Semi-chronic laminar recordings in the brainstem of behaving marmoset monkeys

Thomas Pombergera,b, Steffen R. Hagea,⁎

a Neurobiology of Vocal Communication, Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Otfried Müller-Str. 25, 72076 Tübingen, Germany
b Graduate School of Neural & Behavioural Sciences – International Max Planck Research School, University of Tübingen, Osterberg-Str. 3, 72074 Tübingen, Germany

ARTICLE INFO

Keywords:
Callithrix jacchus
Semi-chronic recordings
Implanted electrodes
Laminar multi-site microprobes
Deep brain recordings
Single-unit recordings

ABSTRACT

Background: Chronic recordings with multi-electrode arrays are widely used to study neural networks underlying complex primate behaviors. Most of these systems are designed for studying neural activity in the cortical hemispheres resulting in a lack of devices being capable of simultaneously recording from ensembles of neurons in deep brainstem structures. However, to fully understand complex behavior, it is fundamental to also decipher the intrinsic mechanisms of the underlying motor pattern generating circuits in the brainstem.

New method: We report a light-weight system that simultaneously measures single-unit activity from a large number of recording sites in the brainstem of marmoset monkeys. It includes a base chamber fixed to the animal’s skull and a removable upper chamber that can be semi-chronically mounted to the base chamber to flexibly position an embedded micro-drive containing a 32-channel laminar probe to record from various positions within the brainstem for several weeks.

Results: The current system is capable of simultaneously recording stable single-unit activity from a large number of recording sites in the brainstem of vocalizing marmoset monkeys.

Comparison with existing methods: To the best of our knowledge, chronic systems to record from deep brainstem structures with multi-site laminar probes in awake, behaving monkeys do not yet exist.

Conclusions: The semi-chronic implantation of laminar electrodes into the brainstem of behaving marmoset monkeys opens new research possibilities in fully understanding the neural mechanisms underlying complex behaviors in marmoset monkeys.

1. Introduction

The marmoset monkey (Callithrix jacchus) has recently garnered considerable interest as a neuroscientific model organism (Miller et al., 2016). The renewed focus on this already established animal model species (Eliades and Wang, 2008a, 2003; Fritsches and Rosa, 1996; Roberts and Wallis, 2000) has primarily been driven by the prospect of developing primate transgenic lines (Sasaki et al., 2009), but also by the potential for transferring a number of rodent neuroscientific techniques to a small primate model system (Miller et al., 2016) that can be used in controlled experimental designs (Song et al., 2016). Furthermore, marmoset monkeys are social and highly vocal New World monkeys making them an ideal model system to investigate cognition and social communicative behavior (Borjon and Ghazanfar, 2014; Burkart and Finkenwirth, 2015).

Recently, several chronic multi-electrode systems have been developed to record from several cortical brain regions, such as the premotor and auditory cortex, in behaving marmoset monkeys (Eliades and Wang, 2008b; Roy and Wang, 2012). These chronic systems have numerous advantages over acute recordings, such as increased yield and improved recording stability, as well as the ability to simultaneously record from a large number of neurons during complex behavior (Eliades and Wang, 2008a). In contrast, there have only been a few approaches to record from brainstem structures in awake and behaving monkeys, and mammals in general (Jürgens and Hage, 2006) – and these systems only measured neuronal activity at most from two positions. However, in light of recent work indicating that complex microcircuits are involved in motor behaviors such as respiration (Anderson et al., 2016; Del Negro et al., 2018; Harris et al., 2017) and vocalization (Hage and Jurgens, 2006; Hage and Nieder, 2016; Jürgens, 2002), as well as in audio-vocal integration mechanisms (Hage et al., 2006; Luo et al., 2018), it is necessary to simultaneously record from an ensemble of neurons within such circuits to fully understand the intrinsic mechanisms of these motor pattern generating brainstem...
structures.

Here, we report a light-weight system to simultaneously measure single-unit activity from a large number of recording sites in the brainstem of marmoset monkeys. It includes (1) a base chamber fixed to the animal’s skull and (2) a removable upper chamber that can be semi-chronically mounted to the base chamber to flexibly position an embedded micro-drive containing a 32-channel laminar probe to record from various positions within the brainstem for several weeks. The upper chamber can be removed and repositioned to record from new brainstem positions. This newly developed semi-chronic recording device combines the advantages of chronic recording systems, including stable recordings and short preparation times, with those of acute approaches, such as the flexibility in the choice of recording sites.

