Accuracy of Subspace Mapping of Spatiotemporal Frequency Domain Visual Receptive Fields

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Submitted 11 November 2004; accepted in final form 10 January 2005

Nishimoto, Shinji, Miki Arai, and Izumi Ohzawa. Accuracy of subspace mapping of spatiotemporal frequency domain visual receptive fields. J Neurophysiol 93: 3524–3536, 2005. First published January 12, 2005; doi:10.1152/jn.01169.2004. Orientation and spatial frequency selectivities are fundamental properties of cells in the early visual cortex. Although they are customarily tested with drifting sinusoidal gratings, a recently developed subspace reverse correlation method may be a better replacement for obtaining a selectivity map in a joint orientation and spatial frequency domain at higher resolution efficiently. These two methods are examined for their accuracy and data compatibility for cells in areas 17 and 18 of anesthetized and paralyzed cats. Peaks and bandwidths of tuning curves from these two methods are highly correlated. However, spatial frequency bandwidths obtained by reverse correlation tend to be slightly narrower for the subspace reverse correlation than those from the drifting grating tests. Consistency between the two methods is improved if the entire duration of data containing signal are taken into account for the subspace reverse correlation rather than using the map only at the optimal correlation delay. Examination of convergence of the subspace mapping process shows that reliable 2-day profiles can be obtained within 5–10 min. for the majority of cells. Temporal dynamics of tuning properties are also examined more directly with the subspace mapping than with the drifting gratings. For many cells, the optimal spatial frequency shifts substantially, measured as a fraction of tuning bandwidth, over the time course of response. In comparison, the optimal orientation remains highly stable throughout the duration of response. Overall, these results suggest that the subspace reverse correlation is a better substitute for the conventional method.

INTRODUCTION

Orientation and spatial frequency selectivities are two of the most prominent characteristics of neurons in the primary visual cortex (Campbell et al. 1969; De Valois et al. 1982; Hubel and Wiesel 1962; Maffei and Fiorentini 1973; Movshon et al. 1978a). As these characteristics of the stimuli determine predominantly the overall strength of a cell’s responses, measurements of these selectivities are essential when one performs electrophysiological experiments in these areas, regardless of the primary experiments that follow. Traditionally, measurements of these tuning characteristics have been performed primarily by using drifting sinusoidal gratings. A substantial body of existing physiological data, both published and unpublished, is in these forms. However, recent advances in receptive-field mapping techniques have made alternative techniques possible for measuring these fundamental tuning properties in the form of subspace reverse correlation (Ringach et al. 1997b), which is fast becoming a new standard for basic characterization of neurons (Bredfeldt and Ringach 2002; Felsen et al. 2002; Mazer et al. 2002; Ringach et al. 1997a, 2003; Sugihara et al. 2004). How accurate is the new method considering the speed at which measurements are completed? How do data obtained by the new methods compare with those obtained by the traditional techniques? And if they differ, how and where do differences arise? Answers to these basic questions are important in establishing the basis on which results from future studies are compared reliably with previous findings.

With the traditional method using drifting sinusoidal gratings, the parameter for one of the dimensions (e.g., orientation) is varied, whereas those of other dimensions (e.g., spatial frequency) are fixed. The orientation and spatial frequency tunings thus obtained (typical forms of these tunings are in Fig. 1, A and B) are, however, essentially cross-sections of a two-dimensional tuning surface of a joint orientation and spatial frequency domain (Fig. 1C). Tuning characteristics for off-the-peak cross-sections have not been measured traditionally [with a few exceptions, e.g., Jones et al. (1987)], and one-dimensional sequential measurements described in the preceding text might miss the overall peak in the surface. Although the entire two-dimensional (2-D) tuning surface can theoretically be obtained by drifting gratings, it simply takes too long to execute in practice. Furthermore, simultaneous measurement from many neurons is increasingly becoming common recently (Alonso et al. 2001; Brown et al. 2003; Maldonado et al. 1997; Norman et al. 2001; Warren et al. 2001; Weliky et al. 2003), but the traditional tuning measurement is not conducive for these situations. This is because the orientation is fixed for measuring the spatial frequency tuning, hence only a subset of neurons, the preferred orientations of which are close to the stimulus orientation, could be excited in a given measurement.

The subspace reverse correlation, exploiting flash grating stimuli of various orientation of spatial frequency, can measure the two-dimensional tuning surface in experimentally reasonable testing time (Mazer et al. 2002; Ringach et al. 1997b, 2003; Sugihara et al. 2004). What remains to be shown is how accurately results of the conventional tests using drifting gratings and those of the subspace mapping technique agree. Do peaks or bandwidths of the parameters match between the two methods? Is there any tendency for the match to be better depending on the cell type or on other properties of neurons, such as direction selectivity? One of the primary purposes of this study is to assess compatibilities between the two methods.
FIG. 1. Typical orientation and spatial frequency tunings are shown for a simple cell in area 17. A and B: orientation and spatial frequency tuning curves obtained by drifting sinusoidal gratings. Error bars show SE. C: a tuning surface in a joint orientation and spatial frequency domain obtained by a subspace mapping for the same neuron.

with the intent of replacing conventional measurements by drifting gratings with subspace reverse correlation mapping. In what follows, we will show that 1) estimates of preferred orientation and spatial frequency are generally matched well for the two methods; 2) bandwidths of the spatial frequency tunings tend to be narrower for the subspace mapping than that for the drifting gratings; 3) Classification of simple and complex cells, traditionally based on the degree of modulation in responses to drifting gratings, is also possible using subspace reverse correlation by a ratio of responses to stimuli of antagonistic phases; and 4) for the majority of the cells, reliable 2-day profiles can be obtained only by 5–10 min of stimulations. Therefore results from the current study provide the basis on which physiological data from new studies may be compared directly with those based on the traditional measurements.

METHODS

All recordings were made from adult cats weighing between 1.5 and 4.4 kg. All animal care and experimental guidelines conformed to those established by the National Institute of Health and were approved by the Osaka University Animal Care and Use Committee.

