NCBI Bookshelf. A service of the National Library of Medicine, National Institutes of Health.

Nicolelis MAL, editor. Methods for Neural Ensemble Recordings. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2008.

Chapter 7 Chronic Recording During Learning

Aaron J. Sandler.

INTRODUCTION

The study of learning has a rich tradition, going back at least to the days of Aristotle, who proposed that the formation of associations between coincident events is the way humans learn. More famously associated with learning, especially in the popular mind, is Pavlov who, in the 1920s, studied what is now known as *classical conditioning* or *Pavlovian conditioning*. In these well-known experiments, an unconditioned stimulus (food), which naturally causes an unconditioned reflex (salivation), was presented along with a neutral stimulus (a bell) with enough repetition that, eventually, the bell began to evoke the salivation even without the presence of food (conditioned response). At that point the bell had become a conditioned stimulus, i.e., a stimulus that, after learning a new association, evokes the conditioned response.

Classical conditioning refers to an environmental stimulus that can elicit a response, but it does not address the ways in which an animal might learn how its own *behavior* could cause an environmental response. Work by Thorndike in the late 1890s began to address this by studying what he termed *instrumental conditioning*. Thorndike observed that a hungry cat could learn through trial and error that rubbing up against the side of its cage would open a latch allowing it access to food. From this he proposed his *law of effect*, in which he argued that the tendency to repeat a behavior is dependent on the consequences that behavior evokes. In a series of studies from the 1930s through the 1950s, Skinner followed up on Thorndike's work, studying primarily pigeons and rats in a wide variety of conditions, including *positive reinforcement* (in which a behavior is more likely to recur if it is followed by a reward such as a piece of food), *negative reinforcement* (a behavior becoming more likely if it is followed by the removal of an aversive stimulus such as an electric shock). Skinner renamed this paradigm *operant conditioning* because a spontaneously emitted behavior (or *operant*) is what elicits the response.

Although the rich psychological history of studying classical conditioning, operant conditioning, and other forms of learning has led to greater understanding of the phenomenology of learning, less is known about the neurophysiological mechanisms of these forms of learning. This is because most studies of cortical and subcortical function have involved either functional imaging, in which case the spatial resolution is too gross to permit the study of precise mechanisms of neuronal learning, or acute single-electrode recording methods, which are inherently limited in their ability to examine functional interrelationships between various cortical areas or to study any changes in neuronal firing that might occur over days or weeks. This inability to track changes across cortical areas has meant that most studies have used highly trained animals who have reached a stable level of performance on a previously learned task, rather than animals learning a new task.

Multielectrode ensemble recording, however, provides the ability to record from a large number of cells simultaneously. The mammalian brain contains many millions of neurons that work in an interconnected manner to produce complex behaviors and thoughts. Understanding the interrelations between many neurons that make up a functional circuit, therefore, requires simultaneous recording from many more than one at a time. Furthermore, because the brain's encoding of a given event (be it sensory, motor, or cognitive) relies on complex interactions between neurons, our ability to understand fundamental neural-circuit mechanisms is greatly improved when one simultaneously records the firing of many neurons, rather than just a single one at a time. Thus, multielectrode recording brings us a step closer to understanding normal brain function.

Another important advantage of multielectrode recording is that it provides a more random sample of the neurons in the implanted area, obviating *a priori* decisions about the cell types of interest and permitting comparison of the contributions of different neurons to the encoding of, for example, a given motor action.

Finally, and most important for the study of learning, chronic implantation of multielectrode arrays allows us to study ongoing processes that take more than a single session to complete. Thus, multielectrode arrays may be implanted in a naïve animal and recordings made throughout the course of learning. This allows the sampling of many neurons each day of the study, even if it lasts weeks, months, or years.

THE IMPORTANCE OF NEURONAL ENSEMBLES

Processing and storing information has long been suspected to require large populations of neurons with dynamic and distributed interactions (Sherrington 1906; Hebb 1949; Lashley 1950; Erickson 1968; Freeman 1975; Fuster 1995; Nicolelis, Ghazanfar et al. 1997). More recently, experimental evidence has accumulated to support this idea (Freeman 1975; Georgopoulos, Schwartz et al. 1986; Georgopoulos, Taira et al. 1993; Nicolelis and Chapin 1994; Wilson and McNaughton 1994; Fuster 1995; Nicolelis, Baccala et al. 1995; Nicolelis, Ghazanfar et al. 1997). When processing sensory stimuli and generating motor outputs, a wide variety of tasks must be accomplished: the sensory information must be processed, converted to percepts, and stored in memory; a response must be selected; and a behavior must be generated. This process is not limited to simple circuits or reflex loops, but rather requires large-scale interactions among widely distributed and interconnected populations of neurons (Nicolelis, Ghazanfar et al. 1997).

Indeed, current evidence suggests that decision making based on sensory cues does involve multiple subcortical and cortical structures (Wise and Murray 2000). This evidence is most plentiful in neurophysiological studies of the oculomotor system in which visual cues instruct eye movement responses (saccades). Areas shown to be involved in oculomotor decision making in nonhuman primates include the medial temporal cortex (Salzman, Britten et al. 1990), prefrontal cortex (Kim and Shadlen 1999), areas of parietal cortex (Quintana and Fuster 1992; Platt and Glimcher 1999; Shadlen and Newsome 2001; Pesaran, Pezaris et al. 2002; McCoy, Crowley et al. 2003; McCoy and Platt 2005), the frontal eye fields (Gold and Shadlen 2003), the supplementary eye fields (Chen and Wise 1995), premotor cortex (Mitz, Godschalk et al. 1991; Boussaoud and Wise 1993; Brasted and Wise 2004), and the superior colliculus (Horwitz and Newsome 2001). Wise et al. found activity in many areas throughout the visuomotor pathway that both reflected sensory information and predicted the monkey's choice (Wise and Murray 2000). In humans, functional magnetic resonance imaging (fMRI) studies of visuomotor tasks have found evidence of task-related activity across several cortical and subcortical areas, including frontal and parietal cortices (Thoenissen, Zilles et al. 2002).

