Journal of Neuroscience Methods 203 (2012) 28–40

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

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

Wireless multi-channel single unit recording in freely moving and vocalizing primates

Sabyasachi Roy [a], Xiaoqin Wang [a,b,*]

[a] Laboratory of Auditory Neurophysiology, Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
[b] Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

ARTICLE INFO

Article history:
Received 23 May 2011
Received in revised form 31 August 2011
Accepted 6 September 2011

Keywords:
Action potential
Free-roaming
Marmoset
Multi-channel
Multi-electrode array
Natural behavior
Neurophysiology
Neural telemetry
Pre-motor cortex
Primate
Single-unit
Vocalization
Wireless

ABSTRACT

The ability to record well-isolated action potentials from individual neurons in naturally behaving animals is crucial for understanding neural mechanisms underlying natural behaviors. Traditional neurophysiology techniques, however, require the animal to be restrained which often restricts natural behavior. An example is the common marmoset (*Callithrix jacchus*), a highly vocal New World primate species, used in our laboratory to study the neural correlates of vocal production and sensory feedback. When restrained by traditional neurophysiological techniques marmoset vocal behavior is severely inhibited. Tethered recording systems, while proven effective in rodents pose limitations in arboreal animals such as the marmoset that typically roam in a three-dimensional environment. To overcome these obstacles, we have developed a wireless neural recording technique that is capable of collecting single-unit data from chronically implanted multi-electrodes in freely moving marmosets. A lightweight, low power and low noise wireless transmitter (headstage) is attached to a multi-electrode array placed in the premotor cortex of the marmoset. The wireless headstage is capable of transmitting 15 channels of neural data with signal-to-noise ratio (SNR) comparable to a tethered system. To minimize radio-frequency (RF) and electro-magnetic interference (EMI), the experiments were conducted within a custom designed RF/EMI and acoustically shielded chamber. The individual electrodes of the multi-electrode array were periodically advanced to densely sample the cortical layers. We recorded single-unit data over a period of several months from the frontal cortex of two marmosets. These recordings demonstrate the feasibility of using our wireless recording method to study single neuron activity in freely roaming primates.

1. Introduction

In neurophysiology as in other scientific fields the ability to investigate a particular system or phenomenon depends on the limitations of the available methodology. Over the last several decades, neuroscientists have successfully applied single electrode neural recording techniques to study the central nervous system. More and more studies have highlighted that the measurable output (behavior) of a biological system such as a motor action or sensory representation is not simply determined by the activity of a single neuron, but by collective activity of networks of neurons. In other words, the brain is an ensemble of neurons that are functionally clustered to produce sensation, action, memory and cognition. To derive realistic models of the cortical neuronal networks, it is crucial to simultaneously record and study multiple neurons in a cortical region of interest during behavior (Nicolelis and Ribeiro, 2002; Buzsáki, 2004). Previous studies have used multi-electrode arrays with sharp metal wires, tetrodes, multi contact silicon probes and microelectromechanical systems (MEMS) electrodes to record neural activity in population of neurons from various animal species (e.g., Ainsworth and O'Keefe, 1977; Gray et al., 1995; Nicolelis et al., 1997; deCharms et al., 1999; Fee and Leonardo, 2001; Hoffman and McNaughton, 2002; Jog et al., 2002; Keating and Gerstein, 2002; Muthuswamy et al., 2005; Suner et al., 2005; Sinha and Moss, 2007; Eliades and Wang, 2008b; Battaglia et al., 2009). However single-unit neural recording over an extended period of time from naturally behaving and freely moving animals has posed a number of technical challenges. Such challenges include: achieving a sufficiently high signal-to-noise ratio (SNR) in recorded neural data in order to capture single neuron activity, maintaining SNR for extended time period (SNR degradation due to glial scarring at the tip of an electrode), developing a neural telemetry system with sufficient bandwidth, high transmission fidelity, small size, lightweight and low power consumption, and finally techniques to perform real-time analysis on the large amount of simultaneously captured neural and behavioral data.

* Corresponding author at: Department of Biomedical Engineering, Johns Hopkins University, 720 Rutland Ave, Traylor 410, Baltimore, MD 21205, United States. Tel.: +1 410 614 4547.
E-mail address: xiaoqin.wang@jhu.edu (X. Wang).

0165-0270/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2011.09.004

The wireless multi-channel single-unit recording technique described in this report was designed to record cortical neural activity from freely roaming and vocalizing marmosets in a controlled laboratory environment. The common marmoset is a New World primate with a rich repertoire of vocalizations (Epple, 1968; Wang, 2000; DiMattina and Wang, 2006; Pistorio et al., 2006). These animals are highly vocal in the wild (Bezerra and Souto, 2008) and remain vocal in laboratory conditions when unrestrained (Aitkin and Park, 1993; Pistorio et al., 2006). Over the last decade, the cortical auditory system of the marmoset has been extensively studied in our laboratory using acute and chronic single-unit recordings in awake and behaving conditions. The responses of neurons in the auditory cortex to vocalizations and other auditory stimuli have been systematically investigated (Lu et al., 2001a,b; Wang et al., 2005; Bendor and Wang, 2005; Eliades and Wang, 2008a) in this species. More recent studies have investigated their natural vocal behaviors (Miller and Wang, 2006; Miller et al., 2009). However, restraining marmoset's movement using a primate chair severely diminishes its vocal behaviors (Eliades and Wang, 2008b). Using a tether (connecting the headstage of an electrode array to a commutator) to record neural signals has its limitations in arboreal animals like marmosets due to frequent interruptions by an animal's movements (Eliades and Wang, 2008b). Here we describe a wireless recording system that combines a previously developed chronic multi-electrode implant array (Eliades and Wang, 2008a,b) with a lightweight, low noise, battery powered wireless neural headstage in order to record multiple single-units from naturally behaving and freely moving marmosets. An important aspect of this recording system is the controlled radio frequency (RF) environment and electro-magnetic interference (EMI) shielded booth that was custom built to ensure an uninterrupted and high fidelity wireless neural data link. In addition, we developed a software based neuro-vocal processing system that integrates multi-channel spike-sorting with the marmoset vocalization detection and audio playback experiment capabilities.

Prototype neural recording systems utilizing telemetry have been previously tested on different animal models (Grohrock et al., 1997; Nieder and Klump, 1999; Mohseni et al., 2005; Jürgens and Hage, 2006; Schregardus et al., 2006; Chen et al., 2008; Chestek et al., 2009; Harrison et al., 2009; Gregory et al., 2009; Gilja et al., 2010; Szuts et al., 2011). These systems are not ideal for use in a highly mobile small primate such as the marmoset with three dimensional movement patterns. There are four major requirements for a telemetry system that will enable single-unit neural recordings from freely roaming marmosets. First, marmosets have a typical body weight of 400 g, which limits the size and weight of the wireless headstage that they can carry. Second, these animals do not (in our laboratory experience) readily wear a jacketed backpack which could be used to house a larger recording system connected to the head mounted multi-electrode array. Even if the marmosets were trained to wear a backpack, the tether from the backpack to the electrode array would limit the animal's head movement and pose a risk of entanglement in the recoding cage. This makes it less feasible for marmosets to use devices with multiple tethered sub-systems that are used in rodents (Szuts et al., 2011). Therefore the headstage and electrode array assembly needs to have a compact form factor (Fig. 1A). Third, being arboreal animals, the marmosets move in a three dimensional space requiring antenna beam patterns that provide good signal coverage with the receiver in different body orientations. Fourth, the wireless headstage should be capable of transmitting high fidelity, multi-channel raw neural data streams so as to match the multi-electrode array channel count and enable single-unit extraction via spike-sorting. The system described in this report meets the above mentioned requirements. This wireless neural recording system could be relatively easily adapted to other animals with similar or larger size.

2. Materials and methods

2.1. Animal preparation and multi-electrode array implantation

The details of the animal head cap implantation and multi-electrode array implantation techniques have been described in previous studies (Lu et al., 2001a,b; Wang et al., 2005; Eliades and Wang, 2008b). We will summarize the main steps in this and the following paragraphs. Over a period of four weeks the marmoset was adapted to the sit quietly in a primate chair. The animal was also adapted to the telemetry chamber inside a plexiglass and nylon mesh recording cage (60 cm × 41 cm × 30 cm) within which it was free to roam. Two head posts were attached to the animal skull using stainless steel screws and dental cement under general anesthesia and sterile conditions. These head posts were used to secure the animal's head at the beginning of an experimental session to mount the wireless transmitter and during electrode pushing sessions. During the head cap implant surgery, the locations of the lateral sulcus on both hemispheres were marked as the landmarks for the subsequent array implantation procedure (Fig. 1B). The surface of the skull covering the frontal lobe including the premotor cortex was covered with a thin layer of dental cement.

