Recruitment in Retractor Bulbi Muscle During Eyeblink Conditioning: EMG Analysis and Common-Drive Model

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Recruitment in retractor bulbi muscle during eyeblink conditioning: EMG analysis and common-drive model. J Neurophysiol 102: 2498–2513, 2009. First published August 12, 2009; doi:10.1152/jn.00204.2009. To analyze properly the role of the cerebellum in classical conditioning of the eyeblink and nictitating membrane (NM) response, the control of conditioned response dynamics must be better understood. Previous studies have suggested that the control signal is linearly related to the CR as a result of recruitment within the accessory abducens motoneuron pool, which acts to linearize retractor bulbi muscle and NM response mechanics. Here we investigate possible recruitment mechanisms. Data came from simultaneous recordings of NM position and multiunit electromyographic (EMG) activity from the retractor bulbi muscle of rabbits during eyeblink conditioning, in which tone and percuticular shock act as conditional and unconditional stimuli, respectively. Action potentials (spikes) were extracted and classified by amplitude. Firing rates of spikes with different amplitudes were analyzed with respect to NM response temporal profiles and total EMG spike firing rate. Four main regularities were revealed and quantified: 1) spike amplitude increased with response amplitude; 2) smaller spikes always appeared before larger spikes; 3) subsequent firing rates covaried for spikes of different amplitude, with smaller spikes always firing at higher rates than larger ones; and 4) firing-rate profiles were approximately Gaussian for all amplitudes. These regularities suggest that recruitment does take place in the retractor bulbi muscle during conditioned NM responses and that all motoneurons receive the same command signal (common-drive hypothesis). To test this hypothesis, a model of the motoneuron pool was constructed in which motoneurons had a range of intrinsic thresholds distributed exponentially, with threshold linearly related to EMG spike amplitude. Each neuron received the same input signal as required by the common-drive hypothesis. This simple model reproduced the main features of the data, suggesting that conditioned NM responses are controlled by a common-drive mechanism that enables simple commands to determine response topography in a linear fashion.

INTRODUCTION

Classical conditioning of the rabbit’s nictitating membrane (NM) response has been extensively studied in two contexts: first, as an exemplar of associative learning (e.g., Dudenev et al. 2007; Gormezano et al. 1983) and, more recently, as a suitable task for investigating cerebellar function in the production of a learned movement (e.g., Hesslow and Yeo 2002; Thompson 2005). Although a major attraction of using the NM response has been its apparent simplicity, accumulating evidence suggests that this simplicity is only relative. For example, although the NM response does indeed have much simpler

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acquisition of the conditioned response, the peak EMG spike rate increased appropriately to drive a larger peak NM response, whereas the time-to-peak of the EMG spike rate and NM response amplitude did not change appreciably.

Therefore the overall effective dynamics that link motoneuron firing to NM movement can be viewed as a “plant simplification” that make conditioned response parameters such as amplitude, duration, and timing much easier to control than the complexities of the plant (i.e., muscle plus globe) would at first suggest. Such a linearized “virtual plant” should result in a lower computational load in the neuronal systems driving the movement and give more accurate motor control. Similar plant simplifications occur frequently in physiological motor control (Angelaki and Hess 2004; Mussa-Ivaldi et al. 1994; Nichols and Houk 1976), so the mechanisms by which they are achieved are of general interest.

Our previous analysis, which treated all EMG spikes as equal, irrespective of their amplitude (Lepora et al. 2007), cast no direct light on the underlying mechanism. Here we extend the analysis to take spike amplitude into account because inspection of retractor bulbi EMG records strongly suggested that spike amplitude and conditioned response parameters were related. Such a relation implies recruitment and, indeed, the analysis of EMG spike amplitudes indicated a simple procedure for recruiting motor units in the retractor bulbi muscle to control conditioned response (CR) amplitude. The same command is sent to all motoneurons in the accessory abducens nucleus pool. Its intensity varies over time with a Gaussian profile, whose peak is related to CR amplitude and width to CR duration. Motoneuron threshold varies with EMG spike amplitude, so that the higher the threshold, the larger the spike. Spike amplitude varies, in turn, with motor unit strength. In this manner, a simple high-level command can generate the forces necessary for the appropriately sized CR, using the common-drive mechanism for motor control previously proposed for a range of movements that use other muscles (De Luca et al. 1982a,b, 1996; Jabre and Spellman 1996; Masakado et al. 1995; Sauvage et al. 2006).

In these previous studies, EMG records allowed identification of the action potentials from individual motor units, so that unit firing rate could be related unequivocally to the amplitude of its action potential. Here, the technical difficulties of recording from the small and mobile retractor bulbi muscle prevent individual motor unit action potentials from being reliably identified. To determine whether the common-drive mechanism could in principle account for the EMG patterns observed in the previous study, we attempted to simulate the data patterns using a computational model. This model was able to characterize various observed features of the EMG records using simple assumptions about the properties of motoneurons receiving common-drive synaptic input. The success of the model is consistent with the view that, because of the technical difficulties of EMG recording and analysis, “computational models are fast becoming the dominant means to establish the mechanism relating EMG to muscle force and neural drive” (Keenan and Valero-Cuevas 2007). Some of the findings previously appeared in abstract form (Lepora et al. 2006).

METHODS

Data collection

Procedures for surgery, training, and data acquisition have been described previously (Lepora et al. 2007). Briefly, data were obtained from two different laboratories, one at University College London (Yeo) and the other at the State University of New York at Stony Brook (Evinger). All procedures carried out at University College London conformed to UK Home Office, Animal Procedures regulations. All procedures carried out at the State University of New York at Stony Brook adhered to federal, state, and university guidelines concerning the use of animals in research.

At University College London, EMG electrodes were implanted in the retractor bulbi muscle of three male Dutch belted rabbits (2.0–2.2 kg), subsequently referred to as RB1–RB3, under halothane general anesthesia. All general surgical procedures were as described in Yeo (1985) and those specific to the EMG electrode implantation are as previously described in detail (Lepora et al. 2007). EMG electrodes were of Teflon-coated stainless steel wire (50-μm diameter bare). In subject RB1, a pair of additional EMG electrodes, similar to those used in the retractor bulbi muscle, were implanted in the orbicularis oculi muscle immediately dorsal to the upper eyelid at a spacing of about 3 mm. Postoperatively, all subjects received buprenorphine twice per day for 3 days to provide postimplantation analgesia and minimize discomfort. Displacement of the NM was measured with an isotonic transducer based on a low-torque potentiometer (Gruart and Yeo 1995) whose output was fed into an A/D converter (CED 1401). For adaptation and training, each subject was placed in a restraining box in a sound-attenuating chamber facing a centrally mounted loudspeaker and the NM transducer was fitted. In the 50-min adaptation session, no stimuli were presented. For acquisition, the conditioned stimulus (CS) was a 1-kHz sinusoidal tone of intensity 85 dB and duration 560 ms (RB1) or 410 ms (RB2, RB3), delivered through the loudspeaker. The unconditioned stimulus (US) was a 60-ms train of three biphasic current pulses of 2 mA delivered to the periorbital region via a pair of 3-mm Michel clips, attached 1–2 mm below the lower lid and 1–2 mm posterior to the lateral canthus, as previously described (e.g., Gruart and Yeo 1995). The intertrial interval between CS presentations varied randomly between 25 and 35 s and 100 trials were presented each session. On every 10th trial the CS was presented alone. On each trial EMG and NM response data were recorded for a 2-s period starting 1 s before CS onset. EMG data were sampled at 5–40 kHz and NM response data at 1 kHz.

After acquisition, RB1 and RB2 were tested with triplets, which were repeating sets of one trial CS alone, one trial CS + US, and one trial US alone. In addition, RB1 was tested with varying CS intensities (65, 75, 85, and 95 dB). In the case of RB3, triplet testing was carried out with an airpuff US, which reduced CR amplitude in comparison with the earlier trials using a shock US that had been analyzed previously (Lepora et al. 2007). However, the triplet CRs were more evenly distributed across the amplitude range and were thus more suitable for analysis here.