2. Material and methods

2.1. Animals

The system has been designed for the use in common marmoset monkeys (Callithrix jaccus). The animals used in this study are housed at the University of Tübingen and were all born in captivity. The facility room was maintained at approximately 26 °C, 40–60% relative humidity, and with a 12 h:12 h light-dark cycle. The marmosets had ad libitum access to water and were fed daily with standard commercial chow and a selection of fruit, vegetables, mealworms, and locusts. Marshmallows and special fruit (e.g., banana, grapes) were used as positive reinforcement. Experimental procedures were approved by the local authorities of Tübingen (Regierungspräsidium) and are in agreement with the guidelines of the European Community for the care of laboratory animals.

2.2. Animal preparation and laminar probe implantation

All surgical procedures were performed under aseptic conditions and general endotracheal anesthesia and were in accordance with the guidelines for animal experimentation and authorized by the Regierungspräsidium Tübingen. For the attachment of the base chamber (Fig. 1A–C), the animal was held using a stereotaxic apparatus (Kopf Instruments). The skin and underlying muscles were excised from the top of the skull. Four small elongated trepanations were drilled (Piezosurgery touch, Mectron, Germany) for the placement of the titanium anchor screws (Fig. 1A and D) around the desired position of the light-weight titanium base chamber (material: Ti-6Al-4V, weight: 1.4 g) as shown in Fig. 1E. The titanium anchor screws (M 1.6) were inserted into the holes, with the flattened head pushed into a position between the skull and dura, turned by 90° to ensure fixation, and fixed with nuts. The remaining openings were closed with bone wax and the screws and skull below the desired chamber position were covered with a thin layer of dental acrylic (Superbond, Sun Medical Co. Ltd., Japan). Next, the base chamber was positioned using the aid of a robotic stereotactic micromanipulator (Neurostar, Dettingen, Germany) with the center of the brain being stereotactically positioned above the center of the area of interest in the brainstem (here: AP = 0, ML = 0 according to the stereotactic brain atlas coordinates (Paxinos et al., 2012)). The stereotaxic placement of the base chamber in combination with the ability to precisely position the microdrive within the base chamber then enables stereotaxic positioning of the laminar probe in the brainstem. The chamber was then fixed to the skull with self-adhesive resin cement (Relyx Unicem, 3M Germany) covering the anchor screws and outer side of the chamber (Fig. 1B and E). Between chamber fixation and subsequent probe implantation, the base chamber was covered with a titanium protective cap (Fig. 1F and G) that was screwed to the chamber.

The light-weight semi-chronic laminar probe device (“upper chamber”, Fig. 1A and B; Neuronexus, USA; material: 3d printed plastic (VisiJet M3 Crystal), weight: 5.3 g) was implanted a few weeks after chamber fixation. In a stereotaxic surgery, a small trepanation was performed within the base chamber at a position just above the stereotactic coordinates at which the laminar probe was to be implanted (Fig. 1B), while the dura was maintained intact. For our experiments, we decided to use 32-channel laminar probes (VI × 32-Edge, Neuronexus, USA; impedance: ~ 1 MΩ, probe dimensions at base: 50 × 175 μm) to be able to simultaneously record from several positions. The upper chamber was then screwed on to the base chamber and the laminar probe was lowered into the brain via the micro-drive until the tip of the probe reached the upper rim of the brainstem. Hereby, the laminar probes could be precisely positioned by turning the thread of the micro-drive in full or partial turns (one full turn lowers the electrode by 250 μm). At the end of the surgery the lower part of the chamber was filled with artificial dural sealant (Dura-Gel, Cambridge Neuretech, UK) to seal the trepanation and the chamber was closed with a removable protective cap (material: Ti-6Al-4V, weight: 2.6 g; Fig. 1A, B, F and G). Following surgery, animals underwent analgesic and antibiotic treatment for three to five days, which were given orally via fruit or marshmallow pieces, to allow optimal recovery. Additionally, and in accordance with a recent study, the behavior of each animal was monitored daily for 20 min for 5 days post-surgery using ethograms (Hage et al., 2014).

