FIRING PROPERTIES OF RAT MOTOR UNITS DURING VOLUNTARY CONTRACTION

By

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Abstract

The properties of motor units have been well studied in anesthetized rats with current injection. Investigators have collected a large amount of data to support hypotheses about the relationship between firing rates and force. However, a very limited number of studies have been done in awake animals doing voluntary contractions. Because it is suggested that discharge properties of motor units mediated by current injection are different from that with natural synaptic inputs in intact animals, the current study aims to identify differences between the two modalities of activation. We recorded motor unit activity using intramuscular electromyography in left hindlimbs of 5 rats after they underwent behavioral training protocol to perform isometric plantar flexion. We found that the minimum firing rates in intact rats are much lower than those in current injection studies, while the positive relationship between firing rate and force still exists. Possible reasons for the results we observed are discussed, and we proposed some modifications for future studies based on limitations faced in the current one.

Introduction

A motor unit consists of a motor neuron and all the muscle fibers it innervates. Previous studies have shown that the intrinsic properties of motor neurons appear to be matched with the mechanical properties of the muscle fibers they innervate (Kernell et al., 1999). For example, when a motor neuron discharges at the lowest rate that it can sustain, the interval between successive spikes appears to closely match the twitch duration of its muscle unit (Bakels and Kernell, 1993; Meehan et al. 2010). This makes mechanical sense because the lowest firing rate would be that needed to just initiate temporal summation of the individual force impulses. A key factor that appears to determine the minimal firing rate of a motor neuron is the duration of the after-hyperpolarization (AHP) following a spike (e.g. Kernell 1965; Meehan et al. 2010).

However, most of the studies that have demonstrated such a match between motor neuron and muscle properties have involved anesthetized and/or reduced animal preparations in which excitation is mediated by current injection through intra-somatic microelectrodes. There are a number of investigators who have suggested that the discharge properties evoked by current injection may be different from that arising in response to natural synaptic input (Destexhe et al. 2003; Kuhn et al. 2004). Therefore, the purpose of this study is to characterize the discharge properties of motor units in intact rats during voluntary contractions and compare our findings with already existed information about the intrinsic properties of rat’s motor neurons and the contractile properties of their motor units.

Method

The experimental set-up and behavioral training protocol were adapted from Larkin (2018) with some modifications.

Set-up

Both male (3) and female (2) Sprague Dawley rats were used in the experiments. Their weights ranged from 235g to 355g in the beginning of the experiment. They were trained to perform voluntary isometric plantar flexion with left hindlimbs to obtain reward. The training started immediately after the rats arrived. A rat was introduced into a clear plastic tube. A black plastic nose cone sat in the front of the tube, which could be adjusted to increase or decrease the length of the tube so that the rat could not turn around in the tube. A flat disc closed the tube in the back with an opening for the tail to remain outside. The rat's hindlimb exited through a hole that was cut out at the bottom of the tube. The hindlimb was extended and strapped onto a pedal with a Velcro. The pedal was connected to a force transducer to measure the force exerted by the hindlimb during the behavior. In order to receive reward, the rat had to exert a force within a target range (typically 5 – 15 grams) that could be adjusted depending on the strength of individual rats. When the exerted force was within the target range, a tone was presented, and the rat received reward (liquid Ensure) via a peristaltic pump and tube positioned in front of the opening in the nose cone. If the rat held the force within the target range for more than 30 s, the delivery of the reward was halted. In order to obtain further reward, the rat had to reduce the force below the target range and then return the force back within the target range.

Behavior training

The rats were trained in a progressive way. Beginning with only 10 minutes in the tube, they gradually learned to tolerate up to 30 minutes with their left hindlimbs extending out of the tube and pushing on the pedal.

Initially, the rats were expected to simply sit in the tube, while the reward was provided ad libitum. They tended to turn around and explore the tube in the beginning and may or may not lick the syringe, but as the dimension of the tube decreased by adjusting the nose cone to the next setting, the rats eventually could not turn any more. Meanwhile, the duration of the training session increased every 2 days. This could be extended, depending on how comfortable the rats were in the tube at each setting. By the middle of the training schedule, the rats were put on food restriction in order to promote reward seeking during a session.

