

Both Corticospinal and Reticulospinal Tracts Control Force of Contraction

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The control of contraction strength is a key part of movement control. In primates, both corticospinal and reticulospinal cells provide input to motoneurons. Corticospinal discharge is known to correlate with force, but there are no previous reports of how reticular formation (RF) activity modulates with different contractions. Here we trained two female macaque monkeys (body weight, 5.9–6.9 kg) to pull a handle that could be loaded with 0.5–6 kg weights and recorded from identified pyramidal tract neurons (PTNs) in primary motor cortex and RF cells during task performance. Population-averaged firing rate increased monotonically with higher force for the RF, but showed a complex profile with little net modulation for PTNs. This reflected a more heterogeneous profile of rate modulation across the PTN population, leading to cancellation in the average. Linear discriminant analysis classified the force based on the time course of rate modulation equally well for PTNs and RF cells. Peak firing rate had significant linear correlation with force for 43 of 92 PTNs (46.7%) and 21 of 46 RF cells (45.5%). For almost all RF cells (20 of 21), the correlation coefficient was positive; similar numbers of PTNs (22 vs 21) had positive versus negative coefficients. Considering the timing of force representation, similar fractions (PTNs: 61.2%; RF cells: 55.5%) commenced coding before the onset of muscle activity. We conclude that both corticospinal and reticulospinal tracts contribute to the control of contraction force; the reticulospinal tract seems to specify an overall signal simply related to force, whereas corticospinal cell activity would be better suited for fine-scale adjustments.

Key words: corticospinal; force coding; reticulospinal; strength

Significance Statement

For the first time, we compare the coding of force for corticospinal and reticular formation cells in awake behaving monkeys, over a wide range of contraction strengths likely to come close to maximum voluntary contraction. Both cortical and brain-stem systems coded similarly well for force, but whereas reticular formation cells carried a simple uniform signal, corticospinal neurons were more heterogeneous. This may reflect a role in the gross specification of a coordinated movement, versus more fine-grained adjustments around individual joints.

Introduction

Movements occur when muscles exert forces on limbs; the control of contraction force is thus fundamental to the control of movement. Increases in force are achieved by the recruitment of additional motoneurons within the pool projecting to a muscle, and also by modulating the rate of firing of motoneurons already recruited (Milner-Brown et al., 1973; Burke, 1981; Enoka and Duchateau, 2017). Increases in both rate and recruitment result

from raised synaptic drive to motoneurons (Fuglevand et al., 1993). Many descending and segmental systems provide synaptic inputs to motoneurons; the relative contribution of these diverse circuits to the modulation of force over its full range remains uncertain.

In primates, the corticospinal tract (CST) is a major source of descending motoneuronal drive. Evarts (1968, 1969) reported that identified pyramidal tract neurons (PTNs) modulated their discharge with force, both in movements against an external load and during isometric contractions. Cheney and Fetz (1980) took cell characterization further using spike-triggered averaging to identify cortico-motoneuronal (CM) cells with direct projections to wrist flexor or extensor muscles. Cell firing rate during static contractions was positively correlated with wrist torque, consistent with these monosynaptic projections contributing some of the varying motoneuron drive required for force modulation. However, subsequent studies reveal more complex relationships.

In a dexterous finger movement task CM cells showed great heterogeneity, with negative as well as positive correlations to

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grip force (Maier et al., 1993). Corticospinal coding of force appears to be task specific: some CM cells that are active during carefully controlled ramp-and-hold contractions are comparatively silent during ballistic movements (Cheney and Fetz, 1980). Similarly, Muir and Lemon (1983) observed CM cells with higher firing rates during precision grip than power grip, although the muscle target of the CM projection showed higher electromyogram (EMG) activity during the latter. This led Muir and Lemon (1983) to conclude that “during power grip their motoneurons must receive synaptic excitation from sources other than the direct corticomotoneuronal connections.”

In addition to the CST, both the rubrospinal tract (Ralston et al., 1988) and reticulospinal tract (RST; Riddle et al., 2009) provide monosynaptic inputs to motoneurons in primates. Recordings from rubromotoneuronal cells reveal tonic discharge that is also modulated by static torque, although to a lesser extent than for CM cells (Cheney et al., 1988; Fetz et al., 1989). Rubromotoneuronal firing rates may instead be better tuned to movement dynamics (Cheney et al., 1988). The relationship between firing rates of RST neurons and force has not been directly explored, yet there is evidence for an important role in force generation. Lawrence and Kuypers (1968a,b) performed sequential lesions of two descending tracts. Loss of both CST and rubrospinal tracts left animals with impairments mainly in fine finger movements, but they retained sufficient strength to climb and run. This capacity was lost after combined CST/RST lesions (Lawrence and Kuypers, 1968b), suggesting that the RST is capable of force modulation independent of corticospinal or rubrospinal function. Furthermore, we have previously demonstrated adaptations in projections from the RST during strength training (Glover and Baker, 2020), suggesting that plastic changes in this tract underlie long-term changes in capacity for force generation.

An important limitation of all prior recordings of neural activity was that only relatively low forces were examined. Direct data on how neural systems control higher forces are lacking.

This study aimed to compare the modulation of firing in the reticular formation (RF) and CST in macaque monkeys trained to perform a weight-lifting task. We explored a wide range of weights; the largest appeared close to the maximum of which the animals were capable. Both CST and reticular cells coded for force, but with important differences in the nature of coding, which suggest distinctive contributions to force control.

Materials and Methods

All animal procedures were performed under UK Home Office regulations in accordance with the Animals (Scientific Procedures) Act (1986) and were approved by the Animal Welfare and Research Ethics Board of Newcastle University. Experiments were conducted with two chronically implanted, purpose-bred rhesus macaques (monkeys N and L; weight range, 5.9–6.9 kg; both female), which were housed together. On training days, food access was restricted in the home cage, and trials of the behavioral task were rewarded with food. On rest days and when trials fell below a threshold value for 2 consecutive days, food was provided *ad libitum*. *Ad libitum* access to water was provided at all times. Both animals were intact before the study, with the exception of monkey N, who had lost parts of two fingers on the right hand in an unrelated incident.

Behavioral task

The behavioral task has been described previously (Glover and Baker, 2020). Briefly, animals were trained to pull a loaded handle toward the body using their right hand. Trials were self-paced, and successful completion was marked by auditory feedback when the handle was moved at least 4 cm from its rest stop. Successful trials were rewarded with food and nucleus accumbens stimulation (see below). A pulley system enabled weights to be attached to the handle, increasing the force required to pull it.

Before this study, the animals were extensively trained on the task until they could perform 50 consecutive trials with at least 6 kg attached to the handle (Glover and Baker, 2020). Animals were head fixed to enable single-unit recordings (see below), and the left arm was held in a restraint to ensure unilateral task performance.

Surgical preparation

As described previously, the animals were implanted with a headpiece, bilateral EMG electrodes in eight upper limb muscles [first dorsal interosseous (1DI); flexor digitorum superficialis (FDS); flexor carpi radialis; extensor digitorum communis; biceps brachii; triceps brachii; pectoralis major (PM); and posterior deltoid muscles], and chronic stimulating electrodes in the pyramidal tract (PT). The headpiece incorporated recording chambers, allowing access to the left primary motor cortex (M1) and right RF. Unrelated to the current study, the monkeys were also implanted with chronic stimulating electrodes in the medial longitudinal fasciculus and cortical epidural electrodes. Full surgical and anesthesia details are provided in our previous report (Glover and Baker, 2020).

In addition to food, stimulation of electrodes implanted in the nucleus accumbens was used as a behavioral reward (Bichot et al., 2011). Monkey L had a preexisting nucleus accumbens electrode implanted at the start of our previous study (for surgical details, see Glover and Baker, 2020). However, this became less effective over a period of several months, and so a second electrode was implanted in this animal at the start of RF recordings and was used successfully in subsequent sessions. A nucleus accumbens electrode was also implanted in monkey N early in the RF recordings, but stimulation did not appear to motivate behavior and so it was not routinely used in this animal. When in use, nucleus accumbens stimulation was delivered every one to three successful trials (biphasic pulses, 1.0–2.5 mA, 0.2 ms/phase; frequency, 200 Hz; train duration, 200 ms).

M1 recordings

Recordings from PTNs were made via a recording chamber mounted on the headpiece above a craniotomy centered over M1. Daily recording sessions were performed with platinum-iridium microelectrodes (Thomas Recording); up to five electrodes were loaded into an Eckhorn microdrive (Thomas Recording). The electrodes were individually advanced through the dura and into the cortex until cell activity was detected; the animals were at rest during this process. Following successful insertion of all electrodes into the cortex, the chamber was filled with agar to stabilize the electrodes for cell identification and the subsequent recording session.

Cells were identified as PTNs if they met the following two criteria: a fixed latency response to single-pulse PT stimulation (biphasic pulses, 0.1 ms/phase), and a constant collision interval (Lemon, 1984). The threshold for a response to PT stimulation, antidromic latency (ADL) of this response, collision interval, and cell depth were noted for each cell. Only recordings from such identified PTNs were considered in the analysis of M1 data.

RF recordings

Following completion of the M1 recordings, the M1 chamber was sealed to reduce the risk of infection, and a craniotomy was opened in the RF chamber. Daily recording sessions were performed with either one or two 32-channel U-probes (Plexon); when two electrodes were used, these were positioned 2 mm apart on the anterior–posterior axis. The electrodes were individually advanced through the craniotomy, toward the brainstem, using a microdrive (Nan Instruments). The motor RF was identified based on a location relative to brainstem landmarks such as the abducens nucleus, and because intracerebral microstimulation produced limb movements [trains of 18 biphasic pulses, 0.2 ms/phase; 3 ms interstimulus intervals; isolated constant current stimulator (model 2100, A-M Systems)].

Daily recording sessions

Recording sessions were performed 5 d/week and followed the same pattern for both M1 and RF recordings. During each daily session, the behavioral task was performed at seven different force levels, defined by

the following weights attached to the lever: 0.5, 1, 1.5, 2, 3, 4, and 6 kg. The animals performed blocks of 10 trials at each weight, with the sequence of weights pseudorandomized within each recording session. Between blocks, there was a brief pause during which the experimenter changed the weights on the task. Monkey N performed few trials with 6 kg during the RF recordings, so all 6 kg of RF data have been excluded from the analysis for this animal.

Waveform recordings from microelectrodes, U-probes or EMG electrodes were amplified (bandpass, 1 Hz to 10 kHz), digitized at a 25 kHz sampling rate by miniature headstages (Intan Technologies), and stored to a computer together with a signal representing the position of the lever and digital markers indicating task events.