Surgical procedure and physiological recordings

After initial preanesthetic doses of hydroxyzine (Atarax; 2.5 mg) and atropine (0.05 mg), each cat is anesthetized with isoflurane (2.5–3.5% in O₂). Electrocardiogram (ECG) electrodes and a rectal temperature probe were inserted, and a femoral vein was catheterized. Then cefotiam hydrochloride (Panpolar, 8.3 μg) and dexamethasone sodium phosphate (Decadron, 0.4 mg) were administered. Subsequently, a tracheostomy was performed and a tracheal tube inserted. Then the animal’s head was secured in a stereotaxic device with the use of ear and mouth bars and clamps on the orbital rim. Tips of the ear bars were coated with local anesthetic gel (Lidocaine). After the securing the animal, a craniotomy was performed directly above the central representation of the visual field in the visual area 17 or 18 (Horsley-Clarke P4 L2.5 for recordings of A17, A3 L3 for A18). The dura was dissected away to allow insertion of microelectrodes. We used tungsten microelectrodes (A-M Systems, 5 MΩ) for recording spike activity extracellularly. Typically, two electrodes were used to increase the chance of encountering cells, and they were mounted in parallel in a single protective guide tube and driven by a common microelectrode drive (Narishige). After lowering the electrodes to the cortical surface, agar was used to protect the cortex, and melted wax was applied over the agar to create a sealed chamber for stabilization. Anesthesia was then switched to sodium thiopental (Ranol, given continuously, 1.0–1.5 mg kg⁻¹ h⁻¹). After stabilization of anesthesia, paralysis was induced with a loading dose of gallamine triethiodide (10–20 mg), and the animal was placed under artificial respiration at the rate of 20–30 strokes/min. The respiration rate and stroke volume were adjusted to maintain end-tidal CO₂ between 3.5 and 4.5%. Artificial respiration was carried out with a gas mixture of 70% N₂O-30% O₂. The infusion fluid thereafter contained Ranol, gallamine triethiodide (10 mg kg⁻¹ h⁻¹), and glucose (80 μg kg⁻¹ h⁻¹) in Ringer solution. Body temperature was maintained near 38.3°C with the use of a servo-controlled heating pad. Pupils were dilated with atropine (1%), and nictitating membranes were retracted with phenylephrine hydrochloride (Neosynesin, 5%). Contact lenses with 4 mm artificial pupils were positioned on each cornea.

To record the activity of single units, electrical signals from the microelectrodes were amplified (10,000 times) and band-pass-filtered (300–5,000 Hz). Then spike sorting was achieved using a custom-built spike sorter (Ohzawa et al. 1996), where each spike was sorted by their waveforms and time-stamped with 40-μs resolution.

Visual stimulation and initial procedures

All the experiment control functions and generations of visual stimuli were performed using custom-written software on two Windows PCs. Visual stimuli were generated by a dedicated PC and displayed on a cathode-ray-tube (CRT) display (SONY GDM-FW900, a resolution of 1,600 × 1,024 pixels, refreshed at 76 Hz). The animal saw the display through a custom-built haploscope, which allows dichoptic presentations of visual stimuli to left and right eyes separately. Distance (total length of light paths) between the screen and the eyes were set to 57 cm, subsuming the visual field of 23 (horizontal) × 30 (vertical) degrees for each eye.

When we encountered isolated action potentials from one or more cells, we performed the following preliminary tests to obtain an accurate center position and a size of the cell’s receptive field. First, we obtained a rough position of the cell’s receptive field by presenting a small circular patch of a drifting sinusoidal grating and controlling the X-Y position of the patch manually by a pointing device (mouse). Then a standard reverse correlation procedure (DeAngelis et al. 1993a; Jones and Palmer 1987), and/or a modified reverse correlation procedure (instead of the conventional black/white bars, a small Gabor patch was presented to enhance the cell’s responses) was performed to obtain the accurate position of the receptive field. Position of the visual stimuli throughout this paper was set to the center of the visual field mapped in these tests.

The primary purpose of this study is to assess compatibilities between drifting sinusoidal gratings and subspace reverse correlation technique. Therefore orientation and spatial frequency selectivities were measured by both subspace reverse correlation and drifting gratings for each isolated cell as described in the following text.
Subspace reverse correlation test

Visual stimulation and analysis in the subspace reverse correlation procedures were equivalent to those used by Ringach et al. (1997b). Sinusoidal gratings of various orientation, spatial frequency and phase and one blank stimulus were presented in a randomized order, typically lasting for 39 ms (3 video frames). Range of orientation was from 0 to 180° in 10° steps. Range of spatial frequency was adjusted for each cell to cover the entire frequency range that evoked responses, sampled regularly in logarithmic scale (typical ranges are 0.02–0.5 cycle/° for cells in area 18 and 0.1–1.2 cycle/° for cells in area 17). Eight to 13 spatial frequencies were used typically. For each combination of orientation and spatial frequency, four phases of gratings, 0, 90, 180, and 270°, were used. Stimulus sets, each of which was a randomized sequence of 577 (8 frequencies × 18 orientations × 4 phases + blank) to 937 (13 frequencies × 18 orientations × 4 phases + blank) gratings, were presented 20–30 times. Evoked responses and the stimulus sequence were then cross-correlated to obtain a 2-D orientation and spatial frequency selectivity map. Spike counts for the blank stimuli were subtracted from the map. We obtain a 2-D orientation and spatial frequency selectivity map. Spike counts for the blank stimuli were subtracted from the map. We calculated the maps for correlation delays from 0 to 300 ms in 15-ms step, and we hereafter analyzed and presented a map obtained at the optimal correlation delay that contains the largest signal as in this example unless otherwise noted.

