Rapid acquisition of novel interface control by small ensembles of arbitrarily selected primary motor cortex neurons

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Law AJ, Rivlis G, Schieber MH. Rapid acquisition of novel interface control by small ensembles of arbitrarily selected primary motor cortex neurons. J Neurophysiol 112: 1528–1548, 2014. First published June 11, 2014; doi:10.1152/jn.00373.2013.—Pioneering studies demonstrated that novel degrees of freedom could be controlled individually by directly encoding the firing rate of single motor cortex neurons, without regard to each neuron’s role in controlling movement of the native limb. In contrast, recent brain-computer interface work has emphasized decoding outputs from large ensembles that include substantially more neurons than the number of degrees of freedom being controlled. To bridge the gap between direct encoding by single neurons and decoding output from large ensembles, we studied monkeys controlling one degree of freedom by comodulating up to four arbitrarily selected motor cortex neurons. Performance typically exceeded random quite early in single sessions and then continued to improve to different degrees in different sessions. We therefore examined factors that might affect performance. Performance improved with larger ensembles. In contrast, other factors that might have reflected preexisting synaptic architecture—such as the similarity of preferred directions—had little if any effect on performance. Patterns of comodulation among ensemble neurons became more consistent across trials as performance improved over single sessions. Compared with the ensemble neurons, other simultaneously recorded neurons showed less modulation. Patterns of voluntarily comodulated firing among small numbers of arbitrarily selected primary motor cortex (M1) neurons thus can be found and improved rapidly, with little constraint based on the normal relationships of the individual neurons to native limb movement. This rapid flexibility in relationships among M1 neurons may in part underlie our ability to learn new movements and improve motor skill.

brain-computer interface; comodulation; neuron ensemble; null space; neural trajectory

THE ADULT PRIMARY MOTOR CORTEX (M1) classically was viewed as a stationary platform of outputs controlling muscles and movements. Research over the past two decades has shown, however, that M1 in both humans and monkeys changes in response to amputation, CNS lesions, and practice of particular movements (Chen et al. 2002; Sanes and Donoghue 2000). At the single-unit level, the activity of M1 neurons becomes modified when normal monkeys adapt to perturbations of previously practiced movements (Li et al. 2001; Mandelblat-Cerf et al. 2011; Paz and Vaadia 2004), indicating that modification of neuronal activity in M1 is a normal part of learning new behaviors.

Nowhere is learning-induced modification of M1 neuron activity more apparent than in studies involving brain-computer interfaces (BCIs). Both animals and humans can acquire control of a BCI through an algorithm that decodes the spiking activity of M1 neuron populations. Early decoding algorithms were developed from neuronal activity recorded as the subject moved its native limb (Carmena et al. 2003; Chapin et al. 1999; Lebedev et al. 2005; Taylor et al. 2002). The decoded neurons, when switched to control the BCI, were expected to be active in the same manner as when controlling the native limb. Investigators soon found, however, that the motor system in general, and the cortical neurons driving the BCI in particular, behaved differently while controlling the BCI than while controlling the native limb (Wolpaw 2010). Native limb movement and muscle activity often ceased, and the tuning of many individual neurons changed, while the subject nevertheless successfully controlled the BCI.

Other studies have shown that effective decoding algorithms also can be developed from neuronal activity recorded as the subject either imagines moving a paralyzed limb (Hochberg et al. 2006) or simply observes the motion of a robotic arm driven by the investigator (Collinger et al. 2013; Velliste et al. 2008). Because the native limb does not move in either of these conditions, the activity of the motor cortex is not the same as during voluntary movement of the native limb. Nevertheless, in all of these studies the subjects found ways to modulate the selected neurons to achieve control of the BCI.

Recent work also suggests that the relationships among M1 neurons involved in BCI control can be nonstationary, changing over days of training and even within single sessions (Taylor et al. 2002). In particular, after monkeys learned to use a decoding algorithm originally derived from recordings made during native limb movements, randomly shuffling the decoder coefficients assigned to the 10–15 neurons initially degraded performance. But over a few days’ practice, the neurons’ directional tuning shifted as the monkeys became proficient at using the shuffled decoder (Ganguly et al. 2011; Ganguly and Carmena 2009). Smaller shifts in preferred directions (PDs) also have been observed in single sessions. For example, after rotating the PDs of 25% or 50% of ~40 spike recordings, the PDs of rotated neurons shifted in the direction of the rotation and their depth of modulation decreased (Chase et al. 2012; Jarosiewicz et al. 2008). Populations of M1 neurons normally involved in controlling native arm movements, after learning to control a BCI, thus are able to shift their PDs to adapt to arbitrary changes imposed by the decoding algorithm. In so doing, individual neurons were found to either increase or...
decrease their modulation depth, altering the magnitude of their contribution to the decoded output.

In taking advantage of the robustness of population decoding, these studies used substantially more neurons than the number of degrees of freedom being controlled. In contrast, other early studies demonstrated that monkeys can control a single degree of freedom with voluntary modulation of a single neuron (Fetz 1969; Fetz and Baker 1973; Schmidt et al. 1977, 1978; Wyler and Prim 1976). Further work showed that monkeys could use one neuron to drive temporarily paralyzed wrist flexor muscles and another neuron to drive wrist extensors, whether or not the neurons had been related to wrist muscles before the temporary paralysis (Moritz et al. 2008). And, using phasic bursts in two different neurons to drive a cursor in X and Y dimensions, respectively, a human subject was able to position a computer cursor and select icons with a burst from a third neuron (Kennedy et al. 2000). Hence up to three M1 neurons each could use its own range of firing rate modulation to control three separate, arbitrarily assigned degrees of freedom simultaneously.

Given the adaptive ability demonstrated in these and other studies (Fetz 2007; Green and Kalaska 2011; Schieber 2011), is there any reason to think that adding more neurons to control arbitrary degrees of freedom might not improve performance? Adding more task-related neurons typically does lead to improved decoding because additional nonredundant information is provided by most individual neurons. However, as noted by Green and Kalaska (2011), “. . .the recorded neurons are embedded in a cortical network whose functional architecture evolved to control the subject’s arm. This synaptic architecture could impose constraints that limit the ability of each neuron to assume any random activation pattern and so limit the ability of subjects to acquire the perfect BCI . . .” It therefore might be thought a priori more difficult, for example, to coactivate two neurons when one has a PD of 0° and the other 180° than when both have a PD of 0°. Furthermore, while averaging the activity of more individually noisy neurons might be expected to reduce the noise of the resulting signal, at the same time averaging across more neurons might make full-range modulation of the output signal more difficult because more neurons would need to be comodulated in parallel. Whether averaging across more arbitrarily selected neurons helps or hinders control of a novel interface therefore remains uncertain.

To bridge the gap between studies decoding the activity of large neuron ensembles to control a multidimensional BCI and other studies assigning individual, arbitrarily selected neurons to control novel degrees of freedom, we examined the single-session performance of rhesus monkeys as they comodulated small ensembles of up to four M1 neurons to control a single degree of freedom. For each session, we selected a different combination of one to four single neurons recorded during an immediately preceding center-out task, but without regard to their modulation depth or directional tuning (Koralek et al. 2012, 2013). Moreover, each neuron’s contribution to controlling the one-dimensional cursor was normalized between 0 and 1, and the equally weighted average across all ensemble neurons then continuously defined the position of the cursor. We examined several features of the ensemble neurons—including PD similarity, verticality of PDs, local outputs to proximal versus distal musculature, and spatial separation in the cortex—that might have been expected to affect performance because of constraints imposed by the synaptic architecture that normally controls voluntary movement of the native limb. Limiting the size of ensembles to no more than four neurons also enabled us to examine the comodulation among many unique combinations of ensemble neurons as performance improved in single sessions. Finally, we investigated the extent to which other, simultaneously recorded M1 neurons that did not contribute to controlling the BCI were modulated along with those that did.

METHODS

All procedures for the care and use of nonhuman primates followed the Guide for the Care and Use of Laboratory Animals and were approved by the University Committee on Animal Resources at the University of Rochester.

Microelectrode Array Implantation

With aseptic technique and isoflurane anesthesia, two male rhesus monkeys (Macaca mulatta; monkey V and monkey M) each were implanted with four floating microelectrode arrays (FMAs; MicroProbes, Gaithersburg, MD) in M1 of the left hemisphere. Each FMA consisted of 16 recording electrodes of different lengths varying from 1 to 6 mm in monkey V and from 1 to 4.5 mm in monkey M. All recording electrodes had a nominal impedance of 0.5 MΩ at 1 kHz at the time of implantation. Two additional low-impedance electrodes on each FMA served as reference and ground. After craniotomy and durotomy, FMA implantation sites were selected by visual inspection relative to the cortical sulci (Fig. 1). Each FMA, held by suction on the end of a 2-ml pipette, was implanted by slowly advancing it into the cortex with a micromanipulator. The durotomy was closed loosely by suturing the dural flap back in place with three to five stitches and overlaying it with a collagen matrix designed for dural closure (Duragen, Integra LifeSciences, Plainsboro, NJ). Methyl methacrylate was used to seal the craniotomy and then to fix the FMA connectors inside a protective chamber. Figure 1 shows the location of FMA electrodes in each monkey as reconstructed from intraoperative photographs. Conventional intracortical microstimulation (ICMS, trains of 12 biphasic pulses at 333 Hz, 0.2 ms per phase, up to 60 μA) subsequently confirmed that all four FMAs in each animal were implanted in the upper extremity representation.

Neural Recording and Data Acquisition

Neural data were recorded with a Plexon Multichannel Acquisition Processor and associated preamplifiers and headstages (Plexon Neu-
rotechnology Research Systems, Dallas, TX). Single-unit activity from each recording electrode was filtered with one-pole 150-Hz high-pass and three-pole 8-kHz low-pass cutoff frequencies, amplified to a final gain of 1,000–32,000, and sampled at 40 kHz. At the beginning of each experimental session, single-unit spikes were sorted online with Plexon SortClient software. Spikes were sorted by \( J \) manually adjusting the gain and threshold settings to isolate spike waveforms from noise and 2) discriminating spike waveforms from noise and/or other neuron spikes by identifying distinct clusters in the waveforms’ principal component space (i.e., the projection of the waveforms onto the first and second principal components). The average waveform from each selected cluster became a template against which subsequent waveforms were compared. Waveforms matching a given template were labeled as spikes generated by the template neuron. All spike times and waveform shapes were stored by SortClient for off-line analysis. For the BCI task described below, spike times from well-isolated neurons were transmitted across an Ethernet connection to a task-control computer using the Plexon PlexNet utility.

