32 Reverse Correlation Methods

BEVIL R. CONWAY AND MARGARET S. LIVINGSTONE

Introduction

Reverse correlation is a method that is often used to characterize the response properties of neurons and to answer the simple question “What is the correlation between the response of a neuron and the stimulus used to elicit a response?” In any given sensory area within the brain, say, primary visual cortex, a given neuron will respond only to a restricted portion of that sensory domain, say, the upper left quadrant of the visual field. This phenomenon is captured by the notion of the receptive field, and our example neuron would be said to have a receptive field in the upper left quadrant of the visual field. In addition to spatial localization, receptive fields also describe the particular stimulus configuration to which the neuron is most responsive. Importantly, different neurons respond specifically to some stimuli and are indifferent or respond poorly to other stimuli. A visual neuron might be responsive to a small black spot on a white background, a line of a particular orientation, or a specific color, for example. Moreover, a given neuron may respond in opposite ways to two different stimuli, with different time courses. For example, a given cortical color cell might respond with excitation at a short latency and suppression at a long latency to one color and suppression at a short latency and excitation at a long latency to the opponent color. Thus, receptive fields have spatial and temporal structure. And a complete description of a receptive field includes both where in a sensory field a neuron is responsive to stimuli and to what stimuli the cell responds best. Although we will focus on visual neurons for this chapter, the concept of receptive field is useful in all sensory systems; for example, one can investigate the response properties of a neuron in somatosensory cortex to different kinds of stimuli applied to a specific region on the skin, the response properties of a neuron in auditory cortex to different frequencies, and the response properties of a neuron in olfactory cortex to different odors. In fact, reverse correlation techniques were originally developed for characterizing cells in the auditory cortex.1,8

The problem of how to characterize a given neuron’s receptive field is not trivial; in fact, it took several decades before neuroscientists appreciated that single retinal ganglion cells in the cat’s eye respond only to small spots of light in a specific location of the retina.19 Previously, scientists had attempted without much success to elicit responses using full-field illumination, which they naively considered to be the "best" visual stimulus. We can sympathize. After all, what good would a visual neuron be if it could not report the difference between the room lights “on” and the room lights “off”? We now appreciate the sophistication of center-surround receptive fields and that these are a rather elegant way to encode the maximum amount of information (contrast borders) about the visual world with the smallest number of neurons—even if center-surround neurons do not respond very well to global illumination changes. In the primary visual cortex, the characterization of receptive fields was an even greater challenge. Having just established that retinal ganglion cells and cells in the lateral geniculate nucleus prefer small spots of light, scientists were rather discouraged to find that these stimuli were largely ineffective at driving cells in the next stage of visual processing, the primary visual cortex (V-1). It took an accident of experimental design to discover that most cells in V-1 actually respond best to oriented lines;15,16 As David Hubel describes it in his Nobel lecture:13

For 3 or 4 hours [of recording from a single V-1 cell] we got absolutely nowhere. Then gradually we began to elicit some vague and inconsistent responses by stimulating somewhere in the midperiphery of the retina. We were inserting the glass slide with its black spot into the slot of the ophthalmoscope [used to stimulate the retina by projecting the spot onto a screen in front of the animal’s eyes] when suddenly over the audiometer the cell went off like a machine gun. After some fussing and fiddling we found out what was happening. The response had nothing to do with the black dot. As the glass slide was inserted its edge was casting onto the retina a faint but sharp shadow, a straight dark line on a light background. That was what the cell wanted, and it wanted it, moreover, in just one narrow range of orientations.

The experimental procedure employed by Kuffler and Hubel and Wiesel in their pioneering studies of the visual system can be characterized as follows:

Place an electrode into an area of the visual system → Flash a stimulus on the retina → Measure the neuron’s response and decide what stimulus to try next

As more and more different stimuli were tested, it became clear that cells are rather specialized, responding really well to some stimulus features (e.g., lines and edges) and not very well at all to others (e.g., different colors). Moreover, a given cell might respond best not just to lines and edges, but to lines and edges of a specific orientation. In fact, one might have one’s electrode immediately adjacent to a
cortical cell and not be able to elicit any response at all from the cell with a 45° line if the cell's preferred stimulus is an 80° line. Thus, in trying to fully characterize both the spatial and temporal response properties of a given neuron, one is faced with two problems: First, the particular stimuli that you choose to use will influence your decisions about what stimuli the cell is responsive to (you might mischaracterize an orientation-selective cell if you test it only with small spots), and second, it takes quite a long time to test a cell's response to every stimulus you can think of (as you would like to, so as not to miss the cell's actual stimulus preferences).