General surgical procedures at the Stony Brook University laboratory were identical. To insert EMG electrodes, a pair of Teflon-coated stainless steel wires (76-μm diameter bare, 140 μm coated; A-M Systems #791000) were inserted into one slip of the retractor bulbi muscle with a 30-gauge hypodermic needle. Five hundred microns of insulation was removed from the tip of each wire and tip separation between the wires was between 1 and 2 mm. To eliminate any postoperative discomfort, rabbits received buprenorphine twice daily for 2 days after the surgery. Data were analyzed from rabbit RB4, a New Zealand white. After initial acquisition of the conditioned response, RB4 was tested under a variety of conditions, including variations in CS and US intensities and extinction.

Once CR amplitude increased beyond the chosen threshold amplitude of 0.5 mm, it reached asymptotic values in a small number of trials (median 45). Since CS alone trials constituted only 10% of all
trials during acquisition, few CRs from acquisition were available for analysis. Thus most of the CRs analyzed here (median 93%) came from the postacquisition phase.

Data selection

The data used for analysis came from trials in which the CS was presented on its own and CR amplitude was >2.5% of the maximum CR amplitude for that subject. About 15% of these trials were subsequently discarded either to avoid spontaneous movement of the NM just before CS presentation or 2) there was interference in the EMG or NM response signals or there was evidence from the EMG recording suggesting electrode movement during the CR.

Data analysis

The occurrence of motor-unit action potentials was estimated from spikes in the EMG records (Sanders et al. 1996) using a threshold crossing procedure so that a spike was registered whenever EMG amplitude increased past a minimum spike amplitude threshold \( \theta \) (Fig. 1A). Given an EMG signal \( x = (x_0, x_1, x_2, \ldots) \) sampled at frequency \( f_{\text{EMG}} \) Hz, [i.e., at discrete time \( n f_{\text{EMG}} = (0, 1/f_{\text{EMG}}, 2/f_{\text{EMG}}, \ldots) \)], this procedure gives a spike sequence \( s \) that is a binary time series of zeros and ones

\[
\begin{align*}
\mathbf{s} &= (s_0, s_1, s_2, \ldots) \quad s_i = \begin{cases} 
1, & x_i < \theta < x_{i+1} \\
0, & \text{otherwise} 
\end{cases} 
\end{align*}
\]

Possible problems with this method were discussed in a previous analysis of the same EMG data set (Lepora et al. 2007). First, high-frequency noise can cause multiple-spike counting artifacts, so the EMG records were downsampled to a frequency of 5 kHz. Second, at high spiking rates there can be significant spike overlap, producing a wavelike interference pattern rather than discrete action potentials (Sanders et al. 1996). The effects of this interference were demonstrated to be minor for the EMG data sets under consideration (Lepora et al. 2007). Third, a suitable threshold \( \theta \) must be chosen for each subject. A signal-to-noise separation procedure was used to estimate the values for \( \theta \), which are reproduced here in Table 1 for each of the four subjects RB1–RB4.

As explained in the introduction, the focus of the present study is EMG spike amplitude. The distribution of spike amplitudes was quantified by placing the peak amplitude \( e \) of each of the spikes found by Eq. 1 in one of \( N_{\text{class}} \) distinct spike amplitude classes. These classes were defined from a set of equally spaced thresholds \( \theta_1 < \theta_2 < \ldots < \theta_{N_{\text{class}}} \), such that a spike of amplitude \( e \) is in class \( k \) (where \( 1 \leq k \leq N_{\text{class}} \))

\[
\theta_{k-1} < e \leq \theta_k = \theta_0 + k \frac{e_{\text{max}} - \theta}{N_{\text{class}}} 
\]

where \( \theta_0 \) is the spike-detecting threshold used in Eq. 1 and \( e_{\text{max}} \) is the maximal spike amplitude across all EMG records under consideration (values for \( e_{\text{max}} \) are given for RB1–RB4 in Table 1). A characteristic amplitude \( \bar{e}_k \) for the spikes in each amplitude class is the midpoint of their range

\[
\bar{e}_k = (\theta_{k-1} + \theta_k)/2
\]

The above procedure results in \( N_{\text{class}} \) spike sequences \( s(k), s(k), \ldots \) with each a binary time series of whether spikes occur in a particular amplitude range—for example, with \( N_{\text{class}} = 5 \) classes, the spike classes \( k = 1, 3, 5 \) can be thought of as signaling when the “small,” “medium,” and “large” spikes occur, as illustrated in Fig. 1. It is important to note that these classes are a convenient measure for dividing spikes by amplitude along a continuum of values, rather than representing true clusters of spikes into distinct classes.

Once spikes had been extracted, their firing rates were calculated from the number of spikes in successive 50-ms time intervals. The noise levels of individual records meant that data had to be pooled across trials, based on similarity of CR amplitude. Three-trial batches were typically used (Lepora et al. 2007) to give a suitable number of spikes for analysis, with the trials ordered by peak NM amplitude to ensure that similarly sized responses contributed to the average. All results in this study were checked for robustness with respect to 5, 6, 7, 8, 9, and 10 classes; results were not found to change appreciably apart from a general deterioration of the results as the number of classes was increased because of too few spikes in each class. Thus for simplicity the number of spike classes was always set to \( N_{\text{class}} = 5 \), a value small enough for the results to have enough spikes in each class and large enough for statistical analysis of the EMG records with respect to spike height.

Previous analysis of the EMG data indicated that for CRs a portion of the total spike rate profiles could be well fitted by Gaussian curves (Lepora et al. 2007) according to the equation

\[
f(t) = h \exp \left[ -\frac{(t - \mu)^2}{2\sigma^2} \right]
\]

where \( f(t) \) is instantaneous firing rate calculated over successive 50-ms intervals, \( \mu \) is the time of the distribution mean, \( h \) its maximum amplitude, and \( \sigma \) is a measure of its width. Fitting was restricted to the

![FIG. 1. Example of spike extraction and classification for electromyogram (EMG) of retractor bulbi muscle (subject RB4). A: a portion of EMG record shown with expanded time base to illustrate threshold crossing criterion for spike extraction and classification for 5 spike amplitude classes. B: entire EMG record for a conditioned-stimulus alone trial (middle trace), showing extracted and classified spikes (bottom trace) and conditioned nictitating membrane response (NMR; top trace).](http://jn.physiology.org/doi/abs/10.1152/jn.00633.2009)
interval from CS onset to one stimulus interval (ISI) after US onset (ISI is the time between CS and US onsets), to exclude a tail in the spike rate profile of some records. This range actually covers most of the spike rate variation of each record because the peak frequency typically occurs significantly before US onset (see Fig. 2, for example). Effectively, this procedure characterized each spike distribution for the muscle in response to the common-drive current. These model motoneurons have firing rates that are linearly proportional to the input synaptic currents above a threshold.

TABLE 1. Parameters describing the observed and fitted EMG spike-rate profile and CR profile for subjects RB1–RB4

<table>
<thead>
<tr>
<th>Subject</th>
<th>Threshold $\theta_0$, mV</th>
<th>Maximum $e_{\text{max}}$, mV</th>
<th>Median Center $\mu$, s</th>
<th>Median Width $\sigma$, s</th>
<th>Peak Range $h$, spikes/s</th>
<th>Median Fit $R^2$</th>
<th>NM Response Amplitude Range $y$, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB1</td>
<td>0.17</td>
<td>2.53</td>
<td>0.45</td>
<td>0.13</td>
<td>0–440</td>
<td>0.92</td>
<td>0–4.4</td>
</tr>
<tr>
<td>RB2</td>
<td>0.15</td>
<td>0.75</td>
<td>0.29</td>
<td>0.06</td>
<td>0–220</td>
<td>0.94</td>
<td>0–2.4</td>
</tr>
<tr>
<td>RB3</td>
<td>0.0275</td>
<td>0.67</td>
<td>0.36</td>
<td>0.10</td>
<td>0–450</td>
<td>0.83</td>
<td>0–3.3</td>
</tr>
<tr>
<td>RB4</td>
<td>0.0375</td>
<td>0.36</td>
<td>0.39</td>
<td>0.07</td>
<td>0–370</td>
<td>0.82</td>
<td>0–6.1</td>
</tr>
</tbody>
</table>

where $f_j$ represents the firing rate of the $j$th bin from a total of $n$ bins, $t_j$ is the time at which that firing rate occurs, and $N$ is the total number of spikes divided by the time width $\delta$ of the bin. The maximum amplitude $h$ is the peak value across the spike rate record. The width $\sigma$ is found by equating the spike total of the record to that for the Gaussian curve (i.e., matching areas under the curves) through the relation

$$\sigma = \frac{1}{h \sqrt{2\pi}} \int_{-\infty}^{\infty} \exp \left( -\frac{(t-\mu)^2}{2\sigma^2} \right) dt = \frac{1}{h \sqrt{2\pi}} \sum_{j=1}^{n} f_j \delta = \frac{N\delta}{h \sqrt{2\pi}}$$