To make an objective criterion for data selection, we estimated the noise level in the data by computing SDs of selectivity maps with noncausal correlation delays, i.e., maps obtained from stimuli after spikes occurred. Specifically, the SDs of maps for noncausal delays are calculated from −300 to −90 ms in 15-ms steps, and we rejected the data when the SD at the optimal delay did not exceed the mean ±5 SD of the noncausal maps. For a subset of neurons, to examine temporal dynamics of spatial frequency tunings accurately, we have performed additional experiments with subspace mappings in a specialized configuration as in the following text. For these experiments, the duration of stimulus presentations was shortened to 26 ms from the standard 39 ms to increase temporal resolution, and the orientation of stimuli was restricted to optimal one, i.e. we have measured selectivity of spatial frequency subspace only (Bredfeldt and Ringach 2002; Nishimoto and Ohzawa 2004; Ringach et al. 1997b). In a typical set of stimulus sequences of this type, a stimulus of a given frequency and phase was presented for 53 times. One experiment for a cell typically contains 15 iterations of a set, lasting ~18 min in total.

Drifting grating test

For conventional tests using drifting sinusoidal gratings, orientation, and spatial frequency tuning curves were obtained separately. Only one parameter, either orientation or spatial frequency, was varied in a given run, whereas the other parameter was fixed. The gratings were presented in a randomized order, and each presentation lasted for 4 s with 1 s of interstimulus interval. Stimuli for each condition were presented five times, and responses were averaged over the trial period. The ranges of parameters were adjusted such that they were sufficiently wide to cover the entire range of stimuli that could elicit any response. Size and positions of the gratings were identical to the one used in the subspace mapping test.

RESULTS

Recordings were made from a total of 371 cells for which the subspace reverse correlation mapping tests were completed (184 cells from area 17, and 187 cells from area 18, in 27 cats). The recorded area is judged based on the coordinates of the electrode penetrations, and cells that may have been recorded from the 17/18 border are not included in the sample in the preceding text. Of these 371 cells, additional orientation tuning measurements using drifting grating stimuli could be completed with sufficient reliability for 241 neurons. Complete basic set of three experiments including the spatial frequency tuning run could be completed for 226 cells. Of these, 72 cells were classified as simple and 154 as complex, according to the standard criteria (Priebe et al. 2004; Skottun et al. 1991).

Comparison of peak parameters

To compare orientation and spatial frequency tuning characteristics measured with traditional drifting gratings and those by subspace reverse correlation, we first determine the locations of tuning peaks obtained with the two methods. Figure 2 shows two typical examples of the analysis. Figure 2A shows a typical 2-D selectivity map for an area 18 complex cell obtained with the subspace mapping procedure. The data obtained with 13 spatial frequencies and 18 orientations (10° steps) are shown as a contour plot. The position of the center of inner-most contour in Fig. 2A indicates that the optimal grating parameters was ~165° for the orientation and 0.2 cycle/° for the spatial frequency. The vertical and horizontal dashed lines in the Fig. 2A show the values of fixed parameters (spatial frequency and orientation, respectively) used in the tests by drifting gratings. The vertical dashed line is offset slightly to the right of the peak of the 2-D map, indicating that our initial estimate of optimal spatial frequency used for the orientation tuning run (the very 1st measurement for this cell) was a little too high. These cross-sections through the two dimensional map may be compared directly with tuning curves obtained by the drifting grating tests (Fig. 2B for orientation selectivity and C for spatial frequency selectivity).

In Fig. 2, B and C, the symbols and error bars (SE) depict data obtained by the drifting grating tests, and — indicates fits to the data by Gaussian functions. The —— indicates curves cut out from the 2-D subspace map (at —— in Fig. 2A). For both orientation and spatial frequency tuning curves, results from measurements using traditional drifting gratings and subspace reverse correlation are closely similar. The peak positions (i.e., the optimal parameters) of the — and — are shown with ↑.

Figure 2, D–F, shows another example of the analysis for a complex cell in area 18. In this case, the tuning curves are similar for orientation, but systematic deviations are found for the spatial frequency tuning in that the responses are greater for drifting sinusoidal grating stimuli than for reverse correlation stimuli at low spatial frequencies. Mainly because of this, the spatial frequency bandwidth also is narrower for the subspace reverse correlation than for drifting gratings.

We have analyzed the distributions of optimal parameters obtained by the two methods for our sample of cells, and Fig. 3 summarizes the results. Figure 3A compares the optimal orientations estimated by the drifting grating tests (horizontal axis) with those from the subspace mappings (vertical axis). The shapes of the symbol distinguish the recorded area (—for area 17 and open circle for area 18). The --- indicates the perfect match between the two methods, and the ——— shows the regression line. As is graphically seen, the results from the two methods are highly correlated (r = 0.99, n = 242). The histogram in Fig. 3B shows the distribution of differences in optimal orientations between the estimates from the two methods. Most of the differences are within 15°, which is equal to
the sampling interval for the drifting grating tests. Similarly, Fig. 3, C and D, shows comparisons of the two methods for estimates of optimal spatial frequencies. Again, the results from the two methods are highly correlated ($r = 0.97$, $n = 226$).

We have also analyzed the distribution of parameters separately for areas 17 and 18 (data not shown). For the optimal spatial frequency, there are systematic differences between the two areas for both drifting grating measurements [0.46 ± 0.31 cycle/° ($n = 106$) for area 17, 0.16 ± 0.08 cycle/° ($n = 120$) for area 18] and those of subspace mappings [0.45 ± 0.30 cycle/° ($n = 184$) for area 17, 0.17 ± 0.07 cycle/° ($n = 187$) for area 18]. The differences in the optimal frequency are significant for both measurements ($2$-sample Wilcoxon test, $P < 0.001$), and the trend that these two areas cover different ranges of spatial frequencies is consistent with previous reports (Issa et al. 2000; Movshon et al. 1978b). However, the magnitudes of differences between the two methods did not significantly differ between areas 17 and 18 for both orientation and spatial frequency measurements (2-sample Wilcoxon test, $P > 0.1$). Likewise, except for the overall range of spatial frequency tunings, cells in areas 17 and 18 exhibit similar characteristics at least for those properties we have examined in this study. For this reason, hereafter, we show combined results from the two areas, except for scatter plots where individual cells are marked differently depending on the area. Results of statistical analyses for area dependency will be provided whenever appropriate in each section.