Reverse correlation methods overcome both of these problems, though they have limitations, which should be appreciated so as not to misinterpret the results. (We will discuss these at the end of the chapter.) Moreover, in addition to providing a full characterization of the spatial and temporal response properties of single neurons, reverse correlation methods have also proved useful in characterizing the receptive fields of multiple neurons simultaneously. We will first describe this powerful technique in general terms, as it is used to study the visual system, and will then use some specific examples to outline the limitations.

**Reverse-correlation methods: The generic case**

The stimulus for a reverse correlation sequence is usually presented on a computer monitor and consists of a random sequence of images. In figure 32.1, each letter represents a unique stimulus. The neuron's action potentials are recorded continuously during stimulus presentation. These spikes are shown as tick marks beneath the stimulus sequences (figure

![Figure 32.1](image_url)

**Figure 32.1** The basic idea of reverse correlation. Top, A random sequence of stimuli (represented by letters) is used to elicit a neuron's response while the neuron's activity (vertical lines) is continuously recorded. Middle, Following thousands of stimuli presentations, one correlates the stimuli that occurred before each action potential, keeping track of the stimuli that occurred one, two, three, four, and so on frames before each action potential. Bottom, One then aligns all of the stimuli sequences that preceded action potentials to reveal correlations between the stimuli and the neuron's response. In this case, the stimulus represented by the letter “A” occurred with a high probability two stimuli before each action potential. We could conclude that this neuron responds well to the stimulus represented by “A,” with a physiological latency of two stimulus frames.
As with real neurons, there is no correlation between the neuron's spikes and the stimuli that occurred at exactly the same time; the spikes coincide with the letters E, L, M, Y, Z, and P in this example. In real neurons, this is because there is a visual latency—a finite amount of time that it takes for the visual stimulus to be encoded into neural activity and transmitted to the cell from which you are recording. But after running an extended sequence of stimuli and collecting the neural activity concurrently, one can determine the prior probability that a given stimulus occurred at a given length of time before a spike. We can look at the stimuli that occurred immediately before each spike (CKMAYL), the stimuli that occurred two stimuli before each spike (AAAAAA), and so on (figure 32.1, middle panel). If one then aligns all the letters that occurred before a spike, one can begin to see patterns emerge. In our example case, it is clear that the stimulus “A” occurs with a very high probability two stimuli before each spike (figure 32.1, bottom panel). Therefore, we would conclude that this neuron has a visual latency of two stimulus frames and responds best to the stimulus represented by the letter “A.” Note that this way of correlating a neuron's activity with a stimulus is different from forward correlation in several ways. First, stimuli are constantly being presented. This means that the number of stimuli that can be presented in a given amount of time is much greater with reverse correlation than with forward correlation. Second, the stimuli are presented randomly, which eliminates scientist-introduced biases. Third, the result is an average stimulus preceding a spike by a given amount of time as opposed to an average response following a spike by a given amount of time (although these are mathematically equivalent because the response is a discrete element: a spike).

The stimuli, represented in figure 32.1 by letters, can be anything you like. They can, for example, be used to map the spatial structure of receptive fields. In this case, each letter might represent a single frame of a computer monitor that has been divided (invisibly) into a grid. Each frame would then have just a few squares in the grid illuminated and would look like a disorganized checkerboard (e.g., figure 32.2A); different frames would have different randomly assigned squares illuminated. An important feature of reverse-correlation mapping is that the configuration of each frame is random with respect to those preceding and following it; that is, there is no temporal structure. After presenting many of these stimuli (a sequence that looks like coarse television “snow”), one can then determine which frames precede action potentials, from which we can determine which are more likely to have caused excitation. We can then generate an “average” frame for a given delay by averaging all the frames that preceded an action potential by that delay. We can do this for several “reverse-correlation” delays to generate a “movie” of average stimuli. (An example is discussed in the next section; see figure 32.2.) This is useful because with one major assumption, the resulting maps can be used to infer the receptive field and to predict the “optimal” stimulus. These maps are often referred to as first-order receptive fields or first-order kernels.