Following the recovery from the head cap implant surgery, the array implantation is performed (Eliades and Wang, 2008b). The animal was sedated with Ketamine (20 mg/kg) and acepromazine (0.75 mg/kg). A craniotomy of 5 mm × 5 mm was carefully made in the appropriate location using a 1 mm drill bit attached to a micromanipulator (SM-11, Narishige). The craniotomy was covered with a layer of silastic (low viscosity silicone, World Precision Instruments, Inc., Sarasota, FL, USA) which is allowed to set for 20 min. The exposed dura is carefully cleaned to ensure a good seal with the silastic. The silastic layer (Fig. 1C) completely seals the dura so as to minimize tissue growth on the dura. The silastic layer also prevents a short circuit of the electrode guide tubes due to cerebrospinal fluid. Additionally, it provides stability to the electrodes necessary for long term single-unit recordings in the free moving condition. The array is gradually lowered in place using a micromanipulator until its base (also covered with silastic) is perpendicular to the cortical surface and touches the silastic covering the dura. This junction of two silastic layers is further covered with an additional silastic layer. The array is then secured to the surrounding head cap using several layers of dental cement. After the array is secured a cylindrical protective chamber made from polycarbonate (Ultem, McMaster-Carr) is placed around the array and similarly secured to the head cap using dental cement (Fig. 1C). This protective chamber is closed with a lid in order to prevent moisture and other contaminants from entering the electrode array when the recording is not in progress. The ground wire of the array is wrapped around the front head screw and secured using a miniature brass clamp. The placement of the arrays was based on anatomical and electrical stimulation studies of the marmoset prefrontal and premotor cortex (Burman et al., 2008; Burish et al., 2008).

2.2. Neural recording with chronically implanted multi-electrode array

Chronically implanted multi-electrode arrays have been increasingly used in neurophysiology experiments with behaving animals (Nicolelis et al., 1997; deCharms et al., 1999; Fee and Leonardo, 2001; Hoffman and McNaughton, 2002; Ainsworth and O'Keefe, 1977; Sinha and Moss, 2007; Eliades and Wang, 2008b; Battaglia et al., 2009). The Warp-16 drive (Neuralynx, Inc., 105 Commercial Dr, Bozeman, MT, USA) shown in Fig. 1A and C is a multi-electrode array loaded with 16 independently movable sharp metal electrodes in a simple yet robust design. The chronic implantation technique using the Warp-16 drive in the marmoset was

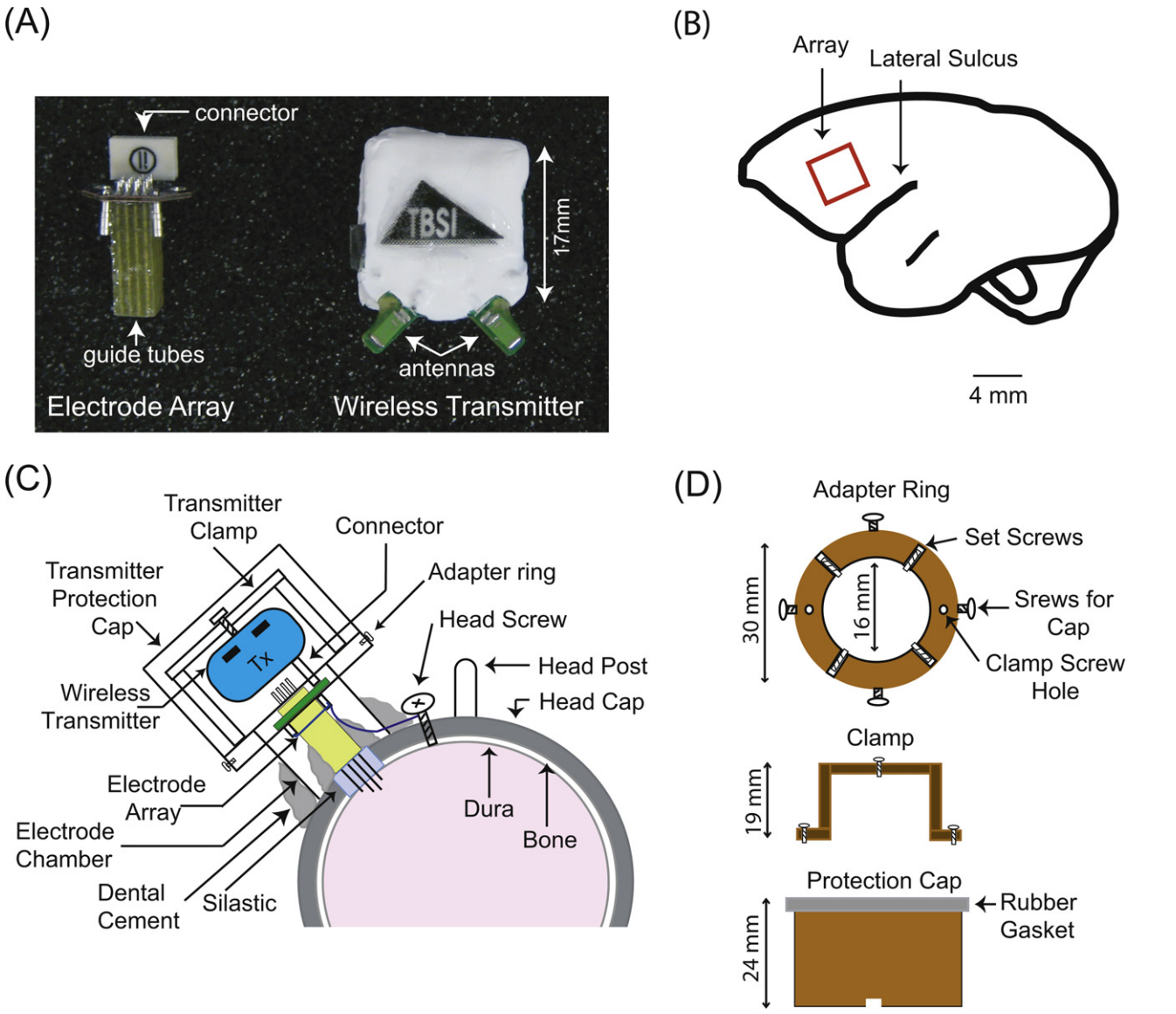

Fig. 1. Wireless neural headstage and multi-electrode array. (A) Warp-16 (Neuralynx) sixteen channel multi-electrode array on the left and W16 (TBSI) sixteen channel wireless neural headstage shown on the right. (B) Approximate location of electrode array in the marmoset left hemisphere. (C) Wireless neural headstage and electrode array assembly is shown along with the protective chamber for use during free-roaming recordings. (D) Individual components of the protective cap for the headstage and multi-electrode array assembly.

established by Eliades and Wang (2008b). Once all the electrodes have penetrated the dura, we use one electrode as a reference channel and record neural action potentials from the remaining 15 electrodes. The individual metal electrodes are pushed using a modified caliper (Manual Cyborg Drive Electrode Pusher, Neuralynx Inc.) which can drive the electrodes in steps of 1 μm. The electrodes make contact with the guide tube via a friction fit achieved by a ~20° bend at the back of the electrode. Using such an array we were able to simultaneously record single-units from multiple adjacent cortical sites over a period of several months after the implantation. This is a significant advantage over electrode arrays with fixed electrodes where the deposition of scar tissue at the electrode tip leads to signal degradation (Polikov et al., 2005). Periodically pushing the individual electrodes ensures well isolated single-unit recordings for several months after the array implantation. Typically, the individual electrodes were pushed 15–50 μm between recording sessions. To minimize dimpling of the underlying tissue, electrodes were pushed in a sequence, waiting about 10 min between each electrode push. The sequence of advancing the electrodes was determined such that adjacent electrodes were not consecutively pushed. Once all the electrodes have been pushed we waited a minimum of 12 h before recording in order to let the tissue settle around the electrodes. An important factor in determining action potential SNR from a multi-electrode array is the choice of metal electrodes. We have used epoxy insulated tungsten electrodes (FHC, 1201 Main st, Bowdoin, ME, USA) with shaft diameter of 100 μm. Electrodes with impedance varying from 2 MΩ to 5 MΩ (catalog no. UEWMDHSE5NNH) were used in the different implanted arrays.

2.3. Multi-channel wireless neural headstage

The miniature, lightweight and low power wireless neural transmitter shown in Fig. 1A (W16, Triangle Biosystems Inc., 2224 Page Rd, Suite 108, Durham, NC, USA) operates in the 3.2 ± 0.1 GHz) frequency band. It weighs 4 g and is powered by a rechargeable battery (2.8 V@11 mA). The individual neural amplifiers in the wireless headstage have a hardware configured gain of 600 and a band

pass filter with the passband frequency range of 300–7000 Hz. The wireless transmitter uses two tuned chip antennas with circular diversity scheme. The range of the wireless transmission is up to 3 m in free space. The transmitter is connected to the warp-16 array using a miniature 20 pin connector (NSD–NPD pair, Omnetics Inc., 7260 Commerce Circle East, Minneapolis, MN, USA). The transmitter protective housing shown in Fig. 1C and D is secured to the array chamber using an adapter ring, a rectangular clamp and an outer cap. The array housing as well as the transmitter protective cap is constructed using a high strength polycarbonate material (Ultem). This protective structure is water-proof and was designed to minimize mechanical shock to the array-transmitter assembly during recording sessions when the marmoset is freely roaming within the plexiglass cage. The light weight and thin walled (1 mm thickness) transmitter protection cap did not degrade wireless signal from the neural headstage during initial system tests. The complete headstage mount including the transmitter (4 g), protection cap (7 g) and array (1 g) shown in Fig. 1C weighs 12 g (3% of marmoset body weight). The 4 g transmitter weight includes a rechargeable battery weighing a mere 1.8 g. Typically our recording sessions were 3 h long which is well within the 5 h limit on a single battery charge. This recording time included the time spent by animal in the primate chair for initial experimental setup. There are external battery options that can extend the recording length to 24 h and even 90 h with batteries weighing 12 g and 44 g, respectively. We did not use these options because of the weight limitations and the lack of space on the animal head cap to house a large battery. We did not notice any significant change in the movement of the animal within the recording cage when implanted with the wireless headstage.

