However, it is worth noting that more reports involving awake mouse (Moore et al., 2016), rat (Kenkel et al., 2016; Madularu et al., 2015a; Madularu et al., 2016; Madularu et al., 2015b) and vole (Yee et al., 2016) imaging have been published lately, due to recent technological advances (i.e. 3D printing) which allow for the fabrication of restraining systems aimed at reducing motion during the imaging session; restrain-related stress and head motion is mitigated by repeated habituation sessions prior scanning in rats (Ferenczi et al., 2016; King et al., 2005).

Building on the advantages of awake rodent imaging, we have developed an imaging system which allows for the scanning of awake mice. As described earlier, awake imaging eliminates the confounding factors brought about by anesthetic agents, which in turn increases BOLD (Ferenczi et al., 2016; Liang et al., 2015a; Liang et al., 2015b; Peeters et al., 2001). Furthermore, the system described below is built to reduce stress to the animal, as no intubation or cannulation is necessary during the procedure. Although awake mouse systems have been previously described (Ferris et al., 2011), where the coil electronics are integrated into the restraining system, the current system is designed to allow for compatibility with commercially available coil systems commonly used for preclinical studies (Bruker Corp., Germany). In addition, this design approach allows for the combination of neuroimaging techniques with other established techniques such as deep brain stimulation, microinfusions or optogenetics, as the top of the mouse's head is accessible, which is not an option in existing awake mouse systems. Finally, this design can be used with home-made surface coils and can be further customized to accommodate other coil designs. As head and body motion are the main concerns during awake rodent imaging in terms of image quality and subsequent usability, these variables were assessed in a cohort of 33 mice during two consecutive 10-min functional scans. In addition, stress markers (i.e. respiratory rate and bolus count) were assessed.

## 2. Methods

### 2.1. Animals

All experiments and procedures were performed in accordance with the guidelines of the Canadian Council of Animal Care and the McGill University/Douglas Mental Health University Institute Animal Care Committee. Thirty-eight adult male C57BL/6 mice were bred in the Douglas Mental Health University Institute Neurophenotyping center, housed in groups of 4–5 in temperature- and humidity-controlled rooms, maintained on a 12-h light–dark cycle (light on at 0800 h), and given ad libitum access to food and water. Thirty-three nice were used for head movement assessment, while a smaller subset (n = 5) were utilized to assess the effects of acclimation on stress responses (King et al., 2005; Sullivan and Gratton, 1999). This subset was further used to assess possible changes in the magnetic field ($B_0$) homogeneity during scans due to body movement. All scans were performed during the animals' light cycle.

### 2.2. Mouse bed design

All technical 3D drawings were performed using the Sketchup Make software (www.sketchup.com), and exported for printing using the CADspan extension (extensions.sketchup.com). All restraint system components were printed on a 4th generation MakerBot Replicator 2X (MakerBot Industries, NY, USA) using ABS filament at 200 μm slices. The final ABS models were briefly dipped in acetone to add strength to the outer shells and to water-seal the surfaces and allowed to dry for at least a week before use.

The restraining mechanism (patent application US62365449) is designed and built with fitting restraining padded plates tightening laterally around the mouse's head (two plates) and the abdomen (two plates), gradually tightened from the ends of the holding bed; this allows to accommodate for differences in mouse head and body size (Fig. 1a).

### 2.3. Habituation and awake scanning

Mice were habituated to the MRI noise and restraining system once daily, for a total of five consecutive days to reduce the restrain-related stress and motion artefacts (King et al., 2005). During these sessions, restrained mice were placed into a mock scanner (Fig. 1b), consisting of an enclosed space and a loudspeaker (Bose Corp., MA, USA) emitting pulse-sequence noise at ~87 $dB_{SPL}$ for a duration of 10 min/day (Supporting Video). Mice were placed back into their home cages after this session.