The assumption that enables us to use these first-order kernels to predict the optimal stimulus is that the underlying neural response is linear. A cell is considered linear if it responds to two spots presented simultaneously in a way that is matched by the sum of the cell's responses to the two spots presented separately. Because the stimuli are presented in quick succession, it is implied either that the response to a given stimulus ends before the following stimulus is presented or that the response to a previous stimulus does not affect the cell's response to the subsequent stimulus. As it turns out, most real neurons are not linear. For most orientation-selective cells, for example, the sum of the responses to a series of stimuli, each a single spot that would make up a line if presented simultaneously, is generally much weaker than the cell's response to an actual line. But the response of the cell to a spot is not negligible, so if the cell is presented with many such stimuli, the average response can be a fair approximation of the response to a complete stimulus and can be used, with caution, to infer the cell's receptive field. Because the stimuli that are used in a reverse-correlation experiment are random with respect to each other, all the nonlinear interactions average out when an adequately long stimulus sequence is used. As we will discuss in the section entitled “Reverse-Correlation Methods: Determining Second-Order Kernels,” some cells appear to perform a function that is decidedly nonlinear. For these cells, the assumption of linearity, along with the first-order kernels that are extracted from a reverse-correlation experiment, are poor, even misleading, characterizations of the cells' receptive fields.

**Mapping the spatial structure of simple cell receptive fields**

Simple cells, first described in primary visual cortex of the cat by Hubel and Wiesel, respond best to a light bar next to a dark bar in just one particular location of the visual field and at only one orientation of bar. Hubel and Wiesel discovered this by astute observation of an accident (see quote above). When cat simple cells were subsequently mapped with reverse correlation, using single spot stimuli or checkerboards in which the black or white value at any given position is randomly determined, they show an average stimulus that matches the optimal stimulus; the average stimulus appears as oriented white region(s) next to oriented dark region(s) (figure 32.2A). In figure 32.2A, the first panel in the top row (beside the example stimulus frame) represents the average stimulus that coincided, at exactly the same time,
Figure 32.2  Spatial first-order response maps of simple cells in primary visual cortex revealed by reverse-correlation techniques. A, left panel, A single frame of a checkerboard stimulus used to stimulate the anesthetized cat simple cell whose response maps are shown to the right. Right panels, The average blurred stimulus that preceded an action potential by 0, 44, 89, and 132 ms (adapted from Reid et al., 1987, with generous permission from R. Clay Reid). White regions in each response map represent a higher probability that the preceding stimulus had a white square at that location; blacker regions represent a higher probability that the preceding stimulus had a black square at that location. From this first-order response map, we can infer that this cell responded best to an almost vertically oriented white next to black next to white bar, with a visual latency of 44 ms. Note that regions that are white at one delay are black at a later delay (arrowhead). See text for discussion. B, left panels, Single frames from single contrast stimuli used to stimulate the alert monkey simple cell whose response maps are shown to the right. Right panels, The average blurred stimulus that preceded an action potential by 0, 13, 25, 38, 50, 63, 75, 88, 100, and 113 ms for the white stimulus (top panels) and black stimulus (bottom panels). Whiter regions represent a higher probability that the given white or black stimulus preceded an action potential at the given delay. The response to white is subtracted from the response to black (bottom panels) to show the spatial antagonism of the white “ON” subregion and the black “OFF” subregion. (Adapted from Livingstone MS, Conway BR: Substructure of direction-selective cell receptive fields in macaque V1. J Neurophysiol 2003; 89:2743–2759.)

with the cell’s action potentials. As you can see, there is not much structure in this panel, which makes sense; the cell’s activity can correlate with the stimulus only after some visual latency. This is because the stimulus has to be detected by the photoreceptors, converted into action potentials by the retinal ganglion cells, and transmitted through the lateral geniculate nucleus to the neuron from which we are recording in primary visual cortex (layer 4). All this takes some time, which we call the visual latency. We can gauge how long the visual latency is by how far before an action potential the average stimulus starts to show structure.