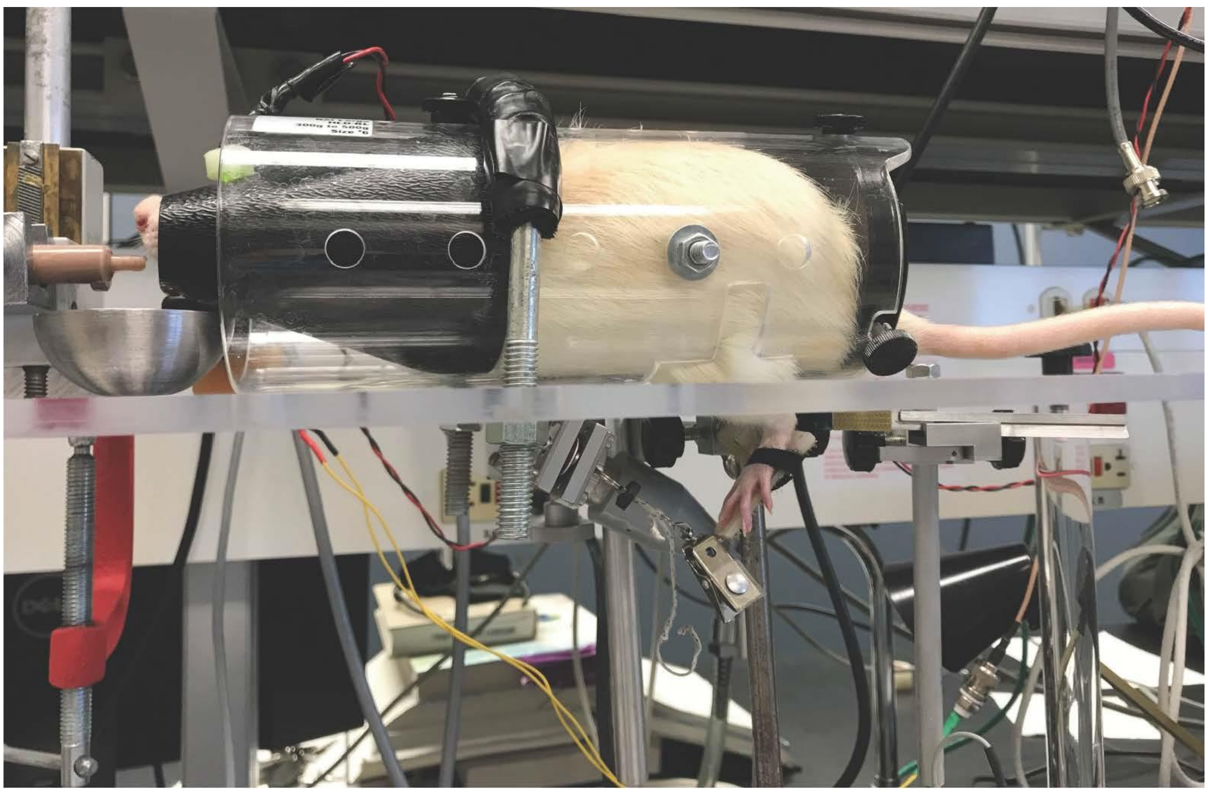

Figure 1: Experimental set-up. The LED light shown in the figure was not used in this study.

The next phase aimed to let the rats learn to extend the leg. We began with gently touching their foot while it stayed in the tube. After the rat became comfortable with touching, the leg was extended outside the tube. First, the foot was still held by hand during a training session. Then, a Velcro straps was introduced. They would not be strapped to the pedal unless they could entirely tolerate the extension and the Velcro. They still had free access to the reward at this point.

During the third phase, they started plantar flexion training with the rat's foot strapped onto the pedal. The reward was no longer provided ad libitum. At first, the experimenters performed the behavior for the rats by manually pushing the pedal and relaxing until they could voluntarily do so.

Motor unit recording

Once the rats were fully trained, we started recording intramuscular electromyographic (EMG) signals from the gastrocnemius or soleus muscle of the left hindlimb using 2 hooked wire electrodes. The wires were threaded through a needle with one end folded to create a hook. In this way, the electrodes could stay in the tissue when inserted. The electrodes were inserted into muscle guided by the needle. A ground electrode was placed at the base of the tail to minimize electrical noise. During the recording session, we sometimes gently tugged the electrodes to get a better recording. All data were recorded and saved using Spike2 program.

