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Longitudinal Functional Magnetic Resonance Imaging in Animal Models

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Abstract

Functional magnetic resonance imaging (fMRI) has had an essential role in furthering our understanding of brain physiology and function. fMRI techniques are nowadays widely applied in neuroscience research, as well as in translational and clinical studies. The use of animal models in fMRI studies has been fundamental in helping elucidate the mechanisms of cerebral blood flow regulation, and in the exploration of basic neuroscience questions, such as the mechanisms of perception, behavior, and cognition. Because animals are inherently noncompliant, most fMRI performed to date have required the use of anesthesia, which interferes with brain function and compromises interpretability and applicability of results to our understanding of human brain function. An alternative approach that eliminates the need for anesthesia involves training the animal to tolerate physical restraint during the data acquisition. In the present work we review these two different approaches to obtaining fMRI data from animal models, with a specific focus on the acquisition of longitudinal data from the same subjects.

Keywords

anesthesia; awake; brain; BOLD; cerebral blood flow; cerebral blood volume; neurovascular coupling; non-human primates; rodents; songbirds

1. Introduction

Functional magnetic resonance imaging (fMRI) has made a remarkable impact on brain research, establishing itself as the most prominent research tool in cognitive neuroscience (1;2) and showing great promise in translational and clinical studies (3). fMRI relies on the neurovascular coupling, a tight relationship between changes in neural activity and local regulation of cerebral blood flow (CBF), volume (CBV) and oxygen consumption (CMRO₂) (4). Functional MRI provides excellent contrast to noise ratio, sub-millimeter spatial resolution, coverage of the whole brain, and relative ease of implementation. On the other hand, the temporal resolution of fMRI is relatively low (particularly with respect to the time

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scale of neuronal events) and the underlying fMRI signal mechanism (5) and its functional specificity (6) are still a subject of research.

The use of animal models has been essential to the development of fMRI techniques. Rodent models were the subjects in the first studies employing the blood oxygenation level dependent (BOLD) contrast (7;8), as well as the first CBF measurements using exogenous MRI tracers, including deuterium (9;10), fluorine (11;12) and gadolinium chelates (13;14), which also allowed estimation of CBV in addition to CBF. The advantages of using an endogenous tracer led to the development of arterial spin labeling (ASL) techniques to measure and quantify CBF non-invasively, with the first demonstrations once again occurring in rodents (15;16). Animal models of functional brain activation have been widely employed to address issues related to spatial localization of the functional signals, the magnitude of signal changes as a function of stimulation parameters, as well as temporal aspects of the hemodynamic response, giving critical insight into the brain's physiology and function (17). Furthermore, the use of animal models in fMRI has been particularly advantageous in preclinical and translational studies of various models of brain disease.

A major practical issue related to performing fMRI in animals relates to compliance. The MRI environment poses stringent restrictions on subject positioning and movement, and typical fMRI studies last from a few to several hours. Notwithstanding the duration of the experiments, because animals are inherently noncompliant, most fMRI performed to date have required the use of anesthesia, which offers the important advantages of ensuring compliance, minimizing movement, and alleviating stress, at the expense of requiring the investigator to monitor and control the systemic physiological status of the animal. Another major disadvantage of the use of anesthesia is that it interferes with both neural activity and neurovascular coupling, thus compromising interpretability and applicability of results to the understanding of human brain function. An alternative choice to the use of anesthesia is to acclimate, condition and train the animal to tolerate physical restraint during the data acquisition. This approach offers the advantage of minimizing the need for physiological monitoring and maintenance. On the other hand, it is difficult to dynamically monitor the level of stress in awake animals and virtually impossible to establish its absence, even with extensive training. Stress can be a significant confound in the study of brain function, particularly when hemodynamic variables are used as surrogate markers of neural activity. Indeed, continued research on training protocols and evaluation of stress indicators is a subject of intense research (18). In the present work we review these two different approaches to obtaining fMRI data from animal models, with a specific focus on the acquisition of longitudinal data from the same subjects.

2. fMRI of Anesthetized Animal Models: From Terminal to Longitudinal Preparations

2.1. Anesthetics for Terminal Experiments

Urethane (19–23) and α -chloralose (24–36) are the most widely anesthetics used in fMRI studies of rodents. A major advantage of either anesthetic is that they preserve the

neurovascular coupling (37). However, both substances are toxic and thus not adequate for longitudinal use, being used only in terminal preparations (38;39).