USING NONHUMAN PRIMATES TO STUDY LEARNING USING CHRONIC RECORDING METHODS

Since the mid-1990s, our laboratory has used microwire arrays to record the activity of large ensembles of single units in multiple cortical areas in behaving nonhuman primates. Nonhuman primates are attractive subjects for multisite, multineuronal recordings during learning for two main reasons: First, primates are capable of performing more sophisticated tasks than are rodents, providing expanded opportunities in conditions for study. Secondly, brain structures in primates tend to be more similar to those in humans, making the findings more directly applicable to human learning. Both of these factors come into play when studying such questions as which neurons and cortical areas are recruited to solve a novel task, such as the operation of a brain–machine interface, a central goal of our lab.

The two types of primates used in our studies are rhesus macaques (*Macaca mulatto*) and owl monkeys (*Aotus trivirgatus*). Regardless of the species chosen, care of nonhuman primates includes appropriate housing in a research animal facility under the direction of full time veterinary staff. The facility must be equipped to take care of any disease problems specific to the animals and general to the primate population. In learning experiments that continue over days or weeks it is also crucial to standardize day-to-day care as much as possible, to avoid introducing uncontrolled variables that may interfere with the animals' learning.

Macaques are the laboratory standard for neurophysiological studies of this type, making them an attractive choice for simple comparison to other studies. In addition, of the laboratory animals used for single unit recordings, the organization of their brain is the closest to that of humans. They are also able to learn quite complicated tasks.

Owl monkeys are New World monkeys, smaller in size than macaques. They have a lissencephalic brain with a very

smooth neocortex. The lack of circumvolutions and sulci allows straightforward access to distinct cortical areas that may be difficult to approach in macaque brain. Additionally, the brains of owl monkeys have been well studied in neuroanatomical and neurophysiological studies, making them an appropriate choice for chronic multielectrode implantation in multiple areas (Kaas 1994). They are also easier to maintain, and cheaper to work with, than other common models, owing to their less aggressive nature and lower level of health risks to humans. One minor complication of working with owl monkeys is due to their being nocturnal animals; this is easily solved by adjusting their light cycle to ensure that all experiments are performed during their awake time.

Perhaps most importantly in the consideration of owl monkeys as experimental subjects, it is possible to obtain excellent neuronal recordings from this species. We have shown that recordings of hundreds of single units can remain viable in owl monkeys for several years, something that has not yet been achieved in any other primate species. In recent years, we have carried out very long-term studies of owl monkeys with multiple implants in primary somatosensory cortex (S1), posterior parietal cortex (PPC), dorsal premotor cortex (PMd), and primary motor cortex (M1). In one of these animals, recordings have been performed for over 5 years. A second animal has been recorded for more than 2 years. After several years, we have continued to record from 50–100 single units and over 100 multiunits per animal (Figure 7.1a-b). The quality of the recordings themselves is as important as the long duration of the implants; even after 2 and 4 years we are able to achieve high-quality recordings from these owl monkeys with good discrimination of single units (Figure 7.1c-d).

Behaviorally, owl monkeys are amenable to learning tasks including somatosensory and visually cued decision-making tasks (Kaas 1994). In our lab, we have developed training methods for owl monkeys that have shown considerable success. In these tasks, our goal is to train monkeys to select reach targets based on a discrimination between several different cues. As they learn to do this, we carry out simultaneous neural ensemble recordings from multiple cortical areas. However, before the experimental learning can begin, the animals must undergo some initial training. We generally start this period of preimplantation training with habituation of the monkeys to the handling procedures and experimental apparatus. They are trained to sit comfortably in a specially designed primate training chair inside a soundproof recording chamber by the use of food and juice rewards over a period of 3 to 4 weeks (Kralik, Dimitrov et al. 2001). The first step in the procedure is training them to leave their home cages voluntarily, by luring them into transport boxes (Primate Products, Miami, Florida) with small pieces of fruit. Once they do this voluntarily and are comfortable enough to take food and drink in the transport box, the monkeys are then transported to the testing room, where a custom-designed restraining chair is attached to the end of the transport box, and the monkeys are again lured into the restraining chair with a small piece of fruit.

The restraining chair we use was made by Crist Instrument Company out of ½ in. thick polycarbonate, and was then substantially modified in our laboratory to fit our purposes. It was designed to be initially in the form of a box that the monkeys were comfortable entering from the transport box. After moving it and the monkey into the sound-attenuating test chamber, the device can be transformed from the box form to the chair form. In this configuration, the chair allows the monkeys to sit comfortably, without being fully restrained while performing behavioral tasks. Their lower extremities are supported on a perch, their head protruding through a hole in the top, and their arms free to reach forward but prevented from reaching up to their implants and connectors. Generally, we restrain one of the monkey's arms and leave the other one free to reach. The head is not restrained, although the front of the body harness is attached to the front of the chair to prevent swiveling of the entire body, which might damage the implants. Once the monkeys are acclimated to the restraining chair, the specific experimental apparatus is introduced.

For experiments studying associative learning or decision making, in which we want to avoid any simultaneous motor learning, we are careful to train the monkeys in the experimental apparatus (without using any cues) before the implantation surgery. This preexperiment training allows us to later study the formation of the association between stimulus and response without the confounding factor of the animal simultaneously having to learn a new motor output. In studies of motor learning, this pretraining is unnecessary.

After a postoperative period of at least 14 days, the animals are reintroduced to the experimental apparatus. Cues are not introduced until neuronal recordings have stabilized (typically 2–3 months in owl monkeys). Monkeys generally

perform 1 session per day, 5 days a week, until the predefined learning endpoint is reached.

ANALYSIS OF SINGLE UNIT RESPONSE PROPERTIES

Quantitative Analysis of Peri-Event Time Histograms

In analyzing any type of neuronal recording, the main goal is to determine the relationship between the firing of the neurons and some event such as a stimulus or a motor response. This can be as simple as looking for a change in firing rate that occurs simultaneously with the appearance of a stimulus. More complex neural events, however, such as the formation or enactment of a motor command, may involve variation in the patterns of neuronal firing. Rather than a simple increase or decrease in firing rate, for example, an individual neuron might show first an increase and then a decrease in firing rate (see Figure 7.2 for examples). It is therefore important to select a method of analysis that can detect more elaborated patterns—an analysis that is able to detect the temporally varying information in the neural spike train.