2.4. Free-roaming neural and acoustic recording setup

The free-roaming neural and acoustic recordings were conducted in an RF/EMI and acoustically shielded chamber measuring 6 m × 3.7 m × 2.4 m (Fig. 2A). The RF/EMI shield is provided by a double-walled 28 gauge galvanized steel chamber (Series 81, ETS Lindgren, 400 High Grove Blvd, Glendale Heights, IL, USA) designed to minimize external electro-magnetic signals from interfering with the wireless neural data transmission. This ensures a highly controlled radio frequency environment within the recording chamber. At the wireless transmission frequency (3.2 ± 0.1 GHz) the RF chamber provides RF/EMI isolation greater than 110 dB. The entire inner surface of the recording chamber is lined with RF absorber cones (EHP-5CV, ETS Lindgren). These cones absorb incident RF energy and significantly reduce (∼35 dB) multi-path reflections within the chamber. Multi path reflections can cause random signal fades and can severely degrade the performance of a wireless communication system. This is especially the case in indoor physiology recording laboratories which are cluttered with large metallic (radio reflective) surfaces. The chamber is equipped with RF/EMI line filters for power supply filtering as well as filtered connector panel for RF/EMI isolation on the data I/O lines. During experimental recording sessions the radio frequency link within the chamber was continuously monitored using a broadband RF spectrum analyzer (R3172, Advantest America Inc., 3201 Scott Blvd, Santa Clara, CA, USA). The RF power spectrum of the transmitted signal was used to adjust the relative orientation of the receiver antenna and recording cage position in order to minimize radio link loss during experiments. The marmoset was free to move within a recording cage (Fig. 2B) measuring 60 cm × 41 cm × 30 cm and made up of plexiglass and nylon mesh. This combination of durable plastic material ensured low acoustic reflections and low attenuation of the line of sight radio signal. At all times the marmoset behavior was monitored using a series of video cameras inside the recording chamber. In addition to RF/EMI control, the recording enclosure was acoustically insulated to attenuate external sound transmission into the chamber. As shown in Fig. 2A and B this was achieved by using a combination of multi layer noise barrier material including acoustic absorber foam (3 in., Pinta acoustics Inc., 2601 49th Ave. N. Suite 400, Minneapolis, MN, USA), mass loaded dense vinyl sheets (0.25 in. thick) and fiberglass insulation (6 in. thick). This design resulted in a sound attenuation of approximately 50 dB (measured at 2 kHz).

2.5. Neural and acoustic data acquisition

The wireless neural headstage is directly connected to the Warp-16 array (Fig. 1A). After pre-amplification, the neural signals are band pass filtered (0.3–7 kHz) and then time division multiplexed. The multiplexed signal is amplified (×600) by a high bandwidth amplifier and modulated onto the 3.2 GHz carrier frequency. The receiver RF frontend includes additional filters and 60 dB gain blocks followed by the FM demodulator, phase lock loop, clock recovery and the de-multiplexer. The individual analog de-multiplexed neural signals are fed into a patch panel (ERP-27, Neuralynx Inc.) for appropriate reference setup (Fig. 2C). The analog signals are then routed to the lynx-8 amplifiers where they are further amplified (×3000) and band pass filtered (0.3–3 kHz). The USB-controlled amplifiers (Lynx-8, Neuralynx) are used to maximize the dynamic range of the first analog to digital conversion stage (NI-PCI-6071e, National Instruments, Austin, TX, USA). Neural signals are sampled at 20 kHz with 12 bit resolution.

The vocalizations of the freely moving marmosets are recorded using a set of 6 directional microphones (K6-C, Sennheiser, Old Lyme, CT, USA) placed inside the chamber. The microphone output is fed to a pre-amplifier (302 dual micphone preamp, Symmetrix, Mountlake Terrace, WA, USA) and filtered to prevent aliasing before being routed to the analog to digital converter (PCI-6052e, National Instruments) card where it is sampled at 50 kHz, 16 bits. The two NI DAQ cards are synchronized using the RTSI protocol which enables the use of a single common clock for both devices. Neural and acoustic data from two chronically implanted monkeys can be simultaneously recorded and monitored using the dual radio system (Fig. 2A and C). To ensure minimum radio interference the two radio transmitters operate at different carrier frequencies (3.2 GHz and 3.0 GHz). A custom Matlab graphic user interface (GUI) optimized to run on a multi-core windows based computer records the synchronized neural (32 channels) and audio (6 channels) into the computer hard drive. This Matlab GUI displays individual electrode signals (raw and sorted spikes) as well as a scrolling window of vocalization and spike firing patterns of user selectable neural channels. This provides the experimenter with real time feedback of neural responses to vocal events. The neural and acoustic data were continuously monitored to ensure high recording quality. Particular attention was paid to the multi-channel raw neural data streams to identify changes in background noise level and occurrence of radio fading. At the beginning of a recording session we checked for radio signal fading or signal glitches (transient signals with amplitude several times higher than rms background level and spike amplitude). These signal glitches when they occur would be common across all neural channels. The relative position of RF receiver antenna and recording cage was adjusted to maximize wireless signal strength as measured by the spectrum analyzer and reduce the occurrence of radio signal glitches.

2.6. Spike sorting

A template based spike sorting method was used to extract the action potential waveforms and their timestamps. This method described below is a modified version of the template matching reviewed in Lewicki, 1998. A Matlab based multi-channel spike

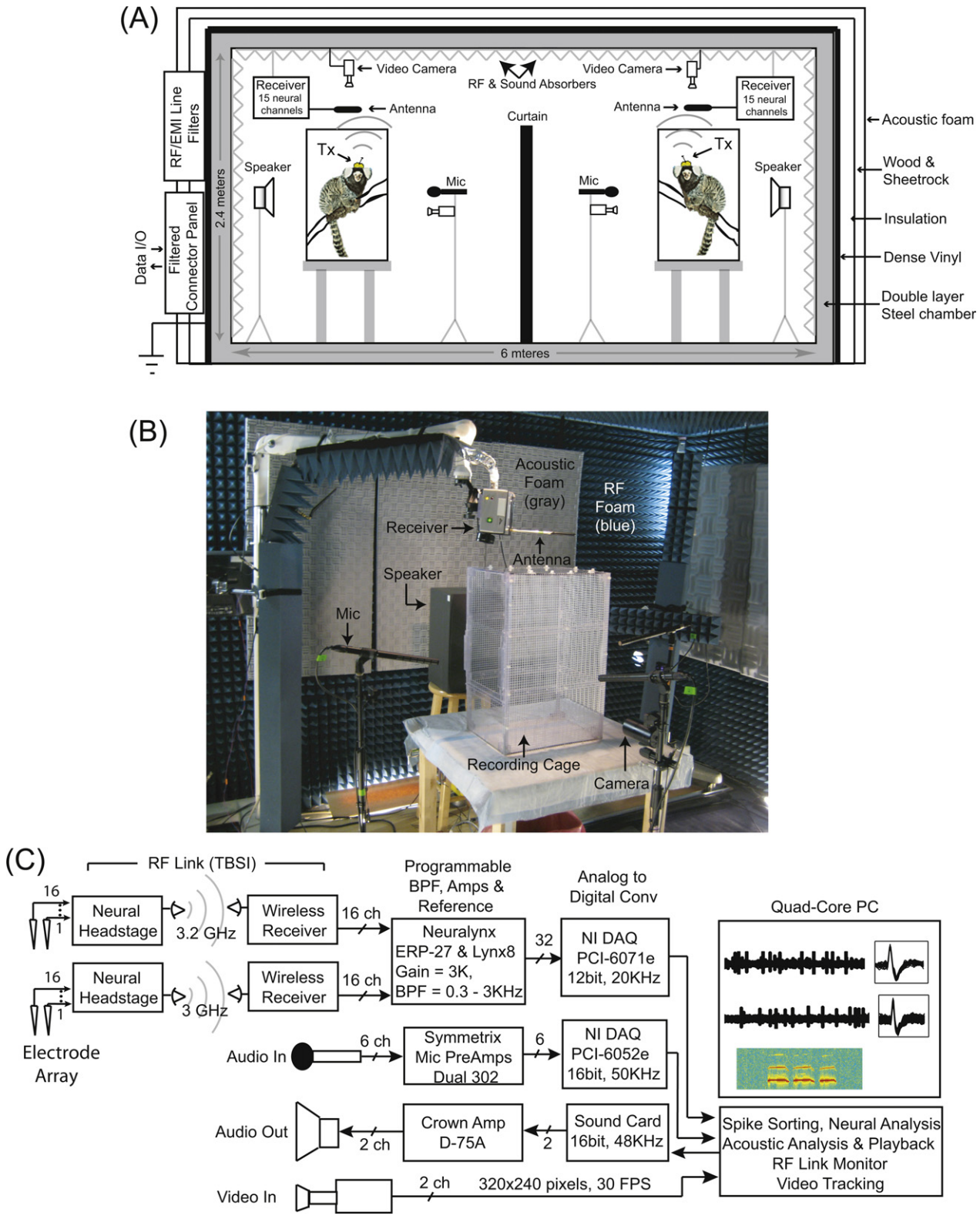

Fig. 2. Wireless free-roaming setup for recording multi-channel single-unit data from multiple marmosets during natural vocal behavior. (A) RF/EMI shielded (ETS Lindgren) and acoustically isolated recording chamber. The custom multi-layered chamber was designed to block external electromagnetic interference, internal radio reflections and attenuate external acoustic noise. The telemetry chamber is designed to record from multiple freely roaming and vocalizing marmosets in separate plexiglass cages. (B) The recording apparatus for a single animal including the plexiglass cage within which the marmoset is unrestrained, the position of the radio receiver, microphones, speaker and the RF absorption foam. (C) Schematic block diagram of the wireless neural recording, wired acoustic recording and wired acoustic playback apparatus.