The goodness of fit for the function in Eq. 4 is then the $R^2$ value

$$R^2 = 1 - \frac{\sum_{j=1}^{n} (f_j - f(t_j))^2}{\sum_{j=1}^{n} f_j^2}$$

which describes the proportion of variance accounted for by the fit of $f(t)$ to the spike data. An associated significance level can be calculated using the variable $R\sqrt{(N-k)(1-R^2)}$, where $n$ is the number of data points and $k$ is the number of fitted parameters. This is distributed as Student’s $t$-test with $N-k$ degrees of freedom (Press et al. 1992).

Here we also applied this Gaussian-fitting analysis to the firing rate profiles of the different EMG spike amplitude classes according to

$$f_j(t) = h_t \exp \left( \frac{-(t-\mu_t)^2}{2\sigma_t^2} \right)$$

where $f_j(t)$ is the firing rate of class $k$. This results in a set of three parameters ($\mu_t$, $\sigma_t$, $h_t$) describing the spike distribution of each of the $N$ classes of spike amplitude classes, with a fitting parameter $r^2$ describing the goodness of fit.

Finally, the relationships between the values for instantaneous total firing rate $f(t)$ and the class firing rates $f_j(t)$ were analyzed for each data set. It proved possible to fit these relations with straight lines of the form

$$f_j(t) = g_k[f(t) - \bar{h}_k]^+$$

where $g_k$ is the gradient of the best-fit line for spike amplitude class $k$, $\bar{h}_k$ is the base total-spike rate representing the intercept above which $f_j(t) > 0$, and the operation $[x]^+$ truncates its argument to positive values.

Simulation of EMG data by common-drive mechanism

The basic structure of the motoneuron pool model is shown in Fig. 2. Motoneurons in the accessory abducens nucleus were represented individually. Each motoneuron had its own threshold and gain, but was driven by an input common to all the motoneurons. EMG spike amplitude was related to motoneuron threshold, so that EMG records could be simulated and then analyzed in the same way as the actual EMG records. The model is a simplified version of EMG models described previously (e.g., Fugl-Eld et al. 1993; Keenan et al. 2006; Zhou and Rymer 2004), with the additional features of 1) a Gaussian-profile common drive and 2) a grouping of motoneuron outputs on the basis of EMG spike amplitude.

The model is first described with parameters appropriate for data set RB1. The manner in which parameters were varied to fit the other data sets (RB2–RB4), and then to test model robustness, are subsequently described.

GAUSSIAN-PROFILE COMMON DRIVE. Analysis of EMG spikes recorded from the retractor bulbis muscle during the production of a set
of conditioned responses at various stages through conditioning indicates that their overall firing rate has a temporal profile that is approximately Gaussian in shape (Lepora et al. 2007). Accordingly, the common-drive input current $I(t)$ to each motoneuron was given a Gaussian profile

$$I(t) = I_0 \exp \left( -\frac{(t - \mu)^2}{2\sigma^2} \right)$$  \hspace{1cm} (10)

where $\mu$ is the time at which the current reaches its peak amplitude $I_0$ and $\sigma$ is the width of the Gaussian function. The parameters $\mu$ and $\sigma$ are independent of the amplitude of the conditioned response, whereas $I_0$ is assumed to be directly proportional to the peak amplitude of the conditioned response (Lepora et al. 2007).

Parameter values for $\mu$ and $\sigma$ were taken directly from the EMG spike firing rates for data set RB1, giving $\mu = 0.43$ s and $\sigma = 0.14$ s (values given in Fig. 6, A and B). However, the third parameter $I_0$ is not specified by available measurements, and so has to be initially assigned a plausible value. Given the maximum possible NM response amplitude is 15 mm (references in Mavritski et al. 2007), a numerically convenient value for the $I_0$ corresponding to that response is 15 nA. The maximum value of $I_0 (= I_{\text{max}})$ for a given data set is then simply determined by the amplitude of the maximum conditioned response in that data set. For RB1 this was 4 mm, giving $I_{\text{max}} = 4$ nA.

**PROPERTIES OF MOTONEURON POOL.** The method for calculating the firing rate of a motoneuron given its synaptic input current and intrinsic properties was taken from previous simplified motoneuron models (Binder et al. 1993; Dean 1997; Heckman and Binder 1990). These models assume that an individual motoneuron starts to fire when its intrinsic threshold current, termed the rheobase $R_i$, is exceeded by the effective synaptic current $f(t)$. Instantaneous firing rate $f(t)$ for a motoneuron is then given by

$$f_i(t) = G_i[I(t) - R_i]^+$$  \hspace{1cm} (11)

where $G_i$ is a constant representing the intrinsic gain of the motoneuron, as indicated by the slope of the relation between firing rate and injected current (Binder et al. 1993), and the notation $[x]^+$ corresponds to $x$ for $x > 0$ and 0 for $x \leq 0$; the index $i$ labels the motoneuron and ranges between one and the total number of motoneurons $n_{\text{MN}}$ (here set to 100, from observations in cat; Lennerstrand 1974).

In accordance with previous studies (Fuglevand et al. 1993; Keenan and Valero-Cuevas 2007; Zhou and Rymer 2004) the gains $G_i$ were assumed to be the same ($= G$) for all motoneurons (cf. properties of abducens motoneurons as discussed in Dean 1997) and the thresholds $R_i$ varied, to give many low-threshold motoneurons and progressively fewer high-threshold motoneurons. An exponential distribution is convenient for this purpose

$$R_i = R_{\text{max}} \frac{e^q_c(I(I_{\text{max}}) - 1)}{e^q_c - 1}$$  \hspace{1cm} (12)

where $R_{\text{max}}$ is the maximum threshold and the exponent $q$ defines the proportion of small to large rheobase currents.

The combined values for $G$ and $R_{\text{max}}$ are constrained by the previous assignment of 15 nA for the value of the input current that produces the maximum possible NM extension of 15 mm. For the initial model $R_{\text{max}}$ was set to 7.5 nA. The last recruited neuron thus starts to fire at this value of input current, and must reach its maximum effective firing rate when the input current reaches 15 nA (i.e., over a range of 7.5 nA). Since the isometric forces produced by motor units in the retractor bulbi muscle saturate at stimulation frequencies of about 150 spikes/s (Lennerstrand 1974), motoneuron gain $G$ must be 20 spikes·s$^{-1}$·nA$^{-1}$. This can be compared with measured values of 20–40 Hz/nA for cat abducens motoneurons (Grantyn and Grantyn 1978). Finally, $q$ in Eq. 12 was assigned the value of 3.45 by trial and error. The relation of this parameter to EMG properties was subsequently explored (details in the following text).