Data analysis

The aim of this study was to compare the firing rates of PTNs and RF cells across a range of weights during a weight-lifting task. All analyses were performed offline using custom scripts in MATLAB and were conducted separately for the two animals.

Task performance. We started by examining measures of task performance to determine whether weight was the only variable that differed between trials. To achieve this, averages of lever position and rectified EMG were constructed across all trials of a given weight in one session. Sweeps were aligned relative to task completion (lever displacement first reaching 4 cm), since this reflects the point at which success was signaled to the animal. For each session, the maximum lever displacement and the latency of this peak were calculated. To investigate the effect of weight on these parameters, linear mixed models were constructed using single-session averages, with trial weight and session ID as crossed factors. This analysis was repeated for single-session EMG peak amplitude and the latency of this peak for each muscle.

Spike discrimination. Waveform recordings from M1 and RF were discriminated offline into the times of single-unit spikes. For M1, this used custom clustering software (Getspike, S. N. Baker); spikes were included only if they had consistent waveforms and interspike intervals >1 ms. Discrimination used records of spike size and shape made during the antidromic identification process to ensure that spikes corresponded to PTNs. For the RF, spikes were discriminated using MountainSort (Chung et al., 2017); this software has the advantage that it can track cells that move electrode contacts because of tissue instability. The MountainSort output was postprocessed using custom MATLAB scripts to ensure that only cells with consistent waveforms and interspike intervals >1 ms were included.

Task-related modulation. The relationship between cell firing rate and the task was examined by constructing perievent time histograms (PETHs; with 10 ms nonoverlapping bins, smoothed by convolution with a Gaussian kernel with a 20 ms width parameter) relative to the task completion marker. Trials were included only if the cell had an average firing rate of >5 Hz (measured from 1.5 s before to 1 s after trial completion) and at least one spike in the “active” window of the task (1 s before to 0.5 s after trial completion); this excluded trials from periods where the cell had been lost from the record. Furthermore, the first trial from each block was also excluded: for this trial, the weight was unknown to the animal, which often led to the monkey producing excessive or inadequate force, depending on whether the previous block used a heavier or lighter weight. After applying these trial exclusion criteria, cells were only included in the analysis if at least five trials per weight remained for all weights, with the exception of RF recordings from monkey N, for which no trials were performed with the 6 kg weight.

To determine whether the firing rate of each cell was related to task performance, a Monte Carlo resampling method was used. For each trial, the interspike intervals were shuffled, and the PETH recomputed. This randomized the spike times; on the null hypothesis that firing was unmodulated by the task, shuffling should not alter the statistics of the PETH. The maximum firing rate was calculated, both for the actual PETH, and after 100 different interspike interval shuffles. For a given weight, a cell was considered significantly modulated if the real maximum rate was larger than at least 95 of 100 of the maxima measured from shuffled PETHs. If a cell was significantly modulated for all but

one weight, it was considered to be task modulated. Only such task-modulated cells were included in the analysis.

Linear discriminant analysis. To assess whether neural firing rate could reliably predict force, we used linear discriminant analysis (LDA) to perform a pairwise classification of trials by weight. The model was trained separately for each cell and each pair of weights using single-trial PETHs (compiled with 100 ms nonoverlapping bins, smoothed by convolution with a Gaussian kernel with a 100 ms width parameter) from 1000 ms before to 500 ms after task completion. LDA performance was assessed with a “leave-one-out” procedure in which each trial was excluded from the training set in turn, and the model was then used to classify the excluded trial. Accuracy was calculated as the number of correctly classified trials expressed as a percentage of the total. To test whether the model performed significantly better than chance (50%) for each pairwise comparison, the number of correctly classified trials was compared with a binomial distribution ($p < 0.05$).

For a cell with a complete dataset of tested weights, comparisons between each pair of weights produced 21 LDA accuracy values, each with an associated p value. To produce a single accuracy value per cell, we averaged the LDA accuracy for all weights compared with the lightest weight (0.5 kg). For monkey L, this was the average of six values (1, 1.5, 2, 3, 4, and 6 kg vs 0.5 kg). For monkey N, there were only five values since, as described above, 6 kg data were not available from the RF recordings in this animal. Thus, the overall model accuracy values are comparable between cell types within the same animal, but not between animals since they incorporate trials at different weights. To test the overall model reliability for each cell, binomial distributions were used to compare the overall model accuracy to chance (50%).

Several summary statistics were computed. We calculated the percentage of cells with better than chance weight coding (“model reliability”) for each pair of weights compared. Similarly, by averaging model accuracy for each pair of weights across all cells, we obtained an accuracy value across the whole population. To limit this accuracy measure to cells in which the model was reliable, we found the average model accuracy for the subpopulation of cells in which overall model accuracy was significantly better than chance. Finally, to provide a statistical comparison between the two cell types for each monkey, we performed unpaired t tests on overall model accuracy values between PTNs and RF cells. This analysis was repeated for the subpopulation of cells in which the overall model accuracy was significantly better than chance.

We next wanted to investigate whether the peak firing rate alone could code for force. For each cell, the latency of the peak firing rate was calculated from the mean PETH across all trials. A 500 ms window was defined centered on this latency and the maximum firing rate (10 ms nonoverlapping bins, see above) for each trial was calculated within this window. Single-trial peak firing rate values were entered into the LDA model described above.

Correlation of firing rate with force. LDA provides valuable insight into the extent to which firing rate codes for force, but it does not describe the nature of this coding; for example, whether there is a positive or negative correlation. To explore the relationship between peak firing rate and force further, we identified the weight associated with the highest peak firing rate for each cell, and the latency at which this peak occurred, relative to task completion. The distribution of peak firing rate latencies was tested for normality using Kolmogorov–Smirnov tests.

Furthermore, for each cell we fitted a linear regression between mean peak firing rates and trial weight. The gradient and significance of the peak firing rate versus force correlation was recorded for each cell. Cells were classified depending on whether they had a significant positive correlation, a significant negative correlation, or no correlation. We compared the gradient of the rate versus force correlation between PTNs and RF cells, and between PTNs with positive and negative correlations, using unpaired t tests.

Relation to anatomical location and conduction velocity of PTNs. We recorded PTNs with a range of antidromic latencies and from a range of depths. Anatomical and electrophysiological studies have revealed that fast-conducting PTNs with monosynaptic CM connections originate mainly from the anterior bank of the sulcus in M1, whereas slower-conducting CM cells and corticospinal cells that terminate on interneurons

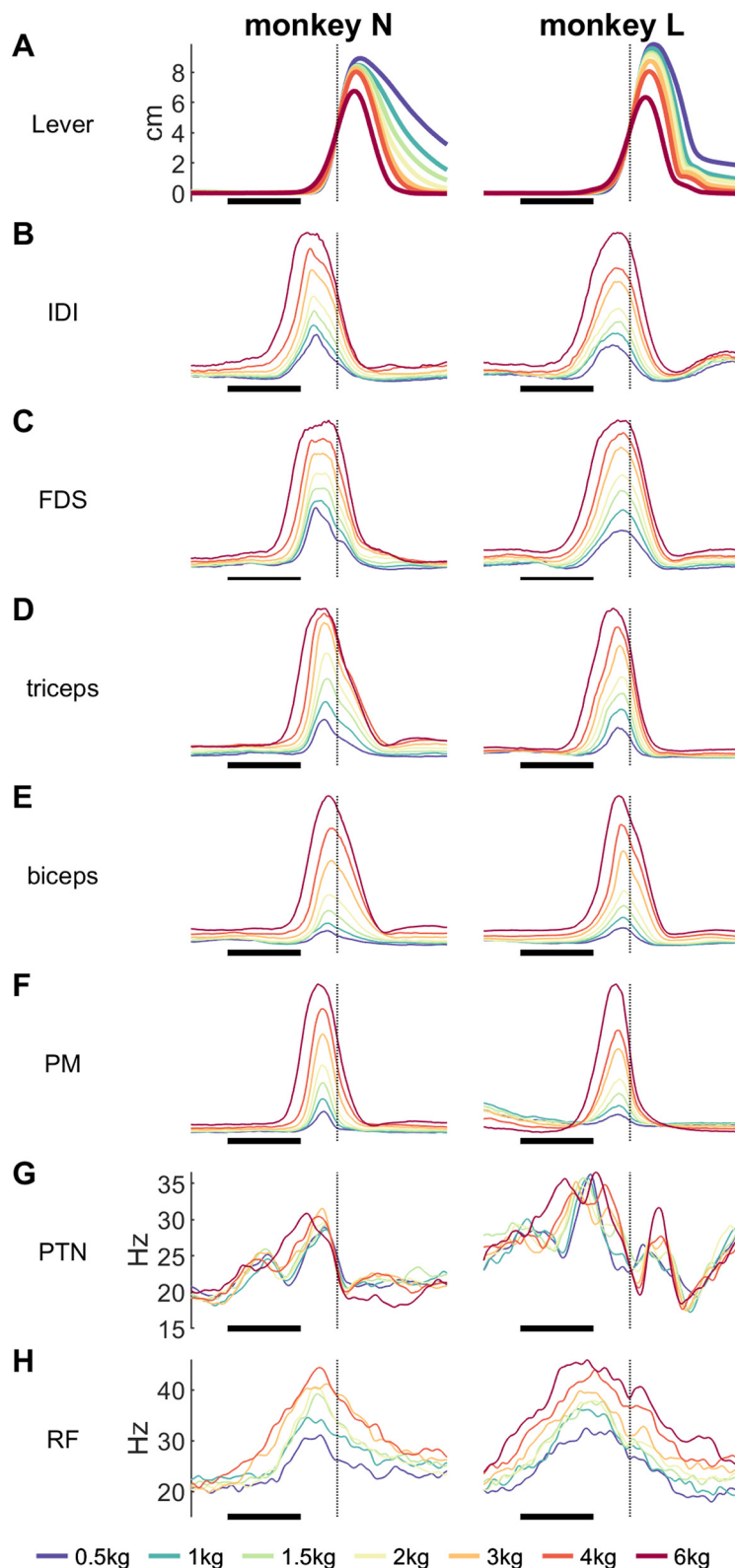


Figure 1. Average muscle activity and cell-firing rates relative to lever movement. Mean sweeps, averaged across all recording sessions for each animal (columns), shown per weight (see legend) and aligned to task completion (4 cm deviation of lever; black dotted line). Calibration: 500 ms. Data shown are averaged across all 36 sessions from monkey N (28 PTN sessions, 8 RF cell sessions), and 21 session from monkey L (16 PTN sessions, 5 RF cell sessions). **A**, Mean lever displacement. Linear mixed models (see Materials and Methods) were constructed to assess the effect of trial weight on the amplitude (monkey N: $F_{(1,242)} = 42.8$, $p < 0.001$; monkey L: $F_{(1,145)} = 71.0$, $p < 0.001$) and latency (monkey N: $F_{(1,242)} = 59.4$, $p < 0.001$; monkey L: $F_{(1,145)} = 143$, $p < 0.001$) of maximum lever displacement. **B–F**, Rectified mean EMG activity. Linear mixed models with single-session data were used to assess the

are found throughout M1 (Rathelot and Strick, 2009; Witham et al., 2016). To investigate whether there was a relationship between conduction velocity and force coding in PTNs, for each animal we fit a linear regression between overall model accuracy and antidromic latency. We also fit linear regressions between peak firing rate or rate/force gradient and conduction velocity. These analyses were repeated for “superficial” (<2.5 mm from first recorded cell in the penetration) and “deep” (>2.5 mm from first recorded cell in the penetration) PTNs. Independent *t* tests were performed to compare the mean model accuracy per cell between superficial and deep PTNs.

