Statistical assessment of the stability of neural movement representations

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33   ABSTRACT
34
35   In systems neuroscience, neural activity that represents movements or sensory stimuli is often characterized
36   by spatial tuning curves that may change in response to training, attention, altered mechanics, or the passage
37   of time. A vital step in determining whether tuning curves change is accounting for estimation uncertainty
38   due to measurement noise. In this study, we address the issue of tuning curve stability using methods which
39   take uncertainty directly into account. We analyze data recorded from neurons in M1 using chronically
40   implanted multi-electrode arrays in four monkeys performing center-out reaching. Using simulations, we
41   demonstrate that under typical experimental conditions, the effect of neuronal noise on estimated preferred
42   direction can be quite large, and is affected by both the amount of data and the modulation depth of the
43   neurons. In experimental data we find that, after taking uncertainty into account using bootstrapping
44   techniques, the majority of neurons appear to be very stable on a timescale of minutes to hours. Lastly, we
45   introduce adaptive filtering methods to explicitly model dynamic tuning curves. In contrast to several
46   previous findings suggesting that tuning curves may be in constant flux, we conclude that the neural
47   representation of limb movement is, on average, quite stable and that impressions to the contrary may be
48   largely the result of measurement noise.
49

50   INTRODUCTION
51
52   Many experiments in neuroscience aim at measuring how experimental manipulations affect the tuning
53   properties of neurons. Tuning curves typically characterize how firing rates depend on a single relevant
54   property of a stimulus or movement. For example, visual neurons are often characterized by their
55   dependence on stimulus orientation, auditory neurons by their dependence on pitch, and motor cortical
56   neurons by their dependence on the direction of movement of an animal's hand. A common method for
57   studying how these coding properties change is to characterize the tuning curve, introduce an experimental
58   manipulation, and characterize the tuning curve again to assess if changes have occurred (Cronin et al. 2010;
59   Li et al. 2001; Rokni et al. 2007; Schummers et al. 2007). Tuning curves in a variety of brain areas have been
60   shown to change during adaptation and learning. In the absence of an experimental manipulation we can ask
61   how stable tuning curves are and whether they drift due to the passage of time. Importantly, determining
62   whether changes in tuning are statistically significant depends on our ability to take into account the effects
63   of noise and limited data.
64
65   Since noise in the recorded data (e.g. Poisson noise in spike counts) will affect tuning curve estimates, it is
66   important to characterize any tuning curve parameters with confidence bounds. Without such bounds it is
67   impossible to determine whether an observed change is due to actual changes in tuning or simply
68   measurement noise and uncertainty in the estimation. We will tend to overestimate the average (absolute)
69   magnitude of any existing tuning curve changes, since the measured change consists of the real change plus
70   apparent changes due to measurement noise. Therefore, measurement noise must be considered in order to
71   obtain reasonable estimates of the magnitude of changes in tuning curves.
72
73   Here we focus on the role of measurement uncertainty in estimating the tuning curves of neurons in primary
74   motor cortex. In particular, we examine the discharge properties of neurons during a well-learned center-out
75   task, where a monkey is trained to reach from the workspace center to several peripheral target locations
76   (Georgopoulos et al. 1982; Kakei et al. 1999; Kalaska and Hyde 1985; Morrow et al. 2007; Scott and
77   Kalaska 1997). Typically, tuning curves that characterize the discharge of neurons in primary motor cortex
78   (M1) as a function of reaching direction are well fit by a cosine function (Georgopoulos et al. 1982;
79   Georgopoulos et al. 1988). The peak of this cosine indicates the direction of movement for which the
80   neuron's firing rate is maximal, the "preferred direction" (PD).
81

A central question in the neural control of movement is whether the preferred directions of M1 neurons are stable during normal behavior, from reach to reach. If tuning curves fluctuate from reach to reach the motor system must either be redundant, such that fluctuations do not affect behavior (Pohlmeyer et al. 2007; Rokni et al. 2007), or must rapidly adapt to allow for stable movement. By recording many trials from the same neurons over the course of several days, a number of studies have shown that neuronal tuning appears to be relatively stable (Chestek et al. 2007; Dickey et al. 2009). However, there is also some contrasting evidence to suggest that neurons fluctuate rapidly on timescales on the order of minutes to hours (Rokni et al. 2007). One recent study found that the preferred directions of movement-related neurons in M1 varied substantially over time with a majority of neurons shifting as much as 30 degrees within 15 minutes (Rokni et al. 2007). These results suggested that neurons may be quite unstable and that there must be substantial redundancy in the nervous system and down-stream processing to ensure stable behavior. However, these estimates were obtained with methods that did not explicitly correct for measurement noise.

In this study, we address these issues using methods which take measurement uncertainty and potential instability directly into account. We analyze data recorded from neurons in M1 using chronically implanted multi-electrode arrays. Using simulations, we demonstrate that under typical experimental conditions, the effect of neuronal noise on estimated preferred direction can be quite large. Using experimental data we find that, after taking uncertainty into account, the average patterns of neuronal activity expressed by tuning curves are quite stable on a timescale of minutes to hours. We then explore ways of explicitly modeling dynamic tuning curves using adaptive filtering methods. These methods reveal that there may be tiny but real fluctuations of the tuning properties of neurons but that such changes are very hard to detect robustly. We conclude that the neural representation of limb movement is actually quite stable, and that impressions to the contrary may be the result largely of measurement noise.