Initial analysis of neuronal recordings typically consists of investigating the response properties of each individual recorded neuronal unit with respect to a particular event of interest. Raster plots and peri-event time histograms (PETHs) can provide a good visual representation of the firing patterns of individual cells around, for example, the delivery of a vibrotactile stimulus or the onset of a movement. PETH analysis involves measuring the average firing rate (spikes per second) in a series of time-sequential bins across an epoch of interest. After each session, offline computer analysis is used to construct peri-event time histograms (PETHs) of the firing of each neuron during trials. The average intensity of neuronal firing within a control background firing period is calculated, and again for each of several user-defined response epochs. The “evoked unit response” or instantaneous firing rate of the neuron can then be expressed as: (1) spikes per second, (2) average instantaneous firing rate over different peri-event epochs, (3) firing rate minus background, or (4) firing rate divided by background (i.e., signal or noise). We then use standard statistical tests to determine whether the differences in firing rate during different epochs are significant, including the Kolmogorov–Smirnov test, student’s t-test, and the cumulative frequency histogram, as appropriate.

Distance Index (DI) Analysis

Despite the advances that firing rate analysis has provided, relying on average firing rate may be significantly limiting (Nicolelis, Ghazanfar et al. 1997). It assumes that the relationship between the neuronal firing rate and the event of interest is simple and unchanging (i.e., stationary), which is often not the case. Firing rate analysis ignores the temporal components of the neuronal encoding. Indeed, patterns of neuronal firing may convey more information than the average firing rate alone (Laubach, Shuler et al. 1999). These temporal patterns of firing (TPF) can be analyzed in a number of ways, including principal component analysis, discriminant analysis, and independent component analysis. To facilitate this analysis, we developed a linear technique that we refer to as the Distance Index (DI) method (Figure 7.3) (Sandler, Kralik et al. 2003).

We have found the results from DI analysis to be very similar to other methods, however, the DI analysis had the advantages of being faster to perform and conceptually simpler. The DI method has since been further validated by other researchers, who came to the conclusion that it works as well as, or better than, other commonly used methods, but is more efficient to use with large data sets and provides superior abilities to investigate time-dependent properties of the neural code (Foffani and Moxon 2004).

The DI method is a linear model in which a neuron’s firing rate is calculated for many small bins (subdivisions) of an epoch of interest. The first step involves normalizing the firing rates (to avoid undue weight given to a tonically more active neuron). An individual neuron’s series of firing rates in each of these bins is then formed into a vector, and the vector formed from the average trial of a given class can be compared with that of another class. The Euclidian difference between those average vectors is calculated; the resulting number is the DI. Statistical methods are then employed to determine whether that DI is significant, i.e., the level of certainty about whether the neuron can distinguish between the classes of trials. We use a bootstrapping shuffle test to determine the significance of the DI without having to make *a priori* assumptions about the distribution of the data. In this method, the different classes of

trials are shuffled randomly a large number of times. For each shuffle, a shuffled DI is calculated to build a distribution of possible DIs for the given data set. The significance of the true DI is then determined by locating it on the histogram of potential DIs. This analysis is performed with all neurons in the sample to determine which ones are involved in a particular function.

ANALYSIS OF NEURAL ENSEMBLE DATA

Studying the development of interactions of highly distributed networks of neurons located across multiple areas of the brain requires a reliable method for quantifying the information available in the firing activity recorded from a given neuronal ensemble. Such a measure is also necessary for the purpose of studying the functional implications of neuronal plasticity over the course of learning the task. With respect to our sensory discrimination tasks, the relevant variable is the monkey's choice of response (reach target). Thus, in a two-choice task, our methods of choice for quantifying neural information about the stimulus take the form of categorization algorithms that classify single trials as "reach to target one" or "reach to target two" on the basis of recorded neuronal firing rates. The resulting metric can be expressed as the percent of trials classified correctly and can also be further analyzed using information analysis.

Using Artificial Neural Networks (ANNs) for Single Trial Classification Based on Neural Ensemble Recordings

Extracting information from the firing patterns of neuronal ensembles is difficult largely due to the combinatorial complexity of the problem, and the uncertainty about how information is encoded in the nervous system. Our previous studies indicated that a large number of neurons are usually active in primate S1, PPC, PMd, and M1 in order to encode tactile, decision making, and reaching information (Nicolelis, Ghazanfar et al. 1998). We also observed that there is a high degree of variability in an individual neuron's spike trials. Nevertheless, at the level of neural ensembles, tactile information can be reliably represented on a trial-to-trial basis. That is, although the number of spikes and the temporal position of spikes of a given neuron can vary from trial to trial, the overall neuronal population response predicts multiple attributes of a tactile stimulus with great precision. Thus, statistical pattern recognition approaches, such as those based on different architectures of multilayer artificial neural networks (ANNs) can be effective tools in the investigation of putative population encoding schemes. Indeed, several groups have successfully applied ANNs to investigate potential neuronal coding schemes in the auditory (Middlebrooks, Clock et al. 1994), visual (Kjaer, Hertz et al. 1994), and somatosensory systems (Nicolelis, Ghazanfar et al. 1998).

LVQ-Based Population Analyses

Previous studies have demonstrated that differences in neuronal activity as a function of task performance can be quantified using a nonparametric method for statistical pattern recognition called learning vector quantization (LVQ) (Ghazanfar, Stambaugh et al. 2000, Krupa, Wiest et al. 2004). This method has been used previously in our lab to study neuronal responses in the somatosensory and motor cortex (Ghazanfar, Stambaugh et al. 2000; Laubach, Wessberg et al. 2000). We have found that for neuronal ensemble data, LVQ outperforms most other modern methods for statistical pattern recognition. LVQ is carried out using MATLAB (The Mathworks) and the MATLAB Neural Network Toolbox in which LVQ is implemented as a two layer artificial neural network. The inputs to the network are single-trial PETHs. The first layer in the LVQ network is called the competitive layer, and its elements are equivalent to the "codebook vectors" in Kohonen's original algorithmic implementation of LVQ (Kohonen 1997). Each competitive neuron is assigned to a class in the training data (e.g., trials with narrow or wide apertures). The LVQ network is trained such that neurons in the competitive layer become matched to typical firing patterns recorded during performance of the behavioral discrimination task. The second layer of artificial neurons essentially computes the distance between single trials in the training or testing data sets and the outputs of the neurons in the competitive layer (i.e., the product of the coefficients for a given competitive neuron and the recorded neuronal ensemble response).