Following habitation, mice (N = 38) were scanned on a 7T Bruker Biospec 70/30 USR (Bruker Corp.), housed at the Douglas Brain Imaging Centre, using a Bruker-issued quadrature driven 86 mm inner diameter volumetric transmit coil and a Bruker-issued receive-only mouse head quadrature surface coil. Prior to the scans, mice were lightly anesthetized for 30–60 s using isoflurane (ISO) to reduce some of the possible stress associated with the setup procedure, which takes approximately 15 s.

Anatomical scans were acquired (RARE: TR/TEff 2900/35 ms, NEX 3, RARE factor 8, Matrix 64 × 64 pts, Slices 22, Resolution 0.3 × 0.3 × 1.2 mm), followed by two consecutive

10 min functional scans (RAREst: TR/TEff 3000/34 ms, NEX 1, Repetitions 200, RARE factor 48, Matrix 64 × 64, Slices 22, Resolution 0.3 × 0.3 × 1.2 mm) for thirty-three mice. Mice were presented with an unfamiliar olfactory stimulus (either methyl benzoate or limonene; Kulkarni et al., 2012), through a polyethylene tube placed immediately anterior to the mouse's snout in order to assess possible motion artefacts in response to novelty. The two 10-min consecutive functional scans consisted of a 3-min baseline period (no olfactory stimulation), 4-min olfactory stimulation period (methyl benzoate or limonene, counterbalanced between scans), followed by another 3-min washout period (no olfactory stimulation).

Body movement during awake mouse scanning can potentially degrade $B_0$ field homogeneity, thus reduce image quality and introduce image distortions (Baldwin et al., 2007; Jezzard and Clare, 1999). Therefore, to evaluate body movement induced $B_0$ field inhomogeneity over time, a subset of mice were subjected to a separate imaging protocol consisting of a vendor-supplied global shim (for 1st and 2nd order shims, eight repetitions), followed by a 3D gradient −echo (GRE) $B_0$ field map sequence (TR 20 ms, TE 1.487/5.293 ms, Matrix 128 × 128 × 128, Resolution 0.23 × 0.23 × 0.23 mm) performed before, and after a 10 min functional scan (RAREst: TR/TEff 3000/34 ms, NEX 1, Repetitions 200, RARE factor 48, Matrix 64 × 64, Slices 22, Resolution 0.3 × 0.3 × 1.2 mm). This protocol was performed twice for each mouse; during the first segment the mouse was awake and under 1–2% ISO during the second session, as summarized in Fig. 2a.

### 2.4. Motion analysis

Bruker native image formats were converted to NIFTI using *pvconv.pl* (pvconv.sourceforge.net). Inter-volume motion within individual fMRI time courses were extracted using *MCFLIRT* (FSL version 5.0.8; Jenkinson et al., 2002). Outlier motion was defined as greater half the voxel dimension (i.e. 0.15 mm in the X and Y dimensions, and 0.6 mm in the Z dimension), from the time series median. Possible differences in movement between baseline/washout and odour presentation were assessed using dependent measures t-test for each scan and dimension. Statistical analyses and graphs were generated using the Prism GraphPad software (GraphPad Software, CA, USA).

### 2.5. Stress response assessment

Stress response during acclimation and scanning procedures was assessed in five mice. Respiration rate was measured as breaths/min and collected every minute throughout the 10-min functional scan. Fecal boli were collected at the end of each acclimation session (days 1–5), as well as after the subsequent scan session (day 6). Data (mean respiration rate and mean fecal boli) were analyzed using repeated measures ANOVA, and Tukey's posthoc analyses were performed where necessary.

### 2.6. Field homogeneity measurements

$B_0$ field maps of the brain covered by ten coronal slices, spanning ± 2.3 mm in the anterior-posterior direction centered ~−2.7 mm from Bregma, were manually extracted using the corresponding magnitude images, and the standard deviation of the whole brain field map was calculated. $B_0$ field variability was then evaluated before and after the 10 min functional

scan, during awake and anesthetized states as described in earlier. All $B_0$ field map analyses were performed using MATLAB (MathWorks Inc, MA, USA), and paired t-tests were used to assess $B_0$ field variability differences before and after functional scans with respect to the functional scan condition (i.e. awake vs. anesthetized).