**Comparison of tuning widths**

Next, we have examined the consistency of the sharpness, or the bandwidth, of tunings obtained with the two methods. To compare the bandwidths quantitatively, half-width at half-height (HWHH) were estimated for each tuning curve. Figure 4, A and B summarizes the orientation bandwidths for our sample of cells. Again, the results from two methods are highly correlated ($r = 0.88$), and there was no trend that the results from a method tend to exceed that from the other (1-sample Wilcoxon test, $P > 0.1$, Fig. 4B).

A different trend is observed for spatial frequency. Figure 4, C and D, shows the comparisons of spatial frequency bandwidth for the two methods. The spatial frequency bandwidth is defined here as the ratio of high-cutoff frequency to the optimal spatial frequency. Note that, in Fig. 4C, there are more symbols below the − − −, meaning that, in contrast to the orientation tuning, the spatial frequency bandwidth for the subspace mappings tended to be narrower than those for the drifting gratings on average (1-sample Wilcoxon test, $P < 0.01$). There was no significant cortical area dependency (17 or 18) in the magni-
tude of differences between two methods (2-sample Wilcoxon test, $P > 0.1$).

**Comparisons of modulation ratio**

How do the subspace mappings differ from drifting gratings in determining other characteristics of neurons, such as the simple and complex cell types? Modulation of responses to drifting sinusoidal gratings at the temporal frequency of drift is an intuitively striking property of simple cells. Partly for convenience and simplicity, this measure has been used for simple/complex classification in studies that use grating stimuli. To quantify the degree of modulation, modulation ratio ($F_1/F_0$ ratio) of cell’s responses to drifting sinusoidal gratings is widely used as a convenient criterion to classify the cells as simple or complex type (Priebe et al. 2004; Skottun et al. 1991). Although this criterion is challenged recently (Mechler and Ringach 2002), it is nevertheless true that the ratio is highly correlated with the classification of simple and complex types by other criteria. To examine whether the modulation ratio can be predicted from the data obtained by the mappings, we have defined a “modulation index” (MI) for a given condition (i.e., for a given combination of orientation, spatial frequency and correlation delay) using the subspace mapping data as follows

$$MI = 2 \times \frac{|R_0 - R_{180}| + |R_{90} - R_{270}|}{R_0 + R_{90} + R_{180} + R_{270}}$$

where $R_0$ indicates spike counts for phase $\theta$ (degrees) for a given combination of orientation and spatial frequency. MI increases when spatially antagonistic stimuli (i.e., $R_0$ vs. $R_{180}$ or $R_{90}$ vs. $R_{270}$) yield antagonistic responses, which is more likely for simple cells. It decreases when stimuli of all the phases elicit similar level of responses, as is common for complex cells. To perform quantitative comparison of the MI and the $F_1/F_0$ ratio, we have calculated a MI for the same spatial frequency and orientation as those used to extract the $F_1/F_0$ ratio in the drifting grating tests. If exactly the same condition was not sampled in the subspace mapping, the nearest sample point was used. Figure 5A shows histograms and a joint distribution of these metrics for the $F_1/F_0$ ratio and MI. Correlation coefficient between the two indices was 0.85 ($P < 0.001$). Figure 5B shows how drifting gratings classify cells to simple or complex compared with the classification by the subspace mapping. Of 91% (205/226) of cells, the classification of simple/complex type was matched for the subspace mappings and drifting gratings. There was no significant cortical area dependency (17 or 18) for the correlation coefficients ($P > 0.1$).

**Efficiency and accuracy of subspace mapping with reverse correlation**

How efficient is the subspace reverse correlation method? This is an important question that essentially determines how long it takes for measuring a receptive field reliably in the frequency domain. Figure 6A shows an example of a progressive refinement of a selectivity map as the subspace mapping progresses over time for a complex cell recorded from area 18. Figure 6A, top left, is a spatial frequency-orientation map obtained from a single iteration of a randomized stimulus set. The second map is computed using data from the first two
iterations and so on. Thus a map at the Nth position is obtained by using data from only the first N iterations. A number above each panel shows correlation coefficient between the map for the panel and the final map obtained by using data from all iterations (the bottom-most panel). Each iteration, as noted in the methods, consists of a complete set of all spatial frequencies, orientations, and phases, which are 13, 18, and 4, respectively for most measurements. Therefore an iteration is typically 37-s long. For this cell, just one iteration of stimulations yields a visible structure in the map, although it is still noisy. After ~10 iterations of the randomized stimulus sequences (~370 s), a smooth structure was obtained with a quality that is casually indistinguishable from the final map (which required ~1100 s). Therefore for this cell, there was only a marginal improvement in the tuning map after ~8–10 min of testing.

To examine convergence of the selectivity maps during measurement to the final one obtained by the all iterations, we have calculated expected values of the correlation coefficients between the selectivity map obtained by a given number of iterations and the map obtained by full iterations. The expected value of the correlation coefficient for a given iteration count, N, is calculated using a form of a re-sampling method as follows. For each iteration count N, maps are re-sampled N times from the entire map samples (typically 20 or 30 iterations were performed), allowing repetitive use of maps. As preliminary treatments for noise reductions, both the re-sampled and final maps were smoothed by a 3 × 3-pixel 2-D Gaussian filter with the SD equal to the pixel separation. Then we have calculated correlation coefficients between the re-sampled maps and the final map. We have performed this procedure 100 times for each iteration count N and defined the mean of the coefficients as the expected value for a given N.

Figure 6B shows the growth of the expected values for the cell illustrated in Fig. 6A as data from more iterations are included in the analysis. Correlation coefficient monotonically increases and come to a plateau after ~10 min. Therefore for this cell, the time necessary for reliable measurement of the complete 2-D tuning surface was ~10 min.

