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Short communication

Behavioral effects of acclimatization to restraint protocol used for awake animal imaging

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HIGHLIGHTS

- We tested the efficacy of a behaviorally uncharacterized awake animal using an fMRI restraint acclimatization protocol.
- We measured 22-kHz vocalizations to assay the affective state of rats throughout the restraint acclimatization protocol.
- We used the forced swim test to determine if habituation to fMRI restraint was due to an engendered learned helplessness state.
- The demonstrated efficacy of this protocol enables increased data interpretation reliability in an awake animal fMRI.

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ABSTRACT

Functional MRI in awake rats involves acclimatization to restraint to minimize motion. We designed a study to examine the effects of an acclimatization protocol (5 days of restraint, 60 min per day) on the emission of 22-kHz ultrasonic vocalizations and performance in a forced swim test (FST). Our results showed that USV calls are reduced significantly by days 3, 4 and 5 of acclimatization. Although the rats showed less climbing activity (and more immobility) in FST on day 5 compared to the 1st day of restraint acclimatization, the difference was not detected once the animals were given a 2-week hiatus. Overall, we showed that animals adapt to the restraint over a five-day period; however, restraint may introduce confounding behavioral outcomes that may hinder the interpretation of results derived from awake rat imaging. The present data warrants further testing of the effects of MRI restraint on behavior.

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1. Introduction

There is an increasing number of studies employing functional MRI in rodents to study brain function and anatomy. Issues arise with the use of anesthetics and paralyzing agents that make the method less attractive for those in neuroscience fields. However, imaging animals while awake and restrained raises concerns regarding distress and the possibility of an ensuing depressive state. Anesthetized preparations are used extensively in fMRI studies in rats. These methods are not suitable for many longitudinal applications under ideal experimental conditions where the same populations of animals are imaged at multiple time points. Many studies utilize surgical methods to compensate for the loss of vital homeostatic mechanisms. Additional paralyzing agents are often required to further reduce movement to levels sufficient for stable imaging. However, these invasive methodologies are not favorable for long-term studies in rats. In addition, there are also significant lines of evidence suggesting that agents typically used to anesthetize animals can significantly suppress neuronal activity and modify specific patterns of neuronal activity and metabolism (Wang et al., 2011; Williams et al., 2010; Masamoto et al., 2009). In addition to neuronal activity, anesthesia can also influence global cerebrovascular response and thus, anesthetics may affect the magnitude of the blood oxygen level dependent (BOLD) signal via disparate effects on cerebral blood flow (CBF), cerebral blood volume (CBV) and other hemodynamic parameters (Sicard et al., 2003). Our laboratory has utilized methods to image awake rats as an alternative to imaging under anesthesia. Prior to imaging, however, the animals must be acclimated to the restraint conditions and MR pulse sequence noise (Fig. 1) (King et al., 2005). The procedures for acclimation were performed for 5 days prior to the collection of imaging data. Acclimation was performed to simulate the actual imaging experimental setup during data collection. Movements during scanning (for example, slow shifts in the head

Abbreviations: BA, sodium butyrate; USV, ultrasonic vocalizations.

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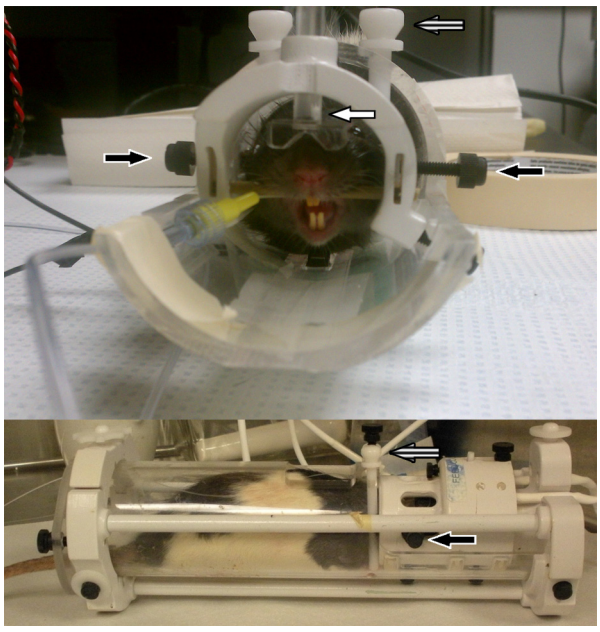

Fig. 1. fMRI restraint set-up. (1) The restraint set-up was designed to restrict head movement in all directions. Screws (black arrows) fixed to the enclosure around the animal's head prevented yaw. The incisors were placed over a bite bar and a plastic nose bar (white arrow) was lowered and locked on top of the snout to prevent pitch. The animal was then placed in a body tube, and plastic bars (striped arrows) were inserted immediately in front of the shoulders to isolate the head from caudal body movement.