## 3. Results

Mean group level linear motion was assessed for each fMRI time series. As voxel size is 0.3 × 0.3 × 1.2 mm (X × Y × Z) and it would take half the voxel edge distance to travel outside the voxel, motion was evaluated for distances at 0.15 mm in the X and Y directions, and 0.6 mm for Z direction in the voxel. The mean group level outlier motion is represented in Fig. 1d. The average motion parameters for the two scans in all three dimensions did not surpass the pre-set threshold, even when a novel olfactory stimulus was presented during the scan Fig. 1e.

When considering gross inter-volume motion, there was no detection of outside voxel motion in the X and Z dimensions. In the Y dimension however, volume motion outside the voxel was detected in five mice, with two movements on average for the first scan; time series considered to show motion if at least one volume (of 200) was outside threshold (Table 1). For the second scan, outside voxel motion in the Y dimension was recorded in three mice. Overall, less than 15% of the time series presented with motion outside the voxel. Statistical analyses comparing motion before and during olfactory stimulus presentation yielded significant differences only in the X dimension, and only for the first scan ($P < 0.01$).

Respiratory rate was significantly reduced on acclimation days 2–5 and scan day (day 6), compared to acclimation day 1 ($P < 0.001$, Fig. 2b); mean fecal boli change was not significant across acclimation and scan days (Fig. 2c).

Average deviations in $B_0$ field variability overtime (before and after a 10 min functional scan) for the awake and anesthetized conditions were 4.6 ± 6.3 Hz and 4.7 ± 4.4 Hz, respectively (Fig. 2d, e); the differences were not statistically significant ($P > 0.05$).

## 4. Discussion

The aim of this methodological study was to develop a system which facilitates awake mouse neuroimaging using commercially-available coils. The system was built using 3D printing technology in combination with open-source 3D design software. Head motion was assessed during two consecutive fMRI scans, and a linear motion cutoff value was set, aiding with outlier identification; motion data are presented in the absence of motion-correcting procedures. Furthermore, potential $B_0$ field variations as a result of body movement of awake mice during scan sessions was assessed with $B_0$ field maps obtained before and after a 10 min functional scan for awake and anesthetized conditions.

As expected, all mice showed a degree of head movement during the functional scans particularly in response to olfactory stimulus presentation, however motion was on average restricted within voxel in all three spatial dimensions. Less than ¼ of the animals showed

movement outside the ±0.15 mm cutoff in the Y dimensions, while none of the mice tested showed movement outside cutoff in the X and Z dimensions (Table 1). There were no apparent differences in movement between the first and second scan in the X and Z dimensions, however motion in the Y dimension was reduced in the second scan compared to first, as shown by the reduced number of mice presenting motion surpassing the threshold. Similarly, there were no differences in motion before and during olfactory stimulation in the Y and Z dimensions during both scans. There was a significant difference in motion before and during olfactory stimulation in the X dimension in the first, but not the second scan, which can be attributed to either fatigue, or to the habituating effects of the first scan (or both). The overall reduced head motion associated with this design deem it to be effective in restraining the animal during functional imaging, even in instances where the animal is exposed to a novel olfactory challenge during the scanning session. Finally, the lack of a significant difference in $B_0$ field variability in awake and anesthetized mice (where head and body motion is minimal) indicates that body motion is unlikely to affect the quality of functional imaging sequences.

Breathing and fecal boli measurements have been used previously in determining stress levels in rodents, with increased levels on these measures associated with elevated stress (King et al., 2005; Sullivan and Gratton, 1999). Respiratory rate significantly decreased from acclimation day 1 compared to subsequent acclimation days. Surprisingly, the decrease was not gradual, as shown previously in rats exposed to a similar procedure (King et al., 2005), suggestive of a possible floor effect on this measure. There were no differences in fecal boli production during the acclimation procedures across the acclimation session, consistent with earlier findings in rats (Sullivan and Gratton, 1999).