For our applications, we modified the code provided by The Mathworks to implement the optimized LVQ (OLVQ) learning rule (Kohonen 1997). This variant of the LVQ algorithm adaptively modifies the learning rate by decreasing the learning rate for each competitive neuron if the selected example is correctly classified and otherwise increasing

it. This results in rapidly decreasing learning rates for competitive neurons that are far from the decision boundaries (i.e., near the center of a subset of data from a given class) and increasing learning rates for competitive neurons near the decision boundaries.

Quantification of Neuronal Responses with LVQ

Single-trial PETHs (50 ms bins) are constructed for the epoch starting 200 ms before presentation of the stimulus until after the reach is complete. The data sets are divided into training and testing subsets. The training data are used to initialize the LVQ networks by setting the coefficients for each competitive neuron dedicated to a given type of trial equal to the mean neuronal ensemble response for that type of trial (plus a small random noise term). We use as many competitive neurons as twice the number of classes of data to be classified. Leave-one-out cross-validation (i.e., iteratively use all but one trial as training data and test performance of the LVQ network on a single "hold-out" trial) is used to estimate error rates and confusion matrices for each data set. Results are quantified in terms of percentage of single trials classified correctly and the informational entropy derived from confusion matrices (Krippendorff 1986).

Population Distance Index (pDI) Analysis for Single Trial Classification Based on Neural Ensemble Recordings

The pDI can be calculated for an ensemble of neurons (e.g., the entire set of neurons recorded from a single cortical area) in much the same way as the DI discussed above is calculated for an individual neuron. In calculating the pDI, vectors consist of appended vectors of the type calculated for the DI analysis, ending up with a vector of length $n * m$, where n is the number of neurons in the neuronal ensemble of interest, and m is the number of bins into which the epoch was divided. Significance is then calculated by using the same bootstrapping shuffle test as for the DI. Classification can be accomplished by using a leave-one-out cross-validation method for training and testing the model (Nicolelis 1998; Kralik, Dimitrov et al. 2001), in which one single trial is removed from the data set and the remaining trials are used to calculate the average vectors (remaining vectors) for the two classes (Figure 7.4). The left-out trial is then classified, based on the shortest distance between it and the two remaining vectors. This procedure is repeated, sequentially leaving out each trial in the sample, in order to estimate the predictive ability of the data set, and thus the potential relationship between the neuronal activity and the stimulus or behavior of interest. Results are quantified in terms of percentage of single trials classified correctly and the informational entropy derived from confusion matrices. In the latter case, information is calculated by determining how much of the uncertainty about the reach direction that exists at the beginning of the trial is reduced by the end of the trial due to the pDI calculations performed on the recorded neuronal firing. Information is measured in bits, and this task is a 1 b problem (the monkey must either reach left or right); therefore, the maximum amount of information that could be obtained from neuronal recordings is 1 b, thus resulting in values between 0 and 1.

Moving-Window Analysis

A "moving-window" analysis is used to assess the time-course of information about the monkey's decision. For this purpose, LVQ or pDI is applied sequentially to a 200–500 ms epoch of neuronal ensemble activity that "moves" in 50–100 ms steps through the course of the trial. This analysis produces a continuous quantitative readout of the recorded population's ability to distinguish between the possible classes of trial.

Over recent years, we have acquired extensive experience with this type of analysis (Nicolelis, Ghazanfar et al. 1998; Ghazanfar, Stambaugh et al. 2000; Krupa, Wiest et al. 2004) and have been able to characterize neural ensemble responses in a way that minimized the number of *a priori* assumptions made about how populations of neurons encode information. The only parameters available to the LVQ ANN and pDI classifiers are the firing rate and the temporal patterning of neuronal firing within simultaneously recorded cortical ensembles.

Cross Correlations to Study Neuronal Interactions

To identify interrelationships between the different neurons (including those in separate cortical areas), we calculate pairwise crosscorrelation functions for all possible pairs of recorded cortical neurons (Carmena, Lebedev et al. 2003). This allows us to identify both time-synchronized neuronal firing and sequential (or time offset) firing.

Partial Directed Coherence (PDC) Analysis

To identify the direction of activity between neurons in different cortical areas at various points in the task, we use the method of partial-directed coherence (PDC) (Sameshima and Baccala 1999; Baccala and Sameshima 2001a; Baccala and Sameshima 2001b; Fanselow, Sameshima et al. 2001). Briefly, PDC is a frequency domain representation of the key concept of Granger causality, which states that an observed time series x(n) Granger-causes another series y(n), if knowledge of x(n)'s past significantly improves prediction of y(n). This relation between time series is not reciprocal, i.e., x(n) may Granger-cause y(n) without y(n) necessarily Granger-causing x(n). Nullity of PDC between two structures at a given frequency suggests the lack of a direct link between those structures. We have previously adapted PDC for multivariate autoregressive modeling of signals derived from neuronal spiking (Fanselow, Sameshima et al. 2001). Because existing direct feedback relationships between each pair of channels are explicitly exposed, PDC allows the uncovering of coactivations among multichannel neuronal recordings by highlighting neuronal groups that possibly drive other neuronal groups.

For the PDC analysis, we use the primary principle component of the activity collected from all of the electrodes in a given cortical area. We find the mean value of coherence across 1–50 Hz for each 2 s analysis time window throughout the recording session. These values are calculated for classical coherence and directed coherence between each pair of cortical areas. They are then correlated with the stimulus location and reach direction. Results for each coherence type and behavior can be normalized to the classical coherence value for the experiment.

Principle Component Analysis (PCA)

PCA is used to reduce the large numbers of original neural signals to a smaller number of derived "components" that account for most of the variance observed in the original data set (Nicolelis, Ghazanfar et al. 1997; Nicolelis 1998; Kralik, Dimitrov et al. 2001). These components represent dimensions of information embedded in the firing pattern of the neural population, and they may reflect functional associations between the neurons in the ensemble. In our experience, neurons with similar functional characteristics, such as those related to a specific sensory input, or neurons located in the same area of the brain tend to have high coefficients on the same principal components, whereas neurons with dissimilar functional associations or from different areas of the brain tend to be clearly separated onto different principal components (Nicolelis, Ghazanfar et al. 1997; Nicolelis 1998).