sorting program was developed to separate the single-unit waveforms from the raw neural data. The initial threshold for an action potential detection was manually set (SNR > 2σ) as shown in Fig. 4A. Using two adjustable vertical windows (green and red vertical bars in Fig. 4B) an initial template of the action potential was created. The first 20 (user defined number) spike waveforms fitting this template were used to generate a more accurate twelve-point template. The user can change the standard deviation for each of the

twelve template points independently. The template can be fixed for the duration of the session or periodically updated. The template matching method was used for both online (during experiment) and offline spike sorting. The online spike sorter had some of its features disabled when recording more than eight neural channels in order to reduce the computational load on the recording computer. For example the offline spike sorter used a ten point spike template whereas the online sorter used a level threshold and a two point spike template.

3. Results

The wireless neural recording system reported here was tested in two adult marmosets (35U and 6207A). Neural spiking activity was recorded under different behavioral conditions such as spontaneous vocalizations and interactive vocal behavior (antiphonal calling) experiments. These recordings were carried out while the marmosets were freely roaming within a plexiglass cage in the telemetry chamber as shown in Fig. 2A and B. One marmoset (35U) was implanted with left and right hemisphere arrays whereas the other (6207A) was implanted with a right hemisphere array. Stable single-unit recordings were obtained from all three arrays over an extended time period of several months (35U-left hemisphere: 460 days, 35U-right hemisphere: 150 days, 6207A-right hemisphere: 270 days).

3.1. Recording quality

An example of wireless neural recording from the frontal cortex of a marmoset is shown in Fig. 3C, along with simultaneously recorded vocalizations produced by the same animal. Fig. 3A and B shows the voltage waveform and spectrogram of a 25 s audio recording from a spontaneously vocalizing and freely moving marmoset. The individual phee vocalizations are clearly identified in

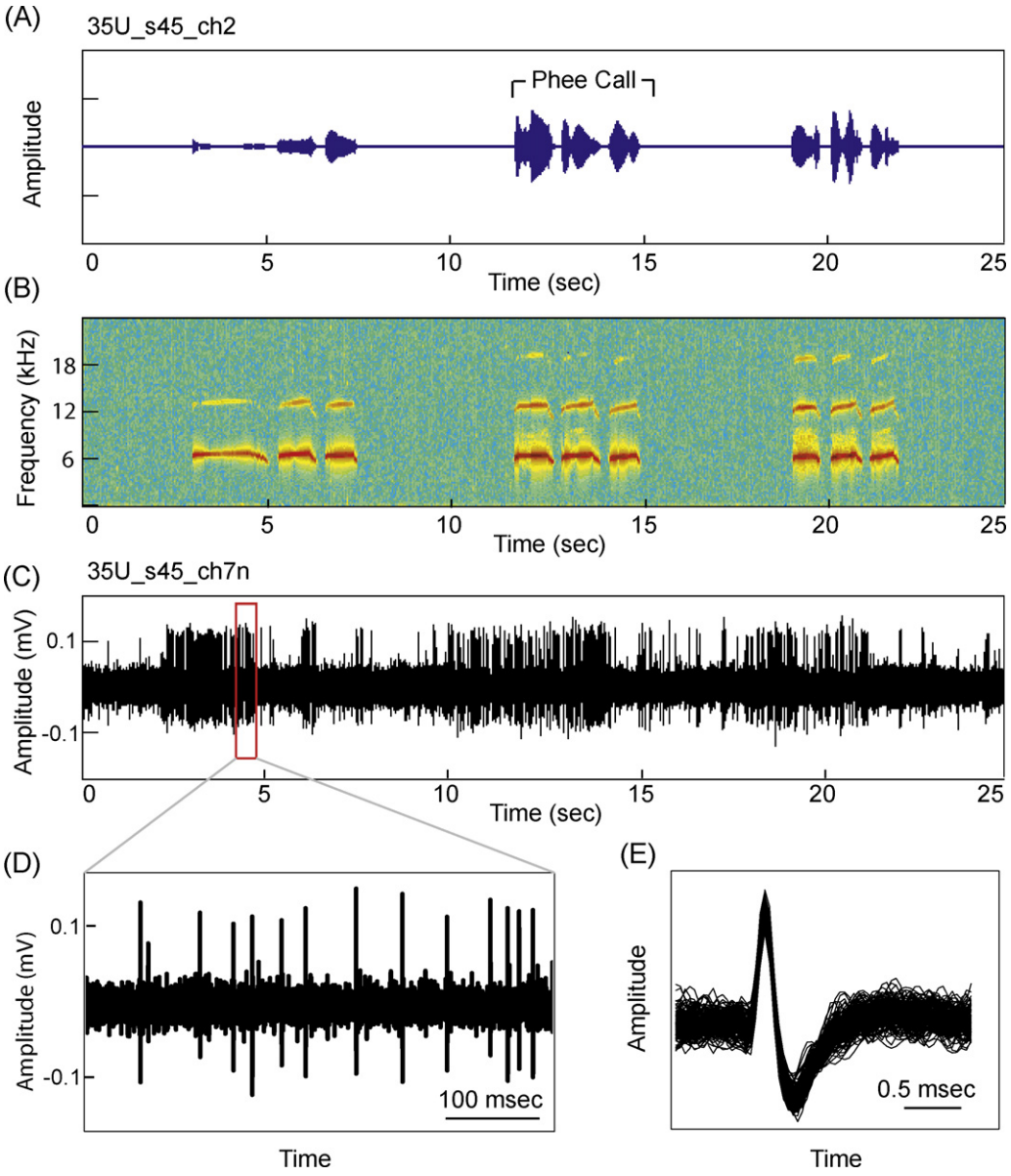

Fig. 3. Simultaneously recorded spontaneous phee vocalizations and neural action potentials from the premotor cortex of freely moving marmoset. (A) Voltage waveforms of three phee vocalizations spontaneously elicited by a marmoset. Each of the phee calls has three phrases in this example. (B) Spectrogram of the vocalizations show that the fundamental frequency for this particular marmoset phee call is 7 kHz. (C) Raw neural data trace from one of the electrodes. (D) Close up view of neural action potentials. (E) Action potentials extracted from the raw neural trace using the template based sorting technique.

the amplitude and frequency time plots. Fig. 3C and D shows a simultaneously recorded raw neural data trace from a single electrode of the Warp-16 electrode array. In Fig. 3E the individual action potential waveforms are plotted after the offline template based spike-sorting.

After the recording session the neural data were further analyzed starting with the offline spike sorting. Units were classified as single-unit or multi-unit based on the SNR and percentage of inter spike intervals (ISIs) less than 1 ms refractory period (threshold of 1% was set for single-units). The SNR is defined as the ratio of action potential peak to peak height and the standard deviation of the background noise over 0.3 ms preceding all spikes ($SNR = 20 \times \log_{10}(AP_{peak-peak}/noise_{SD})$). We chose a SNR threshold of 13 dB based on our data and that reported previously by Eliades and Wang (2008b). Fig. 4C–E shows the single-unit and multi-unit classification criteria using the combination of action potential SNR and percent ISIs < 1 ms.

3.2. Recording quality vs. distance

We further quantified the spike SNR change as a function of the line of sight distance and orientation between wireless headstage and receiver antennas. Signal to noise ratio was measured with a test signal (recorded spikes input to the wireless headstage) in 10 cm increments over a total distance of 5 m within the telemetry chamber. Measurements were taken with three different antenna orientations of 0°, 45° and 90° (angle between receiver antenna and transmitter antenna plane). The spike SNR remained stable within a 3 m separation between headstage and receiver as shown in figure (Fig. 5E). In all three orientations the spike SNR fluctuations were within ±0.25 dB for a range of 0.1–3 m.

3.3. Long term recording viability and yield

Fig. 5A shows four single-units recorded simultaneously eight days after implantation and Fig. 5B shows four different units recorded 260 days after implantation from the same electrodes but at different depths. The electrodes were periodically advanced with a minimum of 15 μm and maximum of 50 μm on a given day. We have analyzed 355 units with a median SNR of 15.5 dB as shown in Fig. 5C. Overall, the action potential SNR ranged from 8 to 26 dB. At the beginning of any given recording session not all the 15 electrodes had a good quality unit. The median number of active electrodes (unit with SNR ≥ 8 dB) was 8 per session and the maximum number of recorded units was 15 per session from a single array as shown in Fig. 5D. The units were classified as single-units if their SNR was greater than 13 dB and if the percentage of ISIs violating the 1 ms refractory period did not exceed 1%. About 69% (245 out of 355 units) of the units recorded using the wireless system were classified as single-units based on the two criteria. Out of the 355 units analyzed, only 14 units (4%) were lost by the end of recordings sessions. This stability and neural signal SNR are comparable to an earlier study using tethered recordings (Eliades and Wang, 2008b).