Although other awake mouse systems have been previously described and successfully used in studies (Moore et al., 2016), the restraining system presented here is novel and in that it was designed to accommodate a variety of clinically-available and/or customized coil systems. Adding to the versatility of this design, this system allows for the passage of microinjection lines or optic fiber for awake opto-fMRI imaging in mice (Supporting Fig. S1). This feature increases the functionality of this system, allowing for more complex imaging protocols by pairing fMRI with established intracranial methodology such as optogenetics, microinfusions and deep brain stimulation. In addition, this approach allows the same restraining system to be used for both training (habituation) and imaging purposes, reducing the costs associated with awake imaging methodology while minimizing the time required for setting up the animal, as one mouse can be prepared while another is scanned. Finally, the approach taken for designing and building this system is modular in nature, such that any mechanical failure would result in the replacement of part of the system, rather than the entire unit as well as having the potential to add more features in the future.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This research was funded by FRSQ Subvention pour projet de développement stratégique innovants 2012–2016 (Grant No. 26679) awarded to MNR, and NIDA (Grant No. R01DA037911) awarded to CF.

## References

Baldwin LN, Wachowicz K, Thomas SD, Rivest R, Fallone BG. Characterization, prediction, and correction of geometric distortion in 3T MR images. Med. Phys. 2007; 34:388–399. [PubMed: 17388155]

Ferenczi EA, Zalocusky KA, Liston C, Grosenick L, Warden MR, Amatya D, Katovich K, Mehta H, Patenaude B, Ramakrishnan C, Kalanithi P, Etkin A, Knutson B, Glover GH, Deisseroth K. Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. Science. 2016; 351:aac9698. [PubMed: 26722001]

Ferris CF, Smerkers B, Kulkarni P, Caffrey M, Afacan O, Toddes S, Stolberg T, Febo M. Functional magnetic resonance imaging in awake animals. Rev. Neurosci. 2011; 22:665–674. [PubMed: 22098446]

Haensel JX, Spain A, Martin C. A systematic review of physiological methods in rodent pharmacological MRI studies. Psychopharmacology. 2015; 232:489–499. [PubMed: 25585682]

Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. NeuroImage. 2002; 17:825–841. [PubMed: 12377157]

Jezzard P, Clare S. Sources of distortion in functional MRI data. Human Brain Mapp. 1999; 8:80–85.

Kenkel WM, Yee JR, Moore K, Madularu D, Kulkarni P, Gamber K, Nedelman M, Ferris CF. Functional magnetic resonance imaging in awake transgenic fragile X rats: evidence of dysregulation in reward processing in the mesolimbic/habenular neural circuit. Transl. Psychiatry. 2016; 6:e763. [PubMed: 27003189]

King JA, Garelick TS, Brevard ME, Chen W, Messenger TL, Duong TQ, Ferris CF. Procedure for minimizing stress for fMRI studies in conscious rats. J. Neurosci. Methods. 2005; 148:154–160. [PubMed: 15964078]

Kulkarni P, Stolberg T, Sullivanjr JM, Ferris CF. Imaging evolutionarily conserved neural networks: preferential activation of the olfactory system by food-related odor. Behav. Brain Res. 2012; 230:201–207. [PubMed: 22343130]

Liang Z, Liu X, Zhang N. Dynamic resting state functional connectivity in awake and anesthetized rodents. NeuroImage. 2015a; 104:89–99. [PubMed: 25315787]

Liang Z, Watson GD, Alloway KD, Lee G, Neuberger T, Zhang N. Mapping the functional network of medial prefrontal cortex by combining optogenetics and fMRI in awake rats. NeuroImage. 2015b; 117:114–123. [PubMed: 26002727]

Madularu D, Kulkarni P, Ferris CF, Brake WG. Changes in brain volume in response to estradiol levels, amphetamine sensitization and haloperidol treatment in awake female rats. Brain Res. 2015a; 1618:100–110. [PubMed: 26032742]

Madularu D, Kulkarni P, Yee JR, Kenkel WM, Shams WM, Ferris CF, Brake WG. High estrogen and chronic haloperidol lead to greater amphetamine-induced BOLD activation in awake, amphetamine-sensitized female rats. Horm. Behav. 2016; 82:56–63. [PubMed: 27154458]