Special effort was put into the development of the upper chamber to allow maximum flexibility in laminar probe placement, i.e., positioning of the micro-drive within the chamber. We therefore designed the upper chamber in such a way that the micro-drive could be flexibly positioned within the upper chamber to position the laminar probe across almost the entire range from the center to the outer rim of the base chamber. This was accomplished by mounting the micro-drive to the upper chamber at three different positions, with the additional possibility to slightly shift the micro-drive back and forth via oblong screw-holes (Fig. 2A). In addition, the upper chamber could be mounted in twelve possible positions to the base chamber with three screws (Fig. 2B). The combination of flexibly positioning the micro-drive and, therefore, the laminar probe within the upper chamber and mounting the latter on the base chamber in multiple positions enables neural recordings from positions encompassing the entire lower brainstem and most of the upper brainstem with a single chamber implantation (Fig. 2B and C).

2.3. Neural and vocal recording setup

Prior to implantation, monkeys were trained to sit in a primate chair in a soundproof chamber. Vocalizations were recorded via a microphone (MKH 8020 microphone with MXZ 8000 preamplifier, Sennheiser, Germany; phantom power for microphone by PAN 48.2, Palmer, Germany) positioned 10 cm in front of the monkey’s head (Fig. 3). Each time the monkey uttered a vocalization, regardless of call type, they received a liquid reward (mixture of water, marshmallow, fruit, Gummi arabicum, and curd cheese) provided by a small metal syringe directly in front of the monkey’s face that was fed by a computer-controlled syringe pump (Pump 11 Elite, Harvard Apparatus, USA). With this approach, we found that monkeys produced a high number of calls. Vocal detection and reward presentation were synchronized and performed automatically with a custom-written program (OpenEX and Synapse, Tucker-Davis Technologies, USA) running on a workstation (WS-8 in combination with an RZ2 bioamp processor and RZ6D multi I/O processor, Tucker-Davis Technologies, USA). Vocalizations were recorded using the same system with a sampling rate of 100 kHz (Fig. 3). Additionally, a loud speaker and a monitor screen connected to a desktop PC were positioned in front of the animal’s head for potential visual and acoustic stimulus presentations in later training stages.

At the beginning of a daily session monkeys were transferred from the animal facility to the experimental setup. For this, animals were trained with positive reinforcement to directly enter the primate chair when attached to their home cage. In the soundproof chamber, the monkey was placed in front of the microphone and a metal syringe was
placed in front of the animal’s head. The laminar probe was lowered to a new recording position. The probe was connected to a neural preamplifier (PZ2-32, Tucker-Davis Technologies, USA) via a motorized commutator (ACO32, Tucker-Davis Technologies, USA; Fig. 3) that was connected to the neural recording system (RZ2, Tucker-Davis Technologies, USA) and synchronized with the vocal recording system (RZ6D, Tucker-Davis Technologies, USA). With this approach animals were able to freely move their head during recording sessions. Sessions lasted approximately 20 min, i.e., the period in which the animals vocalized with high call rates. Monkeys were monitored via a high-resolution webcam (Brio, Logitech, Switzerland) during the entire session. The video signal was also stored on the workstation in synchronization with the vocal and neural data (Fig. 3).

2.4. Neural and acoustic data analyses

Signal acquisition, amplification, and filtering were performed using the Tucker-Davis Technologies system. Spike sorting was performed using standard software (Offline Sorter, Plexon, USA). Data analysis was accomplished using MATLAB (MathWorks, Natick, MA). Call on- and offsets were manually flagged offline using standard software (SASLabPro version 5.2, Avisoft Bioacoustics, Germany). Call duration was calculated as the difference between the beginning and end of the vocalization. Call types were manually classified as reported earlier (Gultekin and Hage, 2018, 2017) and in accordance to the previous literature (Agamaite et al., 2015; Bezerra and Souto, 2008; Takahashi et al., 2015).
In addition to their response to self-generated vocalizations, all recorded neurons were also tested with acoustic stimuli to determine their auditory response properties and distinguish vocal-motor, purely auditory, and audio-vocal neurons. Auditory stimuli included white noise and representative samples of seven marmoset vocalization types (phee, trill, chirp, tsik, ek, twitter, chatter). A neuron was determined to be auditory responsive if it responded to at least one of the above stimuli.

