Journal of Neuroscience Methods xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

The rabbit as a behavioral model system for magnetic resonance imaging

Craig Weiss[a,*], Daniel Procissi[b], John M. Power[c], John F. Disterhoft[a]

[a] Department of Physiology, Northwestern University Feinberg School of Medicine, 303 E. Chicago Avenue, Chicago, IL 60611, USA
[b] Department of Radiology, Northwestern University Feinberg School of Medicine, 303 E. Chicago Avenue, Chicago, IL 60611, USA
[c] Translational Neuroscience Facility & Department of Physiology, School of Medical Sciences, UNSW Australia, Sydney, NSW 2052, Australia

HIGHLIGHTS

- Rabbits tolerate restraint and have a crepuscular circadian rhythm that facilitates imaging of behavior.
- Atraumatic, MR compatible headbolt facilitates positioning across days/among subjects.
- Stereotaxic alignment of the head facilitates use of stereotaxic brain atlases.
- $MnCl_2$ injection (sc) permits imaging activity that occurred outside of magnet.
- MR compatible stimuli and sensors permit eyeblink conditioning to be done in magnet.

ARTICLE INFO

Article history:
Received 8 March 2017
Received in revised form 17 May 2017
Accepted 22 May 2017
Available online xxx

Keywords:
AFNI
Animal models
BOLD imaging
FSL
Functional connectivity
Manganese enhanced magnetic resonance imaging
Stereotaxic atlas
Whisker stimulation

ABSTRACT

Background: fMRI requires that subjects not move during image acquisition. This has been achieved by instructing people not to move, or by anesthetizing experimental animal subjects to induce immobility. We have demonstrated that a surgically implanted headbolt onto the skull of a rabbit allows their brain to be imaged comfortably while the animal is awake. This article provides a detailed method for the preparation.
New method: We took advantage of the rabbit's tolerance for restraint to image the brain while holding the head at the standard stereotaxic angle. Visual stimulation was produced by flashing green LEDs and whisker stimulation was done by powering a small coil of wire attached to a fiber band. Blinking was recorded with an infrared emitter/detector directed at the eye with fiber-optic cabling.
Results: Results indicate that a single daily session of habituation is sufficient to produce adequate immobility on subsequent days to avoid movement artifacts. Results include high resolution images in the stereotaxic plane of the rabbit.
Comparison with existing method(s): We see no degradation or distortion of MR signal, and the headbolt provides a means for rapid realignment of the head in the magnet from day to day, and across subjects. The use of rabbits instead of rodents allows much shorter periods of habituation, and the rabbit allows behavior to be observed during the day while the animal is in its normal wake cycle.
Conclusions: The natural tolerance of the rabbit for restraint makes it a valuable subject for MRI studies of the brain.

Magnetic resonance (MR) imaging has transformed medical research and medical diagnoses due to its noninvasive sensitivity to differences in water content of different tissues. This differential sensitivity yields a contrast in images that provides relatively clear pictures of the tissue or organ being examined. MRI can also be used to infer functional activation of tissue by detecting differences in oxygenated and deoxygenated hemoglobin which have different paramagnetic properties. This blood-oxygen-level-dependent (BOLD) contrast of the MR signal forms the basis for functional magnetic resonance imaging (fMRI) and has been widely used to examine neural activation in the brain. Brain activation has been studied as event related activity to a sensory or pharmacological stimulus, or as a signal related to emotional states or motor commands and movements. These imaging techniques allow detection of hemodynamic transient changes associated with neuronal activation from fixed spatial locations in the brain of the subject in

* *Corresponding author.
E-mail address: cweiss@northwestern.edu (C. Weiss).

http://dx.doi.org/10.1016/j.jneumeth.2017.05.021
0165-0270/

the MRI scanner. A major requirement for allowing accurate representation of these regional functional changes is that the subject does not move during acquisition of the MR images. This has been achieved by either instructing human patients or volunteers not to move, or by anesthetizing experimental animal subjects to induce immobility.

The rabbit is an animal species that naturally becomes immobile in a confined space. The natural tolerance of the rabbit to restraint has made it a favorite experimental subject for many researchers. Rabbits are often given a single day of habituation to restraint for imaging (Schroeder et al., 2016) while rats were given at least one week of habituation (Chang et al., 2016), and mice were given a session of restraint under isoflurane anesthesia followed by four days of awake habituation (Ferris et al., 2014), or seven days of habituation to lower their resting heart rate (Yoshida et al., 2016). Other features that make the rabbit an attractive model system for imaging are its smooth (lisencephalic) cortex, differentiated striatum (caudate and putamen), strong skull, crepuscular nature (rather than being nocturnal), large blood vessels (relative to those of rodents) and a docile nature. The smooth cortex makes localizing activations more reliable because it avoids ambiguity when apparent activation spans a gyrus. The differentiated striatum is more like that of primates than of rodents and is important for studies that involve the striatum, e.g. studies of Parkinson's disease and several psychiatric disorders. The strong skull enables secure attachments by dental or bone cements, or with non-ferrous screws (e.g. nylon screws) for strong attachments to the skull. The crepuscular nature of the rabbit makes it more likely that it will remain awake during functional tests during the day, an advantage that should not be underestimated as rodents may enter sleep states when tested during the typical experiment time of most investigators. The larger blood vessels of the rabbit are important for procedures involved with inducing experimental tumors and for testing potential cancer therapeutics (Jeon et al., 2016; Eifler et al., 2009; Rhee et al., 2007; Larson et al., 2006), and the marginal vein in the ear provides easy access for intravenous administration of substances before experiments and during imaging experiments after securing a catheter line between an MR compatible angiocath in the ear vein and a syringe with the substance of interest. Lastly, the amino acid sequence for rabbit amyloid is the same as for human amyloid and provides a non-transgenic model system for the study of Alzheimer's Disease (Davidson et al., 1992; Bitel et al., 2012). This model system for Alzheimer's Disease (AD) is affected by a high cholesterol diet which induces learning deficits (Sparks and Schreurs, 2003), hippocampal neurodegeneration, 16 pathological hallmarks of AD (Brooks et al., 2017; Schreurs, 2013), gene expression related to mitochondrial oxidative phosphorylation (Liu et al., 2016), and an increase in BACE1 and beta-amyloid (BACE1 cleaves beta-amyloid precursor protein to release beta-amyloid in the brain, Ghribi et al., 2006; Ghribi, 2008). A summary of these qualities is presented in Table 1 which includes a comparison with rodents and the marmoset (a small primate gaining popularity as a model system).

