Recording elicited and voluntary muscle activity of rodents using high-density electromyography arrays

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Abstract

High-density electromyography (hdEMG) enables the study of motor unit (MU) activity by exploiting the spatial information of multichannel recordings. While myomatrix arrays have recently allowed recordings from awake rodents, thin-film electrodes have been restricted to acute or anesthetized preparations. Here we present pilot experiments demonstrating the feasibility of recording MU activity from upper- and lower-limb muscles of rats during natural behaviour using both devices. Electrode functionality was confirmed by compound responses elicited by nerve stimulation, and individual MU action potentials were observed during reflexive and voluntary movements. Results constitute the first demonstration of thin-film arrays in awake rodents and provide valuable insight into surgical protocols, electrode configurations, and acquisition strategies that could guide the development of stable hdEMG approaches for MU decomposition in small-animal models.

1. Introduction

Electrophysiological methods are the gold standard in neuroscience because they reveal the activity of individual neurons at high temporal resolution and in arbitrary brain locations [1]. These methods extend from non-invasive recordings such as electroencephalography, which capture broad population dynamics from the scalp, to intracortical electrodes that penetrate brain tissue to isolate the spiking of individual neurons. However, continued exploration of alternative methods for recording central nervous system (CNS) activity is vital to mitigate the limitations of current technologies while enabling the development of complementary strategies that further enhance safety and efficiency in neurotechnologies. In place of direct brain recordings, signals in the CNS could be investigated by decoding the electrical activity produced at its outputs [2].

Motor neurons (MNs) constitute the output layer of the nervous system driving movement. A typical muscle is controlled by a few hundred MNs whose cell bodies are clustered in the spinal cord or brain stem, and whose axons run in a peripheral nerve to the muscle. When each axon reaches the muscle, it branches an innervates from a few to several thousand muscle fibers. A motor neuron and the muscle fibers it innervates are known as a motor unit (MU), the basic functional unit by which the nervous system controls movement. When synaptic input depolarizes a MN above threshold, it generates an action potential that propagates along its axon to the neuromuscular junction, where it elicits action potentials in the muscle fibers it innervates. Because the action potentials of all fibers within a MU occur nearly simultaneously, their extracellular currents sum to produce a field potential which can be regarded as an amplified reflection of the original MN action potential [3]. By recording the resulting electrical activity at the periphery, it becomes possible to characterize the inputs to MNs and thereby estimate central signals indirectly [2].

Among the available technology to record muscle signals, high-density electromyography (hdEMG) enables the identification of multiple MU spikes through blind source separation [4], [5]. Given controlled, isometric, voluntary contractions, these methods are providing valuable insights into MU behavior in both healthy and pathological human populations. But while human studies have retained a unique role given their ability to shed direct light on the mechanisms underlying the control of voluntary movement, much of the primary knowledge of neural circuits has come from animal experiments [6]. Preclinical models offer high experimental control over both healthy and pathological conditions, provide access to detailed physiological information through invasive techniques, and enable the safe exploration of interventions—such as neuromodulation—whose short-, medium- and long-term effects remain unclear, serving as an important translational bridge toward human neurotechnological applications.

Animal studies of MU behavior initially relied on highly selective electromyography systems that preferentially captured spikes from a single nearby MU rather than the coordinated activity of many. First attempts to record MUs *in vivo* from the muscles of freely moving rats involved the insertion of bipolar fine-wire electrodes into the center portion of the muscles, tunneled under the skin and fixed to

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the skull, then connected to a preamplifier before storing data on tape, for posterior examination with an oscilloscope [7].

Advances in acquisition systems and electrode design have enabled the shift from bipolar recordings toward hdEMG, providing access to the collective activity of larger MU populations [8], [9]. High-density thin-film electrodes placed epimysially on the rat hindlimb have been used to record MU activity *in vivo*, by inducing asynchronous discharges through nerve crush under anesthesia [10]. However, the approach relied on a highly acute preparation and an artificial source of activation, limiting its direct translation to natural motor behavior. More recently, arrays specifically designed for animal use have enabled the recording of MU activity during voluntary behaviors, such as treadmill locomotion and single-pellet reaching [9].