The use of anesthesia makes the animal lose the ability to regulate its own physiology and body temperature, forcing the investigator to monitor and maintain the vital signs of the animal to ensure stable physiology throughout the entire experiment. Therefore, surgical preparation is required. This consists of: (a) induction of anesthesia; (b) oral intubation or tracheostomy; and (c) placement of intravenous, intra-arterial and intraperitoneal catheters. Figure 1 shows a typical surgical preparation station and all associated equipment.

Fifteen to thirty minutes prior to induction of anesthesia it is useful to treat the animals with atropine sulfate (Vedco Inc, Saint Joseph, MO), given subcutaneously or intramuscularly at a single dose of 0.5 mg/kg, to decrease bronchial secretions and salivation during anesthesia, and as an anesthesia adjuvant. Anesthesia induction in rodents usually uses halogenated anesthetics (halothane, sevoflurane, and isoflurane). In contrast, intramuscular bolus of ketamine (either alone, or in combination with its potentiators xylazine, acepromazine or medetomidine hydrochloride) is frequently used for induction in both cats and non-human primates. Following the anesthesia induction, the animals are typically orally intubated or tracheostomized and mechanically ventilated to allow dynamic adjustments of respiratory parameters as to ensure stable physiology - under the effect of the anesthetic - throughout the experiment. It is extremely important to gain monitoring and control of the animals vital signs at this moment. The body temperature must be monitored via a rectal temperature probe, and a heated water pad or another suitable source of heat must be used to keep the body temperature at normal values (e.g., 37.5 ± 0.5 °C for rats; 38.5 ± 0.5 °C for cats and marmosets). Pulse oximetry and capnography, when available, can be started at this moment. Either technique alone or combined together provides a good assessment of the quality of the animal's respiration, giving the investigator the necessary information to adjust the breathing gas mixture and the ventilator parameters to ensure good oxygenation and proper ventilation rate and volume to the animal. Following the start of mechanical ventilation, catheters are placed into the femoral artery, the femoral vein (or the lateral tail vein) and the intraperitoneal cavity. These catheters will allow the administration of anesthesia and other drugs that control the physiological status of the animal, the measurement of arterial blood pressure via a pressure sensor, and the sampling of arterial blood gases.

Once all surgical procedures are completed and all open wounds are sutured, the inhalational anesthetic can be discontinued and the anesthesia can be switched to either α -chloralose (α -chloralose, product #C8091, Sigma-Aldrich, St. Louis, MO) or urethane (urethane, product #U2500, Sigma-Aldrich). Typical dosage for α -chloralose is 80 mg/kg initial IV bolus (37) followed by 27 mg/kg-hr constant IV infusion (33). For urethane, the typical dosage is 1.25 g/kg single dose IP (23), as urethane is known to be a long-acting (8–10 hours) anesthetic. Both α -chloralose and urethane can be dissolved in slightly warmed phosphate-buffered saline (PBS) at a concentration of 10 mg/ml or 100 mg/ml, respectively. However, urethane is a known carcinogen and should only be manipulated in the hood using gloves. A muscular relaxant such as pancuronium bromide (Pavulon, 2 mg/ml, Teva Pharmaceuticals USA,

North Wales, PA) may be periodically administered at a dose of 2 mg/kg/hr IV or IP to aid with immobilization.

2.2. Anesthetics for Longitudinal Experiments

Due to the extensive surgical preparation described above and also to the toxicity and adverse side effects of both α -chloralose and urethane, different anesthetics need to be used for longitudinal studies. These studies require minimal interventions on the animal so that its overall physiological state is stable throughout the duration of the study and especially each time the animal is tested. Because of this, inhalational compounds such as halothane (30) and isoflurane (40–48) are attractive in that they can be administered via a face mask to the animal, thus avoiding the need for catheterizations for vascular access. Furthermore, these agents provide stable physiology and allow for an easy control of the plane of anesthesia and a quick and smooth recovery of the animal upon withdrawal of the anesthetic. However, while both anesthetics are safe to use in repetitive studies in the same animal, they suppress neuronal activity (49) and cerebral metabolism (50) and affect both cerebrovascular tone as well as cerebrovascular reactivity (51), thus greatly influencing the cerebrovascular coupling (52) and making it necessary to redefine the proper stimulus parameters to produce robust activation (46). In fact, the influence of halogenated inhalational anesthetics on neurovascular coupling and cerebrovascular regulation can be long lasting. A recent study on the long term effects of a short hypoxic episode on CBF regulation in isoflurane anesthetized rats showed a dramatic reduction in the CBF response to hypoxia in animals that were exposed to isoflurane 5 days earlier (53).