METHODS

Tasks
Four monkey subjects (designated F, K, C, and R) were trained on an eight-target center-out reaching paradigm. Monkeys F, K, and C were seated in a primate chair, with movement constrained to a horizontal plane. The monkey grasped the handle of a two link planar manipulandum that moved within a 20 cm by 20 cm workspace. Feedback about movement was given on a computer screen in front of the monkey. Handle position was displayed as a circular cursor, 1-2 cm in diameter. The experiments with monkey R used a KINARM device (BKIN Technologies, Kingston, ON, Canada) in which the monkey's arm rested on cushioned troughs secured to links of a two-joint robotic arm (Scott 1999). The shoulder joint was abducted 90 degrees such that shoulder and elbow flexion and extension movements were made in the horizontal plane. A cursor coincident with the handle position of the robotic arm was projected onto a horizontal screen placed above the monkey's arm. All trials began with the acquisition of a square center target that the monkey was required to hold for 0.3-1.1s (0.6-1.1s for monkey F, 0.5-0.6s for monkey C, 0.3-0.5s for monkey K). After this hold period, subjects reached to one of eight equally spaced, peripheral targets. Subjects had approximately 1.25 s to acquire the outer target and were required to hold this outer target for at least 0.2-0.5s. Each success was rewarded with juice or water.

Surgery
Once the monkey was able to perform the center-out task satisfactorily, we performed a surgery to implant a 100 electrode intra-cortical array (Blackrock Microsystems). We made a craniotomy centered above the arm area of M1. After opening the dura, we identified the area on the crown of the precentral gyrus, just medial to the spur of the arcuate sulcus. In some cases we stimulated within this area using a ball electrode array (monopolar, biphasic, 50 Hz, 100 µsec pulse width, < 6mA) to locate proximal limb movements. All surgery was performed under isoflurane gas anesthesia except during intra-operative stimulation. In order to increase

131 cortical excitability, approximately 30 minutes prior to stimulation, we began infusing remifentanil (0.4
132 μg/kg/min, i.v.) while gradually reducing the concentration of isoflurane to 0.25%. We positioned the array
133 within the identified area, taking care to avoid any major surface vessels and inserted it rapidly with a
134 pneumatic inserter (supplied by Blackrock Miscrosystems). The dura was closed over the array, with a piece
135 of artificial pericardium (Preclude ePTFE membrane, Gore and Associates, Inc.) under the dura to prevent
136 the back of the array from adhering to the dura. Another piece of Preclude was placed over the dura. The
137 original bone flap was thinned and replaced, and the skin was closed over the craniotomy. All leads from the
138 array were routed to a percutaneous connector secured to the monkey's skull (Nordhausen et al. 1996).

140 All animal use procedures were approved by the institutional animal care and use committees at
141 Northwestern University (datasets F, K, and C) or the University of Chicago (dataset R).

143 *Tuning curve estimation and simulations*
144 Spike trains from each subject were recorded during center-out reaching for 30-40 minutes resulting in at
145 least 300 successful trials for datasets F, R, and K and 290 trials for dataset K. Neural signals were classified
146 as single-units based on action potential shape and minimum inter-spike intervals of 1.6 ms. Spike sorting
147 was performed off-line by manual cluster cutting. Trial-by-trial spike counts from 100 ms prior to movement
148 onset until 300 ms after movement onset are used throughout the analysis.

150 Following the conventional cosine tuning model, we assume that the firing rate of each neuron depends on
151 the direction of hand movement, θ as

153 $$y = b_0 + b_1 \cos(\theta - \theta^*) \qquad\qquad\qquad (1)$$

155 where the parameters b_0, b_1, and θ^* denote the baseline firing rate, modulation, and the preferred direction
156 (Georgopoulos et al. 1986). Traditionally, these parameters are estimated by minimizing the squared error
157 between predicted and actual firing rates (Swindale 1998). To simplify the optimization, we can use the sum-
158 difference formula for cosines to rewrite (1) as

160 $$y = b_0 + c_1 \cos(\theta) + c_2 \sin(\theta) \qquad\qquad\qquad (2)$$

162 giving $\theta^* = \text{atan2}(c_2, c_1)$ and $b_1 = (c_1 - c_2)/(\cos\theta^* - \sin\theta^*)$. In this form we can efficiently estimate the
163 parameters using linear regression. Note that, since minimizing the squared error is equivalent to maximizing
164 the log-likelihood with a Gaussian noise model, this optimization implicitly assumes Gaussian noise. In the
165 low firing rate limit, other noise models, such as Poisson, may be more appropriate (Cronin et al. 2010), and
166 several studies have suggested that exponential-cosine tuning functions may be a better description of
167 neurons in M1 (Amirikian and Georgopulos 2000; Hatsopoulos et al. 2007). An analysis of these alternative
168 tuning models is presented in the Supplementary Material. However, for the main analysis we assumed
169 cosine tuning with Gaussian noise as a standard model.

171 For simulations, the firing rate is assumed to be constant over the observation window, drawn from a Poisson
172 distribution with a firing rate given by (1). For the example neurons we fixed the parameters by hand to a
173 physiologically plausible range, and for a more complete analysis we incrementally varied the modulation
174 depth. For comparisons to recorded data, the parameters b_0, b_1, and θ^* were estimated from the entire
175 recording session for each recorded neuron. We then simulated "stable" neurons with the same tuning
176 properties, and compared these results, where tuning curves are fixed, with the observed data. This allowed
177 us to test whether results that previously seemed to suggest tuning curve drift might be consistent with noisy,
178 stable neurons. It is important to note that, although tuning curve estimation assumed Gaussian noise, the