3.4. Effect of rapid movements on neural signal

To analyze the effect of rapid animal movements on the neural signal stability and quality we conducted additional experiments with simultaneous recording of audio, video and neural data while the marmoset roamed freely within its cage. Two video cameras were placed 20 cm above the recording cage such that the cage occupied most of the field of view as seen in Fig. 6A. Video was captured at 30 frames per second and a resolution of 320 × 240 pixels of the recording cage (top view) using a C905 video camera (Logitech, Fremont, CA, USA). The video capture from this camera was triggered every 2 s to synchronize it with the audio and neural recordings. The second camera captured continuous video signal and was used to cross check with triggered video captures for dropped frames. Fig. 6A shows the top view of the recording cage with a marmoset inside. The headstage protection cap on the marmoset was covered with red color tape in order to make it easily visible for video tracking between frames. The red rectangle in Fig. 6A indicates the position of the headstage protection cap. The center coordinates of the red rectangle are also shown. A custom Matlab program analyzed the video frames and calculated the marmoset position as well as the velocity of its movements. Fig. 6B shows locations of the marmoset relative to the cage made during a 2 min session. Red arrows indicate five large movements (>4 cm). During this recording session neural data from two electrodes were recorded as shown in Fig. 6C. The red ticks indicate the occurrence of movements greater than 3 cm.

Fig. 6D and E shows the corresponding (x, y) coordinates of the headstage protection cap and the velocity of the marmoset's movement, respectively. As seen from Fig. 6E, there were five instances within the 2 min recording session when the animal made rapid movements (velocity >1 m/s and distance >4 cm). The movement numbers of these five instances are indicated to the right of the black circles in Fig. 6E. Fig. 6F shows 60 ms of raw neural recording traces of the two electrodes aligned to the peak velocity in fifteen epochs when the marmoset made movements (corresponding to black circles in (E)). These neural traces show no signal drop or large transients that are typical when radio links are disrupted due to relative motion between transmitter and receiver antennas. We analyzed a total of 5 h of video and neural recordings and found that in rare cases there were large transients that occurred simultaneously across all the recorded channels. In approximately 6% of the total 480 movements (>3 cm) analyzed (30 transients in 5 h), we observed that the neural amplifier were saturated for brief time periods (10–30 ms) when the animal's head cap collided with the solid plexiglass surface of the recording cage. This signal distortion was easily detected as it simultaneously appeared in all recorded channels and was several times the magnitude of neural spike waveforms. In our data analysis we discarded vocal events and corresponding neural data when data analysis window (from 1 s before call onset to 1 s after call offset) included such motion artifacts.

3.5. Recording stability

We analyzed the signal to noise ratio (SNR) of spikes and the RMS of the background voltage level over the length of the sessions to quantify stability of our neural recordings. A total of 85 neural data streams (each 1 h long) were analyzed while marmosets were freely roaming and spontaneously vocalizing within recording cage. Fig. 7A shows the raw neural trace from two different recording sessions and Fig. 7B shows the close up view of the neural traces over a 2 s window in four segments of the raw data. Fig. 7C and D shows in 3D the evolution of the spike waveform from sessions 1 and 2, respectively. Fig. 7A–D shows no noticeable changes in the raw data traces and the spike waveform over the hour long neural recordings. In addition, Fig. 7E plots the SNR of spikes and Fig. 7F shows the RMS background level recorded over the duration of a session. The SNR of spikes varies within ±0.2 dB range (Fig. 7E) and the RMS background level varies over a ±2 μv range. In Fig. 7G and H we plot the mean and standard deviation of the change in spike SNR and change in RMS background level respectively from their initial values at the start of the sessions. These values were calculated in 60 s bins. In general there were relatively small spike SNR and the RMS background level variations within an hour of recording.

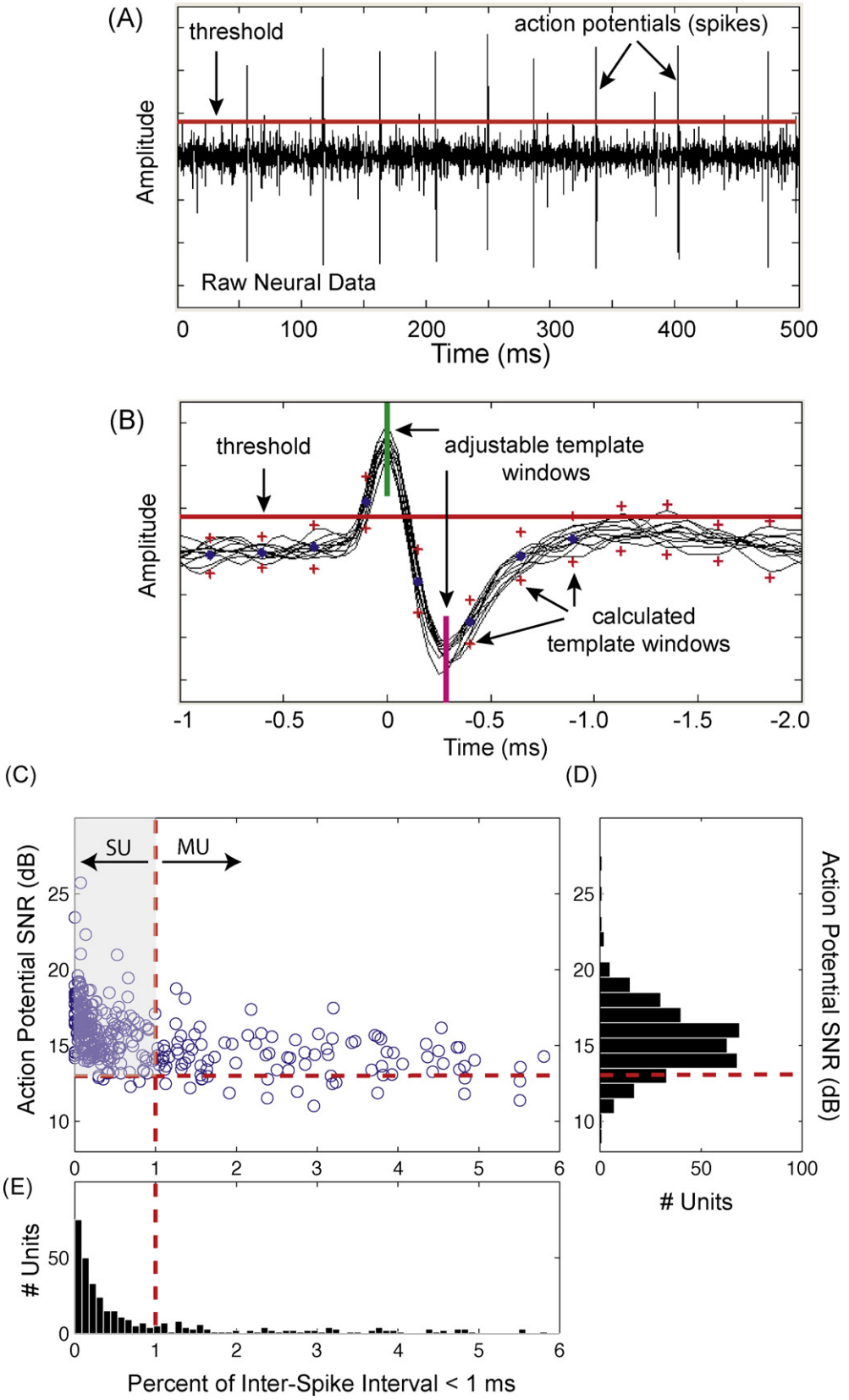

Fig. 4. Template based spike sorting, single-unit (SU) and multi-unit (MU) classification. Classification of unit was based on two criteria. First is the action potential SNR and second the percentage of ISIs that violated the 1 ms refractory period. (A) The 500 ms segment of raw neural data from a single electrode of the multi-electrode array. Action potentials of different amplitudes are indicated. (B) Action potential waveforms extracted from the raw neural data using template based spike sorting. The manually adjustable and automatically calculated spike template windows are shown. (C) Scatter plot of action potential SNR and percentage of ISIs less than 1 ms. We choose a 13 dB SNR threshold for single-units based on our data and that reported by Eliades and Wang (2008b). The maximum allowable refractory period a violation was 1% for single-units. (D) Distribution of action potential SNR with the red dotted line showing the threshold for single-units (≥13 dB). (E) Distribution of ISIs and the red dotted line shows the threshold for the maximum allowable percentage of refractory period violations (ISI < 1 ms). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