Analysis

Segments of the EMG recording showing clear motor unit activity were isolated from the file. The motor units were discriminated using a template-matching algorithm. A customized script was created to extract motor unit firing rates and associated isometric force. Data were categorized into the following classes according to the force and motor unit firing rate: initial firing, ramp up, and steady state. Initial firing means that the firing rate drops lower than 10 impulse/second while the force may or may not fluctuate significantly. During a ramp up, the force increases, and the firing rate is expected to increase as well. At steady state, both the firing rate and force are maintained at a constant level.

Result

Outcome of the training process

The rats gradually started acquiring the behavior in about a week on average. To be fully trained took longer, but eventually all 5 rats were able to perform the behavior correctly without manual help. The entire training process took about 1.5-2 months with 1 session per day and 4-5 days per week. However, it varied depending on the individual animal. It seemed that the sex of the rats was a relevant variable. Generally, it took female rats longer than male rats to be trained. Occasionally, the rats had trouble with relaxation, which was simply fixed by manual help. Most rats had no trouble with the plantar flexion. The training period could be shortened by having 2 sessions per day.

Figure 2 shows a segment of a typical recording session in a female rat after the animal were successfully trained. The bottom trace shows the change in force. When the rat exerted a force above the minimum level, the reward was delivered as shown in the middle trace by the red blocks, which indicate current pulses delivered to the reward pump. Once the rat was timed out (after 30 s in the reward zone) or the force dropped below the minimum, the reward delivery stopped. During a session, the performance was most stable after a few minutes the rat entered the experimental apparatus, and it was possible that the animal became mildly irritated toward the end.

Within a week, the rats tended to perform worse on Mondays after having free access to food at weekends, which made them less motivated to obtain rewards. Later in the week, as they were put on food restriction again, their performance generally improved.

Once trained, the animals were able to sustain the behavior for an extensive period of time, but after another 2-3 months, some of them showed reluctance for entering the experimental apparatus and sometimes for performing the behavior as well. Nevertheless, this training protocol allowed the experimenters to do multiple EMG recording sessions (6 on average) and collect a sufficient amount of data.