179 simulations used here assumed Poisson noise. This is somewhat non-standard from the point of view of
180 statistical inference, but we have used this approach since the Gaussian noise assumption is so commonly
181 used for estimating motor tuning curves.
182
183 *Bootstrapping*
184 To estimate the uncertainty associated with each of the parameters in (1), we used bootstrapping (Davison
185 and Hinkley 1997; Efron and Tibshirani 1997). Briefly, we randomly resampled the original data with
186 replacement and estimated the parameters from each of these resampled datasets. Bootstrapping creates a
187 distribution of parameters consistent with the data, from which confidence intervals can be estimated and
188 significance can be assessed. In practice, the results are robust to the choice of number of bootstrap samples
189 and in this case were nearly identical for >500 samples. In the following analyses we used 1000 bootstrap
190 samples.
191
192 The variability of the bootstrap samples gives an indication of how uncertain the parameter estimates are.
193 Confidence intervals for each of the parameters can then be assessed using the quantiles of the bootstrapped
194 distributions over the parameters. For example, 95% confidence intervals are estimated by the 2.5 and 97.5
195 percentiles of the distribution. After sorting the samples, the lowest and highest 2.5% of the samples are
196 discarded. The range of the remaining samples then defines the error margin. For 1000 bootstrap samples
197 these would be the 26th and 975th samples from the ranked list. Importantly, this method does not need to
198 make the assumption that the distribution is Gaussian, symmetric, or even uni-modal.
199
200 Since the preferred direction parameter is in circular coordinates, special care is needed to estimate the
201 confidence interval. A number of methods exist for dealing with small, <100, sample sizes (Fisher 1996;
202 Otieno and Anderson-Cook). However, in our case (~1000 bootstrap samples, from well-tuned neurons) it
203 suffices first to center the samples by the median, and then apply the quantile procedure described above.
204
205 Estimating confidence intervals allows us to assess statistical significance. Mainly, we want to determine
206 whether the change in preferred direction from one block of trials to the next is statistically significant. In
207 this case we examined the distribution of *differences* between the bootstrap samples for preferred direction
208 from each block. If the 95% confidence interval for this distribution of differences did not contain 0 then we
209 reject the null-hypothesis (that the difference actually is 0) at the 5% significance level. For a previous
210 application of this method to tuning curves see (Churchland and Shenoy 2007).
211
212 In general, the size of confidence intervals is determined by the number of spike observations. For example,
213 the standard error of the mean for a Poisson distributed random variable scales as $1/\sqrt{N}$ where N is the
214 number of observations. For cosine tuned neurons, the confidence interval about the preferred direction is the
215 main quantity of interest. For simplicity we can reparameterize the tuning curves (1) in terms of the total
216 number of spikes emitted by a neuron and the depth of modulation relative to baseline ($M = b_1 / b_0$). Given
217 the assumptions of Poisson noise and cosine tuning, uncertainty about preferred direction is affected only by
218 these two factors.
219
220 To assess how these factors influence the measurement uncertainty, we parametrically varied the total
221 expected number of spikes as well as the modulation depth. For each simulated neuron we sampled spikes
222 from a Poisson distribution in 8 reach directions with the firing rate given by a cosine tuning curve. Without
223 loss of generality, we can assume that the preferred direction is 0 deg. We then used the bootstrapping
224 approach described above to generate bootstrap PD distributions for each modulation depth, spike count
225 combination and determined the 95% confidence bounds of the distribution.
226

227 In order to compare our results with previously published data, we read out the maximal (max) and minimal
228 (min) firing rates across direction, from the graphs published in several papers (Kalaska et al. 1989; Rokni et
229 al. 2007; Wise et al. 1998). In these cases we estimated modulation depth from the tuning curves as
230 $\hat{M} = (\text{max} - \text{min}) / ((\text{max} + \text{min}) / 2)$, and the total number of spikes was estimated as $\hat{N} = Tw(\text{max} + \text{min}) / 2$
231 where T is the number of trials and w is the integration window.
232
233 *Corrected estimate of the variance of changes in preferred direction*
234 In the presence of measurement noise, the differences that we observe in preferred direction between blocks
235 will be due partially to a true (hidden) drift and partially due to the measurement noise. Therefore, we will
236 tend to overestimate fluctuations in preferred direction. Using PD to denote the true, but unobserved,
237 preferred direction and PD_{est} to denote the estimated preferred direction
238
239 $$\text{var}(PD_{1,est} - PD_{2,est}) = \text{var}((PD_1 + \varepsilon_1) - (PD_2 + \varepsilon_2)) \qquad (3)$$
240
241 where ε_1 and ε_2 denote instantiations of the measurement noise, which is assumed Gaussian. Since the
242 variance of a sum of independent random variables is equal to the sum of the variances, the corrected
243 estimate of the fluctuations in preferred direction is given by
244
245 $$\text{var}(PD_1 - PD_2) = \text{var}(PD_{1,est} - PD_{2,est}) - \text{var}(\varepsilon_1) - \text{var}(\varepsilon_2) \quad , \qquad (4)$$
246
247 which captures the excess variance (above the measurement noise) that may be attributed to an actual change
248 in preferred direction. We estimated $\text{var}(\varepsilon)$ empirically by using the variance of the bootstrap samples (mean
249 across the population of neurons). In some cases, the corrected value $\text{var}(\Delta PD)$ was negative. These values
250 were set to 0 to calculate summary statistics, and suggest that there was no detectable PD drift after
251 accounting for measurement noise. The mean and difference statistics reported in the text were all calculated
252 using circular statistics (Berens 2009). However, the equations above use a linear rather than circular
253 coordinate system for correcting the variance. In the results presented here the distribution of ΔPD is narrow
254 enough that (4) and (5) provide an accurate approximation to the fully circular variance correction.
255
256 *Adaptive filtering*
257 Although the methods described above for assessing and describing drift are fairly robust, one potential issue
258 with these approaches is that they assume that tuning curves are stable within each block and that each trial is
259 independent. Ideally, we should use a method that explicitly assumes that tuning curve parameters are
260 drifting over time, from trial to trial. Adaptive filtering is one approach that makes this assumption explicit.
261 Several recent studies have used point-process adaptive filtering to describe time-varying tuning of place
262 cells in hippocampus (Brown et al. 2001; Eden et al. 2004). Here, for consistency with the initial analysis, we
263 analyze trial-by-trial spike counts assuming cosine tuning with Gaussian noise and use least-mean-squares
264 (LMS) or steepest-decent adaptive filtering (Haykin 1996). Briefly, the parameters of the tuning model β
265 are updated every trial following
266
267 $$\hat{\beta}_k = \hat{\beta}_{k-1} - \varepsilon \frac{\partial l_k(\beta)}{\partial \beta}\bigg|_{\beta = \hat{\beta}_{k=1}}$$

$$(5)$$

268
269 where $l_k(\beta)$ denotes the instantaneous, negative log-likelihood (squared error) and ε denotes a learning rate
270 which must be optimized separately. Assuming Gaussian noise and writing (2) in matrix form such that