The properties of the model motoneuron pool produced by Eqs. 11 and 12 with these parameters are shown in Fig. 3. Because Eq. 12 describes an exponential relation between motoneuron number and threshold (Fig. 3A), the actual distribution of motoneuron thresholds is hyperbolic (Fig. 3B). This distribution produces a relationship be-

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**FIG. 3.** Properties of the model of the motoneuron pool with parameters derived from the RB1 data set (details in METHODS). A: exponential relation (Eq. 12) between motoneuron threshold (rheobase current) and the index $i$ of the motor unit, numbered from 1 (smallest threshold) to 100 (largest threshold). For the RB1 data set the smallest input current was 4 nA, so that neurons with a rheobase higher than this value were not recruited. $B$: distribution of motor units with respect to rheobase current, derived from the relation shown in A. The histogram indicates the number of units in each of 5 equal bins over the range of rheobase currents 0–4 nA. The exponential relation in Eq. 12 gives a hyperbolic distribution of motor unit numbers (best-fit hyperbola for a histogram with 5 intervals $x = 41/[1 + 1.86(R - 0.4)]$ shown as black curve on plot), as expected theoretically by integrating Eq. 12 over motor unit number. $C$: linear relation between rheobase current and EMG spike amplitude. The $x$-intercept of this linear relation is the minimum threshold $R_{\text{min}} = 0.17$ mV for EMG spike detection in data set RB1. $D$: relation between common drive input current and total motoneuron firing rate from model Eqs. 11 and 12. Note the weak deviation from linearity for small input currents.
between input current and total pool firing rate that is close to linear over the range of input currents for the RB1 data set (Fig. 3D).

EMG GENERATION. Simulated EMG records were generated from model firing-rate profiles as follows. The shapes of the individual EMG spikes, and possible interference between spikes, were ignored because analysis of the actual EMG records for the retractor bulb muscle suggested that for the data under consideration interference effects were not important (Lepora et al. 2007). The relationship between spike amplitude and motoneuron threshold was assumed to be linear

\[ e_i = \theta_0 + \frac{e_{\text{max}} - \theta_0}{I_{\text{max}}} R_i \]  

(13)

where \( e_i \) is the height of spike produced by the \( i \)-th motor unit, \( \theta_0 \) is the threshold height for spikes to be counted, \( e_{\text{max}} \) the maximum possible spike height, and \( I_{\text{max}} \) is the maximum input current for the RB1 data set. Finally, a sampling parameter \( 0 < P < 1 \) was introduced to convert summed motoneuronal firing to summed EMG spike firing

\[ f(t) = p \sum_{i=1}^{n_s} f_i(t) \]  

(14)

In the absence of any prior evidence on the motor units sampled, it was assumed that the subsample of measured units was unbiased across the motoneuron pool, given the small size of the retractor bulb muscle, so that \( p \) can be regarded as a constant for any given data set of EMG records.

For the RB1 data set \( \theta_0 = 0.17 \text{ mV} \), \( e_{\text{max}} \) the maximum observed spike height of 2.53 mV and \( I_{\text{max}} = 4 \text{ nA} \). These parameters give the linear relation between spike amplitude and motoneuron threshold depicted in Fig. 3C. The sampling fraction \( p \) was set to 0.09, determined by the ratio of the observed peak EMG firing rate (440 spikes/s) to the summed model firing rate for RB1’s largest conditioned response (peak amplitude 4 mm).

FITTING DATA SETS RB2–RB4. A subset of model parameters was varied to fit the remaining three data sets RB2–RB4. These were the parameters \( I_0, \mu, \) and \( \sigma \) for the Gaussian-profile common drive (Eq. 10), the parameter \( q \) for the distribution of motoneuron thresholds (Eq. 12), and the EMG parameters \( \theta_0, e_{\text{max}}, \) and \( p \) (Eqs. 13 and 14). Appropriate values of these parameters gave fits to the data sets RB2–RB4 similar to those shown for data set RB1. Fits for subjects RB1 and RB4 are shown in RESULTS (Figs. 6–8) and fits for subjects RB2 and RB3 in Supplemental Figs. S1–S3.1

1) Experimentally determined EMG threshold \( \theta_0 = 0.15, 0.0275, \) and 0.0375 mV and maximum EMG spike height \( e_{\text{max}} = 0.75, 0.67, \) and 0.36 mV for data sets RB2–RB4 (Table 1). Overall model behavior is unaffected by changing these parameters because the class- and total-firing rates do not depend on these EMG parameters (both the spike amplitudes and class boundaries are scaled identically, so the motor units remain in the same classes).

2) The Gaussian mean \( \mu \) and width \( \sigma \) of the common drive current were set equal to the corresponding values for the mean and intercept width of the data for RB2–RB4, which are \( \mu = 0.28, 0.35, \) and 0.31 s and \( \sigma = 0.10, 0.11, \) and 0.10 s, respectively (Figs. 7 and 8). Because the model Eqs. 10 to 14 depend implicitly on time, the model output remains of the same functional form in Eqs. 16 and 17 but with the precise temporal profiles rescaled by the changes to the model input or common drive current.

3) The motoneuron sampling proportion \( p \) is fixed to the peak model firing rate; peak firing rates for data sets RB2–RB4 are 220, 450, and 370 spikes/s (see Table 1), giving \( p \) values of 0.14, 0.10, and 0.053, respectively. Overall model behavior is again robust to changing the sampling proportion because only the absolute firing rates are affected, not the functional form of Eqs. 16 and 17.

4) The parameter \( q \) specifies the precise exponential relation between motoneuron number and threshold (Eq. 12) and its value determines the parameters \( u, v \) in Eqs. 16 and 17 (see also Figs. 7 and 8) in a complicated manner that depends on the EMG threshold \( \theta_0 \) and maximum EMG spike height \( e_{\text{max}} \). Appropriate values of \( q \) were determined by trial and error for the data sets RB2–RB4, giving 1.8, 4.4, and 1.9 (see also robustness in the following text).

PARAMETER VARIATION: MODEL ROBUSTNESS. Parameters were subsequently varied systematically to investigate the robustness of the model. For this purpose the appropriate parameters were those describing model structure in Eqs. 11 and 12. Nonlinear relations between EMG spike amplitude and rheobase current were also considered, such as exponential and power laws, but no significant improvement to model performance was achieved for the corresponding increase in model complexity.

### Glossary

- **CS**: conditioned stimulus
- **US**: unconditioned stimulus
- **CR**: conditioned response
- **UR**: unconditioned response
- **EMG**: electromyogram
- **MN**: motoneuron
- **MU**: motor unit
- **MUAP**: motor unit action potential
- **NM**: nictitating membrane
- **RB**: retractor bulb
- **\( b_k \)**: base total-spike rate at which \( f_k \) becomes >0
- **\( c_i \)**: strength of motor unit \( i \)
- **\( f(t) \)**: total instantaneous EMG spike firing rate at time \( t \)
- **\( f_k(t) \)**: instantaneous firing rate of model neuron \( i \) at time \( t \)
- **\( f_{\text{EMG}}(t) \)**: instantaneous firing rate of spike amplitude class \( k \) at time \( t \)
- **\( f_{\text{EMG}} \)**: EMG sampling frequency
- **\( g_k \)**: gradient of best-fit line for spike amplitude class \( k \)
- **\( G_k \)**: intrinsic gain of a motoneuron
- **\( h_k \)**: maximum of Gaussian-fit to firing rate for class \( k \)
- **\( h(t) \)**: common drive input current
- **\( I_0 \)**: peak input current for a single trial
- **\( I_{\text{max}} \)**: maximum input current for data set
- **\( f_{\text{class}} \)**: spike amplitude class
- **\( l \)**: gradient of firing rate width \( \sigma_l \) with EMG amplitude \( \tilde{e}_k \)
- **\( m \)**: gradient of base firing rate \( b_l \) with EMG amplitude \( \tilde{e}_k \)
- **\( n \)**: number of time-bins in fit to spike rate
- **\( n_{\text{MN}} \)**: total number of motoneurons in the pool
- **\( N \)**: number of EMG spikes divided by width \( \delta \) of time bin in fit to spike rate
- **\( N_{\text{class}} \)**: number of spike amplitude classes
- **\( p \)**: proportion of sampled motoneurons
- **\( q \)**: exponent defining the proportion of small to large rheobase currents \( R_i \)
- **\( r^2 \)**: square of correlation coefficient
- **\( R_i \)**: rheobase current of a motoneuron
- **\( R_{\text{max}} \)**: highest rheobase current in motoneuron pool
- **\( s \)**: exponent defining the proportion of small to large motor unit strengths \( c \)
- **\( t \)**: time
- **\( t_{\text{EMG}} \)**: times when EMG sampled
- **\( u \)**: exponent of peak firing rate \( h_k \) with EMG amplitude \( \tilde{e}_k \)
- **\( v \)**: exponent of gradient \( g_k \) with EMG amplitude \( \tilde{e}_k \)
- **\( e_{\text{max}} \)**: maximal EMG spike amplitude
- **\( \tilde{e}_k \)**: EMG amplitude in midpoint of range for class \( k \)
- **\( \theta_0 \)**: EMG threshold for spike detection