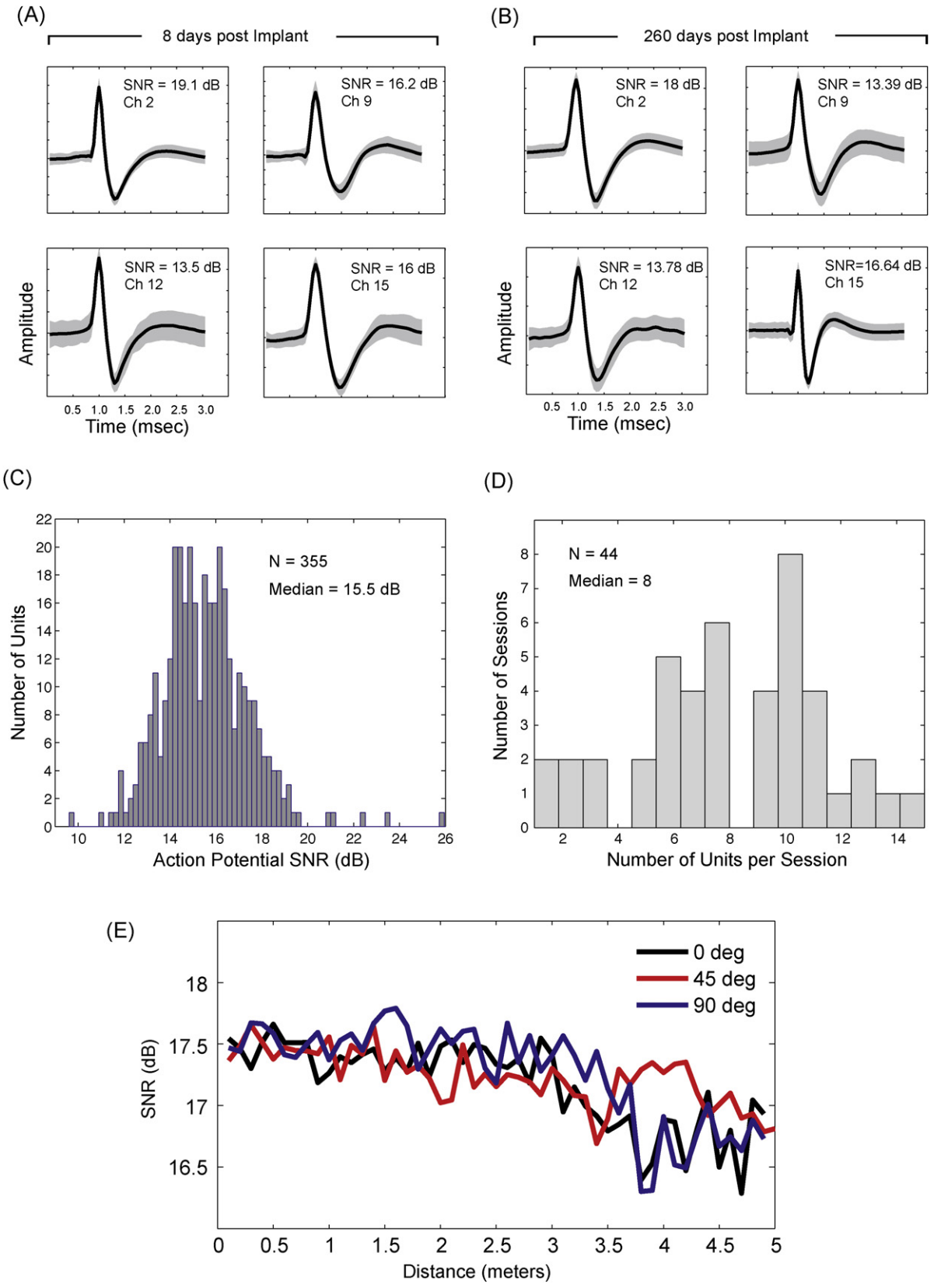

Fig. 5. Long term multi-electrode single unit recording stability. (A) Action potentials recorded from four electrodes from an electrode array eight days after implantation. Waveforms shown were extracted after template based spikesorting and the black solid trace is the mean action potential shape. (B) Action potentials from the same electrodes (different depths) shown 260 days after implantation. (C) Distribution of action potential signal to noise ratio (SNR). Median SNR is 15.5 dB for 355 units recorded using three implanted electrode arrays. (D) Distribution of the number of active electrode per recording session. Median number of units recorded in a given session was 8, slightly more than half the number of available electrodes. (E) Spike SNR as a function of separation between wireless headstage and receiver antenna. SNR was measured every 10 cm with three different antenna orientations (0°, 45° and 90° angle between headstage chip antenna plane and the receiver antenna).

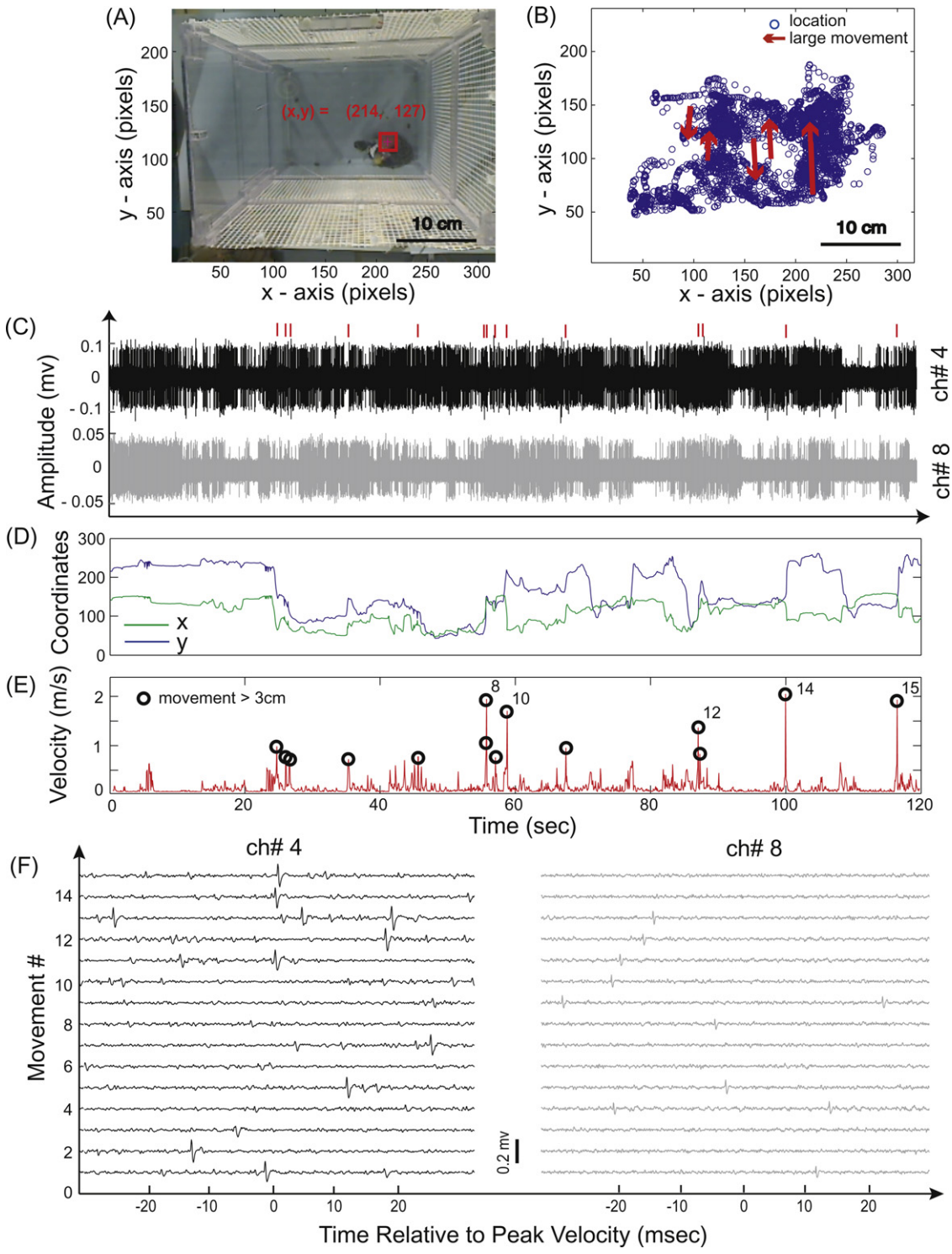

Fig. 6. Effect of marmoset motion on neural signal. (A) Top view of recording cage with the marmoset location indicated by the red rectangle and the center coordinates. (B) The different marmoset locations indicated in blue circles and the red arrows show large movements (>4 cm) within a 2 min recording session. (C) Raw neural data traces from two simultaneously recorded channels. Red ticks indicate the location of marmoset movement (>3 cm). (D) *X* and *Y* coordinates of marmoset red headstage cap (tracked object). (E) Velocity of marmoset's movement shown in red trace. Black circles indicate movements greater than 3 cm. The numbered circles indicate the movement number of the five instances when the animal made rapid movements (velocity >1 m/s and distance >4 cm). (F) The raw neural data traces centered on the movements indicated by the black circles in (E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The past decade has seen a rapid increase in application of multi-electrode neural recordings in free moving animal preparations such as bats, rodents and songbirds. Often, the methodology developed is tailored to the requirements of the particular target animal model or experiment design. For large scale neural ensemble recording from naturally behaving animals, a wireless data link is not just desirable but necessary in some cases. For instance, the marmoset is highly vocal in a laboratory environment but only when unrestrained. Tethered recording techniques have been previously used (Eliades and Wang, 2008a,b) but have major