As an alternative to inhalational anesthetics, injectable agents such as propofol and medetomidine are attractive in providing a satisfactory depth of anesthesia, a quick onset of action and a smooth recovery of the animals. Propofol is an injectable anesthetic with rapid mechanism of action that is increasingly used in fMRI experiments in animal models (40;54–57). The depth of anesthesia under propofol can be readily adjusted by varying the rate of infusion, and the animals quickly recover at the end of the experiment, thus facilitating longitudinal studies. Another agent that has been recently proposed as suitable for repetitive studies is the α 2-adrenoreceptor agonist medetomidine hydrochloride (22;58–61), which has been shown to allow robust fMRI responses (58;59) and the measurement of resting state signal fluctuations in the brain (59–61). The sedative and analgesic effects of medetomidine can be quickly reversed with the application of atipamezole hydrochloride. However, some limitations of prolonged exposures to medetomidine include a gradual rise in blood pressure and heart rate, lower pO₂ values, and a more difficult control of the plane of anesthesia (61).

When using injectable, intravenous anesthetics, special attention must be given to the total infusion volume in comparison with the total blood volume, especially in small animals such as rodents and small non-human primates, so that the infusion does not change blood chemistry. For example, the dose of propofol (10 mg/ml) required to maintain immobilization in rats and marmosets is 0.5 – 1.0 mg/kg/min (3 – 6 ml/kg/hr), which is about five times higher than required in humans (0.05 – 0.2 mg/kg/min). Yet the total blood volume of a 400g rat is ~25 ml (62), circa 1/200 of the total human blood volume. Thus,

intravenous anesthetics have a much greater potential to disturb blood chemistry in small animals than in humans, and drug preparations should be chosen carefully. Propofol, as made by certain manufacturers, has a pH value as low as 4, posing a significant risk of inducing acidosis in small animals, especially in prolonged and/or longitudinal experiments. Like in clinical medicine, concurrent intravenous infusion of Lactated Ringer solution with the anesthetics may partially alleviate the detrimental effects of blood chemical disturbance.

2.3. Placement of the Animal in an MRI Compatible Bed

Magnetic resonance imaging poses stringent requirements on immobilization. After surgery, the animals are thus placed in a stereotaxic-like head holder and strapped to an MRI compatible bed. A number of beds are available from the major vendors of small animal MRI scanners, such as Bruker (Bruker-Biospin, Corp., Ettlingen, Germany) or Varian (Varian, Inc., Palo Alto, CA), or from third-party vendors (e.g., Rapid Biomedical, GmbH, Rimpf, Germany; Ekam Imaging, Inc., Shrewsbury, MA). Figure 2 shows a picture of the bed we use for fMRI of small animals. The bed is an essential part of the experiment, as it integrates, in a single platform, a resting place for the animal, the stereotaxic head holder, the physiological maintenance and monitoring devices, a stage for receive RF coils and preamplifiers, and another stage for the functional stimulation devices. Therefore, significant effort needs to be put on the design, fabrication and adaptation of an animal bed to include and integrate all the features necessary to the successful execution of the experiment.

2.4. Physiological Monitoring

As mentioned above, the use of anesthesia makes the animal lose the ability to regulate its own physiology and body temperature, and it is the responsibility of the investigator to monitor and maintain the vital signs of the animal throughout the remainder of the experiment. As soon as the animal has been moved to the MRI-compatible bed, physiological monitoring must start – or resume if started during animal preparation – according to the available equipment. All essential physiological parameters, including, but not limited to the list below, should be monitored and maintained at normal values.

1. Rectal temperature: via temperature probe inserted in rectum. This is one of the most critical parameters to be monitored and maintained, as anesthesia impedes the animal's own temperature regulation. Normal rectal temperature values for rats are 37.5 ± 0.5 °C, and 38.5 ± 0.5 °C for cats and marmosets.
2. Mean arterial blood pressure (MABP): via pressure transducer connected to a femoral arterial line, if available, or via a pressure cuff wrapped around the tail or thigh. Typical MABP values in rats are 110–120 mmHg (63;64). Typical MABP values in conscious marmosets are 100–110 mmHg (65).
3. Pulse oximetry (SPO₂): via a transducer placed on forelimb or hindlimb. Usually SPO₂ values remain above 90 %.
4. Heart Rate (HR): derived either from MABP trace or from a pulse oximeter. For rats under α -chloralose, HR typically rises above 300 BPM, but stays below that value under other anesthetics. For marmosets under propofol, HR typically

decreases from above 350 BPM in the conscious, awake condition to 150–250 BPM in anesthetized animals.