Each recorded neuron is treated as a separate variable in the PCA. Time series of the firing rates of each neuron (e.g., rates obtained in 10 to 25 ms bins over the trial) are correlated with those of all other neurons in the population, generating a correlation matrix of all neurons. From this correlation matrix, a series of principal components is extracted. Each of these components is formed by the weighted linear sum of the firing patterns of individual neurons, with neurons contributing differentially to the different components, as reflected in the component weights. Note that neither the response properties of the neurons nor their anatomical location are made available to the PCA algorithm; nonetheless, as described previously, these features are often pulled out by PCA.

Principle components can then be used as inputs to the various analyses we have described here, including the LVQ ANN, cross-correlation, and PDC analyses discussed above.

Control Analyses

The accuracy of potential encoding mechanisms employed by cortical and thalamic neural ensembles can be investigated by several manipulations of the original data set (Ghazanfar, Stambaugh et al. 2000). These include sequentially removing neurons, one at a time, from each cortical ensemble in order to measure the variation in discrimination capability as a function of the ensemble size. A power function (Carpenter, Georgopoulos et al. 1999) can be used to estimate the number of neurons needed to achieve a certain level of neuronal ensemble performance (e.g., 99% correct discrimination of single trials) because information capacity changes nonlinearly with an increasing number of neurons. The integration time used to describe each neuron's sensory response (bin size) can also be varied, a procedure designed to alter the temporal structure of each neuron's sensory response and to test the interaction between rate and temporal coding. Procedures for decorrelating the neuronal data are also used, by either shifting the timing of individual spikes or shuffling the trial sequence for each neuron randomly. Finally, S1, PPC,

PMd, and M1 ensembles can be analyzed in isolation or together in order to investigate the potential role of corticocortical interactions.

CONCLUSIONS

New advances in multielectrode ensemble recording, allowing simultaneous recordings from large numbers of neurons, now enable us to study the changing functional interrelationships between various cortical areas over the course of learning. Ongoing learning can now be studied longitudinally, with sufficient data obtained from multiple cortical areas during each session, and across sessions, over a time course that can be as long as years.

We suggest that the techniques of ensemble neuronal recording and advances in analysis of the large amounts of data that can be obtained will facilitate a broader, system-wide understanding of information processing in the brain which will, in turn, aid in the future study of learning and cognition in both the normal and the pathological states.

REFERENCES

1. Baccala LA, Sameshima K. Overcoming the limitations of correlation analysis for many simultaneously processed neural structures. Prog Brain Res. 2001a;130:33–47. [PubMed: 11480285]
2. Baccala LA, Sameshima K. Partial directed coherence: a new concept in neural structure determination. Biol Cybern. 2001b;84(6):463–74. [PubMed: 11417058]
3. Boussaoud D, Wise SP. Primate frontal-cortex—effects of stimulus and movement. Exp Brain Res. 1993;95(1):28–40. [PubMed: 8405252]
4. Brasted PJ, Wise SP. Comparison of learning-related neuronal activity in the dorsal premotor cortex and striatum. Eur J Neurosci. 2004;19(3):721–740. [PubMed: 14984423]
5. Carmena JM, Lebedev MA, et al. Learning to control a brain-machine interface for reaching and grasping by primates. PLoS Biol. 2003;1(2):E42. [PMC free article: PMC261882] [PubMed: 14624244]
6. Carpenter AF, Georgopoulos AP, et al. Motor cortical encoding of serial order in a context-recall task. Science. 1999;283(5408):1752–7. [PubMed: 10073944]
7. Chen LL, Wise SP. Supplementary eye field contrasted with the frontal eye field during acquisition of conditional oculomotor associations. J Neurophysiol. 1995;73(3):1122–34. [PubMed: 7608759]
8. Erickson RP. Stimulus coding in topographic and nontopographic afferent modalities: on the significance of the activity of individual sensory neurons. Psychol Rev. 1968;75(6):447–65. [PubMed: 4883213]
9. Fanselow EE, Sameshima K, et al. Thalamic bursting in rats during different awake behavioral states. Proc Natl Acad Sci USA. 2001;98(26):15330–5. [PMC free article: PMC65029] [PubMed: 11752471]
10. Foffani G, Moxon KA. PSTH-based classification of sensory stimuli using ensembles of single neurons. J Neurosci Methods. 2004;135(1–2):107–20. [PubMed: 15020095]
11. Freeman WJ. Mass action in the nervous system. New York: Academic Press; 1975.
12. Fuster JM. Memory in the cortex of the primate. Biol Res. 1995;28(1):59–72. [PubMed: 8728821]
13. Georgopoulos AP, Schwartz AB, et al. Neuronal population coding of movement direction. Science. 1986;233(4771):1416–9. [PubMed: 3749885]
14. Georgopoulos AP, Taira M, et al. Cognitive Neurophysiology of the Motor Cortex. Science. 1993;260(5104):47–52. [PubMed: 8465199]
15. Ghazanfar AA, Stambaugh CR, et al. Encoding of tactile stimulus location by somatosensory thalamocortical ensembles. J Neurosci. 2000;20(10):3761–75. [PubMed: 10804217]
16. Gold JI, Shadlen MN. The influence of behavioral context on the representation of a perceptual decision in developing oculomotor commands. J Neurosci. 2003;23(2):632–51. [PubMed: 12533623]
17. Hebb DO. The organization of behavior: A neuropsychol theory. New York: Wiley; 1949.
18. Horwitz GD, Newsome WT. Target selection for saccadic eye movements: direction-selective visual responses in the superior colliculus. J Neurophysiol. 2001;86(5):2527–42. [PubMed: 11698540]
19. Kaas JH. Aotus: The owl monkey. Baer JF, Weller RE, Kakoma I, editors. San Diego: Academic; 1994.
20. Kim JN, Shadlen MN. Neural correlates of a decision in the dorsolateral prefrontal cortex of the macaque. Nat Neurosci. 1999;2(2):176–85. [PubMed: 10195203]