1 The online version of this article contains supplemental data.
EMG threshold for spike detection in the spike amplitude class number $k$

mean time of Gaussian-fit to total firing rate $f(t)$

width of Gaussian-fit to total firing rate $f(t)$

mean time of Gaussian-fit to firing rate $f_k(t)$ for class $k$

width of Gaussian-fit to firing rate $f_k(t)$ for class $k$

**RESULTS**

**Analysis of EMG data**

For clarity, the data analysis and modeling results (Figs. 6–8) are presented here for only two of the four subjects: RB1 (a Dutch belted rabbit) and RB4 (a New Zealand white). The corresponding results for the remaining two subjects RB2 and RB3 (both Dutch belted rabbits) are shown in Supplemental Figs. S1–S3. The two subjects shown here are sufficient to illustrate the similarities and differences between the data sets. Results across all four subjects were consistent with the modeling study.

**FIRING-RATE PROFILES FOR TOTAL EMG SPIKES AND RELATION TO CONDITIONED NM RESPONSES.** Previous analysis of retractor bulbi EMG (Lepora et al. 2007) showed that during the production of conditioned responses the firing-rate profiles for total EMG spikes have a particular shape that gradually rises from its onset, reaches a peak, then gradually falls to its offset (representative record shown in Fig. 4A, solid line). The peak times of the firing-rate profiles for individual trials are similar, roughly 50–100 ms before US onset. The shapes of the firing rate profiles across all trials can be well fitted by Gaussian functions (Fig. 4A, dashed line), as explained in METHODS.

The conditioned NM response also has a stereotyped shape, with a steep rise from onset to its peak and then a gradual fall back toward zero displacement (Fig. 4B, black line). Separate trials have a similar latency-to-peak time that coincides with US onset. The NM response profiles can be generated from the corresponding total EMG spike-rate profiles by passing the latter through a first-order linear differential equation

$$F_{\text{EMG}} = Ky + R \frac{dy}{dt}$$

where $F_{\text{EMG}}$ is EMG spike firing rate, $y$ is NM position, $dy/dt$ is NM velocity, and $K$ and $R$ are constants related to the elasticity and viscosity respectively of the overall system. This system is equivalent to a first-order filter with time constant $R/K$ of about 200 ms and with gain fixed across all trials of each data set (Fig. 4, B and C). Thus analysis of total EMG spike firing rates in relation to conditioned NM response profiles suggests that the nictitating membrane plant (retractor bulbi muscle, globe, Harder’s gland, and membrane) behaves effectively as a simple linear system, even though its complicated anatomical structure would incorrectly suggest more complicated dynamics.

**PEAK EMG AMPLITUDE IS PROPORTIONAL TO PEAK CONDITIONED NM RESPONSE.** The analysis referred to earlier does not address the issue of motor-unit recruitment, a process that modeling studies (Mavritsaki et al. 2007) suggest could make an important contribution to plant linearity. To address this issue, we analyzed the properties of EMG spike amplitude. As can be seen from Fig. 5, peak EMG spike amplitude is related to peak conditioned response amplitude in a roughly linear manner over the range of response amplitudes studied (up to ~6 mm). Insofar as EMG spike amplitude is related to motor-unit strength (DISCUSSION), these results are consistent with the view that conditioned NM response amplitude is controlled by recruiting motor units of increasing strength, at least for the range of response amplitudes investigated.

A previous study found that peak conditioned response amplitude linearly relates to peak EMG spike rate over the same range of response amplitudes (Fig. 5; Lepora et al. 2007). Therefore peak EMG spike-rate and peak EMG spike amplitude are also related in a roughly linear manner. The precise relation between EMG spike amplitude and EMG spike rate will be qualified over the next few sections, to suggest the pattern of motor unit recruitment involved in controlling the conditioned NM response. Because the relation between temporal profiles of instantaneous EMG spike rate and NM conditioned response amplitude is described by the linear differential Eq. 15, this motor unit recruitment strategy will be more complex than a simple dependence on NM position during the CRs.

**FIRING-RATE PROFILES FOR EMG SPIKES OF DIFFERENT AMPLITUDES.** Subsequently more detailed analysis of EMG spike amplitudes showed that spikes of different sizes fired in fixed relation to one another. As described in METHODS, EMG spikes were partitioned into five amplitude classes and representative firing-rate profiles for those classes are shown in Fig. 6.

Four regularities of the retractor bulbi EMG are illustrated in these records. First, larger spikes only appear in conjunction
with larger conditioned responses, consistent with the results shown in Fig. 5. Second, when larger spikes do appear, they are always preceded and succeeded by smaller spikes (see also Fig. 1). Third, when spike firing rates are compared across trials, the most prominent feature is that the firing rates of the spike amplitude classes change in step with each other. Finally, just as for overall spike frequency (Fig. 4A), the frequency profiles for individual spike classes can also be fitted by Gaussian functions. The firing rates for each class increase gradually in between the trials shown (see also RELATION TO TOTAL EMG).

The Gaussian fits to the spike rates are convenient because they allow the firing-rate profile for each spike-amplitude class \( k \) to be described by just three parameters: a mean time of spike occurrence \( \mu_k \) (which equals the time of peak firing for a Gaussian profile), a measure of profile width \( \sigma_k \), and the peak firing rate \( h_k \) (METHODS, Eq. 8). This allows the regularities outlined qualitatively above to be described quantitatively in terms of those parameters (Fig. 7). Thus 1) the mean spike times \( \mu_k \) for all the classes are timed about 50 ms before US onset (Fig. 7, A and D), 2) the firing widths \( \sigma_k \) decrease linearly with spike amplitude to zero value at maximum spike amplitude \( e_{\text{max}} \) (Fig. 7, B and E), and 3) the peak firing rates \( h_k \) decrease exponentially with spike amplitude (Fig. 7, C and F). These relations can be summarized by a three-equation system

\[
\mu_k = \mu, \quad \sigma_k = l(e_{\text{max}} - \bar{e}_k), \quad h_k = \exp[-u(\bar{e}_k - \bar{e})] \quad (16)
\]

where \( l \) and \( u \) are an empirical gradient and exponent, respectively (values shown in Fig. 7) and \( \bar{e}_k \) is the midpoint amplitude for the spikes in amplitude class \( k \).

Although the relations in Eq. 16 give good fits to the data shown in Fig. 7, there is still an appreciable amount of scatter around the lines of best fit. This arises partly from random variations in the timing of the conditioned responses themselves (references in Kehoe et al. 2008; Lepora et al. 2007), which give rise directly to variations in the timing of the inferred motor commands (Lepora et al. 2007; Figs. 11 and 12). For example, the time-to-peak conditioned NM response amplitude is known to vary randomly from trial to trial, as is evident in the variation of the Gaussian means in Fig. 7, A and D. However, a second source of scatter comes from pooling data across different CR amplitudes. For example, it can be seen from Fig. 6 that the width of the best-fit Gaussian increases with response amplitude for a given amplitude class. This source of scatter can be reproduced by the deterministic common-drive model described in the following text.

RELATION TO TOTAL EMG SPIKE RATE. Because of these regularities in the structure of the retractor bulbi EMG, the firing rates of each individual amplitude class are related to total spike rate in a systematic manner. This is shown in Fig. 8, with firing rates calculated over 50-ms intervals, as described in METHODS.

The firing rate for each individual amplitude class is approximately linearly related to total spike firing rate (Fig. 8, A and D). The lines of best fit vary with amplitude in two ways. First, the intercept with the \( x \)-axis, which corresponds to the total spike rate at which spikes of the amplitude class appear, increases approximately linearly with spike amplitude (Fig. 8, B and E).
corresponds to the qualitative observation that spikes appear and disappear in strict order of amplitude (Fig. 6). Second, the gradients of the lines decline with amplitude (Fig. 8, C and F), reflecting that high-amplitude spikes always fire more slowly than smaller ones (Fig. 6). This decline is fitted well by a hyperbolic relation, which is consistent with an exponential distribution of motor unit thresholds (see Modeling in the following text).