271    $y = X\beta$ where $X_k = [1 \ \cos(\theta_k) \ \sin(\theta_k)]$ and $\beta = [b_0 \ c_1 \ c_2]^T$ we have the updates

272

273    $$\hat{\beta}_k = \hat{\beta}_{k-1} + \varepsilon(y_k - X_k \hat{\beta}_{k-1}) X_k^T. \qquad (6)$$

274

275   The aim of this analysis is to update the parameters at each time-step to minimize prediction errors. The
276   learning rate for each neuron and parameter is chosen to maximize the total likelihood of the time-varying
277   parameter estimates. The final time-varying parameter estimates then allow us to extract the per-trial drift
278   rate and fluctuations for each parameter. For the analyses presented here we focus solely on the dynamics of
279   the preferred direction. In this case the tuning curve is parameterized following (1), and the baseline and
280   modulation are held constant, while the preferred direction is updated as

281

282    $$\hat{\theta}_k^* = \hat{\theta}_{k-1}^* + \varepsilon(y_k - (b_0 + b_1 \cos(\theta_k - \hat{\theta}_{k-1}^*))) * \sin(\theta_k - \hat{\theta}_{k-1}^*). \qquad (7)$$

283

284   The LMS algorithm works well for processes where the parameters follow a Gaussian random walk. To
285   examine the performance of adaptive filtering we simulated cosine-tuned Poisson neurons with fixed
286   baseline firing rate and modulation, but where the preferred direction followed a random walk

287

288    $$\theta_k^* = \theta_{k-1}^* + \eta \qquad \eta \sim N(\mu, \sigma). \qquad (8)$$

289

290   In this case the preferred direction changes by a random amount η each trial, drawn from a Gaussian
291   distribution with mean μ and standard deviation σ.

292

293   Time-varying tuning can lead to surprising results. For instance, a neuron that appears very broadly tuned
294   using traditional block-estimation techniques may, in reality, be a sharply tuned but drifting neuron (Brown
295   et al. 2001). Finally, it is important to note that when the parameters do not have linear dynamics, but instead
296   change suddenly, recursive methods may be more appropriate to estimate these changes accurately (see
297   (Eden et al. 2004)). Here we used the more basic LMS algorithm as a first step in analyzing trial-by-trial
298   fluctuations in tuning parameters.

299

300   =====================
301   Figure 1 about here
302   =====================

303

304   RESULTS

305

306   *Measuring uncertainty in estimates of preferred direction*
307   In order to illustrate how measurement noise affects estimates of neuronal preferred direction, we simulated
308   the discharge of a typical cosine-tuned Poisson neuron (Fig 1A) during repeated reaches to 8 targets. We
309   assume the simulated neuron has a movement related discharge with a baseline firing rate of 20 Hz and a
310   modulation of 5 Hz. Without loss of generality we assume that the neuron has an actual preferred direction
311   (PD) of 180 degrees. Due to Poisson noise in the simulation, the number of spikes generated during different
312   reaches to the same target differed considerably (Fig 1B). This type of variability is typical of experimental
313   recordings. To characterize measurement uncertainty in this situation we used the well-established technique
314   of bootstrapping. Essentially, bootstrapping is a procedure that produces alternative fits that could have been
315   obtained if one could repeat a given experiment many times. In the case of the simulated neuron in Fig 1, we
316   initially simulated 40 reaches. With bootstrapping, we resampled from these data with replacement, each
317   time computing a tuning curve and preferred direction (Fig 1C). Ultimately, this process can be used to

318 obtain a distribution of possible preferred directions that is be compatible with the original spike data and
319 that can be used to estimate how reliable each tuning parameter is (Fig 1D). In this example, the 95%
320 confidence interval spanned approximately 40 degrees. The parameters we used were well within the range
321 of typical M1 experiments (Georgopoulos et al. 1982; Kalaska et al. 1989; Morrow et al. 2007; Wise et al.
322 1998); however, even after 40 trials there was considerable uncertainty in the preferred direction, and this
323 uncertainty directly determines whether or not a change in tuning can be detected.
324
325 To determine whether the tuning curve of a neuron has changed we can use this same bootstrapping
326 approach on two successive blocks of data. For illustration, we simulated a hypothetical neuron with a tuning
327 curve that changed following an experimental manipulation (Fig 2A). We again assumed that this neuron has
328 a baseline firing rate of 20 Hz and a modulation of 5 Hz. In this case we assumed that the preferred direction
329 changes from 90 deg in the first condition to 135 deg in the second condition. After simulating 40 reaches for
330 each condition (Fig 2B), we calculated distributions over potential PDs for each of the two conditions using
331 bootstrapping (Fig 2C). The two resulting distributions of PDs overlapped only slightly, and the distribution
332 of differences between the preferred directions was significantly different from 0. For this simulation,
333 bootstrapping allowed us to conclude that the PD changed as a result of the experimental manipulation.
334 However, the width of the resulting histograms (~40 degrees) indicates that changes in PD for a given
335 neuron need to be rather large in order to be visible with this amount of data.
336
337 ========================
338 Figure 2 about here
339 ========================
340
341 It is important to understand how measurement uncertainty varies as a function of these simulation
342 parameters. Given cosine tuning and Poisson noise, there are two relevant parameters that determine the
343 precision of PD estimates: the total number of spikes and the modulation depth relative to baseline (see
344 Methods). We used the bootstrapping methods described above to calculate the average confidence interval
345 size as a function of these two parameters (Fig 3). In this case a modulation depth of 1 corresponded to a
346 cosine between 0 and the maximal firing rate, and a modulation depth of 0 corresponded to an un-tuned cell.
347 In general, more strongly modulated neurons yield more precise PD estimates, and as more data are observed
348 the estimates of all tuning curve parameters become more precise.
349
350 Based on these simulated results, the precision of PD estimates in typical studies is likely to vary
351 substantially. Here we have shown total spike numbers and modulation depths for exemplar neurons in
352 several published studies (Kalaska et al. 1989; Rokni et al. 2007; Wise et al. 1998), as well as our own data
353 (Fig 3B). Some of these studies allowed PDs to be estimated with a precision of approximately 10 degrees
354 while others were probably precise only to 50 or 60 degrees. For our data the average modulation depth was
355 0.49±0.02 with 6.23±0.39 spikes/trial on average (400 ms integration window). Mapping these values onto
356 the simulation results suggested an average confidence interval size of 33.5 deg for blocks of 40 trials, 20.6
357 deg for blocks of 120 trials, and 16.9 deg for blocks of 240 trials.
358
359 ========================
360 Figure 3 about here
361 ========================
362
363 Here we have assumed that the only source of variability in addition to directional tuning is Poisson noise.
364 Because there are certainly other noise sources, the values reported in Figure 3 should be seen as a lower
365 bound on the uncertainty. However, this figure can be used to estimate measurement uncertainty both for
366 data that are already published, and also provides a type of power analysis for future studies. Given a