We here build on these developments by taking advantage of the two technologies that have recently become available for hdEMG recordings in rats. As a first step, nerve stimulation was applied to confirm that the arrays reliably captured muscle activity, given the increased susceptibility to noise in small-animal models. We then aimed to determine whether individual MUs could be identified during different behavioral tasks. Finally, we sought to establish a reliable setup to enable stable measurements for the decomposition of MU activity, thereby advancing the use of rodent models for the study of neuromuscular control.

2. Methods

Electromyography arrays were implanted on eight adult Wistar and four adult Long-Evans rats (female, 250-500g). All procedures complied with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and were approved by the Ethics Committee of the Autonomous University of Barcelona (CEEAH 3371).

2.1. High-density electrode devices

'Thin-film arrays' (Fraunhofer Institute, Rostock, Germany) [8], reused after acute intramuscular recordings in humans, and 'myomatrix arrays' (Camber, Emory University, Atlanta, GA, USA) [9] were employed in these experiments; the former consisted of a single thread with 20 contacts per side (40 channels, 40μm $\times$ 140μm contact size, 1000μm inter-electrode distance between contacts on the same face), and the latter of four threads with eight contacts each (32 channels, 100μm $\times$ 200μm contact size, 300μm inter-electrode distance).

2.2. Surgical implantation

Different surgical approaches and electrode configurations were evaluated by implanting the two devices epimysially and intramuscularly in the upper and lower limbs of rodents targeting the deltoid, biceps, triceps, forearm flexors and extensors, and the tibialis anterior muscle. Rodents were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg); confirmed by withdrawal reflex. In acute experiments, arrays were positioned over the epimysium after exposing the target muscle and confirming with electrical stimulation. In chronic experiments, arrays were inserted intramuscularly: a midline incision was made over the skull or dorsum of the rat and over the target muscle to tunnel the arrays subcutaneously between the two points, and electrode thread(s) were guided into one or more muscle bellies with a needle and sutured to the muscle or epimysium. The connector was secured to the skin of the dorsal surface or to the skull. Grounding depended on the array. For thin-film devices, ground and reference were established using bipolar wires or needles placed on the animal or in a skull screw. For myomatrix devices, ground was provided by one contact short-circuited to reference, implanted either at the elbow or in a skull screw. In some preparations, an additional ground was implanted subcutaneously in the interscapular region.

2.3. Experimental approach

In this study we present the results of the acute recordings obtained on days 0 and 1. Muscle activity was recorded from thin-film electrode arrays using a Quattrocento (OT Bioelettronica, Turin, Italy); signals were amplified, band-pass filtered between 200 and 4,400 Hz, and digitized at a sampling frequency of 10240 Hz and 16-bit resolution. An Open Ephys Board (Lisbon, Portugal) was used to record muscle activity from myomatrix electrodes with a RHD 32-channel headstage (Intan Technologies, Los Angeles, CA, USA); signals were amplified, band-pass filtered between approximately 1 and 7500 Hz, sampled at 20000 Hz and digitized with a 16-bit resolution.

To record synchronized MN activity, i.e., compound muscle action potentials (CMAPs), the target peripheral nerve was stimulated using brief, suprathreshold electrical pulses under anesthesia. In awake rats, trains of individual motor unit action potentials (MUAPs) were generated either through the withdrawal reflex or during voluntary behaviours such as locomotion, reaching, or pulling, video-monitored with a camera (Basler acA2040-120um; Ahrensburg, Germany).

2.4. Data analysis

Data analysis was performed in MATLAB (MathWorks, Natick, MA, USA). Signals were visually inspected, and channels with constant amplitude close to zero or dominated by large artifacts were excluded. The signal-to-noise ratio (SNR) was defined as the ratio of 1s spiking activity power—obtained by subtracting baseline noise power from the total power—to 1s baseline noise power, expressed in decibels. Decomposition into constituent sources was performed using the algorithm described by Negro et al. [4], and the resulting MUAP templates were characterized by their mean and standard deviation to assess their consistency over time. Given the exploratory nature of the study, further analyses were tailored to the specific objectives of each recording, with detailed procedures described in the corresponding Results section.

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3. Results

CMAPs consistently verified that the activity from the target muscles was recorded (Fig. 1), with larger responses observed as stimulation intensity increased. The propagation of action potentials and the point of innervation were especially evident over the 2 cm span of the thin-film arrays, compared to the myomatrix arrays which covered ∼0.2 cm.