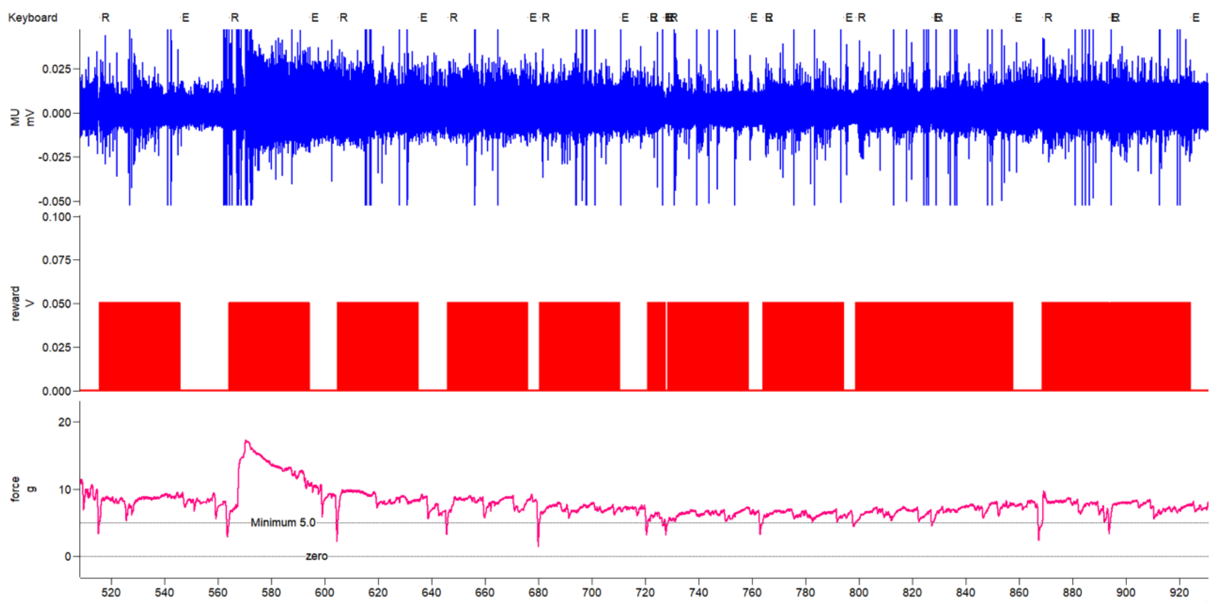

Figure 2: a segment of recording exemplifying the behavior performed by the rat. *Top trace:* EMG recording without discrimination and/or analysis. *Middle trace:* red blocks represent when reward is delivered. *Bottom trace:* force exerted by the rat during the behavior.

Motor unit recording

A total of 10 EMG recordings was collected. After analyzing the discriminated motor unit recording by running the customized script in Spike2, 51 motor units were identified and categorized as steady state, 16 for ramp up, and 22 for initial firing. Figure 3 shows an example of a motor unit recorded during 3 different steady state force levels. As shown in the figure, although the firing rate stays steady within the 4 seconds in each panel, it increases as the force increases.

