5 Introduction to the Physiological Bases of EEG

It seems obligatory for a resource on EEG analyses to discuss the neurophysiological events that produce the EEG signal. There are many excellent resources available on this topic. It would be redundant to rewrite what is already explained in these resources, so this chapter instead provides a brief overview of the important points, along with references for reading more about each specific topic. The following references (among many others) are good starting points for deeper and approachable discussions of the neurophysiology and biophysics that underlie the EEG and MEG signal as well as the neurophysiological mechanisms that underlie oscillations (Buzsaki, Anastassiou, and Koch 2012; Nunez and Srinivasan 2006; Steriade 2006; Wang 2010).

5.1 Biophysical Events That Are Measurable with EEG

EEG reflects mainly the summation of excitatory and inhibitory postsynaptic potentials at the dendrites of ensembles of neurons with parallel geometric orientation. As neurotransmitters activate ion channels on the cell membrane, ions flow into and out of the neuron from and to the extracellular space. This change in potential generates electrical fields that surround the neuron. The electrical field generated by one neuron is too weak to be measured from an EEG electrode several centimeters away, but as neural activity becomes synchronous across hundreds, thousands, or tens of thousands of neurons, the electrical fields generated by individual neurons sum, and the resulting field becomes powerful enough to be measured from outside the head. It has been estimated that between 10,000 and 50,000 neurons, mainly in superficial cortical layers, dominate the EEG signal (Murakami and Okada 2006; Wang et al. 2005). Although the electrical conductivity differs among brain tissue, skull, and scalp, there is sufficient conductivity for the electrical fields to travel from the neural population that generated that field to the top layer of the scalp. Because the electrical conductivity of air is almost zero, a physical electrical bridge must be formed between the skin of the scalp

and the EEG electrodes. This is why scalp EEG electrodes require electroconductive gel, paste, or salt-water-soaked sponges. Magnetic fields are perpendicular to electrical fields and pass through the skull and scalp unimpeded. This is why MEG sensors must be close to the head but do not need to make direct contact with the scalp.

EEG cannot measure all neural events. In fact, most of the events in the brain are not measurable with EEG. If you find that assertion disappointing or disheartening, perhaps you will take some comfort in reading that the same could be said of all brain-imaging techniques, from single-unit recordings to local field potentials, from voltammetry to MR-based imaging. No single brain-imaging technique can record most of the events in the brain. Different techniques are well suited for measuring certain kinds of brain events. This is why you should choose the method that will best answer your research question, and this is why you should interpret results from one method in the context of what can be measured with that method.

EEG cannot measure individual molecular or synaptic events, nor can it isolate events that are produced by a specific neurotransmitter or neuromodulator. EEG cannot measure action potentials or local field potentials generated by small ensembles of a few or dozens of neurons. These spatially small-scale events produce either no electrical fields or weak electrical fields that can be measured only with invasive recordings. Some of these small-scale events may include cortical layer-specific oscillations (Kral et al. 2000; Scheffer-Teixeira et al. 2012; Sun and Dan 2009; Vreugdenhil, Bracci, and Jefferys 2005). If the small-scale events modulate meso- or macroscopic populations that produce large field potentials, EEG may measure the indirect effects of those small-scale events but not those events themselves.

Even some electrical fields that should be powerful enough to be measured from the scalp may not be measured with EEG. For example, field potential fluctuations on opposing sides of a sulcus would cancel if they had similar strengths, and thus not be measurable from the scalp (figure 5.1).

Deep brain sources such as the thalamus, basal ganglia, hippocampus, and brainstem are difficult but not impossible to measure from the scalp. There are two reasons for this difficulty. The first is that field strength decreases as an exponential function of distance, so even very powerful fields generated by deep brain structures have only a small impact on the voltage recorded from the scalp. To measure deep brain activity from the scalp, those deep brain sources must produce powerful fields, and there should be many trials for averaging. For example, studies on brainstem-generated potentials generally have thousands of trials to obtain sufficient signal-to-noise (Stone et al. 2009). This can be compared with the dozens of trials that are typically sufficient for cortex-generated potentials.

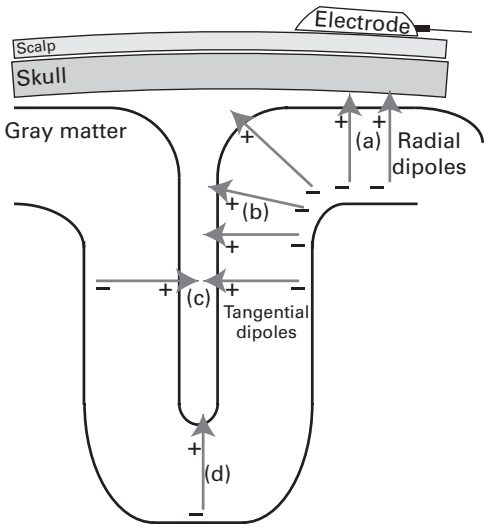

Figure 5.1
Illustration of dipoles in different orientations with respect to the skull. The dipoles illustrated in (a) will contribute the strongest signal to EEG, whereas the dipoles illustrated in (b) will contribute the strongest signal to MEG. The dipoles illustrated in (c) are unlikely to be measured because the dipoles on opposing sides of the sulcus produce electrical fields that are likely to cancel each other. The dipole illustrated in (d) will make a smaller contribution to EEG than dipole (a) because it is further away from the electrode. (This figure is inspired by figure 1 of Scherg 1990.)