### 3. Results

The semi-chronic recording system reported here has been tested in three adult marmoset monkeys (one female: 5 years old, weighing 520 g; and two males: 2.5 and 6 years old, weighing 360 g and 420 g at day of base chamber implantation). The base chambers have been implanted for several months in all three monkeys (monkey S: 502 days, monkey H: 391 days, monkey F: 175 days). Neural activity has been recorded while the animals were vocalizing and chair-restrained in a soundproof chamber (Fig. 3). In all three monkeys, 32-channel laminar probes were semi-chronically implanted enabling stable neural recordings for an extended time period of several months (monkey S: 82 days; monkey H: 90 days, monkey F: 15 days (experiment still in progress)).

#### 3.1. Recording quality

One of the significant advantages of our newly developed system is the capability to semi-chronically record neural activity simultaneously from multiple linear sites in the brainstem of behaving marmoset monkeys. In our approach, we use laminar probes with 32 contact sites, 60 μm apart, allowing neuronal recordings across an almost 2-mm linear range. Fig. 4A and B shows exemplar recordings with the laminar probes in the upper brainstem (Fig. 4C) highlighting several neighboring channels showing stable neuronal activity with well-isolated single unit activity. Due to the close proximity of recording sites, it is possible to record the same neuron at more than one recording site. The
signal-to-noise ratio (SNR) of the spikes with respect to the root mean square (RMS) of the background voltage level was calculated for all recorded neurons with a median value of 19.9 dB (Fig. 4A and B). This enables proper spike sorting as indicated by the small standard deviations shown for all sorted spike waveforms (Fig. 4A and B).

3.2. Recording stability

To quantify the stability of the neural recording, we evaluated the change in the SNR of the spikes with respect to the RMS of the background voltage level over the period of an entire recording session. Fig. 5A shows the raw trace of an exemplar recording in the upper brainstem of a vocalizing marmoset monkey restrained in a monkey chair with a freely moving head. Fig. 5B shows the development of the spike form over the session, Fig. 5C depicts the change in SNR over time, and Fig. 5D the calculated recording position of the neuron shown in Fig. 5A. No noticeable changes were observed in the raw data trace as well as the spike waveform during the recording session. Consequently, the SNR of the spikes varies only in a range of ± 0.3 dB (STD), reflecting small SNR variations throughout the recording session.

3.3. Measurement of event-related neural activity

In our lab, we use the newly developed semi-chronic recording system to record from multiple sites in the brainstem of vocalizing marmoset monkeys. Fig. 6A–C shows an exemplar recording of two neurons at the same recording site in the ventrolateral pontine brainstem. Fig. 6A shows an example of a neuron exhibiting vocalization-correlated activity. The neuron shows an increased firing rate just before the onset of chirp vocalizations that decreases during call production. During chirp-trill call combinations the neuron shows a similar activity with an increase in activity prior to the onset of the call sequence and inhibition during every single chirp and trill call following in the sequence. At the same contact of the laminar probe a second neuron was isolated showing a decrease in activity during the playback of phee vocalizations (Fig. 6B).
Fig. 5. Neural recording stability. (A) Raw neural data trace. (B) Spike waveforms from the session shown in (A). (C) Spike signal-to-noise ratio as a function of time. (D) Calculated position of the recording site shown in (A).