**Center-Out Task**

At the beginning of each session, the monkey performed a standard center-out task. The monkey was seated in a primate chair with its head restrained, facing a 17-in. LCD computer screen. The monkey used a joystick held with its right hand to control the two-dimensional position of a cursor presented on the screen. When centered, the joystick knob was positioned \( \sim 20 \text{ cm} \) in front of and \( 6 \text{ cm} \) below the monkey’s right shoulder. The base of the joystick was inclined toward the primate chair \( 30^\circ \) with respect to horizontal, allowing both monkeys to reach easily in all directions within a 10-cm radius. Each trial of the center-out task began with presentation of a circular central home target on the computer screen (hand-space diameter: 2.5 cm) into which the monkey positioned the cursor, using the joystick. Once the cursor had been held in the home target for 750 ms, one of eight circular peripheral targets (hand-space diameter: 3 cm) positioned at \( 45^\circ \) intervals was presented in a pseudorandom block design. The hand-space distance from the home target center to the center of each peripheral target was \( 8 \text{ cm} \). To earn rewards, the monkey was required to position the cursor in the peripheral target within 600 ms (reaction time + movement time < 600 ms) of target presentation and then hold the cursor in the target for 750 ms. If either of these conditions was not met, the trial was aborted. Trials were separated by an intertrial interval of 250 ms. The center-out task ended after the monkey had performed 20 successful trials per movement direction. Once center-out task data had been collected, the joystick was physically removed from the apparatus, and hence was not present for the monkey to grasp and manipulate during BCI performance.

**Brain-Computer Interface Task**

One to four isolated neurons then were selected to be used as an ensemble for the BCI task. Disregarding other features, the ensemble neurons for each BCI session were selected to meet two criteria: 1) the particular combination of ensemble neurons had not been selected for any previous BCI session, and 2) no current ensemble neuron had been selected for the immediately preceding BCI session.

The firing rate of each selected neuron, \( i \), recorded during the center-out task was transformed in 10-ms time steps as follows: The number of spikes in 10-ms bins, \( s_i(t) \), was counted, and the counts in the most recent 50 bins were convolved with a 50-point normalized half-cycle sinc kernel (Eq. 1), generating a transformed firing rate, \( r_i(t) \):

\[
r_i(t) = 100 \cdot \frac{\sum_{\tau=0}^{50} s_i(t - \tau) \cdot \sin(2\pi\tau/100)}{\sum_{\tau=0}^{50} \sin(2\pi\tau/100)} \text{spikes/s} \quad (1)
\]

The cumulative distribution function, \( \text{cdf}_i \), of these transformed firing rates then was compiled for each neuron across all 10-ms bins recorded throughout the performance of the center-out task (after the first 500 ms). Because a given neuron might not fire at all in some 500-ms periods (the width of the half-cycle sinc kernel), the probability that \( r_i = 0 \) often was greater than zero \( \left[ \text{cdf}_i(0) > 0 \right] \). We therefore computed the transformed, normalized firing rate, \( \text{mFR}_i(t) \), for each selected neuron as

\[
\text{mFR}_i(t) = \frac{\text{cdf}_i(r_i(t) - \text{cdf}_i(0))}{1 - \text{cdf}_i(0)} \quad (2)
\]

Note that this transformation and normalization of each neuron’s firing rate was established by using data collected as the monkey performed the center-out task and then remained fixed as the monkey performed the BCI task. Importantly, because a \( \text{cdf} \) by definition ranges only from 0 to 1, if the firing rate of a neuron during the BCI task exceeded the maximal rate observed during the center-out task then its \( \text{mFR} \) remained limited to 1, and if its firing rate fell below the minimum observed during the center-out task then its \( \text{mFR} \) remained limited at 0.

Throughout a BCI session, the selected neurons directly controlled the vertical cursor position, \( cp(t) \), based on the equally weighted average of their \( \text{mFR}_i(t) \):

\[
\text{cp}(t) = \frac{1}{N} \sum_{i=1}^{N} \text{mFR}_i(t) \quad (3)
\]

where \( N \) is the number of neurons selected for the ensemble controlling the cursor, which we refer to as ensemble size. The cursor did not move in the horizontal dimension.

Figure 2 illustrates the time course of individual trials in a BCI session. Each trial began with the presentation of a rectangular home target at the center of the screen. The monkey then positioned the cursor (\( + \)) in the home target, whereupon the target’s color changed from blue to magenta. After the cursor had been held in the home target for 250 ms, the home target flashed green and disappeared as a rectangular high or low target appeared, selected in a pseudorandom block design. (Only low-target trials are illustrated in Fig. 2.) The high or low target was presented continuously for 2,000 ms, which we refer to as the target presentation epoch. To earn rewards (100 mg food pellets; BioServ, Frenchtown, NJ), the monkey was required to position and then hold the cursor inside the presented high or low target for 250 ms, which we refer to as the target hold epoch. The cursor was allowed to enter and leave any of the targets, or pass entirely through, without penalty. Hence in some successful trials the cursor passed through the target and reentered from the far edge (e.g., Fig. 2C). Trials were separated by intertrial intervals lasting 1,000 ms during which no target was present, although the cursor remained on the screen continuously positioned based on the ensemble’s activity (Eq. 3).

The low target always was centered at a vertical cursor position of \( cp = 0.2 \), the home target at 0.5, and the high target at \( cp = 0.8 \). At the beginning of a BCI session, the high and low targets each spanned a vertical range of 0.35, and the home target spanned the central range of 0.25. Note, therefore, that the low target initially spanned 0.025 to 0.375 and the high target initially spanned 0.625 to 0.975. Hence driving the cursor to the limiting values of 0 or 1 moved it beyond the low or high target, respectively.

To evaluate how consistently and precisely the monkey could acquire targets with a given ensemble, the size of the high and low targets (but not the home target) varied depending on the recent history of successes and failures for that target. Target size varied independently for high and low targets. If the monkey was successful in four or more of the last five trials for a given target (high or low), its vertical size decreased by 5%, continually challenging the precision of the ensemble’s performance. Conversely, if the monkey was unsuccessful in four or more of the last five trials for a given target, its vertical size increased by 5% to minimize frustration. Otherwise,
In contrast to typical BCI control algorithms that weight the contribution of each neuron according to a regression model of its relationship to movement parameters, here the equally weighted averaging of the \( tnFR \)s of all ensemble neurons (Eq. 3) permitted all the neurons of a given ensemble to contribute equivalently to controlling cursor position, whatever their absolute firing rates or relationship to motor parameters. But, consequently, as ensemble size was increased, this averaging over multiple normalized and equally weighted neuron \( tnFR \)s progressively reduced the possibility that controlling a single neuron would enable the monkey to achieve reduction in target size. With a single neuron, variation in its \( tnFR \) from 0 to 1 moved the cursor through the full range of the workspace, from the bottom (0) to the top (1). Suppose, however, that in a two-neuron ensemble the \( tnFR \) of one neuron varied from 0 to 1 but that of the second neuron remained constantly at 0.5. The cursor would move only from 0.25 (= \([0.0 + 0.5]/2\)) to 0.75 (= \([1.0 + 0.5]/2\)), a range nevertheless sufficient to achieve relatively small target sizes. But if the second neuron’s \( tnFR \) rose above 0.76, the lowest the cursor could go would be 0.38 (= \([0.0 + 0.76]/2\)), still above the upper edge of the low target, which started at 0.375, and if the second neuron’s \( tnFR \) fell below 0.24, the highest the cursor could go would be 0.620 (= \([1.0 + 0.24]/2\)), still below the lower edge of the high target, which started at 0.625. So although the first neuron’s \( tnFR \) might vary from 0 to 1, that of the second neuron would need to be controlled to some degree, remaining below 0.76 to acquire the low target and above 0.24 to acquire the high target. What about a four-neuron ensemble? If one neuron’s \( tnFR \) varied from 0 to 1 while that of the other three neurons remained at 0.5, the cursor would move from 0.375 (= \([0.0 + 0.5 + 0.5 + 0.5]/4\)) to 0.625 (= \([1.0 + 0.5 + 0.5 + 0.5]/4\)), i.e., only to the initial closest edges of the low and high targets, respectively. If the \( tnFR \) of any one of the latter three neurons rose at all above 0.5 the cursor would be unable to acquire the low target, and if any fell at all below 0.5 the cursor would be unable to acquire the high target. Furthermore, once the size of the low or high target had decreased at all, at least one other neuron would need to be comodulated to some degree along with the first to acquire that target, and the depth of that neuron’s modulation would need to increase, or else additional neurons would need to be comodulated, too, as the target became progressively smaller. Hence, as ensemble size was increased from 1 to 4, averaging over the \( tnFR \)s of multiple normalized and equally weighted neurons made it progressively more difficult to acquire smaller targets by controlling only one neuron. Consequently, we expected performance to be poorer as ensemble size was increased.

**Data Analysis**

All data analysis was performed with MATLAB (MathWorks, Natick, MA). In reporting \( P \) values, we have followed the convention used in MATLAB of describing values less than the smallest positive double-precision subnormal number \((~9.49 \times 10^{-324})\) as \( P = 0. \)

**Across-trial variability calculation.** As described in RESULTS, we examined the consistency versus variability of ensemble activity by evaluating the trial-to-trial variability of each ensemble’s neurons. Ensemble \( tnFR \) variability was computed at each 10-ms time step during the 250-ms target hold epochs of the first 50 (25 high target + 25 low target) successful trials from each session and of the last 50 (25 high target + 25 low target) successful trials from each session. First, we computed the across-trial variance of each neuron \( n \) at time step \( t \) as

\[
\sigma_n^2(t) = \frac{1}{T}\sum_{i=1}^{T} [tnFR_n(i,t) - \mu_{tnFR}(i,t)]^2
\]

where \( T \) is the number of trials, \( tnFR_n(i,t) \) is the transformed, normalized firing rate of neuron \( n \) during trial \( t \) at time \( t \), and \( \mu_{tnFR}(i,t) \) is the mean transformed, normalized firing rate of neuron \( n \) at time \( t \) computed across the \( T \) trials. For each neuron, we calculated the

**References**

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Ensemble Performance in Single Sessions

Each unique ensemble of neurons was used to control the cursor in only a single BCI session. Figure 3 illustrates the sequential changes in size of the high and low targets relative to their initial vertical height, on a trial-by-trial basis across a two-neuron BCI session. As in most sessions, the size of both the high and low targets decreased progressively through much of the session and finally plateaued and/or increased slightly, presumably as the monkey either reached optimal performance with that ensemble or else became satiated or fatigued. To simplify evaluation of the monkey’s overall performance in each BCI session, we averaged the high and low target sizes relative to their initial vertical height on a trial-by-trial basis (Fig. 3). We refer to this parameter as the average relative target size, aRTS.