These regularities can be summarized in a system of two equations

\[ \tilde{b}_k = m(\tilde{e}_k - \theta) \quad g_k = \frac{g_1}{1 + v(\tilde{e}_1 - \tilde{e}_k)} \]  

where the gradient \( m \) and \( v \) are empirical parameters (values given on appropriate panels of Fig. 8), \( \tilde{e}_k \) is the midpoint spike amplitude for the spike amplitude class \( k \) defined in Eq. 3, and \( g_k \) is the gradient of the class versus total spike rate line.

Simulated EMG records

The simulated EMG records produced by the common-drive model (Fig. 1) were analyzed in the same way as the actual EMG records described earlier. The model was used to generate EMG records for 10 different peak input current amplitudes equally distributed over the range 0–4 nA (METHODS). To aid comparison with results from the EMG data analysis, the model results are shown for subjects RB1 and RB4 in Figs. 6–8 and for subjects RB2 and RB3 in Supplemental Figs. S1–S3.

Properties of simulated amplitude classes. Model EMG spikes were partitioned into five amplitude classes in the same way as the recorded spikes. This procedure resulted in a firing rate \( f_j(t) \) for each of the five amplitude classes.
Results of the simulation (Fig. 6, G–L) are in good agreement with the experimental data, both qualitatively, as comparison with the experimental data indicates (Fig. 6, A–F), and quantitatively (see following text). The four regularities observed in the experimental data are all present in the simulated records:

1) larger spikes appear only in conjunction with larger input currents (corresponding to larger conditioned response);
2) when they do appear, larger spikes are always preceded and succeeded by smaller spikes;
3) when firing rates are compared across trials, the most prominent feature is that the firing rates of the spike amplitude classes change in step with each other;
4) the spike-rate profiles for individual amplitude classes can be fitted by Gaussian functions.

Describing these Gaussian fits in terms of the mean time of spike occurrence, profile width, and the peak firing rate leads to the three quantitative relations (Eq. 16) for the simulated data sets that were obtained previously for the experimental data sets. In particular: 1) the time of peak firing is the same for all amplitude classes (Fig. 7, G and J; first relation in Eq. 16), 2) the widths of the Gaussian fits to firing rate decrease linearly with spike amplitude to zero value at maximum spike amplitude (Fig. 7, H and K; second relation in Eq. 16), and 3) the peak firing rates of the Gaussian fits decrease exponentially with spike amplitude (Fig. 7, I and L; third relation in Eq. 16).

Both simulated and experimental data show scatter around the lines of best fit. In the case of the experimental data, some of this is caused by random variability that is not present in the modeled data. However, there is also variability that is common to the two sets, which appears to arise from lumping together EMG records corresponding to different amplitudes of conditioned response. The similarity of the scatter patterns in experimental (Fig. 7, B and E) and simulated data (Fig. 7, H and G) suggest that the Gaussian fits are a good description of the data.
and $K$) provides further support for the view that the patterns observed in the experimental data are consistent with a common-drive mechanism for generating CRs.

**Relation to Simulated Total EMG Spike Rate.** The experimental results also showed that the firing rates of spikes of different amplitude were related to the total-spike firing rate in a systematic manner (Fig. 8).

It can be seen that the general pattern of simulated results (Fig. 8, $G$–$L$) are similar to the experimental results (Fig. 8, $A$–$F$). The firing rates for each amplitude class are approximately linearly related to total firing rate (Fig. 8, $G$ and $J$). The deviation from linearity seen for the lowest-amplitude spike class arises because the firing-rate for each class in the model is linearly related to input current, whereas the relationship between input current and total spike firing-rate is not precisely linear (Fig. 3D) because of recruitment. There are suggestions of a similar curvilinear relation between the firing rate of the lowest amplitude spike class and total firing rate in the experimental data (Fig. 8, $A$ and $D$), although these data show scatter not seen in the deterministic model arising from random variability in the actual EMG recordings. The relationship between spike amplitude and threshold for firing, expressed in terms of total spike firing rate, is also approximately linear (Fig. 8, $H$ and $K$). Again slight deviation from linearity arises from the model neuron firing-rate being linearly related to input current, not total spike firing-rate. The corresponding plots for the experimental data also show approximately linear relations (Fig. 8, $B$ and $E$), although the scatter is too great to observe any small systematic deviations from linearity. Finally, the relation between the firing-rate gradient and spike amplitude from each class is fitted well by a hyperbolic function (Fig. 8, $I$ and $L$), as expected from an exponential relation between motoneuron number and threshold (Fig. 3), and as observed in the experimental data (Fig. 8, $C$ and $F$).
The relations apparent in Fig. 8, G–L can be summarized by the quantitative relations of Eq. 17 found to describe the experimental data.

MODEL ROBUSTNESS. Most of the parameters that were varied to fit data sets RB1–RB4 related to particular features of the individual data, rather than central aspects of the model itself. To assess the robustness of the model, the effects of varying the main model parameters (Eqs. 11 and 12) were therefore investigated.

1) Number of motor units $n$. Changing $n$ from its baseline value of 100 altered the total- and class-firing rates equally, which rescaled $h_k$ and $b_q$ in Eqs. 16 and 17 while keeping the form of the overall relations shown in Figs. 7 and 8. If the number of units was reduced $\leq 20$ the fits to relations in Eqs. 16 and 17 were degraded by discretization errors in separating the units into spike amplitude classes. Model behavior is therefore robust to changing motor unit number provided a reasonable motor unit number is considered.

2) As explained in METHODS, the magnitude of the input current $I(t)$ is determined by the assignment of a maximum value to the input current ($I_{\text{max}}$) that produces the maximum NM extension. Once $I_{\text{max}}$ is fixed and a value is also given to $R_{\text{max}}$, the highest rheobase threshold in the motoneuron pool, then the motoneuron gain $G$ is also determined (METHODS). The value of $R_{\text{max}}$ in fact determines how much of the force produced by the muscle is produced by recruitment and how much by rate coding. The linear relation between EMG spike amplitude and peak CR amplitude observed for the experimental data (Fig. 3) indicates that for each of the modeled data sets recruitment continues throughout the amplitude range, that is $R_{\text{max}} > I_{\text{max}}$. With this restriction the overall pattern of simulated EMG results is insensitive to the value of $R_{\text{max}}$ (and corresponding value of $G$), provided the sampling parameter $p$ is tuned appropriately to match a given experimental data set. This was demonstrated in simulation (results not shown), but can also be seen directly from the definition of the model because the firing rates of all amplitude classes scale by the same amount on scaling $I(t)$ and $R_i$ in Eq. 11.

3) The final parameter to be varied systematically was $q$, which determines the precise exponential relation between motoneuron number and threshold (Eq. 12). The exponent $q$ in the rheobase distribution determines the parameters $u, v$ in Eqs. 16 and 17 in a complicated manner. We have verified that the model results fit the general form of Eqs. 16 and 17 for $q$ values ranging from 1 to 10.

Additional observation

Subject RB1 had an additional EMG electrode implanted in the orbicularis oculi (OO) muscle, so analysis of OO EMG was also possible for this animal. The pattern of results for spikes of different amplitudes was very similar to that seen for the retractor bulbi EMG.

Discussion

The purpose of the present study was to investigate the control of conditioned NM responses, by analyzing and modeling spike amplitude in EMGs recorded from the retractor bulbi muscle during conditioning. Four main features were revealed and quantified: 1) spike amplitude increased with response amplitude; 2) smaller spikes always appeared before larger spikes; 3) subsequent firing rates covaried for spikes of different amplitude, with smaller spikes always firing at higher rates than larger ones; and 4) subsequent firing-rate profiles were approximately Gaussian for all amplitudes, with parameters related to conditioned NM response parameters so that larger conditioned responses were accompanied by larger spikes. These apparently complex features of the retractor bulbi EMG could be reproduced by a simple model of common-drive–based recruitment in the retractor bulbi muscle, consistent with previous suggestions that the control signal for conditioned NM responses codes the topography of the response in a straightforward manner.