Fig. 6. Vocalization-correlated activity of single neurons in the lower marmoset brainstem. (A) Exemplar neuron showing a significant increase in neural activity prior to and a significant decrease in activity during the production of self-initiated chirp vocalizations (left; n = 100 calls) and chirp-trill sequences (right; n = 10 sequences). Please note that sequences were produced with a different number of trill calls and different inter syllable interval durations. The neural activity, which was directly correlated to the exemplar chirp-trill sequence shown in the upper right spectrogram is highlighted in red in the raster plot on the right. (B) Exemplar neuron showing a significant decrease in response to double phee playbacks (n = 10 playbacks). Upper panels in (A) and (B) show spectrograms of the produced vocalizations and playbacks, respectively. Middle panels show raster plots, lower panels represent the corresponding mean spike density histograms (± standard error) averaged and smoothed trial-wise with window sizes of 25 ms (for left panel in (A)) and 100 ms (for right panel in (A) and (B)). The vertical dark gray lines indicate the onsets and offsets of the produced calls in (A) and playback in (B). The vertical dark gray bars at the end of the calls in (A) indicate the median call/sequence duration and the upper and lower borders of the light gray bars the first and third quartile of the call/sequence distributions, respectively. Insets in (A) and (B) show the mean waveform of the sorted units (± standard deviation). (C) Calculated position of the recorded neurons shown in (A) and (B).
4. Discussion

We developed a semi-chronic recording system with laminar multi-electrodes to record from deep subcortical brain structures of vocalizing marmoset monkeys in a controlled experimental design. The system enables recording from various positions within an implanted titanium chamber using laminar probes attached to a micro-drive. The recording system is therefore capable of flexibly recording from large fractions of upper and lower brainstem regions. This device will help decipher brainstem-based neural networks underlying complex social behavior in marmoset monkeys. Recently, we showed that acoustic perturbation rapidly interrupts ongoing vocal behavior (Pomberger et al., 2018). With the developed system we are able to elucidate if and how vocal motor brainstem circuits are involved in such audio-visual integration mechanisms. Furthermore, future research is now able to investigate ensembles of neurons embedded in complex neural microcircuits underlying other motor behaviors such as respiration (Anderson et al., 2016; Del Negro et al., 2018; Harris et al., 2017) that are predominantly generated by brainstem-based neural networks.

Up to now, only a few systems with chronically implanted multi-electrode arrays have been developed for the use in small primates (Eliades and Wang, 2008b; Roy and Wang, 2012). These systems allow stable recordings from up to 16 electrodes in the cortical hemispheres from behaving marmoset monkeys. However, chronic systems to record from deep brainstem structures in awake, behaving monkeys are virtually absent. To the knowledge of the authors there is only a single study that performed recordings with chronically implanted electrodes in the lower brainstem of behaving squirrel monkeys (Jürgens and Hage, 2006). In this study they were able to simultaneously record from up to two electrodes at a time which were mounted to a micro-drive and the electrodes could be reversibly implanted with the aid of a grid that was attached to the head of the animals. Inspired by the micro-drive design of this study, we used a similar approach in our current study. For more variability in electrode positioning, however, we chose to use a chamber in which the electrodes could be flexibly positioned and implanted.

The developed system combines a number of important features. First, it is lightweight, which is essential because of the small size of the marmoset monkey. Furthermore, with a height of up to 30 mm, it does not unbalance the monkey’s head enabling the animal to freely move around in its home cage with no limitations in between recording sessions. Second, it can perform stable recordings simultaneously from 32 channels via laminar probes enabling dense recording to disentangle the potential intrinsic properties of microcircuits. Third, the probes can be flexibly positioned within the implanted chamber repetitively enabling dense recordings from most brainstem regions within one animal. Fourth, the design of the system is flexible enough to accommodate a wide range of probe designs (e.g., number of recording sites, arrangement of sites on probe) to flexibly adapt the system to the needs of specific experimental approaches. Finally, the developed system will be commercially available (Neuronexus) and useful for research groups lacking sophisticated machining and electronics expertise.

Acknowledgments

We thank the entire team at Neuronexus who developed the recording systems for us in close collaboration and continue to adapt the design to our ever-changing needs. We are grateful to Peter Kronen for his exquisite support in anesthesia and Peter Dicke for assistance during surgeries. We thank John Holmes for proofreading. This work was supported by the Werner Reichardt Centre for Integrative Neuroscience (CIN) at the Eberhard Karls University of Tübingen (CIN is an Excellence Cluster funded by the Deutsche Forschungsgemeinschaft (DFG) within the framework of the Excellence Initiative EXC 307) and the Deutsche Forschungsgemeinschaft (Grant HA5400/3-1).

References