position, sustained or transient leg motion, chewing, vocalizations) and physiological motion (pulsations due to cardiac and respiratory cycles) can significantly reduce the quality of the anatomical and functional MR scans. Although some physiological variables adjust to the daily intermittent restraint conditions involved in acclimatization and there are improvements in image quality (as measured by an increased signal to noise and contrast), no behavioral demonstration of the efficacy of our acclimation protocol exists. In addition, the question still remains whether reductions in motion observed after restraint conditioning are symptomatic of a depression-like state induced by the protocol. We begin to address this point in the present study. Several aversive stimuli have been shown to elicit 22-kHz vocalizations in rats (as reviewed by Knutson et al., 2002). Thus, we used 22-kHz ultrasonic calls as an index of negative affect to behaviorally assay acclimatization. The forced swim test was used to assay depressive-like symptoms at the onset and completion of the restraint conditioning protocol.

2. Methods

2.1. Subjects

Male Long Evans rats weighing 300–400 g were purchased from Charles River Laboratories (Wilmington, MA). The animals were pair housed under a 12-h light:dark cycle (lights off at 19:00 h). Water and Purina rat chow were provided ad libitum. The rats were acquired and cared for in accordance with the guidelines published in the Guide for the Care and Use of Laboratory Animals (8th edition, 2011) and adhered to the National Institutes of Health and the American Association for Laboratory Animal Science guidelines. The Institutional Animal Care and Use Committee at Northeastern University approved the protocols used for this study. The animals were subjected to an acclimatization procedure similar to the one previously reported to minimize motion artifacts during fMRI scanning (King et al., 2005). We recorded USV calls in one group of rats ($n = 11$). Another group of rats ($n = 9$) was subjected to FST on days 1 and 5 of acclimatization. These results were compared to the 9 animals that were tested, but not acclimated and 10 animals acclimated to restraint, but tested two weeks after the final acclimatization session ($n = 8$).

2.2. Acclimation to fMRI restraint procedures

Rats were acclimated for 5 days to MRI restraint procedures (King et al., 2005). On each day, the rats were anesthetized with 2–4% isoflurane gas anesthesia and placed into a replica of the restraint unit used for functional magnetic resonance imaging (fMRI) studies (insightMRI, Shrewsbury, MA) (Fig. 1). A topical anesthetic of 4% lidocaine cream was applied to the skin around the ear canals and over the bridge of the nose before the animal was placed inside the restraint unit. This minimized discomfort on the pressure points around the head. Most of the restraint was around the head and above the shoulders; therefore, the animals were in a hunched posture with their limbs free and unrestrained. The head holder has a bite bar over which the incisors were placed and held by lowering and locking-in a plastic nose bar. Plastic ear bar guides were locked in place by lateral screws, thereby preventing lateral movement. A pair of shoulder bars was lowered behind the animal's neck, thereby further restricting vertical movement. Once the setup was complete, the rat was placed inside a sound attenuation box and USV calls and respiratory rates were recorded. Following the recording session, the animals were removed from the sound attenuation box and placed in a secondary chamber and exposed to a recording of an fMRI imaging to recapitulate the imaging environment. The acclimatization procedure was repeated over 5 days, during which the time spent under restraint was 60 min per day.

2.3. Ultrasonic vocalization recording

The rats were placed inside a sound attenuation chamber (MedAssociates, St. Albans, VT). To minimize the confounding effect of the isoflurane anesthesia used to place the animals in the restraint, scoring of the data did not begin until 5 min after it was evident from the spectrograms that the animals were awake. Ultrasonic vocalizations were recorded using a condenser microphone that was sensitive to frequencies from 10 to 250 kHz (model CM16 Avisoft Bioacoustics, Germany). Sound detection and storage was achieved by interfacing with an UltraSoundGate USB digital-to-analog converter and amplifier (USG 116, Avisoft). A laptop PC running Avisoft RECORDER software was used to store acoustic data as .wav files for analysis of the spectral features. The USVs were continuously monitored using a frequency window between 10 and 70 kHz, and the spectrograms were generated online using a Fast Fourier Transform size of 256, a sampling rate of 140 kHz, and a 16-bit data format. Acoustic datasets were imported into SASLab Pro (Avisoft) and the spectrograms were generated for the analysis of vocalization around the 22-kHz range. Filtering was used to reduce background noise and a trained observer manually quantified the total number of specific spectrogram waveforms that were observed over time. The 22-kHz calls were classified using several criteria: calls with a bandwidth of 1–6 kHz and frequency of 18–32 kHz were considered 22-kHz calls (Brudzynski et al., 1993). We used SASLab Pro (Avisoft), which enabled the accurate determination of call duration, frequency, and bandwidth. All noises shorter than 10 ms, with bandwidths shorter than 1 Hz, or bandwidths greater than 6 Hz, were excluded from the analysis. The 22-kHz call frequency ranged from 18 to 31.5 kHz. The mean call frequency was 23.87 ± 3.79 kHz. Consistent with short 22-kHz calls, the observed call duration ranged from 10.8 to 351 ms.

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