367 baseline firing rate and modulation depth, this figure provides an approximate number of trials necessary to
368 achieve a desired precision in preferred direction estimates. For the purposes of this study, it is important to
369 note that uncertainty in estimates of preferred direction can be of the same order of magnitude as the size of
370 changes reported in previous papers -- changes that have been attributed to either experimental
371 manipulations or instability.
372
373 *Assessing tuning curve stability, taking uncertainty into account*
374 While examples and power analysis illustrate how bootstrapping can be used to assess measurement
375 uncertainty in the presence of simulated Poisson noise, bootstrapping is also a powerful technique for the
376 analysis of actual data. Several recent studies have suggested that the preferred directions of neurons in
377 primary motor cortex may be drifting rapidly over the course of tens of minutes (Carmena et al. 2005; Rokni
378 et al. 2007). However, in describing changes in preferred directions, these studies did not explicitly account
379 for measurement uncertainty. As noted above, estimated changes in preferred direction can easily be inflated,
380 since they include effects due to both any actual changes and measurement noise. Here we used
381 bootstrapping to assess the stability of primary motor cortical neurons during well-learned, center-out
382 reaching.
383
384 To address these issues experimentally, we analyzed how the properties of recorded neurons in primary
385 motor cortex from four animal subjects evolved over the course of a recording session. We recorded four
386 datasets C (79 neurons), F (36 neurons), K (92 neurons), R (78 neurons), each consisting of at least 290
387 successful trials with sessions lasting 30-40 minutes. After collecting spike counts from 100 ms prior to
388 movement onset to 300 ms following movement onset, 70.5% of the recorded neurons (201/285 total --
389 38/79, 34/36, 72/92, and 57/78 in datasets C, F, K, and R, respectively) showed significant cosine tuning
390 across all trials ($\alpha = 0.05$, Hotelling's T^2-test, see Supplementary Note). In the following sections, we limited
391 our analysis to these significantly tuned neurons.
392
393 Following previous approaches (Rokni et al. 2007) we divided our dataset of center-out movements into
394 blocks of trials. For each block, we estimated the neuron's preferred direction, baseline firing rate, and
395 modulation (see Methods), and determined the 95% confidence interval for the preferred direction estimates
396 using bootstrapping. To compare these empirical results to the previous power analysis we first varied the
397 number of trials included in each block (block size) and noted the average confidence interval size. As
398 suggested by the power analysis, the size of the confidence interval decreases as the block size increases (Fig
399 4). In this case, where modulation is measured from the data, confidence interval size dropped approximately
400 as 1/sqrt(number of trials). In close approximation to the power analysis estimates we found that in real data
401 the average confidence interval size is 43.7 deg for blocks of 40 trials, 24.4 deg for blocks of 120 trials, and
402 16.3 deg for blocks of 240 trials. Simulated Poisson neurons that were stable and matched for tuning showed
403 a very similar decrease in confidence interval size as a function of the number of trials (Fig 4, red curves).
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405 =======================
406 Figure 4 about here
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408
409 Keeping in mind that measurement uncertainty (confidence interval size) is related to the block-size, we then
410 assessed stability in preferred direction and examined changes in tuning between blocks. Using
411 bootstrapping analysis and examining successive blocks of 120 trials we found that many of neurons appear
412 to be quite stable (Fig 5A). However, there were neurons in each of the four datasets that appeared to drift
413 and have relatively large changes in preferred direction, modulation, and baseline firing between successive
414 blocks (Fig 5B). To determine whether these changes are real effects or simply artifacts of high measurement
415 uncertainty, it is important to consider whether the observed changes are statistically significant. Greater drift