Some success in multiple sequential trials that could cause target sizes to decrease might have resulted simply from random fluctuations in neuron discharge that caused the cursor to enter the targets. We therefore evaluated the performance that could have occurred if the motion of the cursor had not been actively controlled by the monkey based on the appearance of the high and low targets. For each ensemble, we simulated BCI sessions using the original cursor trajectory from the real BCI session. In a simulated session, however, trial start times were defined by random selection of times from the actual session. For each simulated trial, we then determined whether the actual cursor trajectory, beginning at the random start time, would have resulted in a successful trial. Session simulations progressed over time by appending each simulated trial to the previous sequence of simulated trials and used the same rules for decreasing or increasing the sizes of the high and low targets as used in the actual BCI session. Each simulated session ended when the cumulative duration of simulated trials matched the duration of the actual session. The
light gray area in Fig. 3 indicates the range (1st to 99th percentile) of aRTS attained at each trial time over 100 such simulations for the session illustrated. We then tracked the running minimum (1st percentile) of these 100 simulated sessions across time to generate a monotonically decreasing estimate of the minimum aRTS that might have been attained by the motion of the cursor produced by this ensemble when randomized in time with respect to the presence and size of the high and low targets (dark gray curve in Fig. 3).

Note that because we used the cursor trajectory generated in the actual BCI session to estimate the time-randomized performance of each ensemble separately, our estimate of random performance takes into account properties of cursor motion that might result, for example, from properties of the individual ensemble neurons and their preexisting synaptic connections and might differ from ensemble to ensemble. Comparing performance during the actual BCI session to this estimate of time-randomized performance thus addresses the question of whether each ensemble was controlled actively by the monkey based on the presence and size of the high and low targets. But because segments of the cursor trajectory actually generated by each ensemble were used in estimating random performance, the random performance of different ensembles was not entirely independent of their actual performance. An ensemble that generated smooth cursor motion with little jitter, for example, might not only perform better in the real BCI session than an ensemble that produced a lot of cursor jitter but its time-randomized performance might be better as well. Indeed, we found that the minimum aRTS actually achieved by the monkey with a given ensemble was correlated with the minimum aRTS achieved in time-randomized simulations (monkey V, \( r = 0.32 \); monkey M, \( r = 0.55 \)). The estimates of time-randomized performance used here are thus ensemble specific, and should not be considered to be generalized estimates of chance performance across all ensembles.

As the session illustrated in Fig. 3 progressed, the monkey’s actual performance became better than what might have been achieved randomly for both the high and low targets individually (not illustrated) and for their average size (aRTS). We considered the monkey to have demonstrated voluntary control of the cursor—i.e., performed nonrandomly—when the actual aRTS became less than the running minimum random aRTS and subsequently remained less for >10 consecutive trials. Monkey V performed nonrandomly in 83 of 94 (88.3%) sessions and monkey M in 99 of 106 (93.4%). These sessions included 35 of 49 one-neuron sessions (monkey V, 15; monkey M, 20), 48 of 51 two-neuron sessions (monkey V, 21; monkey M, 27), 50 of 51 three-neuron sessions (monkey V, 23; monkey M, 27), and all 49 four-neuron sessions (monkey V, 24; monkey M, 25). Although ensemble neurons were selected without regard to their relationships to center-out task performance, the monkeys usually were able to voluntarily control the cursor in the BCI task.

Moreover, the monkeys’ actual performance exceeded random performance relatively early in most sessions. In the session illustrated in Fig. 3, random performance was exceeded after 5.0 min, or 81 trials. For those sessions in which the monkey exceeded random performance, monkey V exceeded random performance after 3.5 ± 3.1 (mean ± SD) min and 50.4 ± 36.4 trials and monkey M after 6.2 ± 4.0 min and 89.0 ± 54.8 trials. The arbitrarily selected ensemble neurons thus achieved nonrandom performance relatively rapidly.

After initially exceeding random performance early in a BCI session, the monkeys’ performance typically continued to improve. For the session illustrated in Fig. 3, after initially exceeding random performance the high-target size, low-target size, and their average all continued to decrease. The running minimum of random performance also continued to decrease, but not as fast as the actual aRTS, which reached a minimum of 0.32 after 20.6 min and 333 trials. For sessions in which the monkey exceeded random performance, monkey V achieved minimum aRTS values of 0.36 ± 0.09 after 87.8% ± 14.4% of session time had elapsed and monkey M achieved minimum aRTS values of 0.45 ± 0.11 after 85.7% ± 14.9% of session time had elapsed.

In addition, the minimum aRTS actually achieved by the monkey showed a logarithmic relationship to the time at which performance first exceeded random. Regression of the log-transformed time to exceed random performance as a function of minimum aRTS was significant in each monkey (monkey V, \( P < 10^{-10} \), \( R^2 = 0.43 \); monkey M, \( P < 10^{-7} \), \( R^2 = 0.29 \)). Ensembles that exceeded random performance more rapidly thus also tended to achieve better overall performance by the end of the session.

Factors Affecting BCI Task Performance

What factors affected whether the monkey performed well or performed poorly with a given ensemble in the BCI task? The only factor we controlled experimentally was ensemble size, the number of neurons selected to control the BCI in a given session. A number of additional features of the ensemble neurons that might have influenced the monkeys’ ability to comodulate the ensemble neurons were sampled randomly among sessions, however. Thus, in addition to ensemble size, we examined the following six factors.

Normalized number of ensemble states. Although we often think of a neuron’s firing rate as a continuous variable, single-neuron discharge is, of course, a time series of all-or-nothing events, i.e., a point process. Consequently, the number of spikes counted in any fixed amount of time is a nonnegative integer. Furthermore, whereas a half-sine kernel might be viewed as a continuous function, when evaluated in 10-ms steps over 500 ms a half-sine kernel becomes a discrete time sequence of 50 points where the 25 values on the ascending limb match the 25 values on the descending limb. Hence, while the convolution of 10-ms-binned spike counts with the 500-ms half-sine kernel used in the present study increased the number of possible values each neuron’s \( \text{tnFR} \) could take on, only a finite number of values was available to a given neuron, which we refer to as “neuron states.”

For example, consider a hypothetical neuron that fired at rates from 0 to 10 Hz. In any 500-ms window, this neuron would fire from zero to five spikes. If only one spike occurred in the 500 ms before time \( t \), then the neuron’s transformed firing rate at time \( t \) would be assigned 1 of the 25 values in the half-sine kernel, depending on how long before \( t \) the spike occurred. If two spikes occurred in the preceding 500 ms separated by >10 ms, then the transformed firing rate would be assigned the sum of 2 of the 25 values, the values again depending on when in the preceding 500 ms each spike had
occurred. If the two spikes occurred in the same 10-ms time bin, however, then the transformed firing rate would be assigned twice 1 of the 25 values. While a substantial number of transformed firing rates could be generated given all possible combinations of spike counts and interspike intervals in a 500-ms window, the number of values still would be discrete and finite. Furthermore, the finite number of discrete values is unchanged by the firing rate normalization of Eq. 2.

We therefore estimated the number of states for a given neuron as follows: We created 1,000 evenly spaced bins from 0 to 1, i.e., bins of width = 0.001. For each 10-ms time step in the center-out session, we identified the bin in which the \( t_{nFR} \) of the neuron fell. We then counted the number of bins the \( t_{nFR} \) of the neuron had occupied throughout the center-out session and used this as our estimate of the number of states available to that neuron. The number of neuron states thus could range from 0 to 1,000.

Across all ensemble neurons from both monkeys the number of neuron states ranged from 67 to 549, with a mean of 251 and standard deviation of 79. The distribution is shown in Fig. 4A. As expected, the number of neuron states increased with both the mean (Fig. 4B) and standard deviation (Fig. 4C) of the individual neuron’s raw firing rate, computed across the entire center-out session. In other words, the higher and more variable a neuron’s raw firing rate, the more neuron states it could generate.

We then estimated the number of states available to a given ensemble as the product of the number of states occupied by each of its neurons. Figure 4D illustrates the states available to the two-neuron ensemble of Fig. 3. The \( t_{nFR} \) of neuron 1 had 236 states, each indicated by a vertical line; the \( t_{nFR} \) of neuron 2 had 349 states, each indicated by a horizontal line. Hence this two-neuron ensemble had \( 236 \times 349 = 82,364 \) joint ensemble states, represented by the intersections of horizontal and vertical lines in Fig. 4D. The number of ensemble states in one sense represents the resolution with which the ensemble could access its joint \( t_{nFR} \) space, which in turn might have affected BCI task performance.

The number of ensemble states will increase as a power function of ensemble size, however, because the number of ensemble states is defined as the product of the numbers of individual neuron states. In the present data, the logarithm of the number of ensemble states showed a strong linear relationship to ensemble size \( (R^2 = 0.99) \). To evaluate any effect of the number of ensemble states on BCI task performance independent of ensemble size, we therefore normalized the number of ensemble states from 0 to 1 across ensembles of each size for each monkey separately. The normalized number of ensemble states then was unrelated to ensemble size \( (R^2 = 0.03) \).

**Preferred direction similarity.** In a center-out task, the firing rates of neurons with similar PDs tend to increase maximally during movements in the same direction, whereas the firing
rates of neurons with different PDs increase maximally during movements in different directions (Georgopoulos et al. 1982, 1986). We therefore hypothesized that when PDs were similar, and the monkey already was accustomed to co-modulating the firing rates of the ensemble neurons, BCI task performance would be better than when the ensemble neurons had dissimilar PDs.

To test this hypothesis, we calculated the overall similarity of PD among ensemble neurons as the average across the smaller of the two angles between all possible pairwise combinations of ensemble neuron PDs. Ensembles in which all neurons had similar PDs had average differences close to 0°, whereas the average difference would be larger for ensembles with dissimilar PDs. For ensembles of one, two, three, or four neurons, zero, one, three, or six angles, respectively, were averaged. One-neuron ensembles thus constituted a degenerate case and therefore were not included in this analysis. The maximum possible average difference in PDs is 180° for two-neuron ensembles but 120° for three- or four-neuron ensembles.

Verticality of directional tuning. Some M1 neurons have been shown to discharge more in relation to the location of targets in external space than in relation to the motion of the limb (Alexander and Crutcher 1990; Kakei et al. 1999). Other M1 neurons have been found to discharge in relation to the visually observed motion of a cursor as the monkey watches a center-out task being performed by an unseen agent (Cisek and Kalaska 2004). We therefore considered the possibility that the monkeys’ performance might be better when the ensemble neurons had center-out PDs closer to the vertical direction used in the present BCI task.