Madularu D, Yee JR, Kenkel WM, Moore KA, Kulkarni P, Shams WM, Ferris CF, Brake WG. Integration of neural networks activated by amphetamine in females with different estrogen levels: A functional imaging study in awake rats. Psychoneuroendocrinology. 2015b; 56:200–212. [PubMed: 25827963]

Moore K, Madularu D, Iriah S, Yee JR, Kulkarni P, Darcq E, Kieffer BL, Ferris CF. BOLD Imaging in Awake Wild-Type and Mu-Opioid Receptor Knock-Out Mice Reveals On-Target Activation Maps in Response to Oxycodone. Front. Neurosci. 2016; 10:471. [PubMed: 27857679]

Peeters RR, Tindemans I, De Schutter E, Van der Linden A. Comparing BOLD fMRI signal changes in the awake and anesthetized rat during electrical forepaw stimulation. Magn. Resonance Imaging. 2001; 19:821–826.

Sullivan RM, Gratton A. Lateralized effects of medial prefrontal cortex lesions on neuroendocrine and autonomic stress responses in rats. J. Neurosci. 1999; 19:2834–2840. [PubMed: 10087094]

Yee JR, Kenkel WM, Kulkarni P, Moore K, Perkeybile AM, Toddes S, Amacker JA, Carter CS, Ferris CF. BOLD fMRI in awake prairie voles: A platform for translational social and affective neuroscience. NeuroImage. 2016; 138:221–232. [PubMed: 27238726]

## HIGHLIGHTS

- We designed a restraining system adapted for awake neuroimaging.
- Head motion was assessed in 33 mice, habituated to the procedure.
- Minimal head motion was detected during neuroimaging sessions.
- Body motion during functional acquisition did not affect magnetic field homogeneity.
- Versatile system, as it allows for the use of commercially-available RF coils