5. End-tidal CO₂ (ETCO₂): via a micro-capnometer hooked up to a face mask or to the respiratory line. The distance between the mechanical ventilator and the MRI magnet forces the use of long respiration lines, resulting in substantial mixing between expired and recirculated gas. Therefore, and especially for small animals, the micro-capnometer tends to display significantly attenuated values of ETCO₂. Yet, these values are valid relatives and a significant correlation exists between ETCO₂ and PaCO₂, as shown in Fig. 3.
6. Respiratory pressure: monitored via a pressure transducer in mechanical ventilator. It is important to check the respiratory compliance of the intubated or tracheostomized animal throughout the experiment. The end-inspiratory pressure can be set by adjusting the flow of the air mixture in the ventilator. Typically, end-inspiratory pressures are in the range of 8 – 12 cm H₂O.
7. Arterial blood gases, including pH, PaCO₂ and PaO₂: sampled from the femoral artery, when available. Even though arterial blood sampling in mice is restricted to much fewer samples than in rats, the latest-generation blood gas analyzers are able to work with samples as small as 30 µl of blood (typically 60 µl) (e.g., ABL80 FLEX, Radiometer America, Westlake, OH). Periodic sampling of arterial blood is also performed to yield information on arterial blood gases, hematocrit, and electrolytes. Data collected during unstable physiology is best discarded. Furthermore, large deviations in the arterial blood gases often call for administration of corrective pharmacological agents.

All the physiological parameters listed above can be recorded during the experiment using a multi-channel data acquisition system, such as the system MP150 (Biopac Systems, Inc., Goleta, CA). Figure 4 shows the physiological monitoring graph of a typical fMRI experiment to measure the BOLD and CBF response to electrical stimulation of the forepaws in a rat anesthetized with α -chloralose. Traces of the arterial blood pressure, respiratory pressure, and heart rate were recorded along with the forepaw stimulation epochs, the EPI acquisition times, and the gradient temperature. Monitoring of the animal's physiology during the experiment assures that the data is acquired under stable conditions. For example, no changes in arterial blood pressure are noticed during the forepaw stimulation epochs. Another advantage of acquisition of physiological parameters is the possibility to perform retrospective correction of the influence of either the respiratory or cardiac cycles on the fMRI signal (66).

2.5. Recovery from Anesthesia

In longitudinal experiments in which artificial ventilation is used, the animal's autonomous control of the respiration may be suppressed even after anesthetics are withdrawn. Thus, recovery from anesthesia needs to be closely monitored, and emergency procedures should be planned in advance. Throughout the recovery, artificial ventilation and rectal temperature monitoring should continue, and the intravenous infusion site should remain patent, until the animal is clearly able to breathe on his own after being disconnected from the breathing

circuit. Antidotes for some anesthetics are available, such as naloxone for opioids and atipamezole for medetomidine chloride, and should be administered. In the event of respiratory and/or cardiovascular suspension after removal of the intubation tube, a respiratory stimulant (e.g. Doxapram) and sympathetic stimulant (e.g. Epinephrine) can be administered through the intravenous infusion tube, which should not be removed from the animal until she is fully alert.

3. fMRI of Conscious, Awake Animals

The use of anesthesia for MRI and fMRI studies in animal models has the advantages of effectively ensuring compliance and of minimizing stress via sedation. However, any anesthetic interferes, in different ways, with neural activity and cerebrovascular reactivity, representing a complex confound to the interpretability and applicability of the obtained data to the understanding of human brain function. The alternative to the use of anesthesia as a means to ensure compliance is to condition the conscious, awake animal, to tolerate the rigid head restraint required to ensure the acquisition of good quality data with acceptable levels of motion artifacts. The use of conscious, awake animals in the MRI setting is becoming increasingly popular, as exemplified by studies performed in rodents (18;29;42;55;67–71) and monkeys (71–84). To provide effective, yet relaxed restraint to the animals, the animal bed needs to be designed taking into account comfortable support for their head and body (77;85). Equally important is the development of acclimatization and training procedures to condition the animal to tolerate long periods of restraint with minimal stress (18), as a stressed out animal is as useful to data collection as an unresponsive one!