21. Kjaer TW, Hertz JA, et al. Decoding cortical neuronal signals: network models, information estimation and spatial tuning. J Comput Neurosci. 1994;1(1–2):109–39. [PubMed: 8792228]
22. Kohonen T. Self-organizing maps. New York: Springer; 1997.
23. Kralik JD, Dimitrov DF, et al. Techniques for long-term multisite neuronal ensemble recordings in behaving animals. Methods. 2001;25(2):121–50. [PubMed: 11812202]
24. Krippendorff K. Info theory: Structural models of qualitative data. Thousand Oaks, CA: Sage Publications; 1986.
25. Krupa DJ, Wiest MC, et al. Layer-specific somatosensory cortical activation during active tactile discrimination. Science. 2004;304(5679):1989–1992. [PubMed: 15218154]
26. Lashley KS. Symp Soc Exp Biol. Cambridge: Cambridge University Press; 1950. In search of the engram.
27. Laubach M, Shuler M, et al. Independent component analyses for quantifying neuronal ensemble interactions. J Neurosci Methods. 1999;94(1):141–54. [PubMed: 10638821]
28. Laubach M, Wessberg J, et al. Cortical ensemble activity increasingly predicts behaviour outcomes during learning of a motor task. Nature. 2000;405(6786):567–71. [PubMed: 10850715]
29. McCoy AN, Crowley JC, et al. Saccade reward signals in posterior cingulate cortex. Neuron. 2003;40(5):1031–40. [PubMed: 14659101]
30. McCoy AN, Platt ML. Expectations and outcomes: Decision-making in the primate brain. J Comp Physiol. 2005;191(3):201–11. [PubMed: 15759141]
31. Middlebrooks JC, Clock AE, et al. A panoramic code for sound location by cortical neurons. Science. 1994;264(5160):842–4. [PubMed: 8171339]
32. Mitz AR, Godschalk M, et al. Learning-dependent neuronal activity in the premotor cortex: activity during the acquisition of conditional motor associations. J Neurosci. 1991;11(6):1855–72. [PubMed: 2045890]
33. Nicolelis M. Methods for simultaneous neuronal ensemble recordings. In: Nicolelis M, editor. Methods for neural ensemble recordings. Boca Raton, FL: CRC Press; 1998.
34. Nicolelis MA, Chapin JK. Spatiotemporal structure of somatosensory responses of many-neuron ensembles in the rat ventral posterior medial nucleus of the thalamus. J Neurosci. 1994;14(6):3511–32. [PubMed: 8207469]
35. Nicolelis MAL, Baccala LA, et al. Sensorimotor encoding by synchronous neural ensemble activity at multiple levels of the somatosensory system. Science. 1995;268(5215):1353–1358. [PubMed: 7761855]
36. Nicolelis MAL, Ghazanfar AA, et al. Reconstructing the engram: Simultaneous, multisite, many single neuron recordings. Neuron. 1997;18(4):529–537. [PubMed: 9136763]
37. Nicolelis MAL, Ghazanfar AA, et al. Simultaneous encoding of tactile information by three primate cortical areas. Nat Neurosci. 1998;1(7):621–630. [PubMed: 10196571]
38. Pesaran B, Pezaris JS, et al. Temporal structure in neuronal activity during working memory in macaque parietal cortex. Nat Neurosci. 2002;5(8):805–11. [PubMed: 12134152]
39. Platt ML, Glimcher PW. Neural correlates of decision variables in parietal cortex. Nature. 1999;400(6741):233–8. [PubMed: 10421364]
40. Quintana J, Fuster JM. Mnemonic and predictive functions of cortical neurons in a memory task. Neuroreport. 1992;3(8):721–4. [PubMed: 1520863]
41. Salzman CD, Britten KH, et al. Cortical microstimulation influences perceptual judgements of motion direction. Nature. 1990;346(6280):174–7. [PubMed: 2366872]
42. Sameshima K, Baccala LA. Using partial directed coherence to describe neuronal ensemble interactions. J Neurosci Methods. 1999;94(1):93–103. [PubMed: 10638818]
43. Sandler AJ, Kralik JD, et al. Neuronal correlates of primate somatosensorimotor learning. Society for Neuroscience Annual Meeting; New Orleans, LA. 2003.
44. Shadlen MN, Newsome WT. Neural basis of a perceptual decision in the parietal cortex (area LIP) of the rhesus monkey. J Neurophysiol. 2001;86(4):1916–36. [PubMed: 11600651]
45. Sherrington CS. Integrated action of the nervous system. Cambridge: Cambridge University Press; 1906.
46. Thoenissen D, Zilles K, et al. Differential involvement of parietal and precentral regions in movement preparation and motor intention. J Neurosci. 2002;22(20):9024–34. [PubMed: 12388609]
47. Wilson MA, McNaughton BL. Reactivation of hippocampal ensemble memories during sleep. Science.

1994;265(5172):676–9. [PubMed: 8036517]

48. Wise SP, Murray EA. Arbitrary associations between antecedents and actions. Trends Neurosci. 2000;23(6):271–276. [PubMed: 10838597]