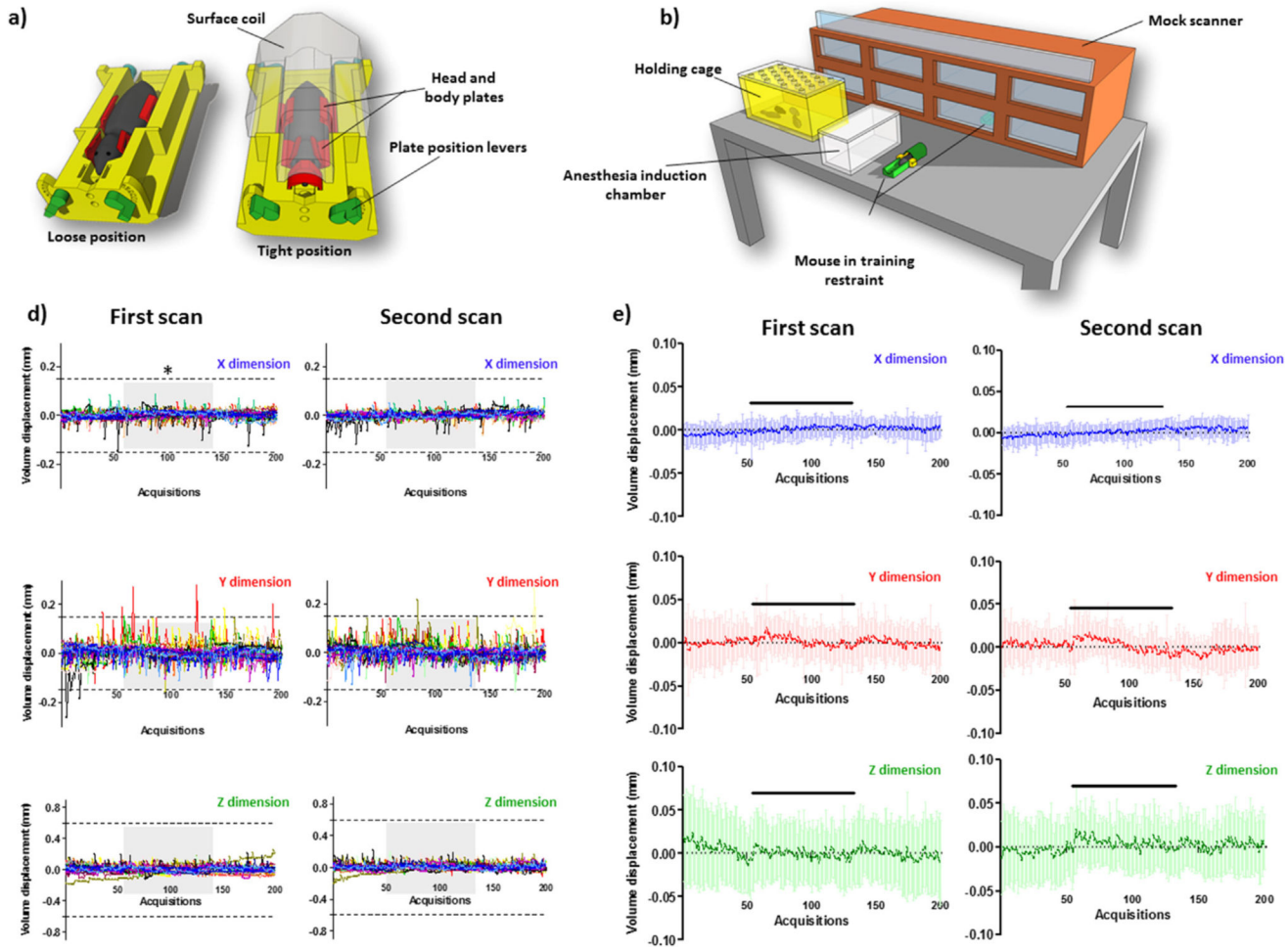


**Fig. 1.**

Mouse restraining system and motion analyses. a) 3D diagram showing a mouse in the restrain system at “loose” and “tight” positions. The tightening of the head and body plates (red) is achieved gradually by rotating the position levers into place (green); b) awake mouse habituation setup, showing the steps included in this process: anesthesia (30–60 sec), placement in restraint (10–30 sec), and pulse sequence noise exposure (10–30 min); d) linear motion analyses in three dimensions (X–Z) for all mice (N = 33, one colour/mouse) during the two consecutive scans. Dashed lines represent cutoff values based on voxel size, grey transparent rectangle represents the onset and duration of olfactory stimulus. * $P < 0.01$ comparing motion before and during olfactory stimulus; e) Mean (±SD) motion for the two scans for all mice. Horizontal line represents the onset and duration of the olfactory stimulus presentation.