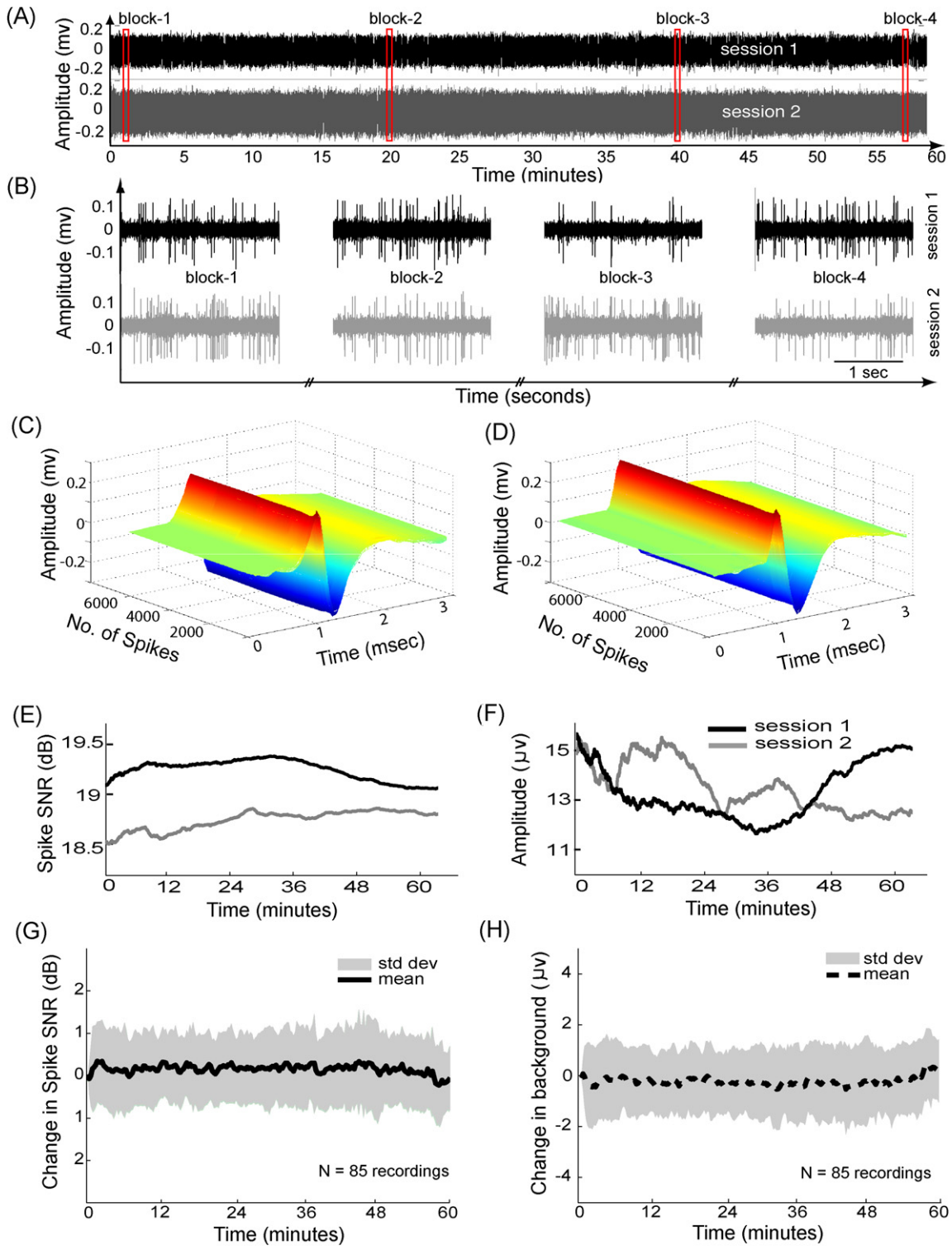

Fig. 7. Neural recording stability. (A) Raw neural data traces from two independently recorded channels. (B) Four close up views of the neural data from A at equally spaced time intervals. (C) Spike waveforms from session 1 shown in (A). (D) Spike waveform from session 2 shown in (A). (E) Spike SNR as a function of recording time of the two channels. (F) Background rms voltage level as a function of recording time. (G) The population mean and standard deviation of change in Spike SNR over the recording length. (H) The population mean and standard deviation of change in background rms voltage over the recording length.

limitations. These limitations arise from frequent interruptions of experiments due to damaged tether and animal getting entangled in the tether, etc. This is especially true if the behavior under study (vocalizations in this case) and the corresponding neural signals have to be recorded over long time periods. The ability of non-human primates to use their hands freely poses another challenge to neural recordings with tethered systems. Our experience with a tethered recording system showed that experimental sessions were frequently interrupted due to the marmoset climbing the tether, getting entangled in the tether or chewing on it. In this study we have demonstrated a wireless recording system that meets the requirements for recording single-units from multi-electrode arrays implanted in premotor cortex of the marmosets. We have successfully used this system to conduct multi-channel

extracellular recordings from two marmosets while they were freely roaming within their cages inside the telemetry recording chamber. Our setup ensures a controlled radio frequency environment necessary for uninterrupted and high fidelity neural signal recordings. The telemetry chamber (Fig. 2A and B) provides high RF/EMI isolation as well as acoustic isolation. One of the challenges with wireless transmission of high bandwidth data in an indoor environment such as a physiology lab or animal colony is multipath resulting due to radio signal reflections from surrounding materials. The RF absorber materials lining the inside surface of the telemetry chamber (Fig. 2A and B) attenuate the reflected (multipath) radio waves and ensure that the primary incident signal to the receiver antenna is the line of sight radio signal from the transmitter. We have successfully demonstrated the feasibility of such a system in long term neural recordings from the frontal cortex of freely moving and naturally vocalizing marmoset. In general, recording sessions were very rarely interrupted (except for battery recharge or adjusting receiver position) and the marmosets continued to produce vocalizations throughout the duration of the recordings. Such uninterrupted recording sessions are necessary to collect sufficient numbers of vocal interactions over the limited duration of experimental session.

4.1. Comparison with existing wireless multi-electrode systems

The Warp-16 implanted electrode array used in this wireless recording system was previously developed for use in marmoset cortical recordings (Eliades and Wang, 2008b), based on a multi-electrode array used in Hoffman and McNaughton (2002). The key features of the Warp-16 array are its lightweight (~1 g), independently movable electrodes and fine control of the recording depth (adjustment in micro meter resolution), flexibility in choosing metal electrode types and parameters and a relatively simple yet rugged drive mechanism. A detailed comparison with other existing drives was provided in Eliades and Wang (2008b). The choice of a suitable wireless headstage (including neural amplifiers and RF transmitter) was a crucial design parameter for the wireless system described here. The W16 (TBSI Inc.) wireless headstage is light, consumes low power and has a antenna design that maintains RF link under the high mobility three dimensional movement pattern of the marmosets. As mentioned before, other existing telemetry systems have subject-specific characteristics that would limit their use in a small, highly mobile and arboreal animal such as the marmoset. Previously developed neural telemetry systems (Hampson et al., 2009; Miranda et al., 2010; Szuts et al., 2011) would be too heavy for our applications (60 g, 114 g and 52 g, respectively). Other systems (Grohrock et al., 1997; Nieder and Klump, 1999; Mohseni et al., 2005; Schregardus et al., 2006) have a low channel count (2, 2, 4, 1 channels per device, respectively) that would under-utilize the 16 available channels of the Warp-16 array. Given the size and the nature of movement in the marmosets, the RF link range of some systems (Mohseni et al., 2005; Yin et al., 2009 with range of 0.5 m and 1 m, respectively) would limit their use in our experimental conditions. Generally the size of the battery is determined by the system power requirements. Systems with high power requirements such as (Hampson et al., 2009; Szuts et al., 2011 with total system power 230 mW and 645 mW, respectively) are too big to fit on the implant head cap and would require a jacketed backpack (Szuts et al., 2011) limiting their usability in the marmosets. Higher power consumption would also generate additional heat over long recording session and require thermal isolation from the tissue surface. Considering the design and experimental requirements for recording multi-channel single-units from freely moving marmosets, the combination of the W16 wireless headstage with the Warp-16 multi-electrode array is ideal.

4.2. Future system improvements

Enhancements to individual components of the wireless recording system reported here will enable a wider range of experiments and increase the yield (units recorded per session) of the current system. First, the relatively sparse spacing of the individual guide tubes (~700 μm) and unidirectional electrode travel could be modified, as discussed in Eliades and Wang (2008b). This limitation is a function of the individual guide tube diameter (30 gauge) and the parallel orientations of the tubes. By using a tapered design, the electrode spacing can be decreased (Sinha and Moss, 2007). Bidirectional electrode travel is possible with a screw-based drive but generally at the expense of additional hardware to translate the spiral motion to linear movement. Screw based drives have lower movement resolution and larger array cross section. Second, the full bandwidth of the RF link could be maximized to increase overall system yield. In the reported experiments, about half of 16 electrodes in the array had spikes with good signal quality (SNR > 8 dB) at any given time. Hence only 50% of the total available bandwidth of the neural telemetry device was used during any given recording session. An adapter that could enable recording from active electrodes from multiple arrays implanted on the same subject could double the yield and avoid using additional headstages. Such an adapter would have to be low profile and enable quick channel interchange without degrading the action potential SNR. Third, the capability to transmit additional data over the RF link such as audio, video from head cap mounted microphones and video cameras would enhance the ability to correlate the surrounding environment with the underlying neural activity. Fourth, longer battery life without significant increase in the size and weight of the headstage would enable longer continuous recording sessions. Currently available external battery options require a tethered connection to a separate jacket making it difficult to use in a marmoset. Our wireless recordings were conducted in a tightly controlled RF/EMI environment but such a controlled space is not always practical. For instance recording within an animal colony with large metal cages makes for a noisy radio transmission environment. Within such an indoor cluttered space it is difficult to prevent multipath fading (from large metal cages) and EMI. Improvements in the antenna design and frontend signal processing solutions to counter such environment specific challenges would add to the flexibility of this system.