In this section, we describe our own experience with obtaining longitudinal fMRI data from conscious, awake marmosets in a 7T horizontal MRI scanner. Traditionally, the use of awake, conscious animals in the MRI requires the surgical implantation of head posts that can be rigidly secured by clamps to a specially designed frame (85). This approach allows maximum restraint of the animals, but has many disadvantages. The head implants typically generate susceptibility artifacts that degrade image quality by introducing geometric distortions and/or signal dropouts in the MR images. In addition, they require constant aseptic cleaning to prevent infections. Furthermore, if an infection appears, the animal needs to be treated with antibiotics and anti-inflammatory drugs that may interfere with neurovascular coupling and confound interpretation of the data. Moreover, the use of a head implant significantly detracts from a major advantage of MRI as a non-invasive technique. An alternative to the use of head implants is to secure the animal to a MRI-compatible stereotaxic head holder by means of ear pieces and a bite bar. To do this, the animals need to be sedated with a short-acting anesthetic, such as medetomidine, so that they can be attached to the stereotaxic head holder, after which they are allowed to wake up by reversal of the anesthesia (e.g., with atipamezole) (18;42;70;80). While it has been shown that animals can be successfully conditioned to not fight the head restraint upon regaining consciousness (18), this approach has the disadvantages of requiring the use of anesthesia and of utilizing a head holder that may potentially hurt the animal due to the presence of the ear bars. Our approach to restrain the head of the animals is different than either of the two above. We opted to eliminate the need for implanting head posts or to administer any anesthetics or sedatives altogether by acclimatizing the animals to be restrained by a custom-fit helmet that

was specifically designed to match the contour of each individual head exactly, providing a comfortable, yet effective restraint.

3.1. Design of the Animal Bed and Restraint Device

As shown in Figure 5, the bed for fMRI of awake marmosets consists of a cylindrical tube of inner diameter 111 mm, made out of fiberglass impregnated with an epoxy resin (NEMA grade G-10, FPI Industries, Arnold, PA), cut in half length-wise, in which the marmoset lays in the prone, sphinx position. Two lateral support bars made out of Delrin are attached to either inner side of the bed to support the helmet and cover pieces. The bed attaches to the sliding mechanism on one end via the hanger (Figure 5), so as to float cantilevered inside the magnet without touching the gradients or the transmit RF coil. Prior to bringing the marmoset to lie in the cradle, a sleeveless jacket (Lomir Biomedical, Inc. Malone, NY) is placed on the marmoset. Next, a plastic semicylindrical cover made of Lexan is attached to the back of the marmoset's jacket using plastic cable ties. The marmoset is then gently placed into the cradle and the cover is secured by screwing attached nylon thumb screws into the bars on the cradle. The animal is now loosely but effectively restrained from sliding out of the cradle anteriorly or posteriorly. The arms, legs and tail of the animal are free to move unimpededly. Additionally, the semi-cylindrical cover is a few centimeters from the marmoset's back, allowing the animal to stretch and to adjust her body position as needed for comfort, as shown in Figure 5.

Because the shape and size of marmosets' skulls vary, individual custom-built helmets are designed for each animal. For this, a 3D spin-echo MRI of the entire head and neck is acquired from each animal. Next, a 3D surface rendering algorithm is applied to obtain the contour of the head, which is then fed into Rhinoceros 3.0 (McNeel North America, Seattle, WA), a 3D modeling program, to design the top (head) and bottom (chin) helmet pieces. After the head and chin helmet pieces are designed (see Figure 6), they are sent to a 3D printer (ProJet HD3000, 3D Systems Corp., Rock Hill, SC), which builds the helmets, layer by layer, from liquid ABS plastic that hardens during the manufacturing process. After the helmet printing is complete, 3mm thick foam is glued onto the inside surface of both top and bottom pieces to provide greater comfort for the animal.

Proper and perfect fitting of the pieces to each animal is guaranteed by design. Since the shape of each animal is used to produce a helmet manufactured specifically for its head, we can ensure that the animal is comfortable yet immobilized in. After the animal is lowered into the cradle and secured by the cover piece, the helmet top and bottom pieces are carefully placed around the animal's head and screwed into the bars.