How efficient are subspace reverse correlation measurements for other neurons in our sample? Figure 7A shows a summary of the convergence analysis for our sample of cells (n = 371) in a format similar to that of Fig. 6A as data from more iterations are included in the analysis. Correlation coefficient monotonically increases and come to a plateau after ~10 min. Therefore for this cell, the time necessary for reliable measurement of the complete 2-D tuning surface was ~10 min.

How efficient are subspace reverse correlation measurements for other neurons in our sample? Figure 7A shows a summary of the convergence analysis for our sample of cells (n = 371) in a format similar to that of Fig. 6A as data from more iterations are included in the analysis. Correlation coefficient monotonically increases and come to a plateau after ~10 min. Therefore for this cell, the time necessary for reliable measurement of the complete 2-D tuning surface was ~10 min. For further clarifying the distributions, Fig. 7B shows distributions of measurement times necessary to achieve a given value of correlation coefficients. Distributions of measurement times are shown for correlation coefficients of 0.8, 0.9, and 0.95, respectively. For example, Fig. 7B (top) shows that correlation coefficient of 0.8 could be reached within 5 min for nearly all cells. However, to reach the level of correlation coefficient = 0.95, some neurons had to be stimulated for >10 min, although more than a half of the neurons still exceeded this level within 5 min. Considering that a complete 2-D joint tuning profile in the orientation-spatial frequency domain consisting of 234 (18 orientations × 13 frequencies) frequency components is obtained in 10–15 min for nearly all neurons, subspace reverse correlation method is remarkably time efficient. For comparison, based on 4-s trials repeated five times per condition, it would have taken 4,680 s.
Overall, subspace reverse correlation in the frequency domain and conventional tuning measurements using drifting sinusoidal gratings produce comparable results. In particular, the estimates of optimal orientation and spatial frequency are in close agreement between the two methods. However, there are some differences that still remain. A part of these differences must be due to inherent variability in neural responses, but it is of interest to examine if there are systematic differences that may hinder straightforward comparisons of new and old results. One factor that may contribute to discrepancies is the difference in the extent of temporal integration of responses. For this study so far, we have estimated the orientation and spatial frequency tuning surface only at the optimal time delay in the subspace reverse correlation. Therefore responses at other time delays are not incorporated into the tuning surface obtained by subspace mapping. However, with drifting grating stimuli, responses are integrated over multiple temporal cycles of stimuli to obtain the response strength. Such a difference does not matter if a neuron's spatial frequency tuning is invariant over the course of the response, as illustrated in Fig. 9A. For this neuron, responses are observed for correlation delays of 25–110 ms, during which the optimal spatial frequency remains nearly constant. However, for another neuron depicted in Fig. 9D, the optimal spatial frequency increases from \(-0.2\) to \(0.4\) cycle/° (an octave) over the correlation delays of 40–90 ms. Similar slants of response maps in the spatial frequency-time domain have been reported by others, both in the cat (Frazor et al. 2004) and the monkey (Bredfeldt and Ringach 2002; Frazor et al. 2004; Mazer et al. 2002). Likewise, there are several reports concerning the temporal changes of orientation tunings (Mazer et al. 2002; Ringach et al. 1997a, 2003; for review, Shapley et al. 2003), although the results are somewhat controversial.

To examine temporal dynamics of orientation and spatial frequency tuning characteristics, we have summarized temporal change of optimal orientation and spatial frequency in Fig. 8, A and B, respectively, for our sample of cells. Each curve indicates changes in the optimal value over the time course of responses for a cell. The horizontal dashed lines indicate the mean bandwidth (±HWHH) for each domain, calculated from our samples (Fig. 4). Figure 8, C and D, shows distributions of these shifts taken at 40 ms after the response onset indicated as the vertical dashed lines in Fig. 8, A and B. The value of 40 ms was selected to cover the initial rising portion of the response that tended to have higher rate of spatial frequency change than later portions of the responses as seen in Fig. 8B. In addition, it could not be made too long for maintaining reasonable cell counts for Fig. 8, C and D. While the majority of temporal shifts for the optimal orientation were restricted to a range as small as \(5°\), the shifts of preferred spatial frequency were substantial and biased for low-to-high sequences. The mean preferred orientation shifts for areas 17 and 18 were \(-1.1 \pm 4.6°/40\) ms and \(-0.16 \pm 4.8°/40\) ms, respectively. Neither of these shifts is significantly different from zero (1-sample Wilcoxon test, \(P > 0.05\)). For the preferred spatial frequency, the mean shifts for areas 17 and 18 were \(0.23 \pm 0.28\) octave/40 ms and \(0.20 \pm 0.28\) octave/40 ms, respectively. Both of these were significantly different from zero (1-sample Wilcoxon test, \(P \ll 0.01\)). There were no significant differ-

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**Possible sources of discrepancies**

One must use caution in interpreting the increase in correlation coefficients noted in the preceding text because the re-sampling procedure itself increases correlation with the final map as more maps are used. Because the degree of overlap between re-sampled maps and the final map will increase with \(N\) (iteration count), the correlation will also increase with \(N\), even if the maps are purely random without any structure. To estimate the degree to which the increase in correlation is caused by the procedure itself, we conducted a simulation by applying the method to synthetic random noise maps. The result of the simulation is shown as in Fig. 7A (\(\delta\) indicates ±1 SD). As expected, correlation coefficient increases with \(N\) up to \(-0.7\). However, the ranges of correlation values observed for the data from cells were far higher from the value obtained from the random noise simulation. Therefore although a portion of the increase of correlation coefficients was due to the re-sampling procedure itself, the correlation coefficients may be used as a reliable measure of convergence to the final map.
ences between the areas 17 and 18 for orientation or spatial frequency shifts (Kolmogorov-Smirnov test, $P > 0.05$).