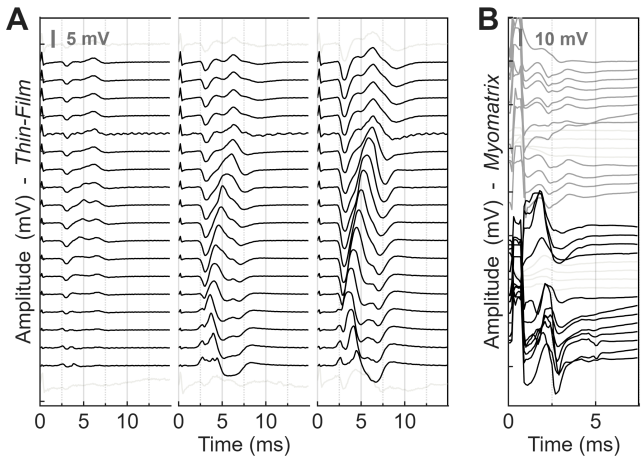


Figure 1. *Arrays can capture electrical activity of target muscles. (A) Raw signal recorded with a thin-film array implanted intramuscularly in the tibialis anterior muscle following direct electrical stimulation of the sciatic nerve at increasing intensities. (B) Raw signal from a myomatrix array inserted in the flexor (black) and extensor (grey) muscles of the rat upper limb following transcutaneous stimulation of the brachial plexus at submaximal intensity.*

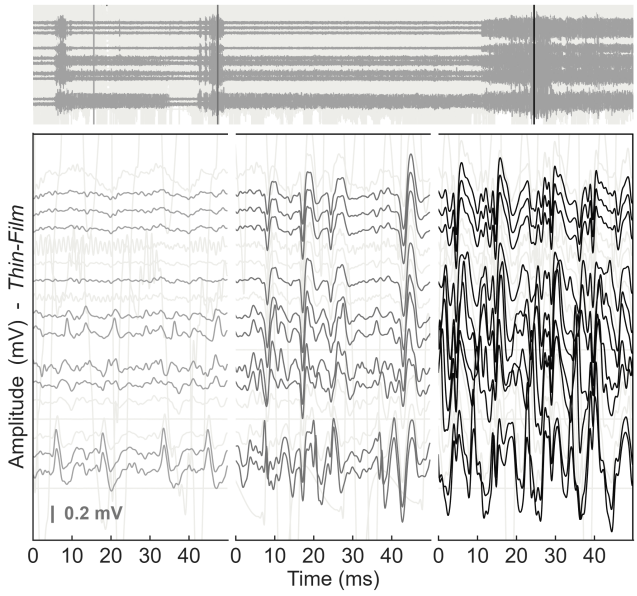


Figure 2. *Three successive withdrawal reflexes evoked with progressively stronger paw pinches in 20 s, recorded from a thin-film array implanted intramuscularly in the tibialis anterior (top). Vertical lines indicate the regions expanded below, with 50 ms windows of the band-pass filtered signal (4th-order Butterworth, 50-1000 Hz) (bottom).*

MUAPs were first observed during reflex contractions in rodents recovering from anesthesia (Fig. 2) and then in fully awake and recovered animals (Fig. 3). As a representative example, SNR (mean ± std, dB) from the recordings shown in the figures, averaged across all channels, were of 8.46 ± 1.53 and 1.27 ± 0.73 for thin-film and myomatrix arrays in monopolar mode, with myomatrix arrays increasing to 8.46 ± 4.88 in differential mode. Weak contractions involved fewer active MUs and allowed clearer differentiation of individual discharges (Fig. 2, left and middle), whereas stronger contractions recruited more units, leading to superposition of spikes and reduced separability (Fig. 2, right). Patterns of activation from antagonistic muscles were captured using the myomatrix arrays, whose multi-thread design allowed concurrent sampling from multiple muscles (Fig. 3). Decomposition yielded a modest number of MUs, some specific to individual bursts and others trackable across neighboring bursts, with relatively stable templates.