The second panel in figure 32.2A shows the average stimulus at a reverse-correlation delay of 44 ms. This was the average preceding stimulus at the optimal delay and can be interpreted as a probability map. Whiter regions indicate regions of the stimulus that were more likely to have been
white at a delay of 44 ms before a spike; conversely, blacker regions represent regions of the stimulus that were more likely to have been black at a delay of 44 ms before a spike. These average stimuli are often referred to as response maps or stimulus-response functions. Note that the gray value (i.e., probability) averaged over an entire response map at any given delay is the same for each average response map at any delay, but in the response maps that correspond to the visual latency of the cell, the probability is distributed non-randomly. We can follow the structure of the probability at different reverse-correlation delays, shown in the remaining panels of figure 32.2A. So we can ask, "How does the average optimal stimulus compare at different reverse-correlation delays?" If we take our simple cell as an example (figure 32.2A), it turns out that regions that are excited at short delays are suppressed at long delays and regions that are suppressed at short delays are excited at long delays (see the arrowhead in figure 32.2A). For some cells, the relationship at different delays is not so simple. This is in fact the case with the cat cell in figure 32.2A. By inspection, you can see that white regions are subtly shifted from one reverse-correlation delay to another: at 44 ms, the dominant white region is centered on the blackened reference square; at 88 ms, it is to the right; and at 132 ms, it is farther to the right. Therefore, we would predict that the optimal stimulus for this cell is not only an oriented white bar next to a black bar, but also one that moves location from right to left across the receptive field. Importantly, the first-order kernels that are produced for this cell, typical of simple cells, correspond well with the receptive field properties of the cell; the cell does in fact respond best to a white bar next to a black bar and, moreover, one that moves. But many, if not most, visual cells are not adequately described by first-order kernels alone (see the section below entitled "Reverse-correlation methods: Determining second-order kernels").

Early technical limitations

Reverse-correlation mapping is an elegant way to describe the spatial and temporal response properties of visual neurons. But it requires a relatively large amount of data storage because one needs to have a continuous record of the stimulus configuration and spike activity. Early attempts at reverse correlation were hampered by the storage limitations of computers. In a creative solution to this problem, Sutter used photographic film to capture the stimuli that preceded action potentials. He would expose the same piece of photographic film to all the stimuli that preceded action potentials. The experimental details are not available (he published this as an abstract to the Society for Neuroscience) but presumably were not optimal because he subsequently developed m-sequences. An m-sequence is a sequence of numbers derived from a simple formula that is used to define the luminance value of each square in a checkerboard. The sequence of stimuli characterized by an m-sequence has no temporal structure, yet each stimulus can be derived from a mathematical formula. Although the whole ensemble of stimulus frames appears as coarse TV snow, a given stimulus frame at a particular time in the sequence can be reconstructed with the m-sequence formula. Thus, one needn't record the stimuli, only the spike history. The cat simple cell shown in figure 32.2A was mapped with m-sequences.

Today, computers are powerful enough that storage capacity is no longer a limitation, and most reverse-correlation studies use randomly generated stimuli, not m-sequences. It should be reiterated, however, that the stimulus sequences that are used will affect how well the stimulus response functions characterize the cell's physiological properties. A tremendous amount of mathematics is involved in determining what sort of stimuli should be used so as to avoid acquiring spurious response functions. M-sequences remain a nice way of doing this. The interested reader is directed elsewhere for details of this mathematics, but it should suffice to say here that the variables for spatial mapping include the number of squares illuminated in any given frame (sparse versus dense) and the way in which each frame relates to the next. Most important, each frame should be random compared to the next so that with very long stimulus sequences, there is no pattern relating the stimuli.

Reverse-correlation methods in the alert animal

The earliest studies of the primary visual cortex were done in alert cats. But as you can imagine, it was not easy to determine where the cat was looking, so it was even harder to determine the correlation between stimulus and neural response. This led to the development of recordings in paralyzed and anesthetized animals, in which eye position can be accurately determined. Although these studies provided valuable insights, one could not rule out the effect of anesthetic on the recordings. But with the development of eye-movement-monitoring systems, one could once again record in alert animals and determine, after the fact, where the stimuli were in retinal coordinates, by maintaining a continuous record of eye position and stimulus position. This could then be coupled with reverse-correlation to enable high-resolution receptive field maps in the alert animal. In these experiments, monkeys are trained to fixate on a small dot presented on a computer monitor. Having the monkeys perform this task eliminates large saccadic eye movements but does not reduce constantly occurring smaller eye movements. The monkeys' eyes are monitored by using an eye coil placed inside a magnetic field coil: An insulated wire loop is saturated to the sclera around the eye, and the ends of the loop are mounted to a connector attached to the monkeys' skull, which allows one to monitor the current through the
wire. The monkey's head is placed inside a magnetic field coil so that a current is induced in the monkey's eye coil every time the monkey moves its eyes. The magnetic flux through the eye coil, and the induced current, changes proportionally with the monkey's eye movements; the system can be calibrated so that by monitoring the current through the coil, one has a remarkably precise measure of eye position.