The SD of the orientation shift was only $\sim 4.7^\circ$ or 17% of the mean orientation bandwidth of 27.5° (HWHH; see Fig. 4A), indicating that for most neurons, the preferred orientation remains very stable over the time course of responses. In comparison, the preferred spatial frequency shifts (in the low-to-high direction) by as much as 0.23 and 0.20 octaves on average for areas 17 and 18, respectively, during the initial 40 ms of the response (see Fig. 8D). This amounts to nearly 40% of the mean spatial frequency bandwidth of 0.56 octaves (see Fig. 4C). If we consider the SD as in the orientation in the preceding text, the fraction is even larger at 50%. The difference between the orientation shifts and those for the spatial frequency is also apparent in Fig. 8, A and B, in that all of the curves for the orientation shifts remain well within the two horizontal dashed lines (Fig. 8A), whereas many curves for the spatial frequency shifts cross and go beyond the top horizontal dashed line (Fig. 8B). These characteristics of temporal shifts of tunings might explain the discrepancy observed for spatial frequency measurements as considered further in the following text but not for orientation tunings.

To examine more precisely whether the temporal integration of tunings can affect discrepancies between the two methods, we have performed additional sets of experiments for spatial frequency tunings. The additional tests were conducted with shorter durations (26 ms) for each grating in a randomized sequence and subspace restricted to spatial frequency (with the orientation fixed to the optimal). All the data in Fig. 9 were from these additional experiments ($n = 39$).

To select the portion of the response time course that contains signals, we have calculated root-mean-square (RMS) response amplitude (i.e., SD of spike counts) of the response for each correlation delay between 0 and 300 ms in 5-ms steps. Spike count for the blank stimulus is subtracted from the map at each correlation delay before computing the RMS amplitude. The correlation delay with the maximum RMS response amplitude ($A_{\text{MAX}}$) defines the optimal correlation delay. The baseline noise level is calculated as the RMS response amplitude for the noncausal portion of the response ($A_{\text{NOISE}}$), which may be considered to contain only noise components (see METHODS). Using $A_{\text{MAX}}$ and $A_{\text{NOISE}}$, we define a criterion response as $A_\text{CRITERION} = 0.3(A_{\text{MAX}} - A_{\text{NOISE}}) + A_{\text{NOISE}}$, which must be exceeded to be counted as signal. The factor, 0.3, is picked somewhat arbitrarily by subjectively comparing the selected response portions and the response maps (similar to Figs. 9, A and D) for all neurons. Other criterion values did not alter the results. These portions of the response containing signal are indicated as red bands above the top margin of Fig. 9. A and D. A black marker in the gray bands indicates the optimal correlation delay. The tuning curve from the multiple correlation delays are then obtained by summing up these

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**FIG. 6.** Refinement of selectivity map during the subspace mapping process is shown for a 2-D spatial frequency and orientation tuning for a complex cell in area 18. A: each panel shows a selectivity map obtained by up to $N_{\text{th}}$ iterations of stimulus presentations (e.g., the top left shows a selectivity map obtained from one iteration of stimulus presentation and the bottom right shows that from 15 iterations of stimulus presentation). Bottom: a selectivity map obtained by all (in this case, 30 times) iterations of stimulus presentations. Response strengths are normalized by maximum values for each map. The number above each panel shows the correlation coefficient between the corresponding map and the final map. B: growth of the correlation coefficients with time (see text) is shown. Error bars indicate the SD.

**FIG. 7.** Summary of the time course of refinement of selectivity maps is shown for all cells. A shows the growth of correlation coefficients for our sample of cells ($n = 371$). The sample size is much larger than those for preceding figures, because only the subspace mapping data are needed. The growth of correlation coefficients examined for random noise map; ii, their SD. The time course and SD for the random noise were average values of 50 times of simulations. B: histograms of stimulation time necessary to attain a given accuracy criterion as defined by the correlation coefficient of 0.80, 0.90, and 0.95 (from top to bottom). The majority of cells reached the criterion of 0.8 within 2 min of simulations. Even reaching the criterion of 0.95 required 5–10 min of stimulation time for the vast majority of neurons.
tuning curves over the selected time period shown by the gray bands.

As expected, for the cell that showed a constant spatial frequency tuning for all correlation delays (Fig. 9A), no difference is found regardless of whether the optimal correlation delay is used (Fig. 9B) or data from multiple correlation delays are integrated (Fig. 9C). In Fig. 9, both B and C, the solid curve depicts the fit to data obtained from drifting grating experiments, whereas dashed curve indicates the spatial frequency tuning obtained by reverse correlation. However, for the cell that exhibited a marked spatial frequency shift over the time course of the response (Fig. 9D), the discrepancy in the tuning curves present in Fig. 9E (only the optimal time slide is used) disappears completely if signals from all significant correlation delays are integrated (Fig. 9F).

Figure 9, G and H, shows a summary of the comparisons between estimations from an optimal correlation delay and that from integrated multiple correlation delays. In Fig. 9G, each symbol, circle or x, with a tick represents a difference of spatial frequency width estimated from drifting gratings and subspace map. For each neuron, the center of the symbol indicates the difference calculated from the map at the peak correlation delay. The style of the symbols represents whether these differences are significant (circle) or not (x) (bootstrap re-sampling, \( P < 0.05 \)) (Efron and Tibshirani 1993). For the spatial frequency bandwidth, 12 of the 39 cells show significant differences depending on whether data at multiple correlation delays are used or not. For the optimal spatial frequency, 18 of the 39 cells exhibited differences. The horizontal solid line shows identity line, i.e., perfect match of parameter estimations from drifting gratings and subspace mappings. Therefore if a tick extends from a symbol outward from the identity line, it indicates improvement of the match by multi-correlation delay integration. On the other hand, if a tick points inward from a symbol toward the identity line, it indicates worsening of the match. An inspection of the plots shows that integrating multiple correlation delay improves, on average, bandwidth estimation of spatial frequency tunings (one-sample Wilcoxon test, \( P < 0.001 \)). However, even after the integration across multiple correlation delays, the trend that bandwidths from subspace mappings are narrower than that from drifting gratings still remains (1-sample Wilcoxon test, \( P < 0.001 \)), as seen by the fact that most of the symbols are above the identity line. For the optimal spatial frequency, analyses from multiple correlation delays also improve estimations on average (1-sample Wilcoxon test, \( P < 0.05 \)).