Timing of firing rate changes. The next analysis aimed to compare the latency of rate changes between PTNs and RF cells. To do this, it was not sufficient to align activity to task completion (as in all analyses above), because muscle activity often preceded lever movement by a few hundred milliseconds. This timing differed between weights, with earlier EMG onset for heavier weights (Fig. 1). Instead, for analysis of timing, we realigned firing rates to EMG onset, as described below.

EMG data were high-pass filtered at 30 Hz, full-wave rectified, smoothed by convolution with a Gaussian (width parameter, $\sigma = 5$ ms), and binned into 1 ms nonoverlapping bins. The frequent presence of baseline activity meant that it was not possible to detect reliably the onset of increased EMG in single trials from individual muscle recordings. Instead, an average was produced for a single trial across the five EMG channels in the right arm that gave clear task-related activity: IDI, FDS, triceps brachii, biceps brachii, and PM. To ensure that this combined EMG sweep was not dominated by a single channel, each channel was first normalized by dividing by its mean value across all trials.

Given that the animals rarely sat completely still before each trial of the task, baseline EMG activity was not defined relative to task completion but instead by finding the quietest 500 ms epoch across the whole sweep (1.5 s before to 1 s after trial completion). For each trial, EMG onset was defined by working backward from task completion to find the first time point at which EMG activity dropped below threshold (1 SD above mean baseline EMG activity). Furthermore, trials were only included if the animals were relatively

effect of trial weight on the amplitude and latency of peak EMG activity. **B**, First dorsal interosseous (monkey N amplitude: $F_{(1,242)} = 903$, $p < 0.001$; monkey N latency: $F_{(1,242)} = 48.6$, $p < 0.001$; monkey L amplitude: $F_{(1,145)} = 586$, $p < 0.001$; monkey L latency: $F_{(1,145)} = 6.41$, $p = 0.012$). **C**, Flexor digitorum superficialis (monkey N amplitude: $F_{(1,242)} = 868$, $p < 0.001$; monkey N latency: $F_{(1,242)} = 2.00$, $p = 0.159$; monkey L amplitude: $F_{(1,145)} = 771$, $p < 0.001$; monkey L latency: $F_{(1,145)} = 0.43$, $p = 0.514$). **D**, Triceps brachii (monkey N amplitude: $F_{(1,242)} = 487$, $p < 0.001$; monkey N latency: $F_{(1,242)} = 3.69$, $p = 0.056$; monkey L amplitude: $F_{(1,145)} = 638$, $p < 0.001$; monkey L latency: $F_{(1,145)} = 1.84$, $p = 0.177$). **E**, Biceps brachii (monkey N amplitude: $F_{(1,242)} = 1578$, $p < 0.001$; monkey N latency: $F_{(1,242)} = 19.6$, $p < 0.001$; monkey L amplitude: $F_{(1,145)} = 1008$, $p < 0.001$; monkey L latency: $F_{(1,145)} = 10.2$, $p = 0.002$). **F**, Pectoralis major (monkey N amplitude: $F_{(1,242)} = 1361$, $p < 0.001$; monkey N latency: $F_{(1,242)} = 0.84$, $p = 0.360$; monkey L amplitude: $F_{(1,145)} = 1213$, $p < 0.001$; monkey L latency: $F_{(1,145)} = 120$, $p < 0.001$). **G**, PETH for PTNs (monkey N: $n = 65$; monkey L: $n = 27$). **H**, PETH for RF cells (monkey N: $n = 34$; monkey L: $n = 16$).

still before EMG onset. This was defined as the 500 ms before EMG onset being below a threshold of 5 SDs above mean baseline EMG activity. Therefore, fewer trials were included in EMG onset-aligned PETHs than in movement onset-aligned PETHs.

To check the validity of EMG onset-aligned PETHs, we repeated the LDA analysis previously performed on movement onset-aligned data. We calculated the average model accuracy per cell and used paired *t* tests to compare these values to the equivalent values from movement onset-aligned PETHs.

To investigate latency effects, firing rate was compared between weights using single-trial, EMG onset-aligned PETHs with 50 ms non-overlapping bins (no smoothing). For each bin, the firing rate for all included trials at a given weight was compared with the firing rate for all included trials at the lightest weight (0.5 kg) using independent *t* tests. This enabled us to calculate the percentage of cells at each time point and each weight that had a significantly different firing rate from the lightest weight. We also identified the first time point at which a significant difference in firing rate was observed relative to the lightest weight for each cell. These values were used to construct cumulative density functions for each cell type and animal to estimate when the population of cells started coding for force relative to EMG onset.

Before the onset of EMG activity, it can be assumed that there is no proprioceptive or cutaneous feedback regarding the weight. Therefore, if the firing rate before EMG onset codes for force, this would suggest that firing rate is set in anticipation of the task requirement. By contrast, the firing rate after EMG onset is likely to be heavily modulated by afferent feedback. We compared the influence of anticipation and afferent feedback by separately performing LDA with firing rates -500 to 0 ms before EMG onset, and 0 – 500 ms after EMG onset. Single-trial PETHs (EMG onset aligned, 100 ms nonoverlapping bins, smoothed by convolution with a Gaussian kernel with a 100 ms width parameter) were entered into the LDA to compare each pair of weights. The overall model accuracy was compared for the “before” and “after” conditions and between cell types with a repeated-measures ANOVA; where significant effects were found, *post hoc* testing was performed between the before and after conditions with paired *t* tests, and between cell types with unpaired *t* tests. To test whether the model accuracy of the cell population was better than chance, for each cell type and monkey we used one-tailed *t* tests to compare the distribution of before and after overall model accuracy values to chance (0.5).

We observed the following two peaks in the EMG-aligned PETHs for PTNs: an early peak at ~ 250 ms before EMG onset, and a late peak at ~ 250 ms after EMG onset. To investigate the nature of these two peaks, for each trial we calculated the maximum firing rate for the early peak (500–0 ms before EMG onset; pre-EMG onset window described above) and the late peak (0–500 ms after EMG onset; post-EMG onset window described above). These early peak and late peak values were entered separately into an LDA model. We also calculated the maximum firing rates of the early and late peaks from mean PETHs per weight per cell and calculated the linear regression between early and late amplitudes for each cell.

Results

Task performance

The weight-lifting task was self-paced, and the lever was free to move beyond the 4 cm target, allowing the two monkeys to adopt their own movement strategies, which varied with force. For example, with light weights both animals frequently pulled the lever beyond the 4 cm target, whereas with the heaviest weights they were more likely to release the lever as soon as the reward tone was heard. Thus, trials were of shorter duration and had smaller lever movements with the heaviest weight (Fig. 1A). The period up to the success tone, which was associated with the greatest EMG activity, appeared consistent across the different weights.

There was a pronounced increase in EMG activity with increasing task load, which was observed across all muscles (Fig.

1B–F). This was expected, as for a larger weight the animal not only had to flex the elbow more strongly, but also grip the handle more firmly to ensure a stable grasp. We did not observe a clear trend between the latency of peak EMG activity and weight across the different muscles, suggesting that the timing of peak EMG activity was consistent relative to task completion.

Firing rate versus force

From an initial dataset of 125 PTNs and 210 RF cells, we excluded 19 PTNs and 124 RF cells because of having recorded activity with insufficient trials (see Materials and Methods), and a further 14 PTNs and 36 RF cells because their firing rates were not task modulated. This resulted in a final dataset of 65 PTNs and 34 RF cells from monkey N, and 27 PTNs and 16 RF cells from monkey L.

PETHs of firing rate relative to task completion averaged over the whole RF cell population demonstrated a clear relationship between firing rate and force (Fig. 1H), whereas a more complex averaged firing profile was observed for PTNs (Fig. 1G). This could arise because RF cells showed a greater correlation between their firing rates and force or, alternatively, that there was more homogeneity in RF cell response. To test this, we compiled averages of the absolute change in rate at a given weight, compared with the lightest (0.5 kg) weight, with the aim of preventing cancellation across a heterogeneous population. Such averages showed clearer gradation with force for both monkeys (Fig. 2A).

A more quantitative comparison of the ability of unit discharge to code force was conducted using LDA. For each cell, a linear model was trained to classify single trials of two different weights. For each pair of weights, we obtained an accuracy level (percentage of trials correctly classified), and whether this was significantly different from chance (50%).

Unsurprisingly, classification reliability increased as the difference between the weights increased. For example, the model performed significantly better than chance in classifying 0.5 versus 6 kg trials for 95.4% of PTNs in monkey N, compared with just 33.8% of cells for 0.5 versus 1 kg trials (Fig. 2B). Overall, the model performed better than chance for 89.2% of PTNs and 76.5% of RF cells for monkey N, and 96.3% of PTNs and 93.8% of RF cells for monkey L (Fig. 2B). Considering only cells where classification was significantly better than chance, there was no significant difference in model accuracy between PTNs and RF cells for monkey L (Fig. 2C; $t_{(39)} = 1.42$, $p = 0.163$), but the model was significantly more accurate for RF cells than PTNs for monkey N (Fig. 2C; $t_{(82)} = -3.31$, $p = 0.001$). By contrast, when looking at model accuracy across the whole population of cells, including those with no better than chance performance (Fig. 2D), there was no significant difference between PTNs and RF cells for either monkey (Fig. 2E). Such a measure represents a convenient summary of overall coding efficiency of a cell population, since it is sensitive both to the fraction of cells which code for force (Fig. 2B) and also to how accurately they code (Fig. 2C). These results suggest that the firing rates of PTNs and RF cells reliably code force to a similar extent at the whole-population level. In monkey N, although a smaller percentage of RF cells reliably coded for force compared with the PTNs (Fig. 2B), force could be predicted from firing rate more accurately in these cells (Fig. 2C). Note that although 6 kg data have been presented for monkey N PTNs, these were not included when making statistical comparisons to monkey N RF cells, where no data were available at this highest force level.