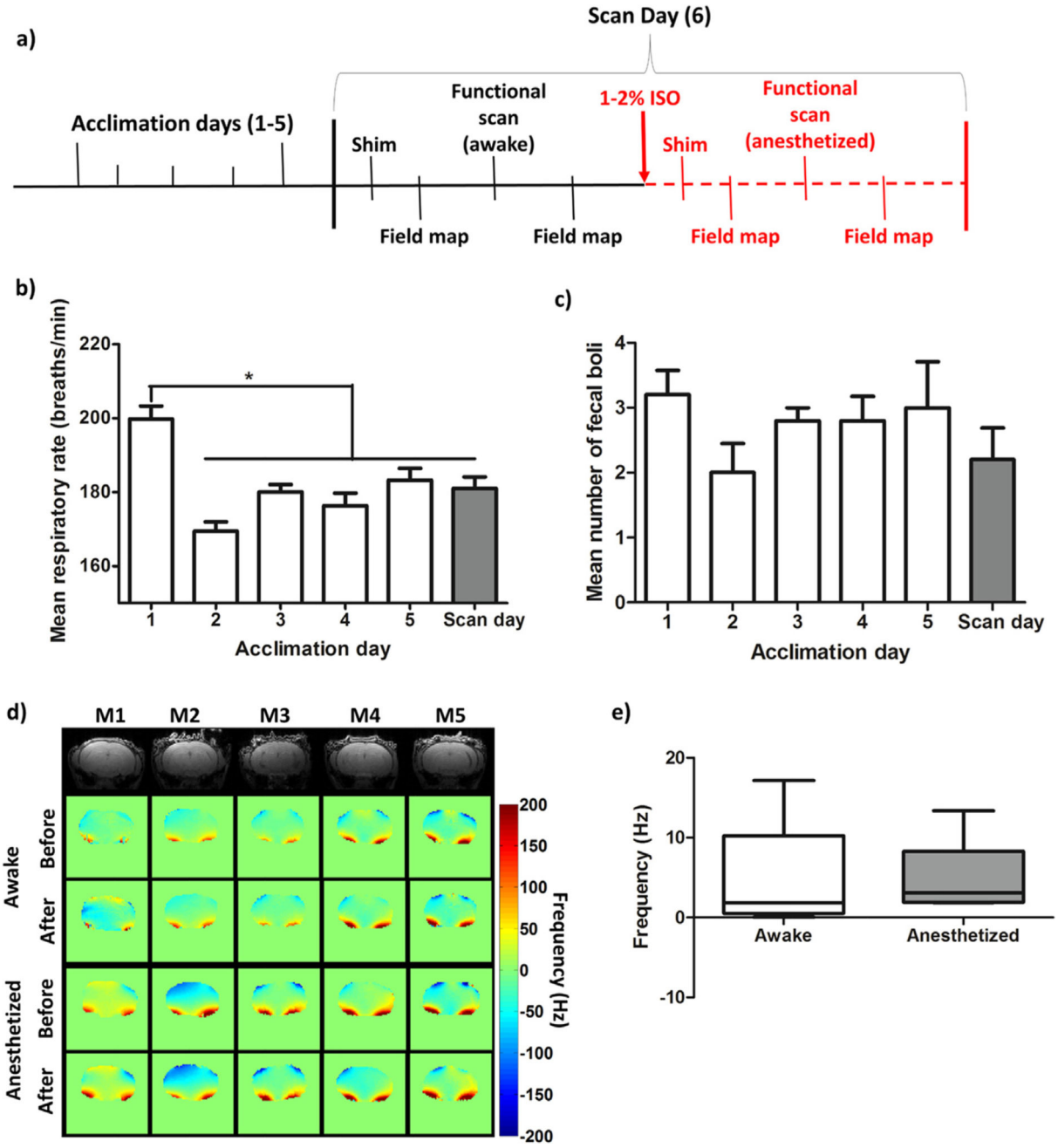


**Fig. 2.**

Assessment of the effects of acclimation on stress responses, and changes in the magnetic field ($B_0$) homogeneity during scans due to body movement. a) Timeline outlining the procedures undergone by a subset of mice, where stress and magnetic field homogeneity were assessed; b) Respiratory rate assessed by mean breaths per minute (±SEM) during the acclimation and scan sessions; c) Mean (±SEM) fecal boli during acclimation and scan sessions; d) Each column illustrates an anatomical slice (~−2.7 mm from Bregma) from each mouse (M1-M5), and field maps corresponding to the anatomical slice. $B_0$ field maps show minimal differences between the “before” and “after” a functional scan for awake and

anesthetized (Isoflurane, ISO) conditions; e) Box plots comparing $B_0$ field map standard deviations for all mice “before” and “after” functional scans for awake and anesthetized scans; the differences were not statistically significant ($P > 0.05$).

**Table 1**

Motion analysis in three dimensions during the two image acquisitions in awake mice.

| Threshold | Scan #/Dimension | # TS with motion/Total TS | % TS with motion |
|---|---|---|---|
| ±0.15 mm | 1/X | 0/33 | 0 |
| | 2/X | 0/33 | 0 |
| | 1/Y | 5/33 | 15.15 |
| | 2/Y | 3/33 | 9.09 |
| ±0.6 mm | 1/Z | 0/33 | 0 |
| | 2/Z | 0/33 | 0 |

TS: Time Series.