Figures

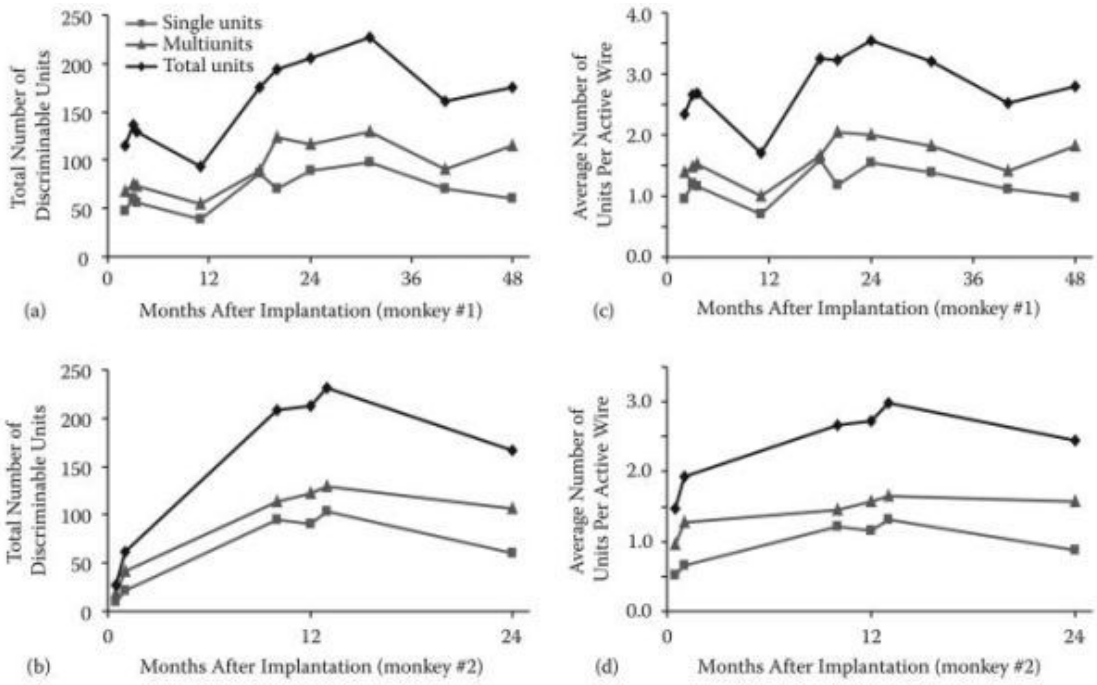

FIGURE 7.1

High quality neuronal recordings were obtained 4 years after implantation in monkey #1 (a) and 2 years in monkey #2 (b). The total number of discriminable single- and multiunits and average number of single- and multiunits obtained per wire are shown for monkey #1 for 4 years after implantation (c) and monkey #2 for 2 years after implantation (d).

FIGURE 7.2

(see pages 131 and 132) (See color insert following page 140.) Complex task-related neuronal responses. Eight different neuronal units that exhibit task-related modulation of neuronal firing other than a simple increase in firing rate are shown (A–H). A method capable of detecting such differences as these must be selected for the quantitative analysis. The Distance Index (DI), which preserves time-dependent information, method fits this criterion. For each unit, neuronal firing is represented both as raster plots where each trial is represented in a row with a tick mark for each action potential (top), and as peri-event time histograms where the average firing rates over trials are graphed and the thicknesses of the lines indicates the standard error (bottom). The x-axis show the time (in seconds) relative to the onset of the vibrotactile stimulus. Trials where the vibrotactile stimulus was applied contralateral to the unit are shown in red. Trials with ipsilateral stimuli are shown in blue. On the right are graphs of the units' waveforms and interspike intervals. The contralateral and ipsilateral trials were randomly interspersed during the sessions. Here they have been sorted to allow visualization of differences between neuronal responses to the two cues. Some units responded to the onset of the task with a decrease in firing rate, as shown here in example units from M1 (A), which showed more inhibition for left reach (blue) than for right reach (red) trials, and PPC (B), which showed similar inhibition for both types of trials near the onset of the stimulus. Other tracings had even more complex patterns. The PPC unit shown in (C) exhibited an initial decrease in firing rate for both vibrotactile stimuli, followed by a rapid rise in firing rate for contralateral stimulus (red) and a slower rise for ipsilateral stimulus (blue). The PMd unit shown in (D) had an initial rise at the onset of both stimuli followed by additional peaks of firing rate approximately midway through the ipsilateral-cued (blue) but not contralateral-cued (red) trials. The firing rate of the PPC unit in (E) increased approximately half a second into both trial types, but rapidly decreased for right reach trials (red) although remaining elevated for another several seconds in left reach trials (blue). The M1 unit in (F) demonstrated elevated firing rate only during the latter half of left reach trials (blue) whereas the M1 unit in (G) demonstrated a decreased firing rate only during the latter half of the right reach trials (red). The PPC unit in (H) showed a slight difference in firing rate between left reach (blue) and right reach (red) trials only near the middle of the trial.