3.2. Acclimatization of the Animal to Body and Head Restraint

Once the individualized helmet pieces are built, the animal is needed to be acclimatized to the bed and to tolerate physical restraint during the MRI sessions. This acclimatization procedure consists of three phases, as illustrated in Figure 7:

1. *Phase 1, Acclimatization of Awake Marmosets to Being Contained in the Bed.* In this phase, the animals are dressed with the jackets, attached to the back cover by zip ties and lowered into the bed. The cover is secured to the bed by nylon thumb

screws. The bed is then inserted into a mock MRI tube and the animal is observed from a distance via a webcam. For phase 1, training begins with 15 minutes on day 1, and progresses to an hour by day 4 (see Table 1). As a reward, 3–6 cc of infant milk formula (Pediasure) and 3–5 mini-marshmallows are given at the beginning and end of each training session.

Prior to, during and after each of the acclimatization phases, a behavioral assessment takes place to provide a measure of the tolerance of the animals to the acclimatization procedures. The scoring of each animal is performed using the Behavioral Assessment Scale shown in Table 2 (86). It is our experience that all animals successfully complete the training in phase 1, starting with an average score of 4 on the Behavioral Assessment Scale and acclimatizing to an average score of 2 by the end of phase 1.

2. *Phase 2: Acclimatization of Awake Marmosets to Being Contained in the Bed in the Presence of MRI Sounds.* In spite of being entirely non-invasive, MRI is, unfortunately, a loud technique. Therefore, it is necessary that the animals get properly acclimatized to the sounds generated by the MRI scanner during imaging. In phase 2, the animals are restrained as in phase 1 for increasing periods (Table 1). While in the mock MRI tube, they are allowed to hear the sounds produced by the MRI scanner, played out at a softer level of loudness than in a real MRI session. This schedule reinforces the adaptation to the body restraint initiated in phase 1, and conditions the animals to ignore the MRI sounds produced by the scanner. As in phase 1, Pediasure and mini-marshmallows are used as rewards at the beginning and at the end of each training session. The same Assessment Scale for Behavioral Responses (Table 2) is used to assess the response of the marmosets to the restraint device in the presence of MRI sounds. It has been our experience that all animals successfully complete this phase of the training, starting again with an average score between 3 or 4 on the first day and moving down to an average score of 2 or better by the end of phase 2. At this point, if an animal proves difficult to train by systematically scoring above the mean score, the investigator may choose to adapt the exact timeline of the acclimatization procedures to the idiosyncrasies of that individual, or may choose to drop the animal out of the study.
3. *Phase 3, Acclimatization of Awake Marmosets to Head Restraint in the Presence of MRI Sounds.* In phase 3, marmosets are restrained to the bed by the body cover, as in the previous phases, and then fitted with their custom-built helmets, which are attached to the MRI bed as shown in Figs. 5–7. The use of the helmet effectively – yet comfortably – restrains head motion. The ears will be fitted with earplugs made of silicon jelly (Insta-Putty, Insta-Mold Products, Inc., Oaks, PA), which are pressed gently into the ear canals by foam pads from both sides of the head. This further restrains head motion and protects the animals from the MRI scanner's noise. In phase 3, the animals are conditioned for increasing periods, as in previous phases (Table 1). While in the mock MRI tube, they allowed to hear the sounds produced by the MRI scanner, played out at the same level of loudness as in a real MRI session (Fig. 7). This schedule reinforces the adaptation to the body restraint of the previous phases, and further conditions the animals to ignore the MRI sounds

produced by the scanner, while enforcing full head fixation. As in previous phases, the animals' response to training will be evaluated by the Behavioral Response Scale shown in Table 2. It has been our experience that all animals are able to successfully complete this last phase of the training, starting again with an average score between 3 or 4 on the first day and moving down to an average score of 2 or better by the end of phase 3.

Out of 14 marmosets trained to date, we only dropped one animal out of the study due to increased agitation during phase 2 of the acclimatization. After the animals have successfully completed the three week training program, they proceed to undergoing the actual MRI studies. Continued exposure to the MRI scanner in actual studies fully consolidates the acclimatization, and all animals are tolerant of all procedures after a couple of MRI sessions. Overall, this new method of restraint is completely non-invasive, comfortable for the animals and of great scientific payoff in eliminating the need to either perform surgery for installation of head-post implants, or to use sedatives to restrain the animals, allowing any marmoset to be acclimatized to restraint and utilized indefinitely in longitudinal experiments.