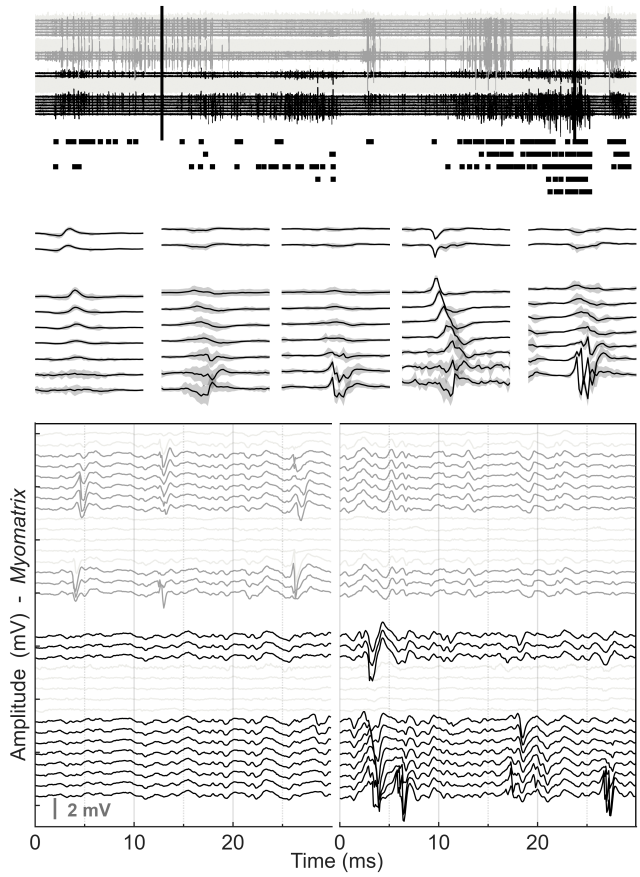


Figure 3. *Signals displayed in differential mode from extensor (grey) and flexor (black) muscles of freely moving rodents (6s), recorded with intramuscularly implanted myomatrix arrays, showing alternating bursts of activity (top). The discharge timings of five motor neurons from the flexor muscle are presented together with their 5 ms average templates (mean ± SD) (middle). Vertical lines mark the two regions expanded below, with 30 ms windows of monopolar signals highlighting periods of predominantly extensor or flexor activation (bottom). All signals were band-pass filtered (4th-order Butterworth, 100–4999 Hz).*

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4. Discussion

In weighing epimysial versus intramuscular configurations, attention should be given to the potential array movement and associated variability in the recorded MUAP shapes, as well as the possibility of tissue damage. The relative size of the array and the muscle is another important factor, given that the recording surface spans ∼0.2 to 2 cm, while the rat hindlimb muscles range from 1.5 to 6 cm in length [11]. Larger recording spans enable the capture of action potential propagation, a characteristic feature of each MU that provides valuable spatio-temporal information for the decomposition, but the array size should be matched to the muscle to ensure appropriate fit. As to the inter-electrode distance: narrower spacing improved resolution at the MU level (generally by reducing the field of view), but overly narrow spacing risked producing duplicate signals across adjacent contacts. Reference and ground were critical for noise reduction; a skull-fixed reference was preferred, but common noise persisted. Differential mode helped mitigate the problem when clear MUAP propagation was observed across adjacent channels, but a less active reference may ultimately provide a better performance.

Signals recorded contained high-frequency components (spikes), as expected from electrodes placed close to active muscle fibers. A temporal shift in spike waveforms across channels was observed, which can be understood as the propagation of action potentials along the fibers. Alternating bursts across multiple muscles were also evident, reflecting the activation of antagonistic groups, from which several MUs were identified. Together, these findings indicate that recordings from freely moving animal can capture accurate electrophysiological information which hold potential for MU decomposition.

As in human studies, movement remained a limitation; dynamic contractions alter the MU signature and hinder its identification, and MU discharge is not regular as in sustained contractions. Chronic recordings, in terms of electrode design and surgical protocol, remain to be explored, so as to maximize the opportunities from hdEMG in animal models.

5. Conclusion

These pilot experiments illustrate the feasibility of recording MU activity from the rat limbs during natural movement, including the first demonstration of thin-film arrays in awake rodents. While preliminary, these findings provide a foundation for future systematic investigations of MU behaviour in small animal models.

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