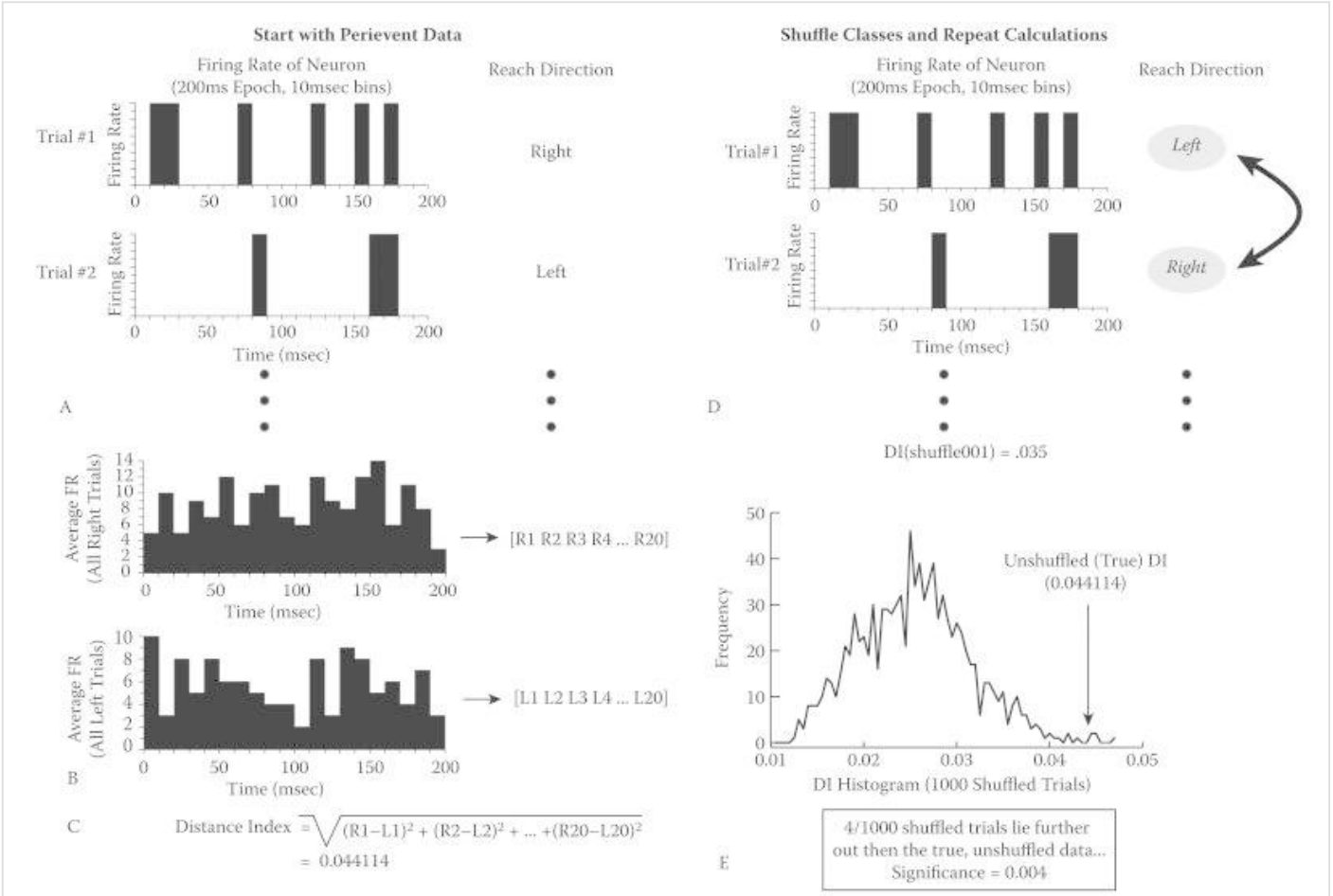

FIGURE 7.3

(see facing page) Schematic of Distance Index (DI) method, a linear model that is used to examine the relationship between the firing of a neuron and behavioral variables, such as reach direction. The DI method takes into account both firing rate and temporal patterns of firing during an epoch of 200 ms, by calculating the firing rate during 20 10 ms bins for each individual trial (a). These firing rates for the average trial of each class (right reach or left reach) are then expressed as a 1 × 20 vector (b). The Euclidian difference between those average vectors is calculated; the resulting number is the DI (c). A bootstrapping shuffle test is then used to determine the significance of the DI without making a priori assumptions about the distribution of the data (d). In this method, the different classes of trials are shuffled randomly a large number of times. For each shuffle, a shuffled DI is calculated to build a distribution of possible DIs for the given data set. The significance of the true DI is then determined by locating it on the histogram of potential DIs (e). By repeating this analysis with all neurons in the sample, the percentages of neurons that respond significantly different from to the two classes of the parameter can be calculated.

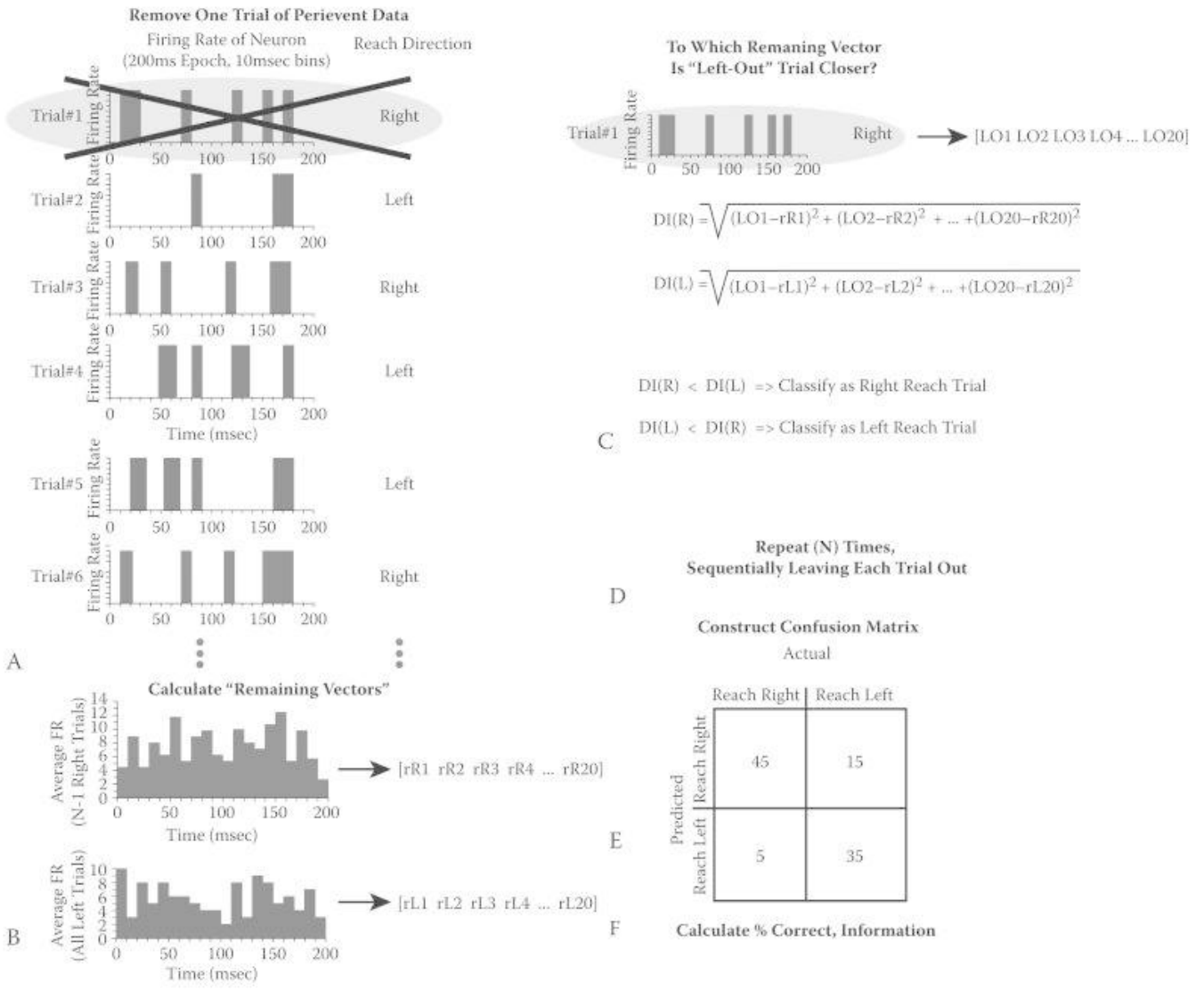

FIGURE 7.4

(see facing page) Schematic of prediction model used to calculate the ability of an individual neuronal unit (or a population of neuronal units) to predict the class of a given single trial. We used the leave-one-out cross-validation method for training and testing the model, removing one single trial from the data set (a) before using the remaining trials to calculate the average vectors (remaining vectors) for the two classes (b). The left-out trial was then classified, based on the shortest distance between it and the two remaining vectors (c). This procedure was repeated, sequentially leaving out each trial in the sample (d), in order to construct a confusion matrix (e). Estimates of the predictive ability of the data set, and thus the relationship between the neuronal activity and the direction of reach, were then calculated both as percent correct prediction and by using information theory (f).

Bookshelf ID: NBK3903 PMID: 21204449