Finally, the biggest challenge of the present multi-channel wireless recording system is the capacity of online data analysis (for both neural and acoustic data) to keep up with the rate of data acquisition. In order to give the experimenter online feedback about the observed neural activity we need the ability to not just isolate multiple single-units but to visualize the combined network level neural signal and correlate it with the ongoing natural behavior. This presents several challenges given the lack of a prior knowledge of the behavior (vocalizations) and the high channel count (32 channels for a dual radio system) of neural data. Performing reliable multi-channel spike-sorting, vocalization (and other behavior) detection and analysis as well as correlating them to the neural activity would require incorporating highly efficient online data analysis and neural network techniques. This would provide immediate useful feedback to the experimenter and greatly reduce offline data analysis therefore saving time and data storage requirements.

Acknowledgements

The authors would like to thank the technical team at Triangle Biosystems Inc., which designed and built the wireless headstage and the team at Neuralynx Inc., for their assistance with system integration. We thank Jenny Estes and Nathaniel Sotuyo for their help with animal care and Dr. Cory Miller for help with initial

animal testing. We also thank Marcus Jeschke, Luke Johnson and Xindong Song for manuscript comments and technical discussions. This research is supported by NIH grants DC005808, DC008578 and P30 DC 005211.

References

Ainsworth A, O'Keefe J. A lightweight microdrive for the simultaneous recording of several units in the awake, freely moving rat. J Physiol 1977;269:8–10.
Aitkin L, Park V. Audition and the auditory pathway of a vocal New World primate, the common marmoset. Prog Neurobiol 1993;41:345–67.
Battaglia FP, Kalenscher T, Cabral H, Winkel J, Bos J, Manuputy R, et al. The Lantern: an ultra-light micro-drive for multi-tetrode recordings in mice and other small animals. J Neurosci Methods 2009;178:291–300.
Bendor D, Wang X. The neuronal representation of pitch in primate auditory cortex. Nature 2005;436:1161–5.
Bezerra BM, Souto A. Structure and usage of the vocal repertoire of *Callithrix jacchus*. Int J Primatol 2008;29:671–701.
Burman KJ, Palmer SM, Gamberini M, Spitzer MW, Rosa MGP. Anatomical and physiological definition of the motor cortex of the marmoset monkey. J Comp Neurol 2008;506:860–76.
Burish MJ, Stepniewska I, Kaas JH. Microstimulation and architectonics of frontoparietal cortex in common marmosets (*Callithrix jacchus*). J Comp Neurol 2008;507:1151–68.
Buzsáki G. Large-scale recording of neuronal ensembles. Nat Neurosci 2004;7: 446–51.
Chen H, Wu J, Hyland B, Lu X, Chen JJJ. A low noise remotely controllable wireless telemetry system for single-unit recording in rats navigating in a vertical maze. Med Biol Eng Comput 2008;46:833–9.
Chestek CA, Gilja V, Nuyujukian P, Kier RJ, Solzbacher F, Ryu SI, et al. HermesC: low-power wireless neural recording system for freely moving primates. IEEE Trans Neural Syst Rehabil Eng 2009;17:330–8.
deCharms RC, Blake DT, Merzenich MM. A multi-electrode implant device for the cerebral cortex. J Neurosci Methods 1999;93:27–35.
DiMattina C, Wang X. Virtual vocalization stimuli for investigating neural representations of species–specific vocalizations. J Neurophysiol 2006;95:1244–62.
Eliades SJ, Wang X. Neural substrates of vocalization feedback monitoring in primate auditory cortex. Nature 2008a;453:1102–6.
Eliades SJ, Wang X. Chronic multi-electrode neural recording in free-roaming monkeys. J Neurosci Methods 2008b;172:201–14.
Epple G. Comparative studies on vocalization in marmoset monkeys (Hapalidae). Folia Primatol 1968;8:1–40.
Fee MS, Leonardo A. Miniature motorized microdrive and commutator system for chronic neural recording in small animals. J Neurosci Methods 2001;112: 83–94.
Gilja V, Chestek CA, Nuyujukian P, Foster J, Shenoy KV. Autonomous head-mounted electrophysiology systems for freely behaving primates. Curr Opin Neurobiol 2010;20:676–86.
Gray CM, Maldonado PE, Wilson M, McNaughton B. Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex. J Neurosci Methods 1995;63:43–54.
Gregory JA, Borna A, Roy S, Wang X, Lewandowski B, Schmidt M, et al. Low-cost wireless neural recording system and software. Conf Proc IEEE Eng Med Biol Soc 2009;2009:3833–6.
Grohrock P, Häusler U, Jürgens U. Dual-channel telemetry system for recording vocalization-correlated neuronal activity in freely moving squirrel monkeys. J Neurosci Methods 1997;76:7–13.
Hampson RE, Collins V, Deadwyler SA. A wireless recording system that utilizes Bluetooth technology to transmit neural activity in freely moving animals. J Neurosci Methods 2009;182:195–204.
Harrison RR, Kier RJ, Chestek CA, Gilja V, Nuyujukian P, Ryu S, et al. Wireless neural recording with single low-power integrated circuit. IEEE Trans Neural Syst Rehabil Eng 2009;17:322–9.
Hoffman KL, McNaughton BL. Coordinated reactivation of distributed memory traces in primate neocortex. Science 2002;297:2070–3.
Jog MS, Connolly CI, Kubota Y, Iyengar DR, Garrido L, Harlan R, et al. Tetrode technology: advances in implantable hardware, neuroimaging, and data analysis techniques. J Neurosci Methods 2002;117:141–52.
Jürgens U, Hage SR. Telemetric recording of neuronal activity. Methods 2006;38:195–201.
Keating JG, Gerstein GL. A chronic multi-electrode microdrive for small animals. J Neurosci Methods 2002;117:201–6.
Lewicki MS. A review of methods for spike sorting: the detection and classification of neural action potentials. Network 1998;9:R53–78.
Lu T, Liang L, Wang X. Neural representations of temporally asymmetric stimuli in the auditory cortex of awake primates. J Neurophysiol 2001a;85:2364–80.
Lu T, Liang L, Wang X. Temporal and rate representations of time-varying signals in the auditory cortex of awake primates. Nat Neurosci 2001b;4:1131–8.
Miller CT, Wang X. Sensory-motor interactions modulate a primate vocal behavior: antiphonal calling in common marmosets. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 2006;192:27–38.
Miller CT, Eliades SJ, Wang X. Motor planning for vocal production in common marmosets. Anim Behav 2009;78:1195–203.
Miranda H, Gilja V, Chestek C, Shenoy K, Meng T, Hermes D. A high-rate long-range wireless transmission system for simultaneous multichannel neural recording applications. IEEE Trans Biomed Circuits Syst 2010;4:181–91.
Mohseni P, Najafi K, Eliades SJ, Wang X. Wireless multichannel biopotential recording using an integrated FM telemetry circuit. IEEE Trans Neural Syst Rehabil Eng 2005;13:263–71.
Muthuswamy J, Okandan M, Gilletti A, Baker MS, Jain T. An array of microactuated microelectrodes for monitoring single-neuronal activity in rodents. IEEE Trans Biomed Eng 2005;52:1470–7.
Nicolelis MAL, Ribeiro S. Multi-electrode recordings: the next steps. Curr Opin Neurobiol 2002;12:602–6.
Nicolelis MAL, Ghazanfar AA, Faggin BM, Votaw S, Oliveira LM. Reconstructing the engram: simultaneous, multisite, many single neuron recordings. Neuron 1997;18:529–37.
Nieder A, Klump GM. Adjustable frequency selectivity of auditory forebrain neurons recorded in a freely moving songbird via radiotelemetry. Hear Res 1999;127:41–54.
Pistorio AL, Vintch B, Wang X. Acoustic analysis of vocal development in a New World primate, the common marmoset (*Callithrix jacchus*). J Acoust Soc Am 2006;120:1655–70.
Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. J Neurosci Methods 2005;148:1–18.
Schregardus DS, Pieneman AW, Ter Maat A, Jansen RF, Brouwer TJF, Gahr ML. A lightweight telemetry system for recording neuronal activity in freely behaving small animals. J Neurosci Methods 2006;155:62–71.
Sinha SR, Moss CF. Vocal premotor activity in the superior colliculus. J Neurosci 2007;27:98–110.
Suner S, Fellows MR, Vargas-Irwin C, Nakata GK, Donoghue JP. Reliability of signals from a chronically implanted, silicon-based electrode array in non-human primate primary motor cortex. IEEE Trans Neural Syst Rehabil Eng 2005;13:524–41.
Szuts TA, Fadeyev V, Kachiguine S, Sher A, Grivich MV, Agrochão M, et al. A wireless multi-channel neural amplifier for freely moving animals. Nat Neurosci 2011;14(2):263–9.
Wang X. On cortical coding of vocal communication sounds in primates. Proc Natl Acad Sci U S A 2000;97:11843–9.
Wang X, Lu T, Snider RK, Liang L. Sustained firing in auditory cortex evoked by preferred stimuli. Nature 2005;435:341–6.
Yin M, Lee SB, Ghovanloo M. In vivo testing of a low noise 32-channel wireless neural recording system. Conf Proc IEEE Eng Med Biol Soc 2009;2009:1608–11.