The details of reverse-correlation mapping using this alert animal preparation are similar to those used in the anesthetized cat except that one accounts for eye position by subtracting it from the stimulus position for any given frame. The resulting maps for a simple cell recorded in monkey primary visual cortex are shown in figure 32.2B. For this particular case, only a single square was illuminated in any given frame, and this square was not constrained to a stimulus grid, as in the m-sequence mapping. The stimuli were either white on a gray background (figure 32.2B, top panel) or black on a gray background (figure 32.2B, middle panel). Thus, unlike m-sequence maps, which use a checkerboard, responses to white and black spots are mapped with separate stimulus runs. The response maps for both black and white spots are shown according to the same grayscale, where whiter regions indicate a higher probability. So white in the black maps shows the part of the receptive field that was excited by black at that delay. Note that at the optimal reverse-correlation delay (the fifth panel from the left), the probability that a white stimulus preceded an action potential is roughly the inverse of the probability that a black stimulus preceded an action potential (compare the boxed regions in figure 32.2B). Thus, the cell was "push-pull". It was excited by white and suppressed by black in one part of the receptive field and was suppressed by black and excited by white in an adjacent part of the receptive field. This can be summarized by subtracting the black response functions from the white response functions (figure 32.2B, bottom row). The size of the stimulus frame was much smaller in the maps of the monkey cell because the monkey cell receptive fields are considerably smaller than those of cells in the cat. The scale bar is the same for both cells, and both cells were recorded at roughly the same eccentricity (within 5 degrees of the fovea/area centralis). Despite the difference in scale, however, the monkey simple cell shows many of the same features of the cat simple cell: White regions at early reverse-correlation delays are replaced by dark regions at late reverse-correlation delays, and this transition is gradual—the black region of the monkey cell gradually shifts to positions more rightward at longer reverse-correlation delays, so one would predict that the cell would respond best to a black bar moving right to left.

Because for some cells, the peak response in the response maps shifts over different reverse-correlation delays (e.g., figures 32.2A and 32.2B), it is sometimes useful to generate space-time maps that display the spatial shift over time in one figure (figure 32.3). To generate the space-time maps shown in figure 32.3, a monkey simple cell was stimulated with optimally oriented black and white bars, presented on a gray background; the bars were moved along a stimulus range, along one spatial dimension (x-axis), through the center of the receptive field. The probability that a given stimulus preceded a spike at a given delay (y-axis) is shown according to a grayscale: Whiter regions indicate a higher probability. You can see that this cell had not only spatial structure (the probabilities for the white and black maps are complementary), but also temporal structure (the spatial distribution of the probabilities for each map shift as reverse-correlation delay is changed), suggesting this cell's optimal stimulus was not just a bar but one that moved over time. This cell and the ones shown in figure 32.2 were in fact directionally selective, though as we will see in the next section, first-order maps give only a weak account of directionally-selective cells' receptive fields, especially those of complex direction-selective cells (see figure 32.4).
FIGURE 32.4 Spatial second-order response maps for a complex, direction-selective cell in monkey visual cortex. A, Direction tuning curve. This cell preferred bars of almost horizontal orientation moving down and slightly to the right. B, First-order response maps to white spots (left) and black spots (right) at the optimal reverse-correlation delay, reflecting the cell's receptive field (rf). The horizontal and vertical axes are in degrees of visual angle, centered on the stimulus range, which was centered on the receptive field. Note that these maps show very little spatial structure and reveal little about the receptive field of the neuron, unlike the first-order maps of the simple cells shown in figure 36.2. C, Second-order response map (Wiener-like kernel). This is derived from the individual maps shown in (D). D, Responses to pairs of spots were extracted from the same spike train used to generate the first-order receptive field maps (A); this involved a long reverse-correlation experiment in which a single white and a single black spot were presented in every stimulus frame, with a random spatial relationship to each other. From this stimulus, four sequences of spots could be extracted: white preceding white, black preceding black, black preceding white, and white preceding black. The maps show the responses to such sequential pairs of spots presented anywhere in the receptive field; one spot was defined as the reference spot, and the other spot as the preceding spot. The reference spot's position, though it could have been anywhere in the receptive field, is normalized to the center. The gray values throughout the map indicate the response of the cell when the preceding spot occupied that location. These maps reflect the direction preference of the cell. For example, this cell was suppressed by preceding white spots located below and to the right of reference white spots (black region in the left most panel) and excited by preceding white spots located above and to the left of reference white spot (white region in the left most panel). Sequential black spots show a similar pattern of second-order interactions (second panel from the left). Interestingly, sequential spots that invert contrast (last two panels) show an inverted pattern of second-order interactions. This pattern of interactions shows that the cell prefers inverting-contrast sequences if the sequences progress in the direction opposite to the cell's actual direction preference. This is the neural correlate of the visual illusion called reverse-phi motion: a stimulus that is made to change contrast in mid movement appears to move in the opposite direction to the physical progression of the stimulus. This shows that the fundamental stage of motion processing is contrast-sign specific. Note that these second-order maps are much more informative than the first-order maps. (Adapted from Livingstone MS, Conway BR: Substructure of direction-selective cell receptive fields in macaque V1. J. Neurophysiol 2003; 89:2743–2759.)
Reverse-correlation methods: Determining second-order kernels