To test this hypothesis, we calculated the verticality of each ensemble as follows. Because increasing firing of each neuron moved the BCI cursor upward, we reasoned that neurons with upward PDs (close to 90°) might be easier for the monkeys to use whereas neurons with downward PDs (close to 270°) would make the BCI task more difficult. Therefore, if a given neuron’s PD was ≤180° we assigned it the absolute value of the difference between its PD and 90°, but if the neuron’s PD was >180° we assigned it the opposite of the absolute value of the difference between its PD and 270°. These values then were averaged across the neurons of a given ensemble, providing an index of verticality that ranged from 0° (upward) to 180° (downward) without regard to the rightward or leftward components of the neurons’ PDs.

Proximo-distal score. In the upper extremity, dexterity commonly is thought to be greater in the hand than in the shoulder, and this dexterity has been harnessed to control novel human-computer interfaces. Human subjects learned to control a two-dimensional cursor using an arbitrary transformation of 19 joint angles in the hand (Liu et al. 2011; Mosier et al. 2005). Moreover, humans learning to control a novel interface by activation of various upper extremity muscles achieved better performance with distal compared with more proximal muscles (Radhakrishnan et al. 2008). We therefore considered the possibility that performance in the present BCI task might be better when the ensemble contained neurons normally related to more distal parts of the upper extremity.

To test this hypothesis, in each monkey we performed ICMS through each electrode after all of the present recording sessions had been completed. Each ensemble neuron then was assigned a rank-ordered proximo-distal score based on the movement evoked by threshold stimulation delivered through the electrode from which the neuron had been recorded: 1, digits; 2, wrist; 3, elbow; 4, shoulder. Neurons recorded through electrodes from which no upper extremity response was obtained with currents up to 60 μA were excluded from this analysis. Each ensemble then was assigned a proximo-distal score by averaging across the scores available for its neurons.

Interelectrode distance. M1 neurons located close to one another are more likely to receive common inputs or have serial connections than neurons located farther apart (Gatter and Powell 1978; Keller and Asanuma 1993; Landry et al. 1980; Matsumura et al. 1996; Smith and Fetz 2009). We therefore considered the possibility that ensemble neurons located close together might be comodulated more readily than if they were distant from one another. To test this hypothesis, we assigned a three-dimensional location to each ensemble neuron based on the estimated three-dimensional location of the electrode tip from which that neuron had been recorded. We then calculated the Euclidean distance between all possible pairs of an ensemble’s neurons and averaged these distances for each two-, three-, or four-neuron ensemble (one-neuron ensembles again being a degenerate case).

Session number. Although we required each monkey to use a unique ensemble of neurons in each session, we considered the possibility that the monkeys’ performance might improve across sessions as the monkeys became increasingly familiar with the structure of the task requirements. For example, because any increase in the firing rate of an ensemble neuron always acted to move the cursor upward, the monkey may have gradually adopted a muscle cocontraction strategy to increase overall M1 neuron firing rates during high-target trials, regardless of the particular ensemble neurons selected. To examine this possibility, we considered the sequential session number in each monkey as a factor potentially influencing performance.

We then used multivariate linear regression models, pooling the data from the two monkeys, to examine whether any of these seven factors affected performance in the BCI task, quantified as the minimum arTTS actually achieved in each session. The full seven-term model was significant and accounted for ~20% of the variance in minimum arTTS (P < 10−5, R² = 0.21). We therefore conducted a factor-dropping analysis, examining models with all possible combinations of from one to six of the seven factors. Although 56 of these 126 models were significant after Bonferroni correction for 126 tests (P < 0.0004), most accounted for less of the variance than the full model. Eight models, however, accounted for somewhat more variance and had substantially lower P values (R² > 0.22, P < 10−10). These eight models all included both ensemble size and the normalized number of ensemble states. Three factors—verticality, proximo-distal score, and session number—each were included in four of the eight models, but none included average PD similarity or interelectrode distance. The highest R² of 0.27 was achieved by the model incorporating ensemble size, the normalized number of ensemble states, verticality, proximo-distal score, and session number. Using ANOVA to calculate partial R² values for this model showed, however, that more variance was attributable to ensemble size (partial R² = 0.15, P < 10−5) and the normalized number of ensemble states (partial R² = 0.10, P < 10−7) than to verti-
comodulation (partial $R^2 = 0.03, P < 0.05$), proximo-distal score (partial $R^2 = 0.0007, P < 10^{-5}$), or session number (partial $R^2 = 0.01, P < 0.05$). Indeed, the more parsimonious model incorporating only ensemble size and the normalized number of ensemble states achieved an $R^2$ of 0.22.

Post hoc, we also examined the trend for each factor separately, using univariate linear regression as illustrated in the scatterplots of Fig. 5. The monkeys performed better when the ensemble included more neurons (Fig. 5A; $P < 10^{-7}$, $R^2 = 0.15$) and had a larger normalized number of ensemble states (Fig. 5B; $P < 0.005$, $R^2 = 0.04$). Significant trends also were present for better performance when neurons were physically farther apart (Fig. 5F; $P < 0.005$, $R^2 = 0.06$) and had more upward PDs (Fig. 5D; $P < 0.01$, $R^2 = 0.03$). Nonsignificant trends ($P > 0.05$) were present for better performance when PDs were dissimilar (larger average difference; Fig. 5C), were located in foci with output to more distal muscles (Fig. 5E), and for later sessions (Fig. 5G).

**Conodulation of Ensemble Neurons**

Of all the factors we considered, multivariate regression showed that ensemble size accounted for more variation in performance than any of the remaining factors. To investigate how larger ensembles contributed to better performance, we examined the simultaneous performance of ensemble neurons. Figure 6 shows the discharge of the individual neurons from the same two-neuron ensemble illustrated in Fig. 3. The first 25 successful high-target trials (Fig. 6A) and the first 25 successful low-target trials (Fig. 6C) are shown on the left; the last 25 successful high-target trials (Fig. 6B) and last 25 successful low-target trials (Fig. 6D) are shown on the right. For each ensemble neuron, rasters of the original spike times are shown above and corresponding trial-by-trial tnFRs are shown beneath as color rasters. The cursor positions resulting from averaging the tnFRs of the two neurons (arrows) are shown as another color raster at the bottom of each panel. These data all have been aligned on the target hold epoch, demarcated by two vertical lines in each raster. Overall, this session included 270 successful and 362 total trials.

Note that because of the transformation produced by convolution with a 500-ms half-sine kernel, the fluctuations in tnFR (color rasters) followed any changes in the neurons’ original discharge (dot rasters) with a nominal lag of 250 ms. In Fig. 6A, for example, the increased discharge of neuron 2 over ~250 ms prior to cursor entry into the high target (left vertical line) produced the increased tnFR during the subsequent 250-ms target hold epoch between the two vertical lines. Use of the 500-ms half-sine kernel produced smoother variation in the tnFRs than would have been obtained with a flat (rectangular) filter, allowing smoother motion of the cursor given the small number of neurons used in any ensemble. Furthermore, the higher weighting of spikes well before the current time step simulated the lead of M1 neuron spiking relative to native limb kinematics (Evarts 1974; Hatsopoulos et al. 2007; Thach 1978).

Although the spike rasters might give the impression that modulation of neuron 2 moved the cursor while neuron 1 discharged tonically, the tnFR color rasters show that both neurons were modulated to control cursor position. While neuron 2 generated higher absolute firing rates than neuron 1,
the normalization process scaled the firing rates of each neuron to range from 0 to 1. Examining the target hold epochs between the two vertical lines in each raster shows that when the cursor was in the high target the \(tnFR\) of both neurons was relatively high and when the cursor was in the low target the \(tnFR\) of both neurons was relatively low. The monkey thus increased the discharge of both neurons to move the cursor to the high target and decreased the discharge of both neurons to move the cursor to the low target.

Figure 6 also illustrates that this pattern of neuron comodulation was present early in the session. In high-target trials, for example, the increase in discharge of neuron 2 that led to its increase in \(tnFR\) during the target hold epoch was present in the first 25 successful trials of the session (Fig. 6A), and the same general pattern was present in the last 25 successful high-target trials (Fig. 6B). By the later part of the session, however, this increase in neuron 2’s firing had become less variable from trial to trial, as can be appreciated by comparing the consistency of colors in the target hold epoch between the two vertical lines of the \(tnFR\) plots for the early versus late trials (Fig. 6, A vs. B). Similar improvement in consistency from trial to trial can be appreciated by comparing the \(tnFR\) of neuron 2 in low-target trials (Fig. 6, C vs. D), as well as for neuron 1 in both high- and low-target trials. Consequently, the consistency of cursor position also increased from early to late in the session, for both high-target and low-target trials. In this two-neuron session, a pattern of comodulation in which both neurons were modulated through their respective ranges thus was established early in the session and became more consistent as the session progressed.

To examine the behavior of ensemble neurons in more detail, we evaluated neural trajectories in the joint \(tnFR\) space of the ensemble neurons. Figure 7A illustrates the simultaneous \(tnFR\) of the two neurons plotted against one another for each of the last 25 successful low-target trials, using the same data shown in Fig. 6D. Colored traces in Fig. 7A show the trajectories of the two neurons’ joint \(tnFR\)s during three 250-ms epochs: immediately before (green), during (red), and after (blue) the target hold epoch.

The joint \(tnFR\) trajectories in Fig. 7A initially might appear to have varied randomly from trial to trial. Further inspection shows otherwise. During the target hold epoch, the trajectories were clustered in the lower right half of the low-target zone. This is demonstrated more clearly in Fig. 7B by plotting the temporal midpoint of the trajectory for each 250-ms epoch from Fig. 7A. Whereas the temporal midpoints for the preceding epoch were highly scattered, those for the target hold epoch were clustered in the lower right half of the target zone, where the \(tnFR\) of neuron 2 was lower than that of neuron 1. Many of the temporal midpoints of the following epoch also were in the region where the \(tnFR\) of neuron 2 was lower than that of neuron 1. Rather than controlling neurons 1 and 2 independently, the monkey thus developed a nonrandom strategy for comodulating the two neurons in which, although the \(tnFR\) of both neurons was decreased to move the cursor to the low target, the \(tnFR\) of neuron 2 was decreased more than that of neuron 1.