The advantages of using awake subjects can be fully appreciated by the high amplitudes and reproducibility of fMRI responses to somatosensory stimuli, as shown in Figure 8. In awake marmosets (Fig. 8A), electrical stimulation of one arm by repeated pulses (1.5 mA, 0.4 ms duration, 1–125 Hz) evoked fMRI responses in a series of brain regions including thalamus, caudate, putamen, primary (SI) and secondary (SII) somatosensory cortices. Responses were significant in contralateral thalamus, SI and SII, as well as in ipsilateral thalamus and SII. While the fMRI responses were much stronger in the contralateral side, occasionally robust ipsilateral responses were also detected in SI. In marmosets anesthetized by propofol (Fig. 8B), however, both the spatial extent as well as the fMRI amplitudes in responsive regions were significantly smaller compared to awake subjects. In particular, responses in ipsilateral thalamus and ipsilateral SI were insignificant in many anesthetized sessions, consistent with previous studies using anesthetized rats. Responses in caudate and putamen were also much weaker, although detectable. Furthermore, the response amplitudes in thalamus and cortical areas in anesthetized subjects were less than half the amplitudes in awake subjects.

Figure 9 shows the fMRI response obtained from the same animal in different MRI sessions in a time span of 10 months. Excellent reproducibility of the amplitude and spatial and temporal characteristics of the BOLD response were obtained (see Table 3), demonstrating that longitudinal fMRI studies can be successfully carried out in awake, behaving animals.

4. Conclusions

In the present chapter, we presented and reviewed different methodological approaches to obtaining fMRI data from animal models in longitudinal studies. When opting for which approach to follow, the reader is confronted with a balance of advantages and disadvantages that may not always point to the same choice, depending on the purpose of the study.

The use of anesthesia has the advantages of ensuring compliance, minimizing movement, and alleviating stress in an effective way. However, anesthesia suppresses many homeostatic

pathways and requires the investigator to monitor and control the physiology of the animal. Another major disadvantage of the use of anesthesia is that it interferes with neural activity and neurovascular coupling, severely restricting the choice of the functional paradigm and compromising the interpretability and applicability of the data to the understanding of human brain function.

An alternative choice to the use of anesthesia is to acclimate, condition and train the animal to tolerate physical restraint during the data acquisition. This approach offers the advantage of minimizing the need for physiological monitoring and maintenance, but must be done in a well-devised and gradual manner so as to eliminate, as much as possible, stress of the animal to the forced restraint. Stress can introduce as much confound to the data as anesthesia, and thus the task of acclimatizing animals to awake studies cannot be taken lightly.

Nevertheless, when performed consistently and correctly, acclimatization can be very effective in conditioning the animal to participate cooperatively in the study, producing results that, in principle, are experimentally as close to mimicking the setup in human studies as possible.

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Fig. 1.
Typical surgical station for preparation of anesthetized animals. (A) Medical gases and vacuum scavenger; (B) Gas flowmeters and mixer; (C) Isoflurane vaporizer and compliance balloon; (D) Small animal ventilator; (E) Pulse oximeter/capnograph; (F) Temperature regulator, heated water bath and water mat; (G) Surgical microscope.

Fig. 2.
(A) MRI-compatible animal bed, containing a stereotaxic head holder with ear pieces and a bite bar (B), to which the head of the animal is secured. Mechanical ventilation is provided by the gas lines. (C) Pulse oximetry sensor and rectal temperature probe. (D) Heated water mat (shown unfolded), which can be wrapped around the animal's body to maintain temperature. (E) Multi-channel receive RF coils. (F) Multi-channel RF preamplifiers.

Fig. 3.

Pooled data plot of ETCO₂ as measured with a capnograph versus PaCO₂ sampled from arterial blood (n= 34 rats, average of 6 points per animal) in normocapnia and hypercapnia. The dashed line is the line of identity, and the correlation coefficient between ETCO₂ and PaCO₂ is 0.77 ($r^2 = 0.59$). Even though the absolute value of ETCO₂ is influenced by the size of the animal relative to the total flow and volume of air in the ventilator, the length of the gas lines, and the flow of expired air into the capnograph, the significant correlation between ETCO₂ and PaCO₂ allows ETCO₂ values to be used as a relative index of changes in PaCO₂.