The firing rate of all identified motor units ranges from 5.9 Hz to 27.2 Hz with a mean of 16.8 Hz (Figure 4A). Due to the nature of the experimental design, the rats only exerted a relatively gentle force during the behavior, and therefore the maximum firing rate recorded here is not equivalent to the actual maximum firing rates of the individual motor units. On the other hand, the recorded minimum is the true minimum firing rate.

As stated previously, we observed a positive relationship between firing rate and force (Figure 3; Figure 5). However, this relationship can linear or non-linear, and the rate of increase in firing rate differs for individual motor units (Figure 5). Nevertheless, based on evidence, the overall trend still exists.

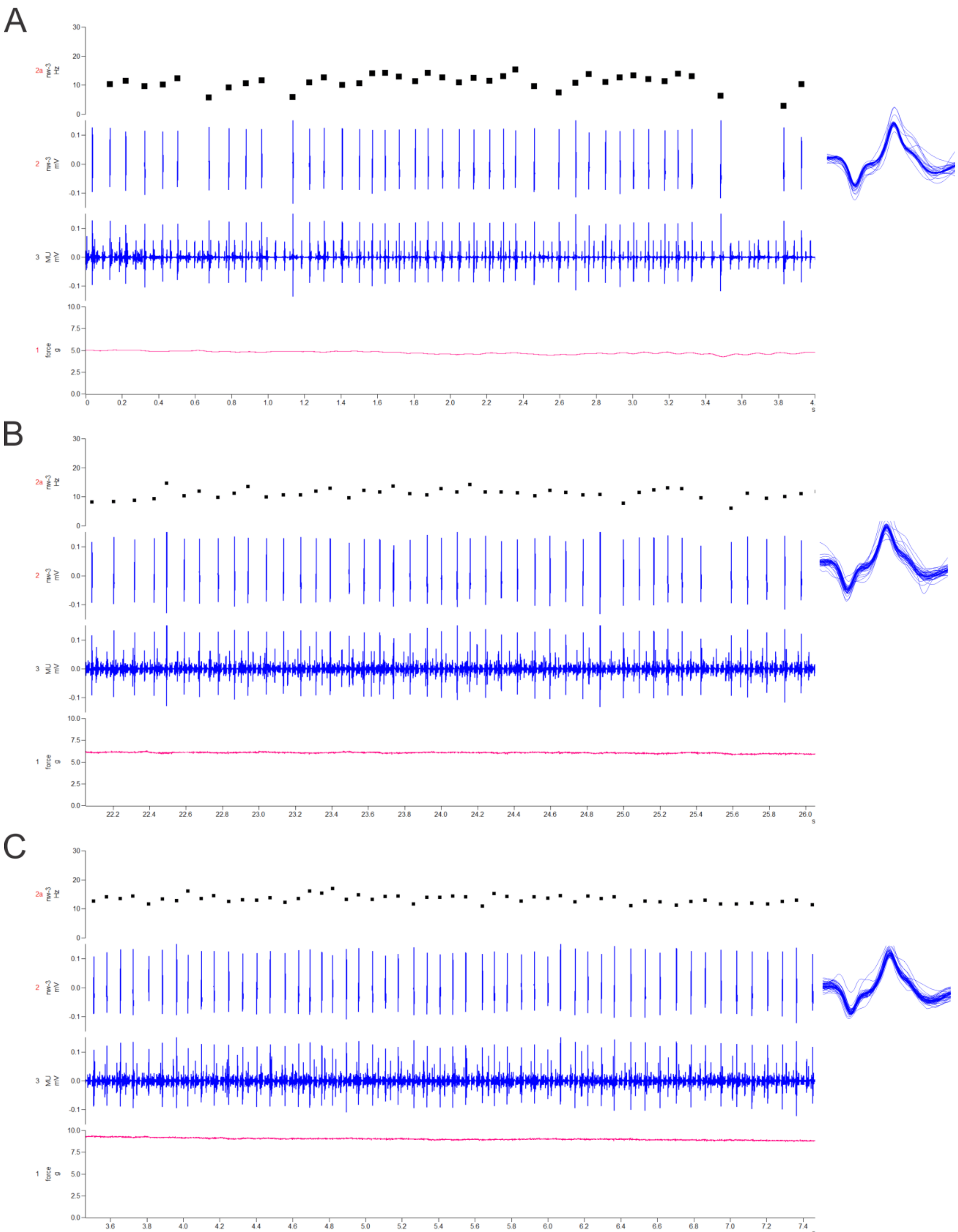
A
B
C
2a rsw-3 Hz
2 rsw-3 mV
3 MU mV
1 force g
s