The temporal patterns of comodulation of the two neurons can be appreciated in more detail by considering the slopes of the joint \(tnFR\) trajectories. When the monkey produced positively correlated changes in the \(tnFR\) of the two neurons, these trajectories had a positive slope, which was more common as the cursor approached the target or left the target. Alternatively, trajectory segments sometimes were vertical or horizontal, indicating that the monkey modulated one neuron while holding the firing of the other relatively constant. When the
cursor was in the target, however, the trajectories often had negative slopes, indicating that the monkey decreased the tnFR of one neuron while increasing that of the other, most often decreasing the tnFR of neuron 2 while increasing that of neuron 1. Note that the joint tnFR trajectory was more likely to have a negative slope during the target hold period than before or after. Although effective strategies for keeping the cursor in the target would have included either holding the tnFR of both neurons relatively constant or comodulating them a small amount in parallel, instead the monkey produced oppositely directed modulation of tnFR in the two neurons. Rather than being limited to comodulating parallel increases and decreases, the monkey thus produced synergistic changes in the correlation between the two neurons over the time course of single trials.

To evaluate the consistency with which the monkeys comodulated ensemble neurons, we quantified the across-trial variability in each session. For each of the 25 time points (10-ms bins) in the 250-ms target hold epoch, we calculated the across-trial variance of each ensemble neuron’s tnFR (Eq. 5), averaged the variance across the neurons of each ensemble (Eq. 6), averaged the ensemble variance across the 25 time steps for high-target trials and for low-target trials (Eq. 7), and then averaged across the high- and low-target trials. We calculated separate averaged ensemble variances for the first 50 (25 high target + 25 low target) successful trials and the last 50 successful trials in each session and then averaged across all ensembles of a given size from both monkeys.

The bar graphs of Fig. 8A show this across-trial ensemble variability, σ'^2_{ensemble(t)}, averaged across all ensembles of a given size. Across-trial variability increased with ensemble size (P = 0, KW test). Furthermore, as is evident for the two-neuron session illustrated in Fig. 6, compared with the beginning of sessions ensemble variability had decreased by the end of sessions (P = 0, KW test). The consistency with which the monkeys modulated ensemble neurons thus increased as a session progressed and performance improved.

To further examine how the monkeys comodulated ensemble neurons, we decomposed the joint tnFR trajectories in the alternate coordinate frame represented by the two diagonals shown in Fig. 7A. As shown mathematically in the APPENDIX, the projection of each joint tnFR trajectory onto the positive diagonal represents the vertical motion of the cursor displayed to the monkey. In contrast, the projection onto the negative diagonal represents motion of the neural trajectory that did not affect the motion of the cursor, a “null” (or “uncontrolled”) dimension (Kaufman et al. 2014; Latash et al. 2007). In the present BCI task, ensembles of any size had a single cursor dimension in their joint tnFR space, and we considered ensembles consisting of one, two, three, or four neurons to have had zero, one, two, or three null dimensions, respectively (see DISCUSSION, however). As described in METHODS, we calculated the across-trial variance of the projection along the cursor dimension (Eq. 8). Subtracting the cursor variability from the total ensemble variability then gave the null-space variability (Eq. 9; see also APPENDIX).

Figure 8B shows across-trial variability in the cursor dimension as a function of ensemble size. Consistent with the smaller aRTS achieved on average with larger ensembles (Fig. 5A), cursor variability decreased as ensemble size increased (P = 0, KW test). And consistent with the improvement in performance and the associated decrease in
target sizes during individual sessions, cursor variability decreased from the first 50 trials to the last 50 trials ($P = 0$, KW test). In contrast to cursor variability, variability in the null space (Fig. 8C) increased as a function of ensemble size ($P = 0$, KW test), accounting for the increase in total ensemble variability (Fig. 8A). But for two-, three-, and four-neuron ensembles, null-space variability also decreased from the first 50 trials to the last 50 trials ($P = 0$, KW test). The decrease of both cursor variability and null-space variability from early to late in sessions indicates that the patterns of comodulation among ensemble neurons became more consistent across trials as performance improved over single sessions.

How much variability would there have been if the ensemble neurons had been modulated independently of one another until the cursor happened to land in the target for 250 ms? Because this variability would depend in part on the discharge properties of the individual ensemble neurons (and the resulting transformation and normalization of their firing rates) and in part on the target size in individual trials, to address this question we made bootstrap estimates of the variability that would have been available had the ensemble neurons been modulated independently. We clipped 2,250-ms epochs of the $tnFR$ of each ensemble neuron separately and shuffled the clips, combining clips from the individual neurons that had occurred at different times, until we found 100 shuffled combinations that produced joint trajectories meeting the actual target size requirements for each of the first 50 (25 high target + 25 low target) and each of the last 50 successful trials of a given session. We can consider these shuffled trajectories to be a random sample of modulation patterns among the ensemble neurons that would have allowed the monkey to succeed at the task had the ensemble neurons been modulated independently of one another.

Figure 7C shows, for example, the three 250-ms segments—before, during, and after the low-target hold epoch—from one of the 100 shuffled trajectories (randomly selected) corresponding to each of the 25 actual trajectories of Fig. 7A. Figure 7D shows the temporal midpoints of these segments of the shuffled trajectories. The shuffled trajectories were more widely scattered in the joint $tnFR$ space than the trajectories actually produced by the monkey. Hence the full range of patterns (joint $tnFR$ trajectories) that would have been available for successful trials had these two neurons been modulated independently of one another was not used, indicating that the two neurons in this session were comodulated (see DISCUSSION).

For each session we averaged the across-trial variability of such shuffled trajectories during the target hold epoch for the first 50 and the last 50 successful trials of each session and then averaged over all ensembles of a given size from both monkeys. The resulting measures of variability available are shown in Fig. 8 as small squares. Note that the variability of the shuffled trajectories—constrained by the target height and the joint-trajectory space—represents the across-trial variability in successful trials that would have been produced by the ensemble neurons if they had been modulated independently. In contrast, if identical joint $tnFR$ trajectories had been produced on all successful trials, then the across-trial variability observed in the actual data would have approached zero in both the cursor and null dimensions for all ensemble sizes. In the cursor dimension (Fig. 8B), all the variability available to independent neurons was used. But in the null dimension (Fig. 8C), the variability actually used (bars) was less than that available (squares) ($P = 0$, KW test), indicating that rather than being modulated independently ensemble neurons were co-modulated in subsets of the available patterns with some degree of consistency.

Moreover, the difference between the ensemble variability actually used and the ensemble variability available increased with ensemble size (Fig. 8A) ($P = 0$, KW test; note that ensemble variability is normalized by ensemble size, see Eq. 6 and APPENDIX). A similar trend was evident for null-space
variability (Fig. 8C). Because null-space variability is not simply normalized by ensemble size, however, we note that the null-space variability actually used became a progressively smaller fraction of the variability available as ensemble size increased. Hence rather than using a fixed fraction of the comodulation patterns available for independent neurons to succeed at the task, a progressively smaller fraction of the patterns available were used as ensemble size increased, even early in the sessions. Increasing ensemble size thus appeared to afford more effective patterns of comodulation. But as ensemble size increased, a progressively smaller fraction of the available patterns were rapidly identified and then reproduced with some consistency from trial to trial. The consistency of these patterns then increased (variability decreased) from the beginning of sessions to the end.

Modulation of Unselected Neurons

Our observations that ensembles did not use all the available null space, and grew more consistent from early to late in individual sessions, indicate that the monkeys actively co-modulated ensemble neurons. One means of achieving such comodulation might have been simply to comodulate most or even all M1 neurons, simultaneously increasing and decreasing the activity of neurons throughout the M1 upper extremity representation. If such were the case, then the neurons we selected to control the cursor would be a sample chosen arbitrarily from a much larger population, all modulated in parallel.

To examine this possibility, we evaluated the modulation of neurons recorded simultaneously with the ensemble neurons but not selected to participate in controlling the cursor for the current session. We normalized and transformed the firing rates of these “unselected” neurons with the same algorithms applied to the ensemble neurons. In comparing ensemble neurons and unselected neurons, we counted each neuron recorded in each analyzed session. A neuron that was recorded in more than one individual sessions, indicate that the monkeys actively co-modulated ensemble neurons. One means of achieving such comodulation might have been simply to comodulate most or even all M1 neurons, simultaneously increasing and decreasing the activity of neurons throughout the M1 upper extremity representation. If such were the case, then the neurons we selected to control the cursor would be a sample chosen arbitrarily from a much larger population, all modulated in parallel.

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We then compared the tnFR modulation depth of unselected neurons with that of ensemble neurons during the 2-s target presentation epoch of all trials, successful or not, from all sessions, whether the monkey exceeded random performance or not. In each 10-ms time step, from 250 ms before target onset until 1 s after target offset, we averaged the tnFR of a given neuron separately in high-target trials and in low-target trials. The high-target average minus the low-target average then provided a measure of that neuron’s modulation depth at that time relative to the target presentation epoch.

Figure 9A shows modulation depth as a function of time averaged across all ensemble neurons and across all unselected neurons in all trials in all sessions from both monkeys; the 2-s target presentation epoch is delimited with vertical dotted lines. Average modulation depths of both ensemble and unselected neurons began to increase ~250 ms after target onset, reflecting both the monkeys’ reaction time and the delay produced by the half-sine kernel. Modulation depth increased more for ensemble neurons than for unselected neurons on average and remained greater for >250 ms after target offset. The black horizontal line below 0 in Fig. 6A indicates the times at which the average modulation depth of ensemble neurons was significantly greater than that of unselected neurons (2-tailed t-tests, \( P < 0.05 \)). Modulation depth was significantly greater for ensemble neurons than for unselected neurons from ~250 ms after target onset until well after target offset.

We also examined the modulation of ensemble neurons and of unselected neurons early versus late in each session to determine whether changes in modulation depth occurred over the 20- to 30-min duration of sessions. For these comparisons we used data from the first 50 and the last 50 successful trials of all sessions from both monkeys, again averaging across all ensemble neurons (Fig. 9B) and across all unselected neurons (Fig. 9C). Whereas the average modulation depth of ensemble neurons increased significantly from early to late in sessions, that of unselected neurons did not.

We conclude, therefore, that comodulation of ensemble neurons was not achieved simply by modulating all M1 neurons equivalently. Although substantial numbers of unselected neurons may have been modulated to varying degrees along with the ensemble neurons, the ensemble neurons used to control the cursor nevertheless were modulated more than unselected neurons, and the modulation of ensemble neurons
increased during sessions while that of unselected neurons did not.