The analysis of Figure 2 performed classification using the entire time course of the PETH response. To examine which

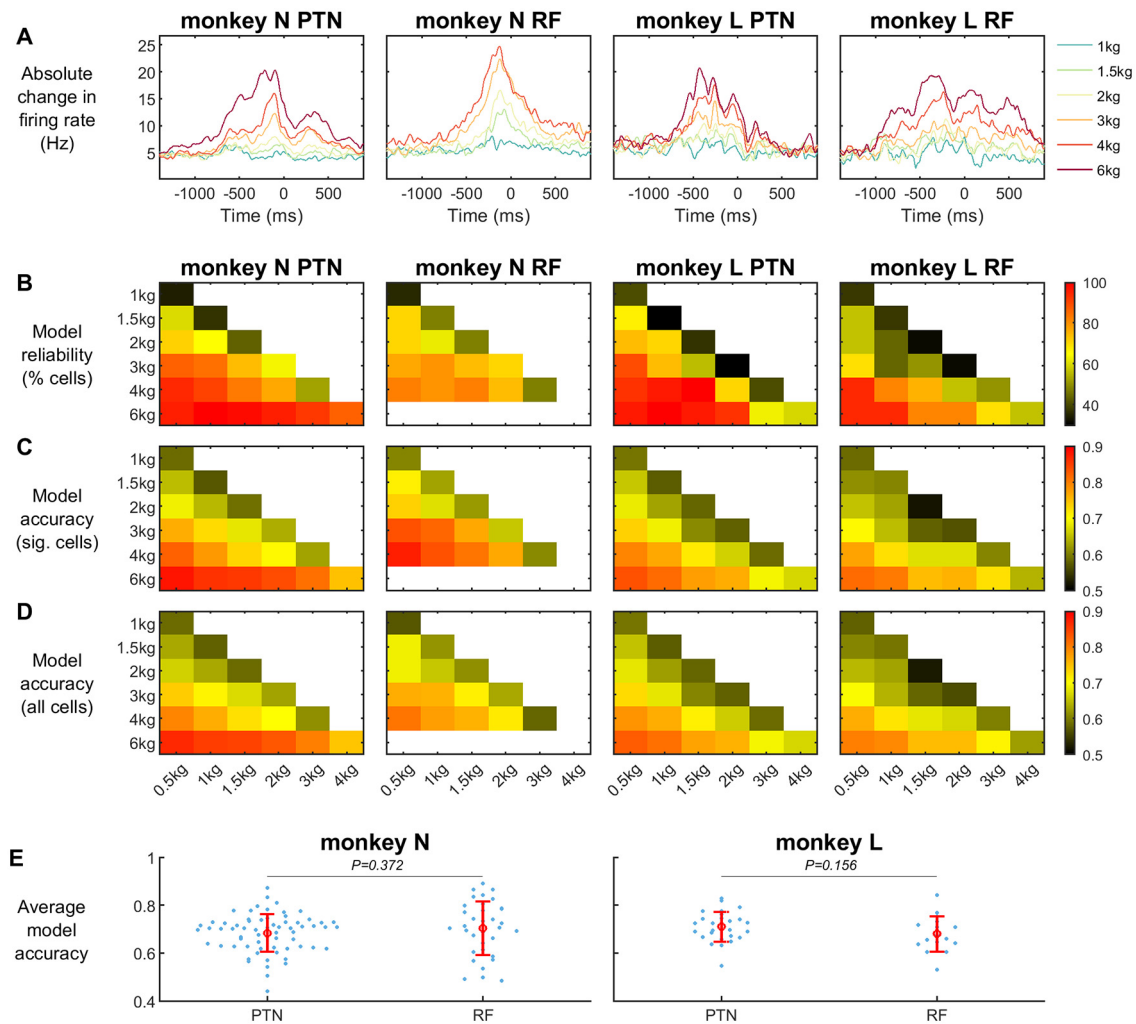


Figure 2. Firing rate across full task duration. **A**, Mean absolute change in firing rate relative to trials performed with the 0.5 kg weight. Sweeps are aligned to task completion and are shown per cell type per animal (subplots) and per weight (see legend). **B–D**, LDA comparing firing rates between each pair of weights, for each cell across the full time course of each trial. **B**, Percentage of cells in which the model correctly predicted the weight of each trial significantly more often than chance (see Materials and Methods). **C**, Mean model accuracy in the subpopulation of cells in which the overall model accuracy was better than chance (see Materials and Methods; monkey N PTN: $n = 58/65$; monkey N RF: $n = 26/34$; monkey L PTN: $n = 26/27$; monkey L RF: $n = 15/16$). **D**, Mean model accuracy in all cells. **E**, Mean model accuracy per cell type, per animal. Blue circles show mean model accuracy for individual cells. Red errors bars show mean and SD across the population of cells. Mean model accuracy was compared between all PTNs and RF cells for each animal with independent two-tailed t tests (monkey N: $t_{(50.3)} = -0.902$, $p = 0.372$; monkey L: $t_{(41)} = 1.44$, $p = 0.157$).

component of the firing rate profile coded for force, we next simplified the LDA model to include only the peak firing rate for each trial. Although we found that this strategy performed better than chance in a smaller percentage of cells (Fig. 3*A*; monkey N: PTNs, 64.6%; RF cells, 88.2%; monkey L: PTNs, 59.2%; RF cells, 81.3%) and was less accurate (Fig. 3*B,C*), it did reveal a significant difference between PTNs and RF cells. Across the whole population, the model using the peak firing rate was significantly more accurate in classifying trials for RF cells than PTNs in both monkeys (Fig. 3*D*). This effect persisted in the subpopulation of monkey N cells in which the model performed significantly better than chance ($t_{(70)} = -2.68$, $p = 0.009$), but not in monkey L ($t_{(27)} = -0.82$, $p = 0.419$).

To investigate the relationship between peak firing rate and force in more detail, we identified the weight that generated the highest peak firing rate for each cell. For PTNs in both monkeys, this was evenly distributed—individual cells could show their largest rate for anywhere from the lowest to the highest weight. By contrast, for RF cells the peak firing rate was often generated by the heavier weights (Fig. 4*A*). To quantify the force–rate

relationship further, we fit a linear regression between peak firing rate and force for each cell, and classified cells according to whether the regression was not significant or significant with a positive or negative slope (Fig. 4*B*). Of the cells with significant regressions, the majority of RF cells (20 of 21 cells) had a positive force–rate relationship, while approximately equal numbers of PTNs had positive and negative correlations (22 vs 21 cells). Figure 4*C* shows the change in peak firing rate at a given weight, compared with the previous weight, where each line represents one cell. Figure 4*D* presents the distribution of the peak firing rate versus weight regression slope. These plots show that the strength of the rate–force relationship was similar for cells with positive and negative correlations (monkey N PTNs: $t_{(22)} = 1.15$, $p = 0.263$; monkey L PTNs: $t_{(6)} = -0.311$, $p = 0.766$; Fig. 4*C,D*), and there was no significant difference between the rate–force relationship of PTNs and RF cells with positive correlations (monkey N: $t_{(25)} = -1.90$, $p = 0.069$; monkey L: $t_{(13)} = -0.325$, $p = 0.751$; Fig. 4*C,D*). Finally, Figure 4*E* plots the latency of the peak in firing rate for the weight with the highest rate for each cell. For RF cells, the latency of peak firing rate formed a normal

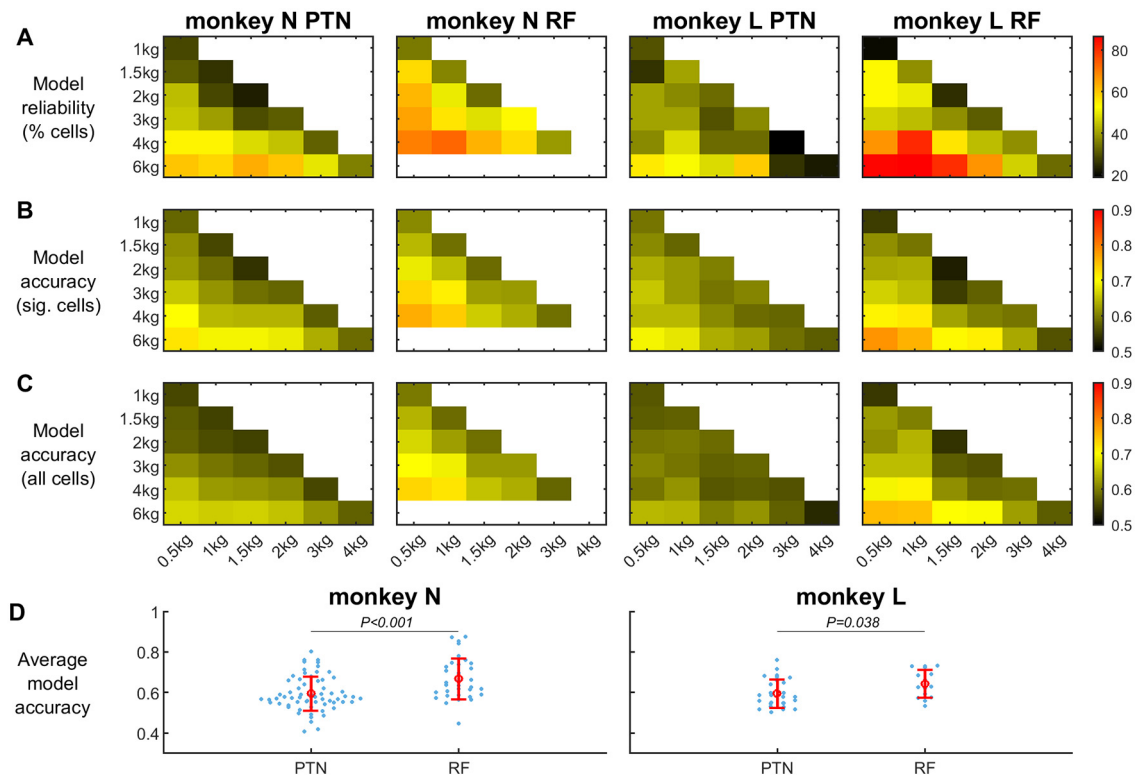


Figure 3. Prediction of trial weight by peak firing rate. LDA comparing the peak firing rate between each pair of weights, for each cell. See Materials and Methods for calculation of peak firing rate. **A**, Percentage of cells in which the model correctly predicted the weight of each trial significantly more often than chance (see Materials and Methods). **B**, Mean model accuracy in the subpopulation of cells in which the overall model accuracy was greater than chance (see Materials and Methods; monkey N PTN: $n = 42/65$; monkey N RF: $n = 30/34$; monkey L PTN: $n = 16/27$; monkey L RF: $n = 13/16$). **C**, Mean model accuracy in all cells. **D**, Mean model accuracy per cell type, per animal. Blue circles, Mean model accuracy for individual cells; red error bars, mean and SD across the population of cells. Mean model accuracy was compared between PTNs and RF cells for each animal with independent two-tailed t tests (monkey N: $t_{(96)} = -3.85$, $p < 0.001$; monkey L: $t_{(40)} = -2.14$, $p = 0.038$).

distribution (monkey N: $D_{(34)} = 0.184$, $p = 0.178$; monkey L: $D_{(16)} = 0.213$, $p = 0.408$) centered just before task completion. By contrast, the latency of peak firing rate in monkey N PTNs did not form a normal distribution ($D_{(65)} = 0.180$, $p = 0.026$) but instead two distinct populations—many cells had a peak firing rate before task completion, but a smaller population of cells had a peak firing rate after task completion (Fig. 4E). A similar trend was observed in monkey L PTNs, although the distribution of peak firing rate latency here was not significantly different from normal ($D_{(27)} = 0.173$, $p = 0.357$).