Figure 3 (previous page): an example EMG recording of a motor neuron at 3 force levels in 4 seconds. In all panels, top trace shows the firing rate of the motor neuron, second trace the discriminated motor unit, third trace the original recording, and the bottom trace the force. From A to C, force increases and so does the firing rate. On the right of each panel shows the overdrawn motor unit. Based on the shape and amplitude, they are identified as the same motor unit.

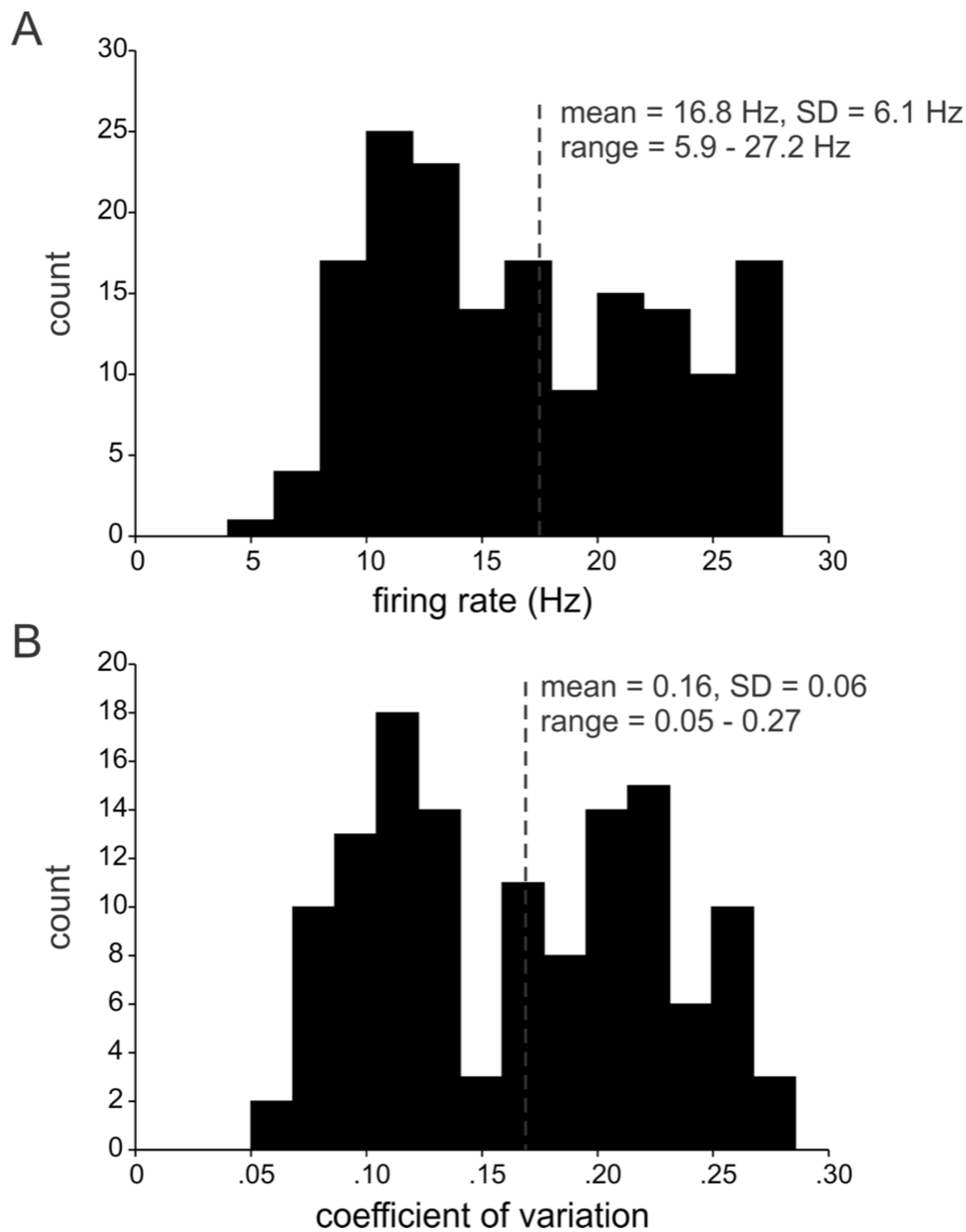

Figure 4: histograms that includes all identified motor units. *A:* number of motor units versus firing rate. *B:* number of motor units versus coefficient of variation.

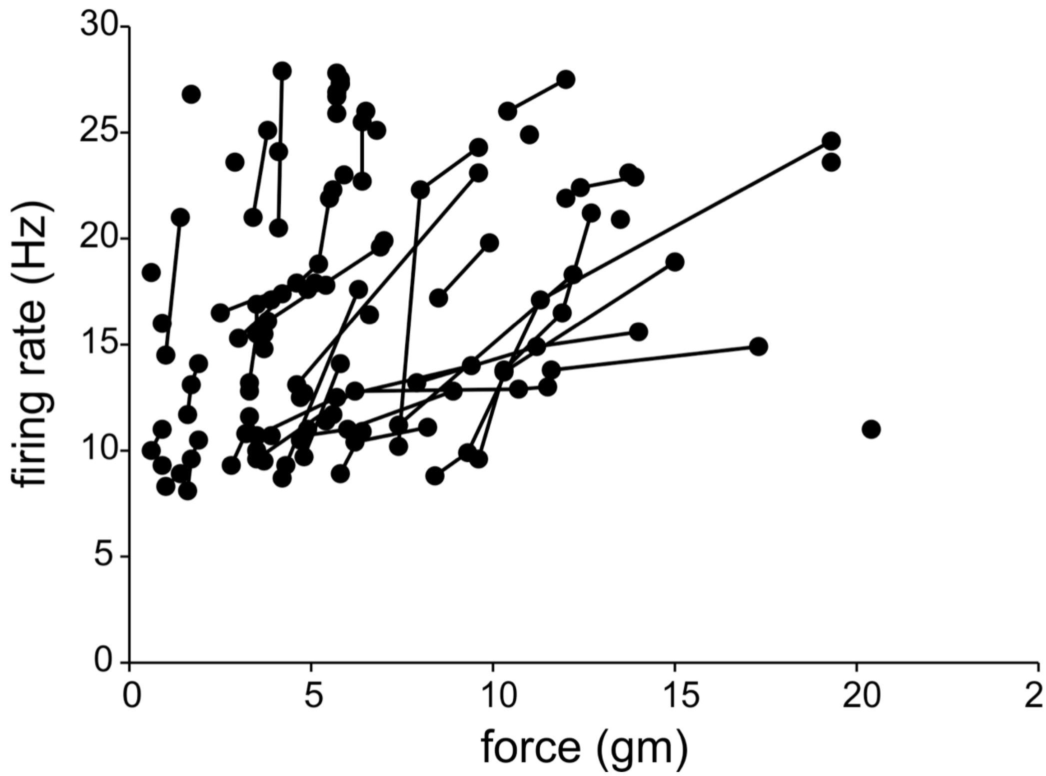

Figure 5: relationship between firing rate and force for all identified motor units