Data analysis

RECRUITMENT AND EMG AMPLITUDE. To our knowledge, spike amplitude in the retractor bulbi EMG has not been analyzed systematically in previous studies. The analyses here indicate a number of respects in which the retractor bulbi EMG resembles that of other muscles. One is that small spikes appear before large ones as muscle activation increases. This observation has been made in many studies (see Goldberg and Derfler 1977 for introduction to earlier literature; Akaboshi et al. 2000; Durkovic 2006; Jabre and Spellman 1996) and is generally interpreted in the context of the size principle, according to which weak (small) motor units are recruited before strong (large) ones (Henneman and Mendell 1981). If the amplitude of an EMG spike reflects the amplitude of a motor unit action potential (MUAP), which in turn is related to the number of fibers in a motor unit and/or the dimensions of individual fibers (Goldberg and Derfler 1977; Henneman and Mendell 1981), then the appearance of small spikes first is consistent with the size principle.

One caveat for this interpretation is that absolute spike amplitude is also heavily dependent on an extrinsic factor, namely electrode type and position. It is relative amplitude that exhibits the robust recruitment effect, which suggests that it is primarily influenced by factors intrinsic to the motor unit, such as the number of fibers it contains (Henneman and Mendell 1981) or the cross-sectional area of individual fibers (Goldberg and Derfler 1977). The precise effects of intrinsic and extrinsic factors in determining EMG spike amplitude have been extensively discussed and modeled (e.g., Akaboshi et al. 2000; Clamann and Robinson 1985; Ertas et al. 1995; Fuglevand et al. 1993; Keenan and Valero-Cuevas 2007; Keenan et al. 2006; Krutki et al. 2008; Nandedkar et al. 1988a,b; Stälberg and Karlsson 2001; Yao et al. 2000). In general terms, it appears that intrinsic factors are likely to be particularly dominant in small muscles, especially if they contain only one type of muscle fiber, as appears to be the case for the retractor bulbi muscle (Alvarado et al. 1967; Crandall et al. 1981; Gurahian and Goldberg 1987; Lennerstrand 1974).

This direct EMG evidence supports a previous conjecture concerning the importance of recruitment in producing conditioned NM responses (Mavritsaki et al. 2007). This study, based on a model constructed earlier by Bartha and Thompson (1992a,b), demonstrated how, in principle, recruitment of motor units in the retractor bulbi muscle could be used to make the NM plant appear more linear to a higher-level controller.
COMMON DRIVE. A second respect in which the retractor bulbi EMG resembles that of other muscles concerns the relationship between the firing patterns of motor units. First, the firing patterns of different amplitude classes covaried and, second, small spikes always fired at a rate higher than that of large spikes (Figs. 6 and 8). This ensemble behavior, sometimes termed an “onion skin” pattern, has been observed in other muscles and interpreted as evidence in favor of the common-drive method of muscle-force control (De Luca et al. 1982a,b, 1996; Jabre and Spellman 1996; Masakado et al. 1995; Sauvage et al. 2006). The key feature of this method is that all motoneurons in the appropriate pool receive the same synaptic drive from a higher-level controller (De Luca and Ernim 1994), so that the differences in firing rates between motor units are determined primarily by motor-unit properties (and, as a consequence, are highly regular).

The patterns of spike firing found in the retractor bulbi therefore offer a potentially important clue about the nature of the control signal sent to the motoneuron pool for conditioned NM responses. They are consistent with a single-valued command for globe retraction that is sent to all the motoneurons equally, in contrast to the multiple signals apparently sent to the extraocular muscles for the control of eye position (Dean 1997; Fuchs et al. 1988; Hazel et al. 2002; McCrea et al. 1986). However, the EMG evidence for the common-drive mechanism referred to earlier typically comes from records in which MUAPs can be assigned reliably to individual units, a process involving sophisticated recording and EMG decomposition techniques (e.g., Stashuk 2001). The data here are clearly different in that (1) only spike amplitude is used, a variable highly influenced by extrinsic factors, especially when spikes run together in interference patterns (e.g., Lewicki 1998); and that (2) spikes are assigned to classes not to individual MUs. The fact that the patterns of EMG spike activity found here resemble those obtained when individual MUAPs are identified is thus not by itself conclusive evidence for common-drive control of conditioned eyelid responses.

There is additional indirect evidence in favor of a common-drive mechanism. The pattern of EMG spike amplitudes found for the retractor bulbi muscle during conditioned responses was also observed for the orbicularis oculi muscle and, when decomposition techniques have been applied to orbicularis EMGs during sustained contractions, they have revealed patterns of MUAPs characteristic of the common-drive mechanism (De Luca et al. 2006). The spike-amplitude patterns observed here are therefore at least consistent with a common-drive command signal. Moreover, overall EMG activity in the orbicularis muscle, which controls the external eyelid, is highly correlated with NM response amplitude during conditioning (Attwell et al. 2002; Lavond et al. 1990; McCormick et al. 1982). It appears that the orbicularis oculi and retractor bulbi pools receive very similar inputs during conditioning, a similarity that could be easily achieved using the simple, single-valued commands required by the common-drive mechanism.

Nonetheless, it was necessary to demonstrate more conclusively whether the patterns of EMG spikes observed in the present study are or are not those produced by a common-drive mechanism. These patterns were therefore modeled explicitly.

Modeling

Comparing the results of data analysis and modeling (Figs. 6–8) shows that the complex patterns of EMG spikes discerned in the data could be well reproduced by a simple common-drive model. An important issue is whether the match between model and data required values of model parameters that were realistic.

PARAMETER VALUES. To our knowledge, the intrinsic gains and thresholds of accessory abducens motoneurons in the rabbit have not been measured. However, indirect evidence suggests that the model values for these quantities are generally reasonable. For cat abducens motoneurons, gains range from 20 to 40 Hz/nA and thresholds from 1.5 to 8.3 nA (Dean 1997; Granty and Granty 1978). The corresponding model values are 20 Hz/nA and 0 to 7.5 nA. The main problem appears to be that the model uses motoneurons with lower intrinsic thresholds than are observed experimentally. However, the low values in the model are to some extent uncertain, since the actual time course of the Gaussian motor commands is not measured directly, but instead inferred from the EMG data themselves. Thus the true value of current threshold at which the lowest threshold motoneuron starts firing is not known. Moreover, there is evidence that the red nucleus, thought to be the immediate source of motor commands for conditioned NM responses (Hesslow and Yeo 2002; Robleto and Thompson 2008; Thompson 2005), contains neurons that fire tonically (Desmond and Moore 1991). Given that inputs from the relevant (contralateral) red nucleus are excitatory, tonic drive from the red nucleus could in effect lower the thresholds of accessory abducens neurons. This possibility is considered further in the following text.

A second potential source of concern with regard to model parameter values is the wide range of EMG spike amplitudes. These can be expressed as the ratios of the largest possible spike heights $s_{\text{max}}$ in the model to the threshold spike heights $\theta_0$ (Eq. 13), and take the values 27–, 14–, 50–, and 12-fold for data sets RB1–RB4 respectively. These figures appear to be consistent with the estimated range of cat RB unit strengths (i.e., 3.5; Lennerstrand 1974) if motor unit EMG spike amplitudes increase faster than their strengths. There is some evidence from skeletal muscle that suggests this is the case (Goldberg and Derfler 1977). It may be also be the case that the range of motor-unit strengths is larger in rabbit than that in cat: no direct measurements appear to have been carried out for rabbit and, although it seems clear that the cat retractor bulbi muscle is relatively homogeneous (Alvarado et al. 1967; Crandall et al. 1981; Gurahian and Goldberg 1987; Lennerstrand 1974), there is a reference to unpublished observations suggesting that there are three fiber types in the rabbit retractor bulbi (Pachtet et al. 1976). For these reasons the wide range of EMG spike amplitudes used in the model appears to be consistent with such data as are currently available.