The technique discussed so far is used to describe the average optimal single frame at any given reverse-correlation delay preceding an action potential. The average optimal stimuli are sometimes referred to as first-order kernels. These first-order kernels show the amount of the cell’s response that is linear and are useful in characterizing the receptive fields of simple cells whose responses are largely linear. But as we discussed in a previous section (“Reverse-correlation methods: The generic case”), most visual cells are not linear; that is, their responses to two spots presented simultaneously or in sequence cannot be adequately predicted by summing the responses to the two stimuli presented independently.\(^{2,6}\) This is epitomized by the so-called complex cells in the cat’s primary visual cortex. A complex cell responds preferentially to a bar of a given orientation, but unlike simple cells, for which the bar needs to be situated in a specific portion of the receptive field, a complex cell will respond to the bar if it is placed anywhere within the receptive field.\(^{15}\) Moreover, complex cells do not respond well to spots; they require a bar to give a good response. Thus, mapping a complex cell with a sequence of spots will not provide an enlightening first-order map (see figure 32.4B). In fact, for most visual neurons, some fraction of the neurons’ response depends on the particular sequence or configuration of frames.\(^{27}\) We can ignore this when we generate a first-order kernel because the sequence of stimulus frames is random—it has no temporal structure. But how then do we adequately study cells that fire well only when a black spot is preceded by a white spot, cells that fire only to a sequence of spots moving through visual space (directionally selective cells), or cells that fire only to two adjacent spots presented simultaneously (complex cells). Such nonlinear cells exist, and the nonlinearities that they encode are certainly important for processing visual information. We would not want to prevent ourselves from studying such cells by looking only at linear properties.

Fortunately, one can use reverse correlation to examine both first-order interactions and second-order interactions. In the schematic shown in figure 32.1, the letters would then represent sequences of frames, not just single frames (if one were interested in mapping sequential interactions), or each letter would represent a pair of spots (if one were interested in mapping simultaneous interactions). Of course, to use the same stimulus run to map the first-order interactions, one still has to make sure that there is no overall temporal structure to the stimulus and that the spatial location of each spot in any given frame is random and independent of the other spot in that frame if pairs of spots are used.

How then, you might ask, can we examine second-order interactions using a stimulus sequence that has no overall temporal or spatial structure? The answer is that the sequence of stimuli used is enormous: it contains every possible combination of spatial and temporal structures, many times over, but no overall spatial or temporal structure. Using our analogy of the letters of the alphabet to represent the stimulus sequence, it is as if all 26 letters are presented, in a random sequence, so many times over that every letter is, at some point in the sequence, preceded and followed by every other letter but no subsequence of letters predominates. The challenge, then, is to extract from the responses to this huge stimulus sequence those responses to all spatial and temporal combinations.