Fig. 4. Physiological and fMRI data acquisition traces recorded during a typical fMRI experiment of electrical stimulation of both forepaws of a rat anesthetized with α -chloralose. The experiment lasted 4 minutes. From top to bottom, the traces show the arterial blood pressure (red), respiratory pressure (green), EPI acquisition tics (blue), stimulation of the left (green) and the right (blue) forepaws, the MRI gradient temperature (magenta) and the heart rate derived from the ABP trace (blue).

Fig 5.

Illustration of a restrained marmoset in the MRI-compatible bed. The body of the animal is loosely attached to a back cover via zip ties secured to a sleeveless jacket worn by the animal. The back cover is screwed to the side bars on the cradle, while the arms, legs and tail of the animal are free to move as she wishes. The head of the marmoset is secured to a two-piece, custom-built helmet made specifically for that individual alone. The chin piece on the bottom supports the chin of the animal, and the head piece on the top prevents head motion. Note that the helmet pieces are lined up with foam on the inside to provide a comfortable support to the entire head. The animal sits in the sphinx position looking out towards the back of the magnet. The bed is secured to the bed sliding mechanism on one end via the hanger.

Fig 6.

Detail of the construction of the custom-fit helmet. Based on a 3D MRI of the entire head of the marmoset, a 3D model of the helmet is created consisting of two pieces: the chin piece (blue) to support the chin of the animal, and the head piece (red) that supports the head and prevents motion. Once the model is created it is sent to a 3D plastic printer that creates the helmet.

Fig. 7.

Illustration of the three phases of training. In Phase 1 (top), the body of the animal is loosely attached to the MRI bed, and the animal is conditioned to staying in the bed for increasing periods of time. In phase 2 (middle), reinforcement of the training in phase continues while the animal gets used to MRI sounds. In phase 3 (bottom), the individualized helmet is introduced to restrain the head of the animal.

Fig. 8.

Map of fMRI response evoked by electrical stimulation of the left arm of the marmoset. Eight contiguous coronal slices immediately posterior to the anterior commissure (AC) are shown in a representative session under awake (A) and propofol anesthesia (B). Robust BOLD responses can be detected in the thalamus (Tha.) and in the primary (SI) and secondary (SII) somatosensory cortex, although the responses are much more significant in the awake than in anesthetized animals.

Fig. 9. fMRI response obtained from the same animal in different MRI sessions in a time span of 10 months. (A) T-map demonstrating the main active areas of the brain, including SI, SII, and caudate putamen. (B) BOLD time-course in response to a 2 s electrical stimulus of both hands (2 mA, 0.3 ms, 64 Hz), obtained at five different times (0 weeks, 3 weeks, 6 weeks, 2 months and 10 months) post-acclimatization. Excellent reproducibility of the amplitude and temporal characteristics of the BOLD response is achieved.

Table 1

Three-Week Acclimatization Schedule

Phase	Monday	Wed	Friday	Sunday	Procedures
1	0:15	0:30	0:45	1:00	Jacket
2	1:00	1:20	1:40	2:00	Jacket + MRI sounds
3	1:00	1:20	1:40	2:00	Jacket + MRI sounds + helmet

Table 2

Behavioral Assessment Scale

Score	Behavior
1	Quiet: marmoset calm and relaxed
2	Mostly quiet, agitated only initially
3	Mostly quiet, with brief, intermittent mild agitation
4	Quiet after initial struggle, increasingly agitated over time
5	Mild agitation for about half of the restraint period
6	Moderate agitation during half of the restraint period
7	Restless and agitated during most of the restraint period
8	Extremely agitated during most of the restraint period

Table 3

The amplitude % signal changes, standard deviation (SD) of baseline, time-to-peak (TTP), and full width at half maximum (FWHM) of the BOLD response from a representative marmoset undergoing longitudinal fMRI (2 s stimulus duration, 2 mA, 0.3 ms pulse width, 64 Hz) of the somatosensory cortex.

	First scan	3 weeks	6 weeks	2 months	10 months	Mean ± SD
Signal increase (%)	1.95	2.50	2.69	2.57	2.47	2.44 ± 0.28
SD of baseline (%)	0.08	0.14	0.17	0.17	0.21	0.15 ± 0.08
TTP (s)	3.84	4.41	3.41	3.80	3.78	3.85 ± 0.36
FWHM (s)	3.92	4.70	4.34	4.14	4.55	4.33 ± 0.31