The results presented above suggest that the RF cell population was relatively homogeneous, whereas the PTNs showed more heterogeneity. Because PTNs were identified antidromically, we were able to investigate whether this heterogeneity was associated with differences in corticospinal axon conduction velocity, measured by the ADL (Fig. 5A). There was no significant correlation between LDA model accuracy (as measured in Fig. 2E) and ADL (Fig. 5B). There was also no significant relationship between ADL and the slope of the peak firing rate versus weight relationship (Fig. 5C).

An alternative way of classifying PTNs is by the depth of the recording site (Koz̄elj and Baker, 2014), which indicates whether a cell is likely to be in the New M1 or Old M1 subdivisions of Rathelot and Strick (2009). Here, we defined the border between deep and superficial PTNs at 2.5 mm below the first recorded cells in that penetration (Fig. 5D). There was no significant correlation between model accuracy and PTN depth (Fig. 5E), and no significant difference between the model accuracy of superficial versus deep PTNs (monkey N: $t_{(63)} = -0.470$,

$p = 0.640$; monkey L: $t_{(19,7)} = -0.836$, $p = 0.413$). Similarly, there was no correlation between the peak firing rate/weight slope and PTN depth (Fig. 5F). These results suggest that the heterogeneity observed in our PTN population cannot be explained by differences between PTN conduction velocity or by the location of cells within the different subdivisions of M1.

Latency effects

All of the analysis described above was conducted on PETHs aligned to task completion. This marks successful performance of the task goal and allows the measurement of firing rates. We were also interested in examining the timing of cell firing relative to muscle activity, but, as shown in Figure 1, muscle activity started earlier relative to the task completion marker for heavier weights. We therefore also constructed PETHs aligned to EMG onset. Figure 6A shows PETHs averaged across cell populations with this alignment. We reran the LDA classifier, replicating the analysis of Figure 2E, but with this new alignment (Fig. 6B), and compared the average model accuracy values with those previously obtained values. LDA showed significantly worse classification using EMG-onset aligned trials for PTNs (monkey N: $t_{(63)} = 3.48$, $p < 0.001$; monkey L: $t_{(26)} = 2.46$, $p = 0.021$), but was not significantly different for RF cells (monkey N: $t_{(33)} = 0.642$, $p = 0.525$; monkey L: $t_{(14)} = -0.951$, $p = 0.358$). However, similar to the finding from task completion-aligned PETHs, there was no significant difference between classifier performance with PTNs and RF cells for either monkey with the EMG-onset aligned trials (Fig. 6B).

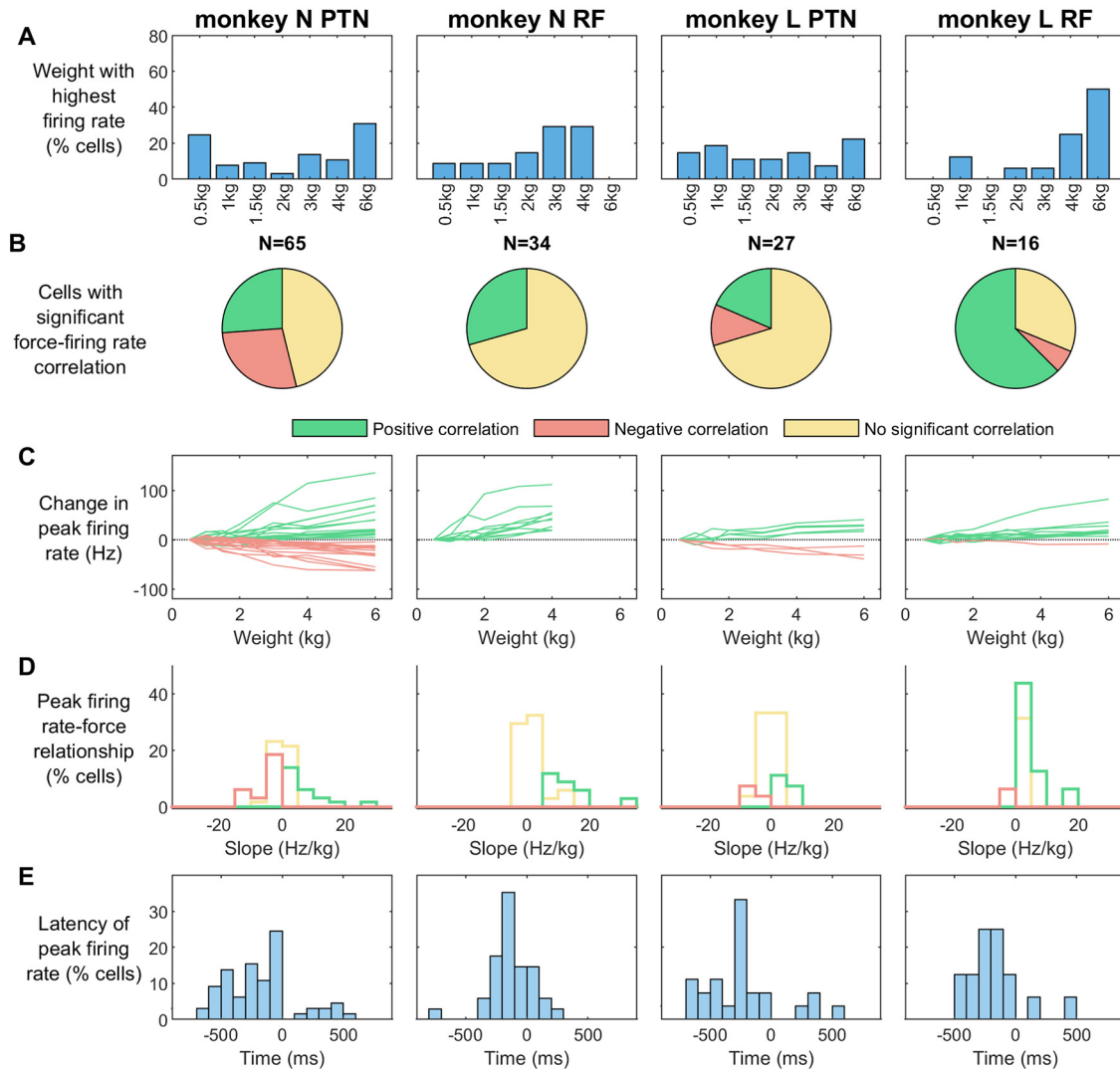


Figure 4. Peak firing rate. **A**, The weight that generated the highest firing rate for each cell, expressed as a percentage of cells per weight. **B**, Proportion of cells with a significant positive or negative correlation (see legend) between peak firing rate and trial weight. **C**, Change in peak firing rate with weight, limited to the subpopulation of cells with a significant positive or negative correlation between peak firing rate and trial weight. **D**, Histogram showing the slope of the peak firing rate versus the force for each cell, grouped by the direction and significance of the relationship (see legend). **E**, Histogram of the latency of peak firing rate, expressed as a percentage of cells.

To analyze how force coding developed in time, for each cell we compared the firing rate at a given moment between each weight and 0.5 kg, and tested for a significant difference. Figure 6C shows how coding evolved with time across the cell population by plotting the number of cells with a significant difference at each time point. For each cell, we then found the first 50 ms bin in which firing rate was significantly different from the 0.5 kg; the distributions of these times, which reflect the onset latency of force coding, are shown as cumulative distributions in Figure 6D. A repeated-measures ANOVA revealed no significant effect of weight (monkey N: $F_{(4,296)} = 2.03$, $p = 0.090$; monkey L: $F_{(5,160)} = 1.05$, $p = 0.392$) or cell type (monkey N: $F_{(1,74)} = 0.14$, $p = 0.710$; monkey L: $F_{(1,32)} = 0.543$, $p = 0.467$) on the latency of the first significant change in firing rate relative to trials at 0.5 kg.

Depending on the cell class, animal, and force, between 31.3% and 79.4% of cells had an onset of force coding before the onset of EMG. Such coding must reflect an aspect of the central command for movement; after EMG onset, there could also be a contribution from afferent feedback. To investigate this further, we compared the ability of the LDA model to classify trials using

firing rates restricted to before EMG onset (before) versus after EMG onset (after). LDA reliability was significantly better when using after firing rates compared with before firing rates for PTNs, but there was no significant difference in before and after model reliability for RF cells (Fig. 7A,E). We also compared LDA accuracy in the subpopulation of cells in which classification was significantly better than chance (Fig. 7B,F) and found that the LDA performed significantly better with after firing rates compared with before firing rates for both cell types, in both animals. When we repeated this analysis including all cells (and not just those where coding was significantly better than chance), we saw the same result (monkey N, Fig. 7C,D; monkey L, Fig. 7G,H). Despite the worse performance of the LDA with before firing rates, we still found the classification accuracy of the cell populations to be significantly better than chance (monkey N, PTNs: $t_{(63)} = 13.7$, $p < 0.001$; monkey N, RF cells: $t_{(33)} = 11.9$, $p < 0.001$; monkey L, PTNs: $t_{(26)} = 9.76$, $p < 0.001$; monkey L, RF cells: $t_{(14)} = 5.98$, $p < 0.001$).