Discussion

The behavioral training protocol was effective at training the rats to perform voluntary isometric plantar flexion, which allowed the experimenters to record motor unit activity in awake animals. Based on data collected by EMG, we observed a positive relationship between firing rate and force, coinciding with many existing studies. The minimum firing rate recorded in this study was 5.9 Hz. Since the recorded maximum firing rate is not a reliable measurement, it will not be discussed here.

Minimum firing rate recorded during voluntary contractions vs. that of current injections

According to existing studies, minimum firing rates from a current injection in anesthetized rats are 24 Hz (Button et al. 2006; Meehan et al. 2010) and 35 Hz (Button et al. 2008; Meehan et al. 2010). These numbers are much higher than what we recorded in this study. This could be caused by the fact that current injections alter the nature of electrical activity of motor neurons. A motor neuron receives both excitatory and inhibitory pre-synaptic inputs from the central nervous system. The summation of these signals determines the magnitude and properties of the post-synaptic potentials. The combination of excitatory post-synaptic potentials (EPSPs) and inhibitory-post-synaptic potentials (IPSPs) creates a fluctuating electrical signal as opposed to the constant input from current injections. The experimenters can control the magnitude of the current injected into a motor neuron to stay at the threshold and generate a train of spikes. The firing rates recorded with the current injection method therefor have a very low coefficient of variation. In an intact animal, when the post-synaptic potential exceeds the threshold is more random. There is a smaller chance to cause a spike, resulting in a lower firing rate.

The anesthetics may play a role in increasing the minimum firing rate in current injection studies perhaps by altering the activity of the potassium channels responsible for the afterhyperpolarization. In addition, some anesthetics may decrease the activity of inhibitory pre-synaptic neurons, and therefore render the minimum firing rates higher during a current rejection. In contrast, in an awake animal, the nervous system is not under influence of the anesthetics and has intact signal transduction. The addition of inhibitory pre-synaptic input might lower the post-synaptic output, causing a lower minimum firing rate that is seen in the current study. Although the influence of anesthetics on the nervous system can be lowered by choosing one that does not completely impair the activity of neurons (Meehan et al. 2010), the physiological condition of the animal is still altered, which consequently affect the electrical signals recorded by EMG.

Limitations and future directions

Although a total of 51 motor units were identified in this study, only in relatively few were we able to track their activity at multiple force levels. These data only allowed us to observe a general trend in the relationship between firing rate and force. However, we did not have enough sufficient evidence to make a more specific and quantitative conclusion about this relationship. Previous studies in human subjects have suggested a rising exponential relation between firing rate and force (Fuglevand et al. 2015). We would need more samples of firing rate at multiple force levels to confirm the existence of such a relation in rats. We could attempt to record more trials of motor unit activity from the animals, but it is not guaranteed that we could record from the same motor units during repeated electrode insertions. A possible solution to this problem is to increase the length the session. In this way, we could record activity from the same motor units over a longer time period, increasing the probability that more force levels would be exerted. However, we observed that the rats generally became frustrated toward the end of the session and started pushing harder than we wanted. Having them stay in the experimental apparatus longer may not provide us much additional useful data. Also, we could change the choice of electrodes. One way is to use tungsten microelectrodes, which allow the experimenters to adjust the position of the electrode back and forth during the recording and to collect data from multiple sites. A disadvantage of a tungsten electrode is that it is less comfortable for the animal than the hook wire electrodes we used in the current study, which may shorten the recording session. Perhaps, a better navigation of electrodes during insertion could be developed.

Modifications proposed above can be applied in future studies, but one way or another, limitations exist. The experimenters need to apply one that is the best fit for their goals.

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