TONIC INPUTS TO ACCESSORY ABDUCENS REGULATE NM REFLEX EXCITABILITY. The excitability of accessory abducens neurons, and as a result the threshold and gain of the NM reflex will depend on levels of tonic excitatory and inhibitory inputs. In rabbit the motor command for conditioned NM responses is thought to travel from the anterior interpositus nucleus to the accessory abducens nucleus via the red nucleus (Christian and
Thompson 2003; Hesslow and Yeo 2002; Yeo and Hesslow 1998). Consistent with this view, the red nucleus is known to be essential for the production of conditioned NM responses (Bracha et al. 1993; Robleto and Thompson 2008; Rosenfield and Moore 1983). This nucleus provides a significant tonic excitatory input to the accessory abducens that is phasically modulated to create conditioned NM responses (Desmond and Moore 1991).

However, tonic excitatory input from the red nucleus to the accessory abducens nucleus should decrease the thresholds of targeted motoneurons. Consequently inactivation of the red nucleus would be expected to have a secondary effect, namely some reduction in amplitude of unconditioned responses elicited via trigeminal inputs to the accessory abducens. Such amplitude reductions have been reported following inactivations of the red nucleus (Bracha et al. 1993) and of the cerebellar interpositus nucleus, which provides excitatory drive to the red nucleus (Welsh and Harvey 1989). Consistent with this interpretation, elevating red nucleus tonic activity by applying gabazine to interpositus neurons involved in trigeminal reflex blinks increases the amplitude and duration of reflex blinks in the external eyelid system (Chen and Evinger 2006). In evaluating evidence from studies of the mechanisms and sites for generating conditioned responses, the importance of changes in these tonic modulatory influences must be considered. Excitability changes that lead to changes in the expression or performance of the reflex and conditioned responses must be dissociated to reveal mechanisms specific to learning (Hesslow and Yeo 2002; Yeo and Hardiman 1992).

Finally, it is important to note that, in addition to red nucleus inputs and trigeminal inputs, accessory abducens probably receive other modulatory inputs. There is evidence for a diversity of synaptic types in these motoneurons (Destombes et al. 1983) and facial motoneurons receive serotonergic input (Aghajanian and McCall 1980) that may modify their levels of excitability (e.g., Hounsgaard et al. 1986). So, even though conditioned responses may be produced by a common drive, there are probably other inputs to accessory abducens motoneurons that could produce effects not modeled here.

EVIDENCE FROM MOTONEURON FIRING RATES. The common-drive mechanism for motoneuron recruitment described earlier predicts the firing patterns of motoneurons during CRs, given that the relationship between total EMG-spike firing rate and CR parameters is described by the simple first-order Eq. 15. Above its individual threshold, the firing rate for an individual motoneuron will be approximately linearly related to combined NM position and velocity (right side of Eq. 15).

At present the data required to test this prediction are not available, since the firing rates of motoneurons in the rabbit accessory abducens nucleus during the production of conditioned NM responses have not been recorded systematically (Lepora et al. 2007). Data are available for cat, but it appears that accessory abducens motoneurons in this species do not fire in relation to CRs, at least for the CSs used in the study (Trigo et al. 1999). In the case of orbicularis oculi (OO) motoneurons in the facial nucleus, we have previously shown that their general firing patterns during both URs and CRs are consistent with Eq. 15 (Lepora et al. 2007; their Fig. 13). However, details of individual motoneuron thresholds in relation to eyelid position and velocity have not been presented explicitly.

Trigo (1999) showed linear fits to the firing rates of OO motoneurons as a function of eyelid position during CRs (see Fig. 16C in Lepora et al. 2007), but it appears from the fits that the motoneurons would be tonically active when the eyelid is in its resting position. Whether these data are in fact consistent with the common-drive mechanism cannot be determined.

As mentioned earlier, the first-order model derived by Lepora et al. (2007) could be used to fit firing-rate data for OO motoneurons for both CRs and URs. Analysis of the relationship between EMG and UR parameters was not possible in the present study because most of the trials used a shock US with consequent interference with the EMG record from the shock artifact. The interesting question of whether URs are also controlled by a common-drive mechanism, or in some other manner (Bartha and Thompson 1992a,b), remains to be addressed.

Control signal for conditioned eyeblink responses

The results from the present modeling study suggest that, as conjectured previously (Lepora et al. 2007; Mavritsaki et al. 2007), conditioned NM responses are generated by a relatively simple motor command that is converted into the appropriate pattern of motoneuron firing by the recruitment properties of the motoneuron pool. This idea of a simple command is consistent with evidence that very similar signals for conditioned responses are sent to both retractor bulbi and orbicularis oculi muscles (Attwell et al. 2002; Lavond et al. 1990; McCormick et al. 1982). It appears that, despite the complexities of the NM plant, conditioned NM responses are in fact relatively easy to control because the plant has been effectively simplified. The creation of a simplified “virtual plant” probably uses a combination of mechanisms (see Discussion in Lepora et al. 2007), in addition to the recruitment process proposed here. The overall result is that a common-drive Gaussian signal sent to the accessory abducens nucleus is straightforwardly related to NM response parameters. In electrophysiological investigations of the mechanisms underlying classical conditioning using the NM response system, this straightforward relationship should simplify parts of the analysis, especially at control sites with relatively direct, oligosynaptic projections to the motoneuron pool.

Although the firing rate properties of eyeblink neurons resemble those of ocular motoneurons to some extent (Trigo et al. 2003), recruitment seems rather different in the two systems. The behavior of ocular motoneurons appears not to result from a common-drive mechanism, but rather from differences in synaptic drives to different motoneurons (Dean 1997; Hazel et al. 2002). The more complex commands required by the oculomotor system probably reflect both its structure, with multiple types of motor unit, and the variety and precision of the responses it controls with respect to both eye velocity and position (e.g., Büttner and Büttner-Ennever 2006). In contrast, the role of the accessory abducens and orbicularis oculi motoneurons is much more straightforward, since their only job is to produce rapid movements of the nictitating membrane and eyelid, followed by a return to their starting position at the end of the blink.

In terms of wider applicability, however, the common-drive mechanism suggested here for conditioned NM responses has been described for numerous other skeletal-muscle responses (e.g., De Luca et al. 1982a,b, 1996; Jabre and Spellman 1996;
Masakado et al. 1995; Sauvage et al. 2006). This suggests that a general principle of making movement control as simple for higher-level neural structures may be followed when possible and, indeed, a variety of mechanisms for creating simplified “virtual plants” have been described (e.g., Demer 2006; Mussa-Ivaldi et al. 1994; Nichols and Houk 1976). In this context, the method of analyzing EMG records described here may be convenient for identifying putative common-drive mechanisms for movements not so far investigated.

Role of cerebellum

The role of the cerebellum in the production of CRs has been the subject of extensive investigation and dispute (Hesslow and Yeo 2002; Thompson 2005). It appears to be generally agreed that in the specific case studied here—i.e., delayed conditioning of the NM response in rabbit—the CR is controlled by signals sent to the accessory abducens nucleus from the anterior interpositus nucleus via the red nucleus (Hesslow and Yeo 2002; Thompson 2005). The question then arises of how cerebellar output could deal with the complexities of the NM plant (Introduction). The present results suggest a mechanism that would allow the parameters of cerebellar output signals with a Gaussian temporal profile to be related simply to the parameters of the CR (Lopora et al. 2007). This mechanism may also be used for CRs of the eyelids, given the evidence summarized earlier that the same control signal may be applied to both NM and external eyelid CRs in delay conditioning in rabbits. A detailed model of the eyelid plant is needed to indicate how it too might be linearized.

It is important to note, however, that the nature and origin of the control signals used in trace conditioning, which depends on forebrain structures such as the hippocampus, may be significantly different. When considering conditioned eyelblink studies in cats and mice (Delgado-García and Gruart 2006), it is important to remember that there are other strong drives to the orbicularis oculi motoneurons, such as those from the motor cortex. These permit voluntary blinking and are absent in the rabbit NMR system. Nonetheless, it is likely that EMG analysis of the kind used here will be helpful for elucidating whether, in accordance with the general principle of plant simplification outlined earlier, the common-drive mechanism for motoneuron recruitment is used in these other cases of learned eyelid responses as well.

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