We next wanted to investigate whether the cell activity that occurred before and after EMG onset was part of the same

phenomenon or was driven by different processes. In support of the latter, in monkey N PTNs (Fig. 6A) there were two clear peaks in the population firing rate, with one before and one after EMG onset. To quantify how well these separate epochs coded for force, we reran the LDA based on peak firing rate (Fig. 3), first with single-trial peak firing rates restricted to the time before EMG onset (−500 to 0 ms; early peak) and then in a separate analysis after EMG onset (0–500 ms; late peak). Across both monkeys and cell types, LDA was significantly more accurate in classifying trials based on rates from the late peak (Fig. 8A). To investigate whether peak firing rate in these two periods was part of the same phenomenon, we looked at the correlation between the early and late peak rates (Fig. 8B). For each cell, we fit a linear regression between the early and late peak rates and compared the R^2 values between cell types with unpaired t tests (Fig. 8C). In monkey L, R^2 values were significantly higher for RF cells compared with PTNs. This higher degree of correlation suggests, as can be appreciated from Figure 6A, that in RF cells the changes in firing rate that occur before and after EMG onset are part of the same effect, whereas the lower degree of correlation for PTNs suggests that cells may behave differently before and after EMG onset. However, we observed no significant differences between PTN and RF cell R^2 values for monkey N.

Discussion

The role of the CST in force coding is well established (for review, see Cheney et al., 1991). PTN discharge is related primarily to force rather than displacement (Evarts, 1968, 1969; Humphrey et al., 1970), and discharge rates show both positive and negative correlations with force (Cheney and Fetz, 1980; Maier et al., 1993). Our findings extend this previous work to high forces—our biggest load was comparable to the body weight of the animal; pulling this with one arm probably required a near-maximal contraction. Firing rate was a strong predictor of force; approximately equal numbers of PTNs had peak firing rates positively or negatively correlated with force (Fig. 4B). Furthermore, in contrast to previous studies that investigated force coding in isolated movements, our results reveal that PTN firing rate and force are also related during a gross movement involving cocontraction of multiple upper limb muscles.

The RST provides both monosynaptic and disynaptic inputs to upper limb motoneurons (Riddle et al., 2009) and, hence, is capable of modulating motoneuron firing rate to generate different forces. We have previously demonstrated RST involvement in strength training (Glover

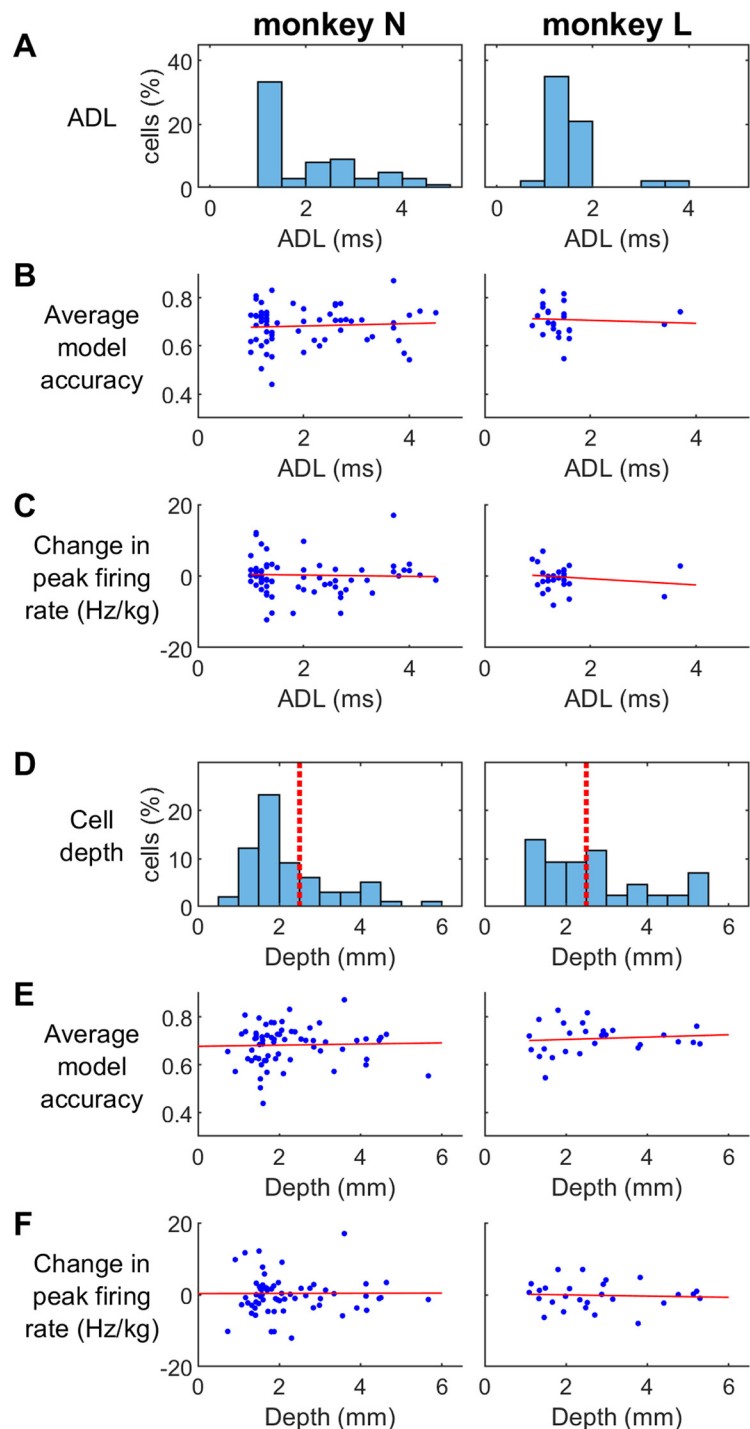


Figure 5. PTN properties. **A**, Histogram of ADL for PTNs, shown separately for each animal (columns). **B**, Correlation between average LDA accuracy (Fig. 2) and ADL. Blue circles, Individual cells; red line, linear regression (monkey N: $R^2 = 0.004$, $p = 0.618$; monkey L: $R^2 = 0.004$, $p = 0.749$). **C**, Correlation between peak firing rate/force slope (Fig. 4D) and ADL. Blue circles, Individual cells; red line, linear regression (monkey N: $R^2 < 0.001$, $p = 0.825$; monkey L: $R^2 = 0.022$, $p = 0.456$). **D**, Histogram of cell depth for PTNs, shown separately for each animal (columns). Red dotted line, The boundary between superficial and deep PTNs (2.5 mm). **E**, Correlation between average model accuracy (Fig. 2) and cell depth. Blue circles, Individual cells; red line, linear regression (monkey N: $R^2 = 0.001$, $p = 0.804$; monkey L: $R^2 = 0.011$, $p = 0.609$). **F**, Correlation between peak firing rate/force slope (Fig. 4D) and cell depth. Blue circles, Individual cells; red line, linear regression (monkey N: $R^2 < 0.001$, $p = 0.977$; monkey L: $R^2 = 0.003$, $p = 0.766$).

and Baker, 2020), indirectly implicating this pathway in force generation. In support of this, similar to PTNs, we found that the RF cell firing rate was highly predictive of force.

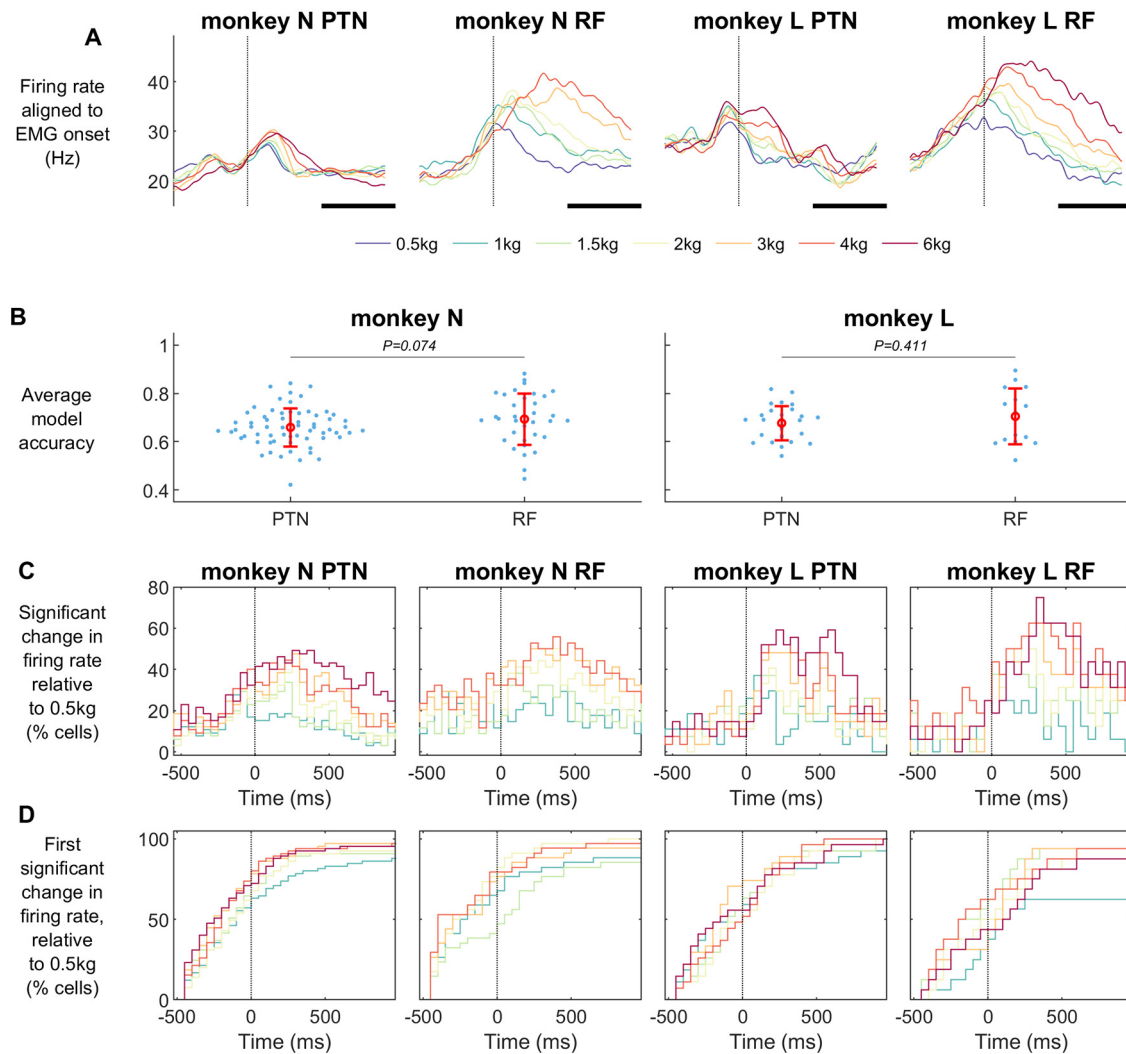


Figure 6. EMG onset-aligned firing rate. **A**, PETHs, averaged across all recording sessions for each animal and cell type (columns), shown per weight (see legend) and aligned to EMG onset (black dotted line; see Materials and Methods). Calibration: 500 ms. **B**, Mean LDA accuracy comparing firing rates between each pair of weights, for each cell across the full time course of each EMG onset-aligned trial. Blue circles, Mean LDA accuracy for individual cells; red vertical error bars, mean and SD across the population of cells. Mean LDA accuracy was compared among all PTNs and RF cells for each animal with independent two-tailed t tests (monkey N: $t_{(96)} = -1.81$, $p = 0.074$; monkey L: $t_{(20)} = -0.841$, $p = 0.411$). **C**, Percentage of cells with a significant change in firing rate compared with trials at 0.5 kg, shown per weight (see legend) and a 50 ms bin. Black dotted line, EMG onset. **D**, Cumulative distribution of the first 50 ms bin with a significant difference in firing rate compared with trials at 0.5 kg, shown per weight (see legend). Black dotted line, EMG onset.