DISCUSSION

In the present study, monkeys used ensembles consisting of one to four arbitrarily selected M1 neurons to control a one-dimensional BCI. Unlike prior studies in which small numbers of neurons each controlled separate degrees of freedom, here up to four neurons were modulated in some manner to control a single degree of freedom. Selected neurons controlled the position of the cursor through a novel transfer function that smoothed each neuron’s raw firing rate with a 500-ms half-sine kernel and normalized the smoothed firing rate in a 0 to 1 range. This firing rate transformation, followed by averaging across the arbitrarily selected set of ensemble neurons, might have limited the monkeys’ use of any previously learned patterns of comodulation among the selected neurons. Yet both monkeys were able to perform nonrandomly in >90% of sessions, typically exceeding random performance in the first few minutes and improving further over the next ~20 min. Although we had expected, given preexisting synaptic architecture, that requiring the monkeys to modulate more arbitrarily selected neurons might make controlling the single degree of freedom more difficult, performance instead improved with more ensemble neurons. Other, unselected M1 neurons recorded simultaneously showed less modulation depth than ensemble neurons and did not increase their modulation depth from early to late in sessions, indicating that the ensemble neurons were to some extent under selective voluntary control.

Rapid Flexibility

During the BCI task, the firing rate of each neuron first was smoothed with a 500-ms half-sine kernel and then normalized from 0 to 1 based on a cumulative distribution function compiled from that neuron’s firing during performance of a standard center-out task. Although the same algorithm was used for all neurons, the resulting nonlinear transformation thus differed from neuron to neuron. The transformed, normalized firing rates (tnFRs) of all ensemble neurons then were simply averaged, without other weighting, to determine the instantaneous cursor position. Hence all ensemble neurons, whether phasic or tonic, whether firing at rates up to 20/s or up to 200/s, had an equal share in controlling cursor position.

These features of the control algorithm may have reduced the likelihood that the monkeys could comodulate ensemble neurons using previously learned patterns. Indeed, BCI performance was not significantly better when ensemble neurons had similar versus dissimilar directional tuning. We cannot know all the patterns a monkey had ever practiced previously (Hwang et al. 2013). We can state, however, that after only a few minutes of working with a new ensemble in each session, and receiving only the continuous visual feedback of the cursor’s position, the ensemble neurons were comodulated such that their performance typically exceeded our estimates of random performance. The monkeys thus were able to rapidly identify a usable pattern of comodulation among the arbitrarily selected ensemble neurons. While not necessarily the best possible, the patterns used were sufficient for the monkeys to succeed quickly at the present BCI task and to continue to improve thereafter over the remainder of a session, as evidenced by the ability to acquire progressively smaller targets. While the present study focused on the rapid improvement in performance during initial sessions with new ensembles, we consider it likely that further improvement would have occurred had we allowed the monkeys to practice with the same ensemble for multiple sessions (Ganguly and Carmena 2009).

Ensemble Size, Number of Ensemble States, and Null Spaces

Ensemble size—the number of neurons used to control the BCI in a given session—had more effect on performance than any of the other factors examined. The number of ensemble states, even after normalization for ensemble size, also had an effect on performance. To understand how these factors might have influenced performance, we examine two different views of the joint neural trajectory space involving the ensemble neurons. More specifically, we consider the null space, i.e., the subspace of the joint tnFR trajectory space that had no effect (projection) on the cursor.

From one point of view, the cursor and null dimensions together are equal to the number of ensemble neurons. As noted by Jarosiewicz et al. (2008), a “...‘braincontrol’ paradigm is unique in that the behavior, cursor movement, is solely the result of neural activity in the population under study. Thus, any mismatches between desired cursor motion and decoded cursor motion can only be corrected by altering the activity of these recorded neurons.” In other words, only the ensemble neurons can have direct effects on the cursor. (Any other neurons in the nervous system can influence the cursor only indirectly, through synaptic inputs to the ensemble neurons.) As the number of ensemble neurons is increased beyond the number of cursor dimensions being controlled, the number of cursor-redundant null dimensions increases in parallel. In the present BCI task with only one cursor dimension, ensembles consisting of one, two, three, or four neurons would have zero, one, two, or three null dimensions, respectively, in this “ensemble-specific” view of the joint-trajectory space comprised of only those neurons directly controlling the cursor.

From an alternate point of view, the joint-trajectory space could be considered to include all the neurons in the motor cortex (or perhaps in the entire brain). This total number of neurons is constant and would not change depending on the ensemble size used in a particular session. Rather, changing the number of neurons in the ensemble controlling the cursor would be described mathematically as a rotation of the cursor dimension in the space of all neurons, such that different numbers of neurons would have projections on the cursor dimension. The number of null dimensions then would be the constant difference between the total number of neurons and the number of cursor dimensions in this “all-neuron” view of a joint-trajectory space.

To appreciate the differences between these two views of the joint-trajectory space, consider a hypothetical, simplified system with only two neurons and one cursor dimension. First consider the situation when only neuron 1 is controlling the one-dimensional cursor. From the ensemble-specific point of view, this one-neuron ensemble has no null space (Fig. 10A); that is, there is no modulation of neuron 1 that does not cause a change in cursor position. If the cursor is to be positioned in a low target covering 10% of the one cursor dimension cen-
Fig. 10. Advantage of a null dimension: schematic neural trajectory spaces. A hypothetical 2-neuron system is depicted. If only neuron 1 controls the cursor, the relevant neural trajectories can be viewed as including either just neuron 1 (A, ensemble-specific) or both neurons together (B, all-neuron). If both neurons control the cursor, the ensemble-specific and all-neuron viewpoints are equivalent (C). In all 3 cases, gray shading indicates the zone in which the cursor would be within a low target spanning 10% of the cursor dimension and dark gray dots mark ensemble states that position the cursor in that zone. Colored arrows depict various types of possible neural trajectories among the ensemble states in the target zone: 1) magenta: neuron 1 is modulated while the firing of neuron 2 remains constant; 2) cyan: neuron 2 is modulated while neuron 1 is constant; 3) red: neurons 1 and 2 are modulated in parallel; 4) green: the firing of neuron 2 decreases faster than the firing of neuron 1 (positive slope); 5) dark blue: the firing of neuron 1 increases while that of neuron 2 decreases (negative slope).

From the all-neuron point of view, while only neuron 1 controls the cursor, the activity of the other neuron in the system, neuron 2, constitutes a null dimension (Fig. 10B). Because the cursor dimension is aligned only with neuron 1 and is orthogonal to neuron 2, neuron 1 still must be kept within the appropriate 10% of its tnFR range to keep the cursor within the 10% low-target zone, whereas the tnFR of neuron 2 can vary in any fashion from 0 to 1 (colored arrows). To make it clear that the number of states of the two neurons can differ, we have assigned 21 possible neuron states to neuron 1 spanning its tnFR range from 0 to 1. The one-neuron ensemble therefore has 21 ensemble states. Only three of these states (dark gray dots) lie within the 10% low-target zone.

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In contrast, from the all-neuron point of view where both neuron 1 and neuron 2 were considered before, the dimensions of the joint-neuron space have not changed. Instead, the cursor and null dimensions have been rotated by 45° such that both neurons now have projections on both the cursor and null dimensions. Neuron 1’s tnFR can be more variable while keeping the cursor in the 10% low target (just as in the ensemble-specific point of view), but neuron 2’s tnFR now must be constrained. On changing from a one-neuron ensemble to a two-neuron ensemble, the number of ensemble states available to position the cursor in this low target would have decreased from 33 to 24, resulting in fewer potential joint trajectories that can keep the cursor in the 10% low target.

In summary, from the ensemble-specific point of view increasing ensemble size results in more ensemble states in the low-target zone, whereas from the all-neuron point of view increasing ensemble size results in fewer ensemble states in the low-target zone. The same would be the case for the high-target zone. Experimentally, we found that better performance in the present BCI task correlated both with larger ensemble size and with larger numbers of ensemble states, normalized for ensemble size. These experimental observations are more consistent with the ensemble-specific view of the joint neural trajectory space and the corresponding null space than with the all-neuron view.

Active Control of Joint Neural Trajectories and Improvement in Performance

A fundamental question regarding the present BCI task is whether the ensemble neurons were comodulated in some nonrandom fashion by an active process or instead simply
underwent unrelated, independent increases and decreases in mFR that by chance occasionally caused the cursor to enter the target for 250 ms. Three lines of evidence indicate that an active process comodulated ensemble neurons. First, our estimates of random performance demonstrated that beginning quite early in the sessions the ensemble neurons were modulated actively to move the cursor to the target in a nonrandom fashion (Fig. 3). Hence the monkeys actively attended to the targets and moved the cursor to acquire them, as was additionally evidenced by the progressive decrease in target size in most sessions. Moreover, we found that the modulation depth of ensemble neurons was significantly greater than that of unselected neurons, and that the modulation of ensemble neurons increased from early to late in sessions while the modulation of unselected neurons did not. The relatively selective increase and decrease in ensemble neuron mFRs following the appearance of high versus low targets indicate that an active process modulated the ensemble neurons.

Second, in theory, nonrandom performance might have been accomplished simply by coactivating or deactivating the ensemble neurons, with no other specific relationship between their discharge. However, consideration of the across-trial variance of the ensemble neurons shows that such was not the case. By shuffling ensemble neuron mFRs and finding combinations that allowed the cursor to be in the target for 250 ms (Fig. 7, C and D), we estimated the across-trial variability that could have occurred if ensemble neurons simply had been independently coactivated and deactivated until the cursor passed through the target (Fig. 8). For two-, three-, and four-neuron ensembles, the across-trial variance actually used by the ensemble neurons, even early in the sessions, was significantly less than what would have occurred had the ensemble neurons simply been independently coactivated and deactivated (Fig. 8A). This indicates that rather than using the full range of patterns available to independent neurons to place the cursor successfully in the target, a given ensemble’s joint trajectories tended to use only a subset of these patterns. Such relative consistency of ensemble joint trajectories could not have occurred without some actively produced relationship(s) among the ensemble neuron mFRs.

Third, the subset of patterns used by ensembles was not a random subset. Suppose the five colored arrows in Fig. 10C represent five different patterns of comodulation available for successful trials. Compared with using all five of these patterns, across-trial ensemble variance could be reduced by randomly eliminating any two of the five. Note, however, that the green and dark blue trajectories provide different time courses of projection along both the cursor dimension and the null dimension. Eliminating these two patterns would reduce across-trial variance in both the cursor and the null dimensions. In contrast, the magenta, red, and cyan arrows provide the same time course of projection along the cursor dimension but different projections along the null dimension. Hence eliminating the magenta and red trajectories would reduce across-trial null variance more than cursor variance. Experimentally, we observed that the present two-, three-, and four-neuron ensembles used all the across-trial variability available in the cursor dimension (Fig. 8B) while using less than the variability available in null dimensions (Fig. 8C). The subset of patterns used by ensembles thus was not random, but rather was biased toward patterns that reduced null variability more than required for the corresponding degree of cursor variability (e.g., Fig. 7B). Such a nonrandom selection of the subset of trajectories used by ensembles again indicates an active process of comodulation.