We have also examined possible dependence of discrepancies between subspace mapping and drifting grating tests for other characteristics of neurons, specifically, correlations with modulation ratio (F1/F0), direction selectivity (DSI), signal-to-noise ratio (S/N), and peak firing rate (Fig. 10). As noted earlier, the modulation ratio is highly correlated with simple and complex type classification. As with tuning shift of spatial frequency over time, direction selectivity is also closely related to spatiotemporal integration of responses, and highly direction selective neurons may be expected to exhibit larger discrepancies. Examination of S/N and peak responses is intended for estimating the degree to which variability and responsiveness influence our measurements of orientation and spatial fre-
results will be considered in the following text. Variabilities in neural responses. Possible implications of these
mates are for the two methods. These latter two correlations
consistent the optimal orientation and frequency bandwidth esti-
dependences on the S/N. The higher the S/N, the more con-
difference of spatial frequency width, as indicated by the
are also found for the difference of peak orientation and the
for simple cells than for complex cells. Significant correlations
nations of bandwidth of orientations tend to be more consistent
bandwidth and the modulation ratio, indicating that determi-
correlation is found between the difference of orientation
Contrary to our expectation, significant correlations are
found only for three cases. A small but significant negative
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Possible sources of discrepancies

One of the most obvious discrepancies found between the
two methods is that the tuning widths tended to be narrower for
the subspace reverse correlation than for the drifting gratings
tests. Superficially, this seems to be inconsistent with the
results of comparison between the orientation tunings obtained
by the Fourier transform of simple cell receptive fields from
m-sequence stimuli (equivalent to reverse correlation) and by
traditional drifting gratings tests (Gardner et al. 1999). In their
study, the tuning widths from the reverse correlation were
broader nearly by a factor of two than those by drifting
gratings. Similar biases were found in the spatial frequency
tuning as well (DeAngelis et al. 1993b). There are a few factors
that are different between these previous experiments and the
present study. One is that the simple cell RF mapping by
reverse correlation, used in the previous studies, obtains an

Discussion

We have shown that spatial frequency and orientation tuning
characteristics measured by a relatively new subspace mapping
methods generally agree with those obtained by traditional
drifting grating measurements. This study therefore establishes
a bridge between new and traditional data, assuring validity of
comparisons with the accumulated traditional data with new
ones obtained now and in the future. In some cases, however,
there are differences between the estimates by the two
methods. We will consider possible factors that may cause these
discrepancies and examine any potential problems.

Possible sources of discrepancies
estimate of a linear receptive field that is independent of output nonlinearity (Anzai et al. 1999; Gardner et al. 1999), whereas the subspace reverse correlation in the frequency domain is not a linearized measurement, as the method simply sums responses to all four phase conditions for a given combination of frequency and orientation. A power-law output nonlinearity (with an exponent of $\frac{1}{2}$) that follows a linear receptive field has been shown to sharpen the orientation tuning (Anzai et al. 1999; Geisler and Albrect 1995). For the case of subspace reverse correlation, such a nonlinearity is always in the measurements. Such power-law output nonlinearities also affect measurements by drifting grating stimuli. Therefore additional factors may be needed to account for generally sharper tuning for subspace reverse correlation mapping. One such possibility is that drifting grating of optimal parameters is a stronger stimulus for the cell, which is able to drive the neuron above the threshold for firing to a greater degree than drifting gratings. Briefly flashed (40 ms) grating stimuli of the same optimal parameters may excite the neuron relatively weakly, making the response more susceptible for the effects of the nonlinear threshold. Consequently, the sharpening of tuning curves may be more pronounced for the subspace reverse correlation than for drifting gratings.

For determining possible causes for discrepancies between the two methods, we have examined dependence of differences in the estimated parameters on other characteristics of neurons, as shown in Fig. 10. Of 20 cases examined, only 3 cases showed statistically significant correlation. One is the difference in the orientation tuning bandwidth, which was found to be dependent on F1/F0 ratio, the degree of temporal modulation in response to drifting sinusoidal gratings. The greater the degree of modulation, the more consistent the results from subspace mapping and drifting gratings were. It is puzzling how such a dependence can arise, and we currently do not have a reasonable explanation for this correlation.

Two other cases of dependence of discrepancies between the methods are found for the signal-to-noise ratio (S/N) for neurons. The greater the S/N, the better the match was for the estimates of preferred orientations and spatial frequency bandwidths by the two methods. This perhaps simply indicates a straightforward relationship where parameter estimation is better for neurons that respond more strongly and consistently. Therefore from the analyses of Fig. 10, we did not find any other systematic dependence of the degree of match even for such properties as the direction selectivity.

**Convergence tests**

The results of the convergence tests (Figs. 6 and 7) provide an objective criterion for considering how long we should record cell activities to obtain reliable 2-day profiles. Our results show that, for the majority of cells, 5–10 min of stimulations is enough to acquire reliable profiles, and the longer stimulations do not substantially improve the profiles.
The efficiency of the subspace mappings is examined by a simulation study in Ringach’s original paper (Ringach et al. 1997b) and our results provide the first experimental examinations to show the efficacy in terms of actual testing time. The equivalent tests can be applied to another classes of reverse correlation and can essentially be performed on-line. The results from these tests might be useful information for determining when to terminate recordings from a given set of neurons and thus to further reduce testing time.

Although we have adopted the correlation coefficient as the convergence metric in this study, this type of convergence tests may be preformed using other metrics such as the optimal orientation in principle. However, we did not adopt such a method because the convergence speed depends critically on tuning bandwidths. Neurons with narrower tuning bandwidths tend to converge faster, whereas those with broader bandwidths tend not to converge. Therefore it would be difficult to distinguish whether the convergence is due to the true growth of S/N or merely reflects dependence on the bandwidths.