Relative roles of CST and RST in force generation

The observation that the firing rate of both PTNs and RF cells codes for force raises the question of their relative roles. Although this could reflect redundancy in the motor system, our observations highlight differences in brainstem and cortical force-coding strategies.

When considering the complete time course of the task, firing rates of PTNs and RF cells predicted force similarly. However, when analysis was limited to peak firing rates, RF cells coded force better than PTNs. One interpretation is that RF cells provide a gross drive to motoneurons, which can be well summarized by peak firing rate. By contrast, PTNs may play a more sophisticated role, involving close modulation of rates to fine-tune movement. This can be subjectively appreciated from the population-averaged PETHs (Fig. 1G,H): the RF rate increased steadily before task completion, while PTN firing had a complex profile with multiple peaks. Furthermore, of the cells with a significant rate–force correlation, approximately equal numbers of PTNs showed positive and negative correlations, while all but

one RF cell had positive gradients. This again suggests a role for the RF cells in the gross specification of force, compared with the fine-tuning of movement by PTNs. This may be task dependent. Muir and Lemon (1983) reported higher firing rates during a precision grip task than a power grip task, although the latter activated muscles more. Similarly, in an alternating wrist flexion/extension task, PTN firing modulated more when the direction of the load changed (requiring activation of different muscles) than with force changes in one direction (Schmidt et al., 1975).

It might be argued that our task was especially suited to control by the RST as it generated substantial cocontraction over multiple upper limb muscles. The CST seems most suited to producing highly fractionated movements (Zaaimi et al., 2018), reflecting the limited divergence of individual axonal projections to different motoneuron pools (Shinoda et al., 1981; Buys et al., 1986). By contrast, the extensive collateralization of the RST (Peterson et al., 1975; Matsuyama et al., 1997) makes it better suited to gross movements (Davidson and Buford, 2004, 2006; Baker and Perez, 2017; Zaaimi et al., 2018). However, regardless

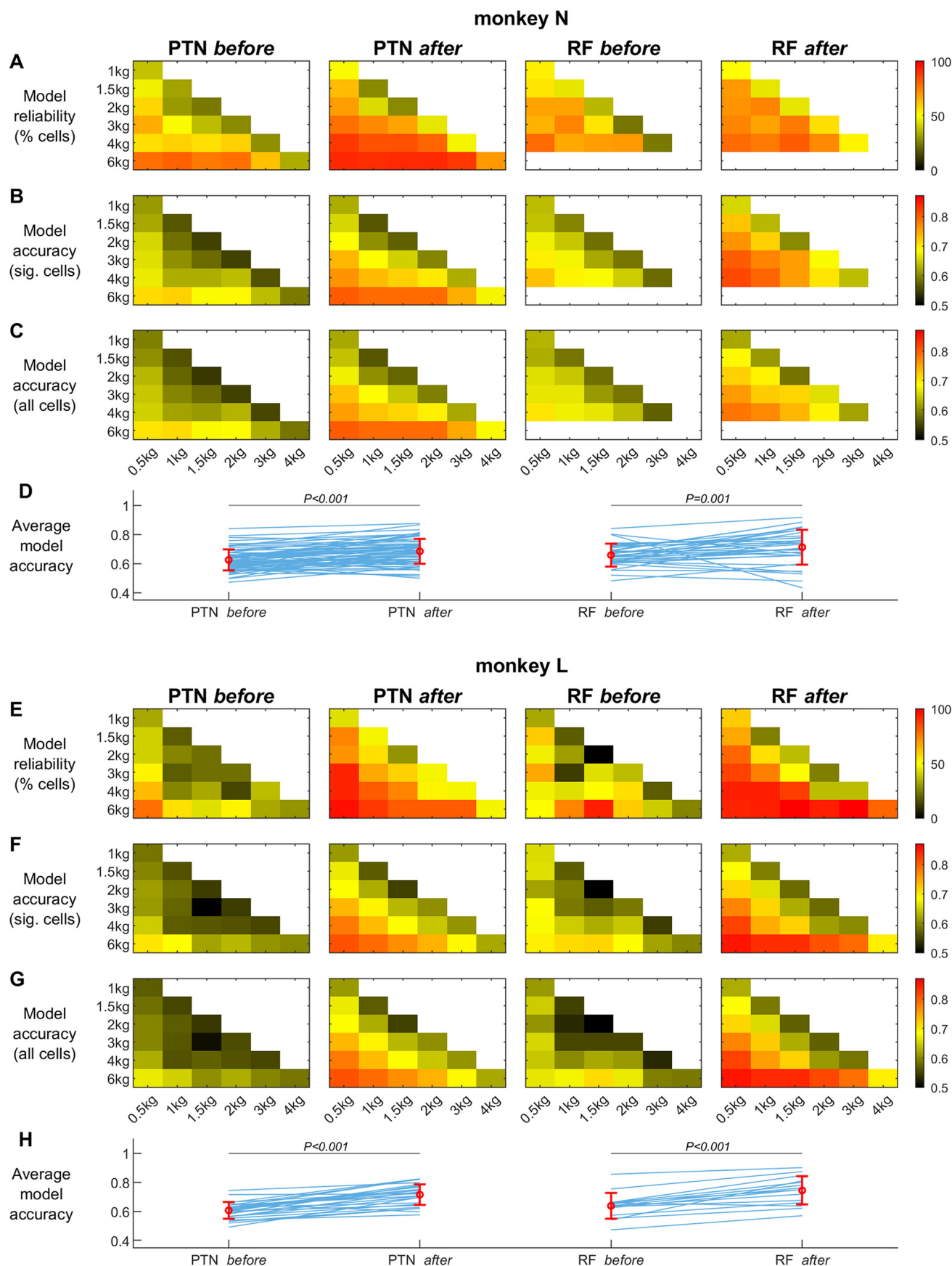


Figure 7. Comparison of firing rate before and after EMG onset. LDA comparing the firing rate between each pair of weights for each cell, performed separately for firing rate before EMG onset (–500 to 0 ms; columns 1 and 3) and after EMG onset (0–500 ms; columns 2 and 4) for EMG onset-aligned PETHs (see Materials and Methods). **A–H**, Results are shown separately for monkey N (**A–D**) and monkey L (**E–H**). **A, E**, Percentage of cells in which the model correctly predicted the weight of each trial significantly more often than chance for each pair of weights. McNemar's test compared the

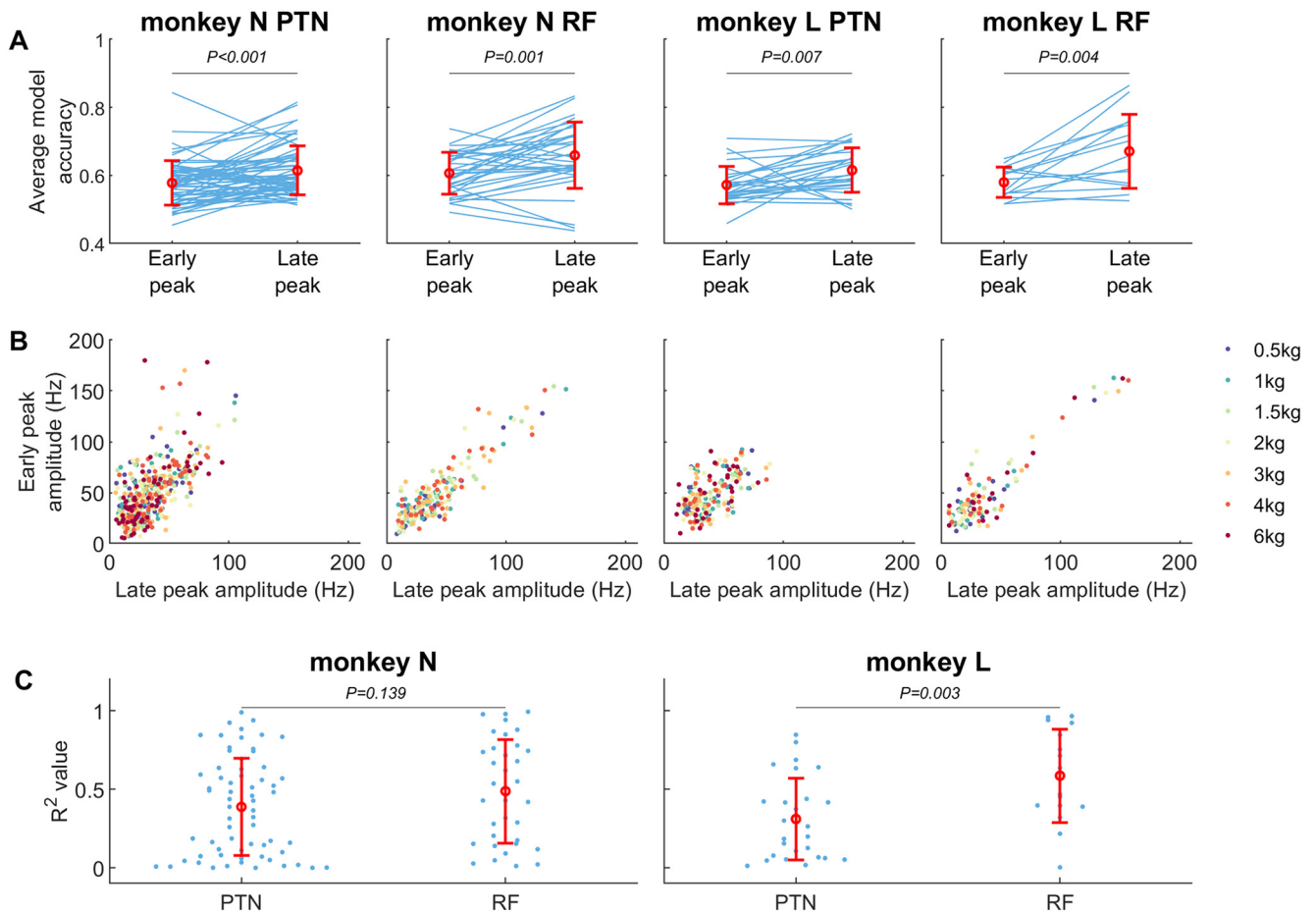


Figure 8. Comparison of early and late peaks. **A**, Mean LDA accuracy per cell type and time period (early or late peak). Blue lines, Mean model accuracy for individual cells, for peak firing rate in the early and late periods; red errors bars, mean and SD across the population of cells. Mean LDA accuracy of the early peak and late peak were compared for each animal and cell type with paired two-tailed t tests (monkey N PTNs: $t_{(63)} = -3.72$, $p < 0.001$; monkey N RF cells: $t_{(33)} = -3.58$, $p = 0.001$; monkey L PTNs: $t_{(26)} = -2.93$, $p = 0.007$; monkey L RF cells: $t_{(14)} = -3.50$, $p = 0.004$). **B**, Correlation of peak firing rate in the early and late periods for each weight (see legend). Individual points show the peak firing rate for each weight, for each cell. **C**, R^2 values for the correlations between peak firing rate in the early and late periods for each cell. R^2 values were compared between PTNs and RF cells for each monkey using unpaired t tests (monkey N: $t_{(97)} = -1.49$, $p = 0.139$; monkey L: $t_{(41)} = -3.19$, $p = 0.003$).