Several studies have shown that trial-to-trial variability in kinematic trajectories decreases over days of practice as motor skill improves in both monkeys and humans (Georgopoulos et al. 1981; Latash et al. 2007; Shmuelof et al. 2012). Decreased across-trial variability (standard deviation) of spike counts in the reach-related bursts discharged by rat motor cortex neurons also has been described after several days of practicing a reaching task (Kargo and Nitz 2004). In the present BCI task, to encourage the optimal performance of each ensemble, the sizes of the high and low targets each decreased around their respective, constant, center positions as performance improved during the session. These decreases in target size gave the monkey visual feedback about the current precision of its cursor control. Success in terms of achieving a reasonable rate of rewards, however, required neither increasing precision nor decreasing variability in cursor trajectories. Indeed, target size was increased whenever the monkey failed four of five sequential trials. The monkeys could have, and sometimes did, continue to achieve rewards throughout the session with little if any decrease in target size. Rather than being necessary for success in the present BCI task, the decreasing across-trial variability of the joint mFR trajectory in both the cursor and the ensemble-specific null dimensions thus accompanied acquisition of a skill that increased the rate at which the monkeys obtained rewards.

Other Factors Affecting Performance

In multivariate analysis, ensemble size and the number of normalized ensemble states accounted for ~20% of the variance in performance of the present BCI task. The other factors we examined—PD similarity, verticality, proximo-distal score, interelectrode distance, and session number—all taken together accounted for an order of magnitude less variance. If the preexisting synaptic architecture in M1 for control of the native limb had constrained the monkeys’ ability to comodulate arbitrarily selected M1 neurons, we would have expected these factors to have influenced performance more strongly. Our findings suggest instead that the preexisting synaptic architecture responsible for the natural correlations in firing exhibited during normal behavior of the native limb does not limit the rapid adaptation of small numbers of M1 neurons to control a novel interface.

Nevertheless, given that roughly 80% of the variance in performance remained unaccounted for by our multivariate analysis, additional contributory factors probably remain to be identified. Some of these might reflect existing synaptic architecture representing features not examined in the present study, including various types of somatosensory input to individual M1 neurons (Cheney and Fetz 1984; Fetz et al. 1980; Kaneko et al. 1994; Lemon 1981; Lemon et al. 1976; Rosen and Asanuma 1972) and other inputs specifying forces and movement dynamics (Ashe 1997; Georgopoulos et al. 1992; Sergio et al. 2005), both of which may be altered during control of a BCI when the subject does not make normal movements of the native limb.

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Selective Voluntary Modulation of BCI Neurons

In early studies of voluntary control of M1 neurons, monkeys were found to be able to modulate a selected neuron while another neuron recorded through the same electrode remained unmodulated, or while the other neuron was modulated voluntarily in the opposite direction (Fetz and Baker 1973). More recently, monkeys activated two different M1 neurons at different times, one to drive temporarily paralyzed wrist flexor muscles and the other to drive the wrist extensors (Moritz et al. 2008). When provided with BCI feedback concerning the discharge of particular M1 neurons, monkeys thus modulated M1 neurons selectively and differentially. Moreover, in larger populations of neurons controlling a two- or three-dimensional BCI, differential alterations in the decoding algorithm for different neurons result in differential changes in the activity of different neurons controlling the BCI (Ganguly and Carmena 2009; Jarosiewicz et al. 2008).

What about the activity of unselected M1 neurons, those not contributing to control of the BCI: are they modulated just as much by the process of voluntary control? When individual selected (“trained”) neurons in the parietal reach region (PRR) were required to flip their PD in a BCI task, unselected (“untrained”) neurons showed a similar inversion of activity (Hwang et al. 2013). And in the first days of learning to control a BCI cursor in a two-dimensional center-out task using 10–15 neurons from M1 and/or the dorsal premotor cortex (PMd), unselected (“indirect”) neurons were found to be modulated as much during BCI control as during manual control (Ganguly et al. 2011). Only after 2–3 days of training did unselected neurons become less modulated during BCI control, with their average modulation depth falling to approximately half that of the BCI ensemble (“direct”) neurons. But during the present ~20-min BCI sessions, unselected neurons on average were less modulated than the ensemble neurons (Fig. 9). Moreover, whereas the modulation depth of ensemble neurons increased from early to late in sessions, the modulation depth of unselected neurons did not. These differences in the activity of unselected neurons between the present and previous studies may have resulted in part from the difference in the number of neurons used to control the BCI. Assuming that a subset of unselected neurons is modulated in parallel with each BCI neuron, using more BCI neurons could lead to modulation of more unselected neurons.

In addition, this difference in the activity of unselected neurons may reflect differences in the cortical regions sampled. Hwang and colleagues (2013) sampled neurons from PRR; Ganguly and colleagues (2011) sampled neurons from the PMd and the M1 arm representation on the crown of the precentral gyrus. In contrast, the present study sampled neurons largely from the anterior bank of the central sulcus, “new,” caudal region of M1 contains virtually all the cortico-motoneuronal (CM) cells that make monosynaptic connections to spinal α-motoneurons; few if any CM cells are found in “old,” rostral M1, on the crown of the precentral gyrus, or in PMd (Rathelot and Strick 2009). New M1 likely plays a major role in making highly dexterous, individuated movements (Schieber and Poliakov 1998). With voluntary effort, neurons in new M1 thus may become controlled differentially more rapidly than those in old M1, PMd, and PRR.

Conclusions

We found that monkeys were able to acquire control of a novel interface rapidly by comodulating the activity of small ensembles of neurons selected arbitrarily from the M1 upper extremity representation. Rather than performance declining when the monkeys were required to comodulate more neurons, the monkeys’ performance improved with the addition of more ensemble neurons, which can be interpreted as a consequence of the increase in variability afforded to each ensemble neuron and to their joint trajectory, as the ensemble-specific null space expanded in proportion to the number of neurons included in the control of a one-dimensional output. Patterns of voluntarily comodulated firing among small numbers of arbitrarily selected M1 neurons thus can be found and improved rapidly, with little constraint based on the normal relationships of the individual neurons to native limb movement. This rapid flexibility in relationships among M1 neurons may in part underlie our ability to learn new movements and improve motor skill.

APPENDIX

Variance of Ensemble Neurons

Consider an ensemble of $N$ neurons. For brevity, their transformed, normalized firing rates ($tnFRs$) at time step $t$ here will be denoted simply as

$$\{r_i(t)\}_{i=1}^{N}. \quad (A1)$$

Using $X$ to denote the expectation value of $X$, the mean of the $i$th neuron’s $tnFR$ across trials at time $t$ is

$$\mu_i = \langle r_i(t) \rangle \quad (A2)$$

and the variance of the $i$th neuron’s $tnFR$ at time $t$ is

$$\sigma_i^2 = \langle (r_i(t) - \mu_i)^2 \rangle \quad (A3)$$

$$= \langle (r_i(t))^2 \rangle - 2 \mu_i \mu_i + \mu_i^2 \quad (A4)$$

$$= \langle (r_i(t))^2 \rangle - \mu_i^2 \quad (A5)$$

The covariance matrix of the ensemble neurons’ $tnFR$s can be expressed as

$$\sum_{ij} = \langle (r_i(t) - \mu_i)(r_j(t) - \mu_j) \rangle \quad (A6)$$

Note that the diagonal elements of $\sum_{ij}$ are the individual $\sigma_i^2$ of Eqs. A3–A5 above.

Ensemble averages (as opposed to expectation values) will be denoted with an overbar:

$$\bar{\mu} = \frac{1}{N} \sum_{i=1}^{N} \mu_i \quad (A7)$$

$$\bar{\sigma_i^2} = \frac{1}{N} \sum_{i=1}^{N} \sigma_i^2 \quad (A8)$$

We will use this ensemble average of the individual neuron $tnFR$ variances as our measure of the variance of the ensemble

$$\sigma_e^2 = \bar{\sigma}^2 = \frac{1}{N} \sum_{i=1}^{N} \sigma_i^2 \quad (A9)$$

Note that this measure of ensemble variance is the trace of the covariance matrix (sum of the diagonal values) normalized by $N$, the number of ensemble neurons. Hence it allows comparison of ensemble variance across ensemble sizes.
Variance of Cursor

The cursor position, or amplitude of the cursor, $A_c$, at time $t$ is defined as the average of the ensemble neuron mFRs. Rewriting Eq. 3 from Methods in the present notation,

$$A_c(t) = \frac{1}{N} \sum_{i=1}^{N} r_i(t) \quad \text{(A10)}$$

The expectation value of the cursor amplitude therefore is the average of the expectation values of the individual neuron mFRs:

$$\mu_c = \langle A_c(t) \rangle \quad \text{(A11)}$$

$$= \left( \frac{1}{N} \sum_{i=1}^{N} r_i(t) \right) \quad \text{(A12)}$$

$$= \frac{1}{N} \sum_{i=1}^{N} (r_i(t) - \mu_i) \quad \text{(A13)}$$

$$= \frac{1}{N} \left[ \left( \sum_i (r_i(t) - \mu_i) \right) \left( \sum_j (r_j(t) - \mu_j) \right) \right] \quad \text{(A14)}$$

$$= \frac{1}{N^2} \sum_i \sum_j \left( (r_i(t) - \mu_i)(r_j(t) - \mu_j) \right) \quad \text{(A15)}$$

which thus is also the ensemble average of the individual mean mFRs. The variance of the cursor at time step $t$ is then

$$\sigma_c^2 = \langle (A_c(t) - \mu_c)^2 \rangle \quad \text{(A16)}$$

$$= \left( \frac{1}{N} \sum_{i=1}^{N} (r_i(t) - \mu_i) \right)^2 \quad \text{(A17)}$$

$$= \left( \frac{1}{N} \sum_{i=1}^{N} (r_i(t) - \mu_i) - \frac{1}{N} \sum_{i=1}^{N} (r_i(t) - \mu_i) \right)^2 \quad \text{(A18)}$$

$$= \frac{1}{N^2} \left[ \left( \sum_i (r_i(t) - \mu_i) \right) \left( \sum_j (r_j(t) - \mu_j) \right) \right] \quad \text{(A19)}$$

$$= \frac{1}{N^2} \sum_i \sum_j \left( (r_i(t) - \mu_i)(r_j(t) - \mu_j) \right) \quad \text{(A20)}$$

$$= \frac{1}{N^2} \sum_i \sum_j (\Sigma_{ij}) \quad \text{(A21)}$$

$$= \frac{1}{N^2} \left[ \left( \sum_i \sigma_i^2 + \sum_{i \neq j} (\Sigma_{ij}) \right) \right] \quad \text{(A22)}$$

Cursor variance is thus the sum of the entire covariance matrix—the trace plus the off-diagonal terms—normalized by the number of terms in the matrix, $N^2$.