416   in preferred direction is correlated with smaller baseline firing rates (Fig 5C) as well as smaller modulation
417   (Fig 5D). In general, the neurons with larger apparent changes were the ones that also had large confidence
418   intervals and high uncertainty about preferred direction (Fig 5E).
419
420   ========================
421   Figure 5 about here
422   ========================
423
424   In all datasets we found that the average change in preferred direction from one block to the next was small.
425   For a block size of 40 trials the mean change was -0.8±1.1 deg, and for blocks of 120 trials was 2.3±1.5 deg
426   for all neurons. Despite the fact that the average PD change is close to 0, there was substantial variability in
427   the changes, which, at first glance, appears to be an indication that preferred directions fluctuate (Fig 6). The
428   standard deviation of PD changes was 39.0±2.2 deg for blocks of 40 trials and 22.9±3.2 deg for blocks of
429   120 trials. However, based on the bootstrapping analysis only a small number of these changes were
430   significant (5.8% for 40 trials and 5.5% for 120 trials, bootstrapping test, $\alpha = 0.05$, not correcting for
431   multiple comparisons). In fact, simulated Poisson neurons that were stable and matched for tuning had a
432   distribution of changes from one block to the next that was almost identical to the distribution of observed
433   PD changes. These results suggest that the majority of neurons are stable on timescales on the order of
434   minutes or, if they are unstable, drift at a rate that is undetectable given the amount of measurement noise
435   (<43.7 deg over 40 trials and <16.3 deg over 120 trials).
436
437   For the population, we can attempt to estimate the true variability in PD changes using a corrected measure
438   of standard deviation that attributes the observed fluctuations to a combination of real changes in PD and
439   measurement noise (see Methods). Using this method we found that the corrected standard deviation for
440   blocks of 40 trials was 0.4±1.1 deg and for blocks of 120 trials was 1.8±2.5 deg, compatible with absolutely
441   stable preferred directions. Our findings are thus consistent with the results from several past studies that
442   incorporated enough data to reduce measurement errors (Chestek et al. 2007; Ganguly and Carmena 2009)
443   and provide statistical insights into previous findings of apparent instabilities.
444
445   ========================
446   Figure 6 about here
447   ========================
448
449   *Explicitly modeling dynamic tuning curves with adaptive filtering*
450   In the methods described above, tuning curves are assumed to be static within each block with changes
451   occurring only between blocks. So far our results have suggested that any changes in preferred direction that
452   do exist are below the level of measurement uncertainty (16.3 deg over 120 trials on average). These block-
453   by-block methods gain statistical power by incorporating more trials, but, at the same time, by including so
454   many trials, they average over any potential fluctuations that might exist. Ideally, we would like to use a
455   method that explicitly models *dynamic* tuning curves that change from trial to trial. Adaptive filtering is one
456   approach for modeling exactly this type of relationship, and several recent studies have used adaptive
457   filtering to model dynamic place fields in hippocampal data (Brown et al. 2001; Eden et al. 2004). Here we
458   use a basic version of adaptive filtering techniques to assess dynamic tuning curves in primary motor cortex.
459
460   Rather than look for differences in tuning between blocks of trials, adaptive filtering methods update the
461   tuning curve parameters after each observation. At each time-step, the parameters are adjusted to reduce the
462   size of prediction errors. Here we used the least-mean-squares algorithm (LMS) based on steepest-descent
463   adaptive filtering. Larger errors result in larger parameter changes, and the size of the parameter updates is
464   determined by a learning rate that is optimized separately. By adjusting the tuning parameters dynamically,

adaptive filtering allows potential changes in tuning to be tracked from trial to trial. Here we assumed that only the preferred direction changes from trial-to-trial while the baseline firing rate and modulation are constant (see Methods for details).

Adaptive filtering is able to track changes in the preferred directions of simulated cosine tuned neurons. The analysis is built on the assumption that the process of spike generation is noisy and that neurons undergo both drift (fixed changes) and diffusion or fluctuation (random changes). In simulation, cosine tuned neurons with a constant drift in preferred direction were accurately tracked for a range of drift rates (Fig 7A). For a drift rate in the range ±2 deg/trial and no fluctuations, the estimated drift rate had an RMS error of only 0.07 deg/trial (Fig 7B). Fluctuations in preferred direction could also be tracked, albeit less accurately (Fig 7C). For fluctuations with a standard deviation between 0 and 20 deg/trial the estimated fluctuation had an RMS error of 2.5 deg/trial (Fig 7D). It is important to note that, while adaptive filtering excels at tracking large changes in the parameters, estimates of small fluctuations are substantially less accurate and sensitive to the learning rate. With an RMS error of 2.5 deg/trial it will be difficult to detect the 0.16 deg/trial variation (1.8±2.5 deg over 120 trials) suggested by the corrected standard deviation analysis above. However, these simulation results suggest that if neurons in primary motor cortex are truly unstable with large, random fluctuations in preferred direction, adaptive filtering methods may reveal this instability.

=====================
Figure 7 about here
=====================

In actual data, adaptive filtering revealed some degree of fluctuation in the preferred directions of a subset of primary motor cortical neurons. Of the 201 significantly tuned neurons from our four datasets, 177 neurons were best fit by a stable rather than dynamic tuning curve. The remaining 24 neurons (~12%) showed some degree of fluctuation, with a median fluctuation of 2.1±0.3 deg/trial (Fig 8A). Assuming that preferred directions follow a Gaussian random walk, results from Rokni et al. as well as the uncorrected block-by-block analysis (Fig 6) suggest fluctuations on the order of 2.1 to 2.2 deg/trial on average (22.9±3.2 over 120 trials for our analysis and 29±3 deg over 160 trials (Rokni et al. 2007)). At first glance, this subset of neurons thus appears to be fluctuating consistent with previous results. However, even in this minority of neurons, it is possible that the observed fluctuations are false positives.

The fact that these estimates are close to the RMS error observed when estimating fluctuations in simulated data suggests that some care is needed in interpreting these values. The fluctuations found by adaptive filtering did improve spike prediction. The average log likelihood ratio for the dynamic tuning model was 8.66±1.6 bits/trial relative to a homogenous Poisson process, while the average log likelihood ratio for the static tuning model was 8.56±1.6 bits/trial relative to a homogenous Poisson process (both on training data). However, with any finite amount of data, adaptive filtering methods can find small false-positive fluctuations even for simulated, stable neurons.

We examined whether the observed fluctuations may have been false positives by again simulating stable cosine tuned neurons of varying modulation depth. In this case, after applying adaptive filtering, ~4-5% of the stable neurons are mistakenly identified as fluctuating. Using these false positives we constructed a null distribution and compared this distribution with the subset of observed fluctuating neurons (Fig 8B, grey). The fluctuations observed in 12% of the recorded neurons are consistent with the results from stable neurons, suggesting that they may indeed be false positives. The null-results suggest that, for typical physiologically realistic tuning curve parameters, fluctuations have to be rather large before they are detectable by adaptive filtering (~5 deg/trial).