Theoretically, this has been solved by Wiener\(^{28}\) and Marmarelis and Marmarelis,\(^{29}\) who stipulated not only the appropriate stimulus sequence that should be used (Gaussian white noise, much like television “snow”), but also a very powerful analysis that extracts the first-order and second-order kernels. (It actually can extract the third-order, fourth-order, fifth-order, and so on kernels too.) Wiener’s method is analogous to the cross-correlation method shown in figure 32.1. Wiener’s analysis also tells you how much of the cell’s response is accounted for by each kernel. Simple cells, for example, would have a lot of their response accounted for by first-order kernels (figure 32.2), while complex cells would have more of their response accounted for by second- or third-order kernels (figure 32.4). The method described by Wiener and Marmarelis and Marmarelis is potentially extremely powerful because it allows one to fully characterize the receptive field of a neuron without prior hand mapping. (Hand mapping is the crude form of forward correlation that Kuffler and Hubel and Wiesel used to determine receptive field location, orientation and/or direction-preference.) One needn’t, for example, just acquire the first-order kernel as a means of quantifying a simple cell; rather, one can use the technique to demonstrate that a given cell is in fact simple by showing that the majority of the variance of its response is accounted for by the first-order map. Unfortunately, the white noise stimuli prescribed by this rigorous approach would require an almost infinite amount of time to execute, which is practically not feasible and further confounded by the fact that visual cells adapt rather quickly to white noise. Simpler stimuli, such as the checkerboards or spots that were used to map the cells in figure 32.4, have proven more useful. Such stimuli yield “Wiener-like” kernels,\(^{10}\) which have proven enlightening in understanding the mechanism underlying direction selectivity, in which the neuron’s job is to compute the spatial configuration of the stimulus across time (figure 32.4).\(^{5,20}\) One can see just on cursory inspection that the second-order Wiener-like kernels for a complex direction-selective cell have much more structure than the first-order kernels (compare figures 32.4B and 32.4C). Modifications of the technique have also been very useful in determining the substructure of complex cell receptive fields.\(^{26,32}\)
Mapping multiple stimulus dimensions: Orientation and color

Reverse-correlation techniques have also been used to investigate the mechanisms of orientation selectivity \cite{29} (figure 32.5), color selectivity \cite{31,32,33} and depth selectivity. In these cases, the letters in the schematic in figure 32.1 represent bars or sine-wave grating stimuli of different orientations; different colors, either equiluminant (i.e., roughly the same value if reproduced in grayscale) or cone isolating (i.e., selectively changing the activity of a single cone while maintaining constant activity of the remaining two cone classes); or bars presented independently to the two eyes.

When orientation is tested with reverse correlation, the response maps show that cells in layer 4B have an orientation preference, \cite{29} a nice confirmation of the results obtained with forward correlation. \cite{19} The peak response of these first-order kernels occurs around a reverse-correlation delay of 53 ms (figure 32.5). Conversely, when the cone inputs to color cells are examined with reverse-correlation, the spatial first-order response maps at the optimal reverse-correlation delay show that the cells are both spatially and chromatically, or “double,” opponent. \cite{4} In these maps, high probability of cell firing is represented by whiter regions. For each cone class, the response profile shows that the cell is suppressed by stimuli in one location and excited by the same stimuli in a different location, while a comparison of the cone maps shows that in any given location the cell is excited by one cone class (L) and suppressed by the opponent cone classes.

![Figure 32.6](image_url)  

**Figure 32.6** Spatial structure of the first-order response maps, at the optimal reverse-correlation delay, of a cone-opponent cell in monkey visual cortex using reverse-correlation and cone-isolating stimuli. These maps show that the cell's receptive field is both spatially and chromatically opponent. Whiter regions indicate a higher probability that the given stimulus preceded an action potential; blacker regions indicate a lower probability. The maps predict that the optimal stimulus for this cell is a red spot surrounded by a green annulus. Such cells likely underlie spatial color contrast. (Adapted from Conway BR: Spatial structure of cone inputs to color cells in alert macaque primary visual cortex [V1]. J Neurosci 2001; 21:2768–2783.)
(M + S). Such “double-opponent cells” are found in the cytochrome oxidase blobs in layers both above and below layer 4 in the monkey. As with the simple cell receptive fields that showed opposite responses to opposite contrast spots at different reverse-correlation delays, oriented cells and color cells are depressed at long reverse-correlation delays by stimuli that cause excitation at optimal reverse-correlation delays. This phenomenon is loosely described as rebound.

Potential pitfalls of reverse correlation

One must be careful in interpreting the results of a reverse-correlation experiment. One potential pitfall of spatial maps involves the interpretation of regions in a stimulus-response function that correspond to the absence of a spot—the dark regions in the response function for the monkey color cell, for example. These regions show that the average optimal stimulus X ms before the cell’s spikes did not contain a spot at that location. This is often interpreted as “spots at these locations cause suppression of cell firing,” which is sometimes misstated as “spots at these locations cause inhibition of cell firing.” The potential difficulty in interpretation arises because the dark regions might simply represent the cell’s complete disinterest in the stimulus—neither excitation nor suppression. If one stimulated a cell in the visual system with auditory stimuli, it would be unlikely to show any correlated response or any response at all. This does not mean that the auditory stimuli cause suppression. So how does one determine whether a region is actually suppressed by a given stimulus, on the basis of the reverse-correlation response profile?