overall model reliability for each cell between the before and after condition (monkey N PTNs: $p = 0.020$; monkey N RF cells: $p = 0.317$; monkey L PTNs: $p = 0.014$; monkey L RF cells: $p = 0.083$). **B, F**, Mean model accuracy in the subpopulation of cells in which the overall model accuracy was better than chance (monkey N PTNs before: $n = 54/65$; monkey N PTNs after: $n = 61/65$; monkey N RF cells before: $n = 30/34$; monkey N RF cells after: $n = 28/34$; monkey L PTNs before: $n = 21/27$; monkey L PTNs after: $n = 27/27$; monkey L RF cells before: $n = 12/16$; monkey L RF cells after: $n = 15/16$). A repeated-measures ANOVA compared mean model accuracy between the before and after conditions (monkey N: $F_{(1,77)} = 9.98$, $p = 0.002$; monkey L: $F_{(1,31)} = 15.9$, $p < 0.001$) and cell type (monkey N: $F_{(1,77)} = 4.91$, $p = 0.030$; monkey L: $F_{(1,31)} = 2.85$, $p = 0.101$). *Post hoc* testing compared mean model accuracy between the before and after periods for each animal and cell type with paired two-tailed t tests (monkey N PTNs: monkey N: $t_{(51)} = -8.44$, $p < 0.001$; monkey N RF cells: $t_{(26)} = -4.82$, $p < 0.001$; monkey L PTNs: $t_{(20)} = -7.95$, $p < 0.001$; monkey L RF cells: $t_{(11)} = -6.45$, $p < 0.001$). In monkey N, *post hoc* testing compared model accuracy between cell types for each period (before: $t_{(77)} = -2.71$, $p = 0.008$; after: $t_{(77)} = -1.47$, $p = 0.144$). **C, G**, Mean model accuracy in all cells. **D, H**, Mean model accuracy per cell type and time period (before or after EMG onset) for all cells. Blue lines, Mean model accuracy for individual cells, before and after EMG onset; red errors bars, mean and SD across the population of cells. A repeated-measures ANOVA compared mean model accuracy between the before and after conditions (monkey N: $F_{(1,97)} = 16.8$, $p < 0.001$; monkey L: $F_{(1,41)} = 6.94$, $p = 0.012$) and cell type (monkey N: $F_{(1,97)} = 0.80$, $p = 0.372$; monkey L: $F_{(1,41)} = 1.71$, $p = 0.199$). *Post hoc* testing compared mean model accuracy between the before and after periods for each animal and cell type with paired two-tailed t tests (monkey N PTNs: $t_{(64)} = -9.60$, $p < 0.001$; monkey N RF cells: $t_{(33)} = -3.59$, $p = 0.001$; monkey L PTNs: $t_{(26)} = -9.17$, $p < 0.001$; monkey L RF cells: $t_{(15)} = -5.00$, $p < 0.001$).

of the specific nature of the task, high-force contractions typically involve substantial and unavoidable coactivation, often bilaterally (Zijdewind and Kernell, 2001). A task that generated strong but isolated activation of a single muscle would be impossible to implement and poorly reflect the reality of real-life high-force tasks. A further limitation of our task was that contractions were brief and did not include a sustained holding phase; the neural substrates controlling sustained versus phasic contractions may have important differences (Albert et al., 2020). However, CST neurons tend to reduce their activity markedly during steady holding (Baker et al., 2001), suggesting that the inclusion of a hold phase would be unlikely to alter the balance between CST and RST seen here.

It is also important to consider the connections between descending neurons and motoneurons. The highly phasic and brief contractions in the present study precluded identifying cells with monosynaptic connections to motoneurons with spike-triggered averaging (Fetz and Cheney, 1980; Lemon et al., 1986). Nonetheless, it is unlikely that the PTN heterogeneity is explained solely by separating into cells with monosynaptic versus polysynaptic projections to motoneurons (Rathelot and Strick, 2009; Witham et al., 2016). A previous study examining only CM cells similarly showed that PTN firing rates can correlate positively or

negatively with force (Maier et al., 1993). That article reported 6 of 17 correlated CM cells (39%) had negative slopes, compared with 21 of 43 PTNs (49%) reported here; these proportions do not differ significantly ($p = 0.34$, χ^2 test). Likewise, Griffin et al. (2015) reported that CM cells recorded in a two-dimensional wrist movement task fired in preferred directions that were not necessarily aligned to the direction of action of the target muscle, leading the authors to conclude that individual CM cells can control a muscle not only when it is acting as an agonist, but also in situations when it functions as antagonist, synergist, or fixator. Additionally, we found no relationship between the force/firing rate gradient and either the recorded depth or ADL of PTNs. Given that fast-conducting PTNs with monosynaptic projections are predominantly found superficially within M1 (Rathelot and Strick, 2009; Witham et al., 2016), we would predict divergence in firing rate characteristics with these properties if PTN heterogeneity could be explained by their projections. Another relevant aspect of descending connectivity is whether an axon contacts inhibitory interneurons (Jankowska et al., 1968, 1976), which would manifest in spike-triggered averages of EMG as a postspike suppression (Kasser and Cheney, 1985). Such cells might be expected to show negative correlations with force, although, as shown by Maier et al. (1993), CM cells with direct, excitatory projections to motoneurons can also, unexpectedly, have negative correlations.

Force specification

Our task was performed in blocks of 10 trials at the same weight. The animals typically generated inappropriate force for the first trial per block, while subsequent trials were performed with more control, reflecting an accurate motor plan (Johansson and Westling, 1988). The first trial was accordingly excluded from all analysis. Firing rates that modulate with force before EMG onset must reflect internal storage of the required force and a centrally generated motor command. By contrast, rate modulation after EMG onset could be generated in response to sensory feedback from proprioceptive or cutaneous afferents. In that case, activity might still contribute differential drive to motoneuron pools, but would not signify the causal spark that ignites the specific movement.

To investigate these possibilities, we examined the coding of force by firing rate before and after muscle activity onset. Unsurprisingly, for both PTNs and RF cells firing rate after muscles became active was a better predictor of force than before. However, the decoding of force from firing rate was still significantly better than chance before EMG onset for both areas, suggesting that force is specified before movement by both PTNs and RF cells.

The finding that force is specified in M1 before movement agrees with much previous work. In humans, the disruption of M1 by repetitive transcranial magnetic stimulation prevents subjects using prior experience to generate appropriate force levels (Chouinard et al., 2005; Berner et al., 2007), implying cortical involvement in weight storage. In monkeys, a small proportion of M1 neurons shows significant force coding before a reach and grasp task (Hendrix et al., 2009), supporting a cortical role in force planning. Similarly, RF cells are active in the preparatory phase of a reaching movement (Buford and Davidson, 2004; Schepens and Drew, 2004). Cortical projections (including from M1) converge extensively onto the RF (Fisher et al., 2020); many of these corticoreticular projections are PTN collaterals (Keizer

and Kuypers, 1989). Force coding before muscle activity onset in RF neurons could therefore be caused by descending instructions from the cortex.

After movement onset, sensory feedback fine-tunes the pattern and force of muscle activity. Both M1 and RF receive sensory inputs (Rosén and Asanuma, 1972; Leiras et al., 2010); feedback from cutaneous and proprioceptive receptors will influence firing rates in both regions. Modern conceptions of the motor program emphasize the importance of the integration of sensory feedback (Todorov and Jordan, 2002). The disruption of sensory feedback produces profound acute motor deficits (Cole and Katifi, 1991; Darian-Smith and Ciferri, 2005), which can be characterized clinically as weakness (Ng and Baker, 2021). Temporary deafferentation modifies M1 activity in both monkeys (Lewis et al., 1971) and humans (Galán et al., 2015). Our finding that PTN and RF firing rates can predict force both before and during movement suggests that force coding likely occurs through a combination of internal storage of object weight and afferent feedback. In this context, we should note that one of the animals studied had lost part of two digits on the hand in an unrelated incident before beginning training on the task. Results did not appear to differ between this animal and the one with an intact hand, but we cannot exclude that altered afferent feedback could have modified both M1 and RF activity in this monkey.

Modulation of motoneuron excitability

Increased descending drive to motoneurons can modulate muscle force through the recruitment of additional motoneurons (Henneman, 1957) and/or the increased firing rate of motoneurons (Monster and Chan, 1977). Motoneurons are also regulated by spinal circuits. For example, C-boutons, which provide cholinergic inputs to motoneurons (Witts et al., 2014), are likely necessary for high-force outputs since their genetic inactivation reduces muscle activity (Zagoraoui et al., 2009). Motoneuron gain may also be regulated by persistent inward currents, which can amplify the response to synaptic inputs (Binder et al., 2020). Such mechanisms may tune motoneuron responses to a given input, but are unlikely to overcome the need for descending inputs to generate different forces. Indeed, descending inputs are required to configure these systems (e.g., descending monoamine pathways such as the raphe spinal tract, which activate persistent inward currents), so that part of the impact of the rate modulation may occur via these spinal circuits, rather than by a direct action on motoneurons.

Summary

Firing rates of both PTNs and RF cells can predict force output. However, it is unlikely that these represent identical, redundant routes for force control. The results are consistent with RF neurons providing a simple gross drive to motoneurons, while PTNs fine-tune activation according to the detailed requirements of the movement. For both PTNs and RF cells, firing rates code the required force output before the activation of muscles, but also after the onset of muscle contraction, when firing could be modulated by sensory feedback.

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