514   =======================
515   Figure 8 about here
516   =======================
517
518   DISCUSSION
519
520   We have presented results from two approaches aimed at detecting fluctuations in the preferred directions of
521   cosine tuned neurons in primary motor cortex. Using bootstrapping on simulations of stable neurons we have
522   quantified how measurement uncertainty or confidence interval size is affected by modulation depth and the
523   amount of data available. In real data, bootstrapping allows us to estimate changes between blocks of trials
524   and test for significance in a way that directly captures the measurement uncertainty. Finally, we used
525   adaptive filtering techniques to model trial-by-trial changes in preferred direction explicitly. After comparing
526   the results with those from simulated, stable neurons we find no evidence for large fluctuations in preferred
527   direction using either bootstrapping or adaptive filtering. Small fluctuations in preferred direction may exist,
528   but detecting these changes is difficult in the presence of spiking noise.
529
530   When examining the properties of neural discharge, the experimental manipulations as well as the statistical
531   approaches used for analysis will influence the interpretation of the results. In this report, we have
532   demonstrated that the uncertainty related to estimates of preferred direction is directly related to the total
533   number of spikes as well as the neuron's modulation depth over different reach directions. The greater the
534   modulation depth and the more data available (number of spikes), the more certain one can be about the
535   tuning of an individual neuron. For typical experiments with limited amounts of data, the uncertainty about
536   preferred direction can be quite large in comparison to the typical effects of a manipulation -- both on the
537   order of a few tens of degrees. Measurement noise is thus of central importance when estimating how tuning
538   curves change over time.
539
540   Using bootstrapping we can determine confidence intervals for each of the tuning curve parameters. For the
541   observed neurons the confidence intervals for preferred direction were rather large: 16.3 deg over 120 trials
542   on average. This value fundamentally limits how well we can detect changes in preferred direction. For
543   instance, the standard deviation of changes in preferred direction between blocks of 120 trials was 22.9±3.2
544   deg -- the same order of magnitude as the confidence interval. Comparing these changes to those from stable,
545   simulated neurons with matched tuning curves and using a corrected measure we find that the standard
546   deviation of changes is likely closer to 1.8±2.5 deg over 120 trials. Adaptive filtering estimates PD changes
547   in a small subset of neurons on the order of 2.1 deg/trial, but after comparing these results to stable simulated
548   neurons we find that these changes are again consistent with stable preferred directions.
549
550   A previous study using large blocks of data reported similar stability of tuning parameters (Chestek et al.
551   2007). However, this study used free reaching rather than a manipulandum and analyzed neurons primarily
552   from PMd, leading the authors to speculate that the differences between their results and those of Rokni et al.
553   could be due to experimental design or the specific population of neurons. The experiments presented here
554   are far closer in design to those by Rokni et al., and yet we find no significant PD changes after corrections
555   for measurement noise. Several studies have examined the stability of neural activity in the context of brain-
556   machine interfaces and observe that firing properties may be highly variable (Carmena et al. 2005) or
557   relatively stable (Ganguly and Carmena 2009) depending on training. However, interpreting these results can
558   be difficult, since it is often unclear how a given decoding scheme relates to the tuning properties of
559   individual neurons. Variability in the decoded behavior tends to be much higher than during normal
560   reaching, and brain-control itself may introduce certain constraints on movement coding.
561
562   As a practical matter in data analysis, anything that is not part of the model but affects neural signals is

considered noise. We considered only Poisson noise, which is compatible with the Fano factor typically observed in cortical recordings (Stevens and Zador 1998; Zacksenhouse et al. 2007). However, there are many other factors beyond the monkey's hand direction that potentially influence firing rates (Johnson et al. 2001), including other kinematic variables such as reach speed (Chestek et al. 2007) and limb posture (Caminiti et al. 1990; Scott and Kalaska 1997), as well as, added loads (Kalaska et al. 1989), cortical waves (Rubino et al. 2006), neuromodulator concentrations (Ahern et al. 2002), oxygen concentration (Jiang and Haddad 1994) and circadian rhythms (Barnes et al. 1977). Modeling these other sources of variability, whether they are observed (Paninski et al. 2003; Saleh et al. 2010; Truccolo et al. 2005; Wu and Hatsopoulos 2006) or unobserved (Kulkarni and Paninski 2007; Stevenson et al. 2010), would likely improve the estimates of PD stability. These uncontrolled sources of variability could inflate estimates of both real fluctuations and measurement uncertainty, and the results here thus provide only an upper bound for the instability of PDs of neurons in primary motor cortex. If other sources of measurement noise could be accounted for, the estimated changes in preferred direction may very well be even smaller.

While our results suggest that preferred directions are substantially more stable during normal reaching than some previous reports, there is also convincing evidence that the relationship between a given neuron's activity and hand direction does change over time. Tuning to hand direction changes on very short timescales due to changing kinematics and dynamics (Churchland and Shenoy 2007; Hatsopoulos et al. 2004; Sergio et al. 2005) as well as over longer timescales during sensorimotor learning (Jarosiewicz et al. 2008; Li et al. 2001; Paz and Vaadia 2004). While several studies have shown that tuning curves are sensitive to the measurement epoch and specific task constraints (Hamel-Pâquet et al. 2006; Sergio et al. 2005), here we focused on a fixed, specific portion of the reaches (100 ms prior to, through 300 ms after movement onset). This type of analysis ignores the short-timescale kinematics and dynamics of reaching, and is aimed to test whether tuning curves are stable on longer timescales during a well-learned task. Additionally, we have focused primarily on the stability and uncertainty in estimates of preferred direction. Both the modulation and baseline firing rate of cosine tuned neurons may show a higher degree of instability (Chestek et al. 2007).