Ensemble Neurons as a Vector Space

Now consider an $N$-dimensional Euclidean space. In this space, we define an ensemble vector, $\hat{\beta}(t)$, as

$$\hat{\beta}(t) = \frac{1}{\sqrt{N}} \begin{pmatrix} r_1(t) \\ r_2(t) \\ \vdots \\ r_N(t) \end{pmatrix} \quad \text{(A23)}$$

In this vector space, the cursor dimension is the direction of the positive diagonal, which is the direction of the unit vector, $\hat{e}$:

$$\hat{C} = A_c(t) \hat{e} \quad \text{(A24)}$$

Hence in this Euclidean space the cursor can be represented as a vector with amplitude $A_c(t)$ and direction $\hat{e}$:

$$\hat{C} = A_c(t) \hat{e} \quad \text{(A25)}$$

Note that the projection of the ensemble vector, $\hat{\beta}(t)$, in the direction of $\hat{e}$ is defined by their dot product:

$$\overrightarrow{\beta} \cdot \hat{e} = \left( \frac{1}{\sqrt{N}} (r_1(t) r_2(t) \cdots r_N(t)) \right) \cdot \left( \frac{1}{\sqrt{N}} (1) \right) \quad \text{(A26)}$$

$$= \frac{1}{\sqrt{N}} \sum_{i=1}^{N} r_i(t) \quad \text{(A27)}$$

$$= A_c(t) \quad \text{(A28)}$$

Hence the projection of $\overrightarrow{\beta}$ in the direction of $\hat{e}$ is the amplitude of the cursor, i.e., the cursor position.

The Moments of $\overrightarrow{\beta}$

The mean of vector $\overrightarrow{\beta}$ is a vector:

$$\overrightarrow{\mu}_\beta = \langle \overrightarrow{\beta}(t) \rangle \quad \text{(A29)}$$

$$= \frac{1}{\sqrt{N}} \begin{pmatrix} \langle r_1(t) \rangle \\ \langle r_2(t) \rangle \\ \vdots \\ \langle r_N(t) \rangle \end{pmatrix} \quad \text{(A30)}$$

We consider the variance of $\overrightarrow{\beta}$ to be

$$\sigma_\beta^2 = \langle (\overrightarrow{\beta}(t) - \overrightarrow{\mu}_\beta)^2 \rangle \quad \text{(A31)}$$

$$= \left( \frac{1}{N^2} \right) \begin{pmatrix} r_1(t) \cdots r_N(t) \end{pmatrix} \cdot \begin{pmatrix} r_1(t) - \mu_1 \\ \vdots \\ r_N(t) - \mu_N \end{pmatrix} \quad \text{(A32)}$$

$$= \frac{1}{N^2} \sum_{i=1}^{N} (r_i(t) - \mu_i)^2 \quad \text{(A33)}$$

$$= \frac{1}{N^2} \sum_{i=1}^{N} \sigma_i^2 \quad \text{(A34)}$$

$$= \sigma_c^2 \quad \text{(A35)}$$

Hence the variance of our ensemble vector, $\overrightarrow{\beta}$, is identical to the ensemble variance derived above in Eq. A9.

The Moments of $\hat{C}$

The mean of vector $\hat{C}$ is a vector:

$$\overrightarrow{\mu}_c = \langle A_c(t) \hat{e} \rangle \quad \text{(A36)}$$

$$= \langle A_c(t) \rangle \hat{e} \quad \text{(A37)}$$
The variance of $C$ is

$$\sigma^2 = \langle (\overline{C} - \overline{C})^2 \rangle$$

(A43)

$$= \langle (A_i(t) - \overline{\mu})^2 \rangle$$

(A44)

$$= \sigma_n^2$$

(A46)

which is the same as Eq. A16 above.

**Dot Products**

It will become useful to have scalar expressions for four dot products of the vectors $\overline{\beta}$, $\overline{C}$, $\overline{\mu}$, and $\overline{\varepsilon}$.

1) $\overline{\beta} \cdot \overline{C} = \overline{\beta} \cdot (A_i \overline{\varepsilon}) = A_i (\overline{\beta} \cdot \overline{\varepsilon}) = A_i \sigma_n^2$ (using Eq. A28)

(A47)

2) $\overline{\beta} \cdot \overline{\mu} = \overline{\beta} \cdot (\overline{\mu} \overline{\varepsilon}) = A_i \overline{\mu} (\overline{\beta} \cdot \overline{\varepsilon}) = A_i \overline{\mu}$ (using Eqs. A28 and A41)

(A48)

3) $\overline{\mu} \overline{\beta} \cdot \overline{C} = \overline{\mu} \overline{\beta} \cdot (A_i \overline{\varepsilon}) = A_i (\overline{\mu} \overline{\beta} \cdot \overline{\varepsilon})$

(A49)

$$= A_i \left( \frac{1}{\sqrt{N}} \sum_{i=1}^{N} \mu_i \right)$$

(using Eqs. A24 and A30)

$$= A_i \frac{1}{\sqrt{N}} \sum_{i=1}^{N} \mu_i \cdot 1$$

(A50)

$$= A_i \overline{\mu}$$

(A51)

which we note

$$= \overline{\beta} \cdot \overline{\mu}$$

(using Eq. A48)

(A53)

4) $\overline{\mu} \overline{\beta} \cdot \overline{\mu} = \overline{\mu} \overline{\beta} \cdot (\overline{\mu} \overline{\varepsilon})$ (using Eq. A41)

(A54)

$$= \overline{\mu} \left( \frac{1}{\sqrt{N}} \sum_{i=1}^{N} \mu_i \right)$$

(using Eqs. A24 and A30)

$$= \overline{\mu} \frac{1}{\sqrt{N}} \sum_{i=1}^{N} \mu_i \cdot 1$$

(A55)

$$= \overline{\mu}^2$$

(A57)

**The Null Vector**

Now we introduce the null-space vector, $\overline{n}$, defined as

$$\overline{n} = \overline{\beta} - \overline{C}$$

(A58)

The dot product of the null vector and cursor vector is

$$\overline{n} \cdot \overline{C} = (\overline{\beta} - \overline{C}) \cdot \overline{C}$$

(A59)

$$= \overline{\beta} \cdot \overline{C} - \overline{C} \cdot \overline{C}$$

(A60)

$$= \overline{\beta} \cdot (A_i \overline{\varepsilon}) - (A_i \overline{\varepsilon}) \cdot (A_i \overline{\varepsilon})$$

(A61)

$$= A_i \overline{\beta} \cdot \overline{\varepsilon} - (A_i \overline{\varepsilon})^2$$

(A62)

$$= A_i (\overline{\beta} - (A_i \overline{\varepsilon})^2$$

(using Eq. A28)

(A63)

$$= 0$$

(A64)

Hence $\overline{n}$ and $\overline{C}$ are orthogonal and constitute an orthogonal decomposition of $\overline{\beta}$. We thus identify $\overline{n}$ as the component vector of $\overline{\beta}$ that points in a direction that is null with respect to $\overline{C}$, as illustrated in Fig. A1.

**The Moments of $\overline{n}$**

The mean of vector $\overline{n}$ is a vector:

$$\overline{\mu}_n = \langle \overline{n} \rangle$$

(A65)

$$= \langle \overline{\beta} - \overline{C} \rangle$$

(A66)

$$= \langle \overline{\beta} \rangle - \langle \overline{C} \rangle$$

(A67)

$$= \overline{\mu}_\beta - \overline{\mu}_C$$

(A68)

and we consider the variance of $\overline{n}$ to be

$$\sigma_n^2 = \langle (\overline{n} - \overline{\mu}_n)^2 \rangle$$

(A69)

$$= \langle (\overline{\beta} - \overline{C} - \overline{\mu}_\beta + \overline{\mu}_C)^2 \rangle$$

(using Eqs. A58 and A68)

(A70)
\[
\begin{align*}
&= \left( \left( \hat{\beta} - \hat{\mu}_\beta \right) - \left( \hat{c} - \hat{\mu}_c \right) \right)^2 \\
&= \left( \left( \hat{\beta} - \hat{\mu}_\beta \right)^2 - 2 \left( \hat{\beta} - \hat{\mu}_\beta \right) \cdot \left( \hat{c} - \hat{\mu}_c \right) + \left( \hat{c} - \hat{\mu}_c \right)^2 \right) \\
&= \left( \hat{\beta} - \hat{\mu}_\beta \right)^2 - 2 \left( \hat{\beta} - \hat{\mu}_\beta \right) \cdot \left( \hat{c} - \hat{\mu}_c \right) + \left( \hat{c} - \hat{\mu}_c \right)^2 \\
&= \sigma^2_\beta - 2 \left( \hat{\beta} \cdot \hat{c} - \left( \hat{\beta} \cdot \hat{c} \right) \right) + \sigma^2_c \\
&= \sigma^2_\beta - 2 \left( A \cdot \hat{c} - \left( A \cdot \hat{c} \right) \right) + A \cdot \hat{c} + \left( A \cdot \hat{c} \right) + A \cdot \hat{c} + \left( A \cdot \hat{c} \right) \\
&= \sigma^2_\beta - 2 \sigma^2 + \sigma^2 \\
&= \sigma^2_\beta - \sigma^2 \\
&= \sigma^2_c - \sigma^2_c \\
&= \sigma^2 \cdot \sigma^2_c \
\end{align*}
\]

Hence the variance of the null vector is equal to the ensemble variance minus the cursor variance, as stated in METHODS (Eq. 9).

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: A. J. L. and M. H. S. drafted manuscript; A. J. L., G. R., and M. H. S. edited and M. H. S. interpreted results of experiments; A. J. L. and M. H. S. prepared A. J. L. performed experiments; A. J. L. and M. H. S. analyzed data; A. J. L., G. R., and M. H. S. prepared A. J. L., G. R., and M. H. S. wrote the manuscript; A. J. L., G. R., and M. H. S. approved final version of manuscript.

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