There are of course disadvantages to using the rabbit (a lagamorph) instead of the mouse (a rodent): The genome of mice is much more easily manipulated than that of the rabbit (although Renova Life, Inc. advertises that they will make transgenic rabbits upon request). Rabbits require more space for housing and more food to eat so they are more expensive to keep. The brain is larger and requires more chemicals and supplies for processing as compared to mice. Rabbits exhibit fewer behaviors than rodents and because they are a covered species according to the USDA their use is more rigorously monitored and has more restrictions than experiments with rodents.

We proposed several years ago that the rabbit would be a good animal model subject for fMRI studies of brain activation and began our studies by demonstrating BOLD activation of the visual cortex in awake and drug-free rabbits (Wyrwicz et al., 2000). We then showed learning related changes in the cerebellum and visual cortex of eyeblink conditioned rabbits as opposed to control rabbits (Miller et al., 2003, 2008), and we described the resting state networks of the awake rabbit (Schroeder et al., 2016a), and how pretrial functional connectivity of those networks was related to the expression of conditioned blink responses (Schroeder et al., 2016b).

Here we describe the methods used to obtain good structural and functional magnetic resonance images from awake rabbits, and how the methodology can be integrated with those for eyeblink conditioning, one of the better understood paradigms for learning and memory from a neurobiological perspective (Weiss et al., 2006; Disterhoft and Weiss, 2017). The basis of the preparation involves the stereotaxic placement of nylon bolts upon the skull so that the head can be closely repositioned from day to day after swaddling the rabbit in a cloth wrap. The headbolt also serves to secure a coil in place to receive the MR signal. The combination of fixing the head and coil in place within a cradle that is placed within the magnet allows the brain to be imaged from day to day with relatively little correction of image alignment, and fiber-optic cables enable MR compatible detection of conditioned and unconditioned blinks (Miller et al., 2005).

1. Preparation of headbolt

Our original headbolt was prepared using the top of a 35 mm film canister to contain a batch of dental cement. Those formerly ubiquitous pieces are now a rare commodity so we machined a mold out of a piece of 1.25" diameter aluminum stock (Fig. 1a). The mold includes 4 clearance holes (size 27 drill, 0.1440") to accept 6–32 x ¾" nylon machine bolts with a hexagonal head. The holes are spaced 0.75" apart (center to center distance) and are centered within a 7/8" square that is 3/16" deep. The mold includes a 1/16" indentation at the center of each edge to facilitate adhesion to additional cement that is applied at the time of surgery, and it includes a 1/16" high ridge along the center of the mold to accept a capillary tube during imaging sessions (to identify the center of the headbolt and verify stereotaxic alignment). A nylon support was machined out of a 1.25" piece of stock and was narrowed to 1" diameter (Fig. 1B) to fit inside the bottom of the aluminum mold. The height of the 1" diameter narrowing was adjusted so that the head of the 6-32 bolt was elevated 1/16" above the base of the mold (Fig. 1C); this allows cement to flow over the top of the head of the bolt and prevents the bolt from being pulled out of the mold when the headbolt is secured to the imaging cradle. We also score the center of each side of the bolt with a heavy duty wire cutter to provide additional friction against the cement.

The inside and top of the aluminum mold are coated with petroleum jelly, as are the center of the four holes. The mold is placed upon the support, the nylon bolts are inserted, and the cement is mixed and poured over the bolts until the tops of the bolts are covered. After the cement has partially cured we use a large diameter wire to create a slight indentation perpendicular to the groove that will be at the top of the headbolt. The indentation is to accommodate the rise in the skull along the midline. We have used both Dentsply's Grip Cement and Hygenic's Perm Reline & Repair Resin to make the headbolts. Both materials were tested in the MRI scanner to evaluate their effect on the images (i.e. presence of artifacts associated with the magnetic properties of the material). Both performed well with little or no artifacts on the MRI brain images. An aliquot of the mixed cement is kept aside and used to evaluate the curing process. Once completely hardened the headbolt is removed by placing the mold upside-down upon a

Please cite this article in press as: Weiss, C., et al., The rabbit as a behavioral model system for magnetic resonance imaging. J Neurosci Methods (2017), http://dx.doi.org/10.1016/j.jneumeth.2017.05.021