Temporal dynamics of tuning characteristics

We have examined the dynamics of optimal orientation and optimal spatial frequency over the time course of responses and have shown that the preferred spatial frequency shifts upward for substantial number of neurons in both areas 17 and 18. This was not the case for orientation tunings. How do our results compare with those reported by others? Bredfeldt and Ringach (2002) and Mazer et al. (2002) report relevant results regarding spatial frequency shifts over the time course of the responses. The results from these studies (for the monkey) and ours (for the cat) all agree that substantial number of cells in V1 exhibit low-to-high shift of optimal spatial frequency. Unfortunately, the data from these two previous studies are not mutually comparable due to different ways in which they quantified the degree of frequency shifts. Mazer et al. (2002) report the rate of spatial frequency shift in cycles per degree per millisecond without noting the initial spatial frequency. This makes the conversion to octave-based metric used by Bredfeldt and Ringach (2002) and by us impossible.

There is also a minor difference between our frequency shift metric and Bredfeldt and Ringach’s. They report the total frequency shift in octaves for the entire duration of the response, whereas we calculate essentially the rate of frequency shift evaluated over the initial 40 ms of the responses. We have chosen this metric because the rate of change of spatial frequency is the key parameter for relating neural responses to, for example, the rate of expansion of visual patterns (although such questions are beyond the scope of this study). However, to compare our results to Bredfeldt and Ringach’s, we have recalculated the frequency shift metric to match theirs. Based on our results shown in Fig. 8B, total frequency shifts for the entire duration of responses was 0.22 ± 0.38 (SD) octaves, 0.24 ± 0.37 octaves, for areas 17 and 18, respectively. This is ~35% of 0.62 ± 0.69 (1SD) octaves reported for monkey V1 (Bredfeldt and Ringach 2002). Therefore although all studies including ours agree that there is a clear and significant optimal spatial frequency shift from low to high over the time course of the responses, the amount of shift for the cat area 17 neurons were ~1/3 of that for the monkey. It is not known whether there are any contributing factors for this difference other than the species difference.

Frazor and his colleagues (Frazor et al. 2004) also examined the temporal shifts of spatial frequency tunings by presenting static sinusoidal gratings with longer duration (200 ms) and interstimulus intervals. Their results show that the mean ratio of spatial frequency shifts was ~0.05 octaves/ms, or 2.0 octaves/40 ms in a metric compatible to ours. This is an extremely high value compared with our results (0.23 octaves/40 ms for area 17 and 0.20 octaves/40 ms for area 18) and even those from Bredfeldt and Ringach (2002), which are about three times larger than ours as noted in the preceding text. As Frazor and colleagues have pointed out in their paper, the difference in the stimulation protocol and the linearity assumption used in the reverse correlation procedure might be the primary factors in explaining the discrepancy. Due to rapid successive presentations of stimuli in the reverse correlation procedure, responses to multiple stimuli inevitably overlap in time thereby allowing nonlinear interactions, whereas stimuli used by Frazor et al. were delivered with sufficiently long intervals with little possibility of nonlinear interactions. (See DISCUSSION of Frazor et al. 2004). Such nonlinear interactions in the spatial frequency domain will be a topic for further studies.

For orientation dynamics, Ringach and his colleagues (Ringach et al. 2003) provided the distributions of the preferred orientation shifts over the response time courses for monkey V1 (Fig. 8 of Ringach et al. 2003). A compatible analysis of our data shown in Fig. 8A yields the mean ± 1 SD of the shifts as 0.41 ± 4.7 and −0.61 ± 5.3° for areas 17 and 18, respectively. These SD values appear somewhat smaller than that for monkey V1, based on inspections of their summary histogram (the exact value was not given for the monkey).

Other tuning properties

In this study, we have limited our analyses to the consistency of spatial tuning parameters between the subspace reverse correlation method and traditional drifting grating measurements. Ideally, the comparison should not stop there and should also be extended to temporal aspects of dynamical neural responses. As Ringach and his colleagues showed in their original paper (Ringach et al. 1997b), a spatiotemporal receptive field may be predicted from a spatiotemporal subspace map under a linearity assumption for simple cells. From these data, we should be able to predict temporal frequency tunings and even forms of PSTHs to the drifting grating stimuli by convolving the receptive field and the stimulus sequences. Unfortunately, we did not obtain temporal frequency tuning curves using drifting grating stimuli necessary for such comparisons. In addition, predictions of relevant spatiotemporal receptive fields are not possible for complex cells and are difficult for many simple cells that possess substantial nonlinearities. This is because the spatiotemporal tuning properties of complex cells are largely determined by linear subunits, and requires second-order analyses (Movshon et al. 1978c; Szulborski and Palmer 1990). Such comparisons of actual data and predictions might provide further insights into nonlinearities embedded in cells’ response generation mechanisms and will be a topic of further studies.

Taken together, our results suggest that the new subspace reverse correlation mapping is able to provide results that are
generally as consistent as the traditional method based on drifting grating stimuli. We have shown that the results from the new method are directly comparable to the substantial amount of existing data obtained by the traditional method. In conclusion therefore given the high efficiency in terms of recording time and generality of stimuli that are applicable for all neurons that are recorded from multi-electrode arrays, the subspace reverse correlation method is highly desirable as a superior replacement for traditional drifting grating stimuli for initial characterization of joint orientation and frequency tuning in studies of early visual cortex.

ACKNOWLEDGMENTS

We thank our laboratory members, H. Tanaka, T. Sanada, R. Kimura, K. Sasaki, M. Fukui, and M. Iida, who participated in recording sessions.

GRANTS

This work was supported by Grant 15029230 and the Project on Neuroinformatics Research in Vision through special coordination funds for promoting science and technology from the Ministry of Education, Culture, Sports, Science, and Technology and by Grant 13308048 from Japan Society for the Promotion of Science.

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