The stability of neuronal properties is of central importance to many computational theories. If presynaptic neurons change rapidly then the motor system must either be redundant to the extent that fluctuations do not affect behavior (Rokni et al. 2007) or postsynaptic neurons must adapt to allow for stable movement and decision making. Many behavioral models (e.g. Cheng and Sabes 2007; Wei and Körding 2009) have suggested that learning is an ongoing process where errors are constantly being corrected even during apparently stable behavior. This might suggest that the fluctuations in tuning, if they exist, may actually be functional. Rather than being an artifact of redundancy in the cortical representation of movement, fluctuations may be a reflection of ongoing attempts to correct small reaching errors. While measurement noise makes it difficult to distinguish between these hypotheses, methods are being developed to isolate the effects of reach errors (Chase et al. 2010; Scheidt et al. 2000) and better understand redundancy in the motor system (Jarosiewicz et al. 2008). Statistical techniques that allow modeling of non-stationary data (Kim et al. 2006; Wu and Hatsopoulos 2008) as well as experimental techniques that allow for longer-term recordings (Dickey et al. 2009; Tolias et al. 2007) should both serve to reveal the more detailed structure of tuning curve dynamics during learning as well as stable reaching.

607   FIGURE LEGENDS
608
609   Fig 1: Measurement noise for a simulated neuron with known tuning. (A) Idealized cosine tuning function of
610   a neuron with typical movement-related discharge (20 Hz) and a modulation (5 Hz). (B) 40 trials simulated
611   during movement in 8 directions with Poisson spike noise. (C) Three example bootstrap samples from the
612   initial set of observations in (B). Note that there is substantial variability in the estimated preferred direction
613   (arrows). (D) Using many bootstrap samples we build a distribution of preferred directions that allows
614   estimation of confidence intervals. In this case the 95% confidence interval spans approximately 40 degrees.
615
616   Fig 2: Measurement noise affects the ability to detect PD changes. (A) Cosine tuning curves for a pair of
617   simulated neurons having preferred directions of 90 (black) and 135 (gray) degrees. (B) 40 trials simulated
618   from each of these tuning curves during movement in 8 directions with Poisson spike noise. (C) Bootstrap
619   distributions for the preferred direction based on the data in (B). Note that there is overlap between these
620   distributions, and the differences between the two distributions allow us to assess whether there is a
621   significant tuning difference between the two conditions.
622
623   Fig 3: Uncertainty in preferred direction as a function of the number of spikes and modulation depth of a
624   simulated neuron. Uncertainty about the preferred direction decreases both with increasing number of
625   observations and increasing modulation. (A) Contour plots illustrating the width of the 95% confidence
626   intervals. Levels denote one-sided 95% confidence intervals in degrees. For instance, for zero observations
627   or a modulation of zero the confidence intervals span ±180 deg. (B) Color plot of same values with
628   representative examples from three published studies -- square: (Rokni et al. 2007), filled circle: (Kalaska et
629   al. 1989), triangle: (Wise et al. 1998). The average confidence intervals for the data used here are shown as
630   open circles for blocks of 40, 120, and 240 trials (from left to right).
631
632   Fig 4: Uncertainty in preferred direction with increasing numbers of trials for four sets of data collected from
633   four different monkeys. Average size of the 95% confidence interval for populations of actual (black) and
634   simulated neurons (red). Simulated data were generated by estimating stable, non-drifting tuning curves from
635   the recorded neurons and simulating spiking with Poisson noise. The average number of spikes per neuron is
636   shown to relate these results directly to Fig 3. Dashed lines denote ±SEM across neurons.
637
638   Fig 5: Changes in tuning across blocks of 120 trials. Tuning curves for the first (black) and second (red)
639   blocks of 120 trials for the five most stable neurons (A) and the five least stable (B) from each of the four
640   datasets. Error bars denote mean firing rate ±SEM, solid lines denote cosine fits. The size of the PD change
641   is negatively correlated with the baseline firing rate (C) as well as modulation (D). Higher uncertainty in the
642   estimate of preferred direction is correlated with larger apparent drift (E). These correlations suggest that
643   estimated changes in preferred direction could be due to measurement noise.
644
645   Fig 6: Estimated changes in preferred direction. (A) Histograms of PD changes for each of the datasets
646   separately and for all neurons for a block size of 40 trials. (B) PD changes for a block size of 120 trials.
647   Black blocks denote changes that were significant at the 95% level by bootstrapping. Note that for blocks of
648   40 trials the variability of changes between blocks is substantially higher than for blocks of 120 trials,
649   suggesting that measurement noise plays a large role in determining the magnitude of PD changes. Red
650   curves denote the PD changes observed when simulating stable Poisson neurons that were matched to have
651   the same tuning as the observed data.
652
653   Fig 7: Adaptive filtering for detecting drift and fluctuation. (A) True and estimated preferred direction for
654   three simulated neurons with PDs drifting 0.9, 0.45, and 0.07 deg/trial. Dashed lines denote the true,
655   underlying preferred direction. (B) Estimated PD drift as a function of the true drift for 512 simulation runs

656   using data from 400 trials. (C) True and estimated preferred direction for three simulated neurons with no
657   mean drift, fluctuating 9, 4.5, and 0.7 deg/trial. (D) Estimated PD fluctuation as a function of the true
658   fluctuation for 512 simulation runs. For data from 400 trials the degree of fluctuation is estimated fairly well,
659   but much less accurately than the mean drift.
660
661   Fig 8: Adaptive filtering for actual data. For this analysis we combined neurons from all four datasets (C, F,
662   K, and R) and use only the significantly tuned neurons (n = 201). Only 12% (n = 24) of these neurons
663   showed some degree of fluctuation. (A) Trial-by-trial PD changes estimated by adaptive filtering for the
664   subset of neurons that appeared to be fluctuating. Note that 177 of the 201 neurons were better fit by a stable
665   rather than a dynamic tuning curve. (B) Estimated fluctuation as a function of the modulation for the subset
666   of fluctuating neurons (black) and simulated stable neurons (grey). The fluctuations revealed by adaptive
667   filtering are consistent with false positives.
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669
670

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672

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793
794

A

Firing rate (Hz)

B

Firing rate (Hz)

C

Ressampled tuning curves

D

Counts

Degrees

A

Firing rate (Hz)
40
20
0

0   180   360

B

Firing rate (Hz)
40
20
0

0   180   360

C

Counts
15
10
5
0

0   180   360

Degrees

A

Observed Fluctuations

n = 24 (of 201)

B

— Observed
— False Positives (Simulated Stable)