For the maps shown in figure 32.6, one can compare the response probability in a region under question to the response probability that one knows is well outside the receptive field, where stimuli do not correlate with the cell’s activity. Thus, we can see that the dark regions in figure 32.6 that form an annulus in the “L” map and the center of the annulus in the “M” and “S” map do correlate with a much lower probability of firing than the average probability outside the receptive field. Therefore, we can safely conclude that these stimuli, when located in particular configurations corresponding to the black regions, cause suppression of firing. But we have to be even more careful. We still do not know whether the suppression is attributed to an inhibition of the cell from which we are recording. It may be inhibition of a cell in the retina or lateral geniculate nucleus. Therefore, even though these regions represent significant suppression of firing, because we cannot record a firing rate of less than zero spikes per second, we cannot attribute this suppression to the mechanism “inhibition.” To establish this, we would have to record from the cell intracellularly.

Similarly, what does one make of the reduced firing of a cell to nonoptimal oriented stimuli at the optimal reverse-correlation delay (compare the response to 180° at the reverse-correlation delay of 35 ms with response at 53 ms; see figure 32.5)? Can we conclude that these nonoptimal stimuli inhibit the cell because the response probability is lower at the optimal reverse-correlation delay? Just as in the spatial maps in which the probability averaged across a given response map at any given delay is the same regardless of the delay (i.e., the average grayscale is the same for all panels in figure 32.6), the average response for all orientations at any given delay will be the same. Thus, at reverse-correlation delays that are shorter than the visual latency, the response for any given orientation will be roughly equal to the response for any other orientation; any orientation could have coincided with an action potential because there is no correlation between the action potential and the randomly presented stimulus. But as one approaches the cell’s visual latency, the probability distribution shifts, revealing the cell’s optimal orientation. This necessarily means that the probability at nonoptimal orientations will go down and does not mean that the cell is inhibited, or even suppressed, by these nonoptimal orientations. But the temporal evolution of the response profile shows not only that the cell is suppressed by optimal stimuli at long reverse-correlation delays (a rebound at 71 ms; see figure 32.5), but also that it is suppressed by nonoptimal stimuli at long delays. This is shown as the so-called Mexican hat response profile at a reverse-correlation delay of 59 ms. This might be evidence that inhibition of a cortical origin, which has a longer visual latency than the feedforward excitation that establishes orientation selectivity, sharpens orientation selectivity.

Finally, one must be careful not to use reverse correlation to blindly assign function to neurons in the visual system. One could imagine, for example, measuring the orientation-tuning response of a strongly cone-opponent cell and achieving some weak orientation bias. This need not mean that the cell is using the orientation bias to encode information about the visual scene. Thus, one is left with a compromise between two experimental approaches. On the one hand, there are advantages and disadvantages to studies that examine all neurons with the same battery of stimuli (e.g., cone-isolating, orientation, spatial luminance, moving). These studies can produce nice population results that can be used to categorize cells. But these sorts of studies are cumbersome because one usually is not able to maintain recording from a single cell long enough to allow one to measure the responses to all the stimuli. Moreover, for a given stimulus parameter (say, orientation), one usually has to use a less than optimal stimulus for any given cell (say, nonoptimal spatial frequency) to allow one to use the same stimulus (differently oriented bars of a fixed spatial frequency) to test every cell. On the other hand, there are advantages and disadvantages to screening cells with hand-mapping before testing with reverse correlation. The major disadvantage is that one cannot make very secure conclusions about how a
given aspect of the visual world (say, color) is processed by a population of cells. But screening does permit one to reach more accurate and in-depth conclusions about how a given visual attribute is processed. Fortunately, different investigators have their biases, so both kinds of studies continue to be done.

Conclusion

In summary, reverse correlation is a powerful tool that has provided insight into the mechanisms of visual processing by permitting a thorough characterization of the spatial and temporal properties of the receptive fields of cells in the early visual system along multiple stimulus dimensions, including spatial structure, luminance, orientation, direction, and color. Undoubtedly, reverse correlation will assist us as we begin to study more closely the transformations of this information in higher visual areas.

ACKNOWLEDGMENTS We gratefully acknowledge Chris Pack and Tom Davidson for useful comments on the manuscript.

REFERENCES