The second reason why activity from deep brain structures is difficult to measure from the scalp is that populations of neurons in subcortical structures are not often arranged in a geometrically parallel orientation. This means that when there is synchronous population-level activity, the electrical fields generated by individual neurons are likely to cancel each other out at the macroscopic scale rather than summing and becoming powerful enough to be measurable from the scalp. If you would like to measure activity from deep brain structures using scalp EEG, you should use a task that is known to elicit activity in that brain region (e.g., as demonstrated with fMRI), have many trials to increase signal-to-noise ratio (hundreds or thousands, if possible), and, if you will perform source localization to provide support for the origin of the deep brain source, use many electrodes and subject-specific anatomical MRIs to increase spatial accuracy of the source reconstruction results.

Very slow fluctuations (<0.1 Hz) can be difficult but not impossible to measure with EEG. This is not due to the biophysics of the brain but rather to limitations of most modern EEG amplifiers and data acquisition systems. Most amplifiers have built-in high-pass filters that

attenuate very slow fluctuations because they may cause amplifier saturations. However, there are DC-coupled amplifiers that are suitable for recording fluctuations below 1 Hz. If you would like to measure very slow activity, check whether your amplifier is well suited for this and check whether there are high-pass filters that are applied during the recording.

Very fast fluctuations (>100 Hz) are also difficult but not impossible to measure. High-frequency activity generally has low power and thus is more difficult to distinguish from noise. MEG is better than EEG at recording high-frequency activity because the magnetic fields are unimpeded by the differing conductances across the brain, skull, and scalp. For EEG, activity above around 80 Hz should be carefully inspected to make sure it is not driven by noise spikes, EMG, eye movements, or other artifacts.

5.2 Neurobiological Mechanisms of Oscillations

An oscillation is a rhythmic alternation of states. Oscillations can occur in time or in space, and are commonly seen in physical and biological systems. In the brain, the term oscillation refers to rhythmic fluctuations in the excitability of neurons or populations of neurons. Neural oscillations are observed on many spatial and temporal scales (Varela et al. 2001) and have been linked to many neurobiological events ranging from long-term potentiation to conscious perception (Buzsaki 2006; Engel, Fries, and Singer 2001; Herrmann, Frund, and Lenz 2010; Kistler, van Hemmen, and De Zeeuw 2000; Klimesch et al. 2008; McBain and Kauer 2009). Oscillations can also be seen in unfiltered ("raw") scalp EEG data. As discussed in section 2.6, one of the major advantages of conceptualizing EEG as comprising oscillations is that it provides a link to a large literature on in vitro, in vivo, and computational mechanisms of oscillations.

The neurobiological mechanisms that produce oscillations are fairly well understood (Buzsaki, Anastassiou, and Koch 2012; Wang 2010), although uncertainties remain in the extent to which different factors contribute to the signal recorded by EEG, in part due to the complexity of the models and the difference in spatial scale between individual neurons and scalp EEG (Coombes 2010; Deco et al. 2008; Whittingstall and Logothetis 2009). There are three basic physiological mechanisms that produce oscillations of hundreds to hundreds of thousands of neurons. One mechanism involves interactions between inhibitory (GABAergic) interneurons and excitatory pyramidal cells. When a population of pyramidal cells becomes active (e.g., from a volley of inputs from the thalamus or other cortical area), their excitation increases as they continue exciting each other. Interneurons within this population also become activated, and as the activity of the inhibitory interneurons increases, the

This is how oscillations occurs in brain

excitatory cells become inhibited. The activity of the interneurons then decreases, allowing excitation of the pyramidal cells to increase again. This shifting balance between states of excitation and inhibition produces oscillations. This process provides the basic skeleton of an oscillation; there are many additional factors that modulate the frequency, amplitude, and phase of the oscillations. This alternating balance between excitatory and inhibitory neurons is thought to be the mechanism that dominates the oscillations observed with EEG. Oscillations can also be produced by purely excitatory networks and by purely inhibitory networks. If you are interested in reading more about the mechanisms of neural oscillations and their involvement in neural computations, you could start with the references cited above, in the opening paragraph of this chapter, and the book *Rhythms of the Brain* (Buzsaki 2006).

5.3 Phase-Locked, Time-Locked, Task-Related

A distinction can be made among phase-locked, non-phase-locked, and background activity (figure 5.2). Phase-locked activity (also sometimes called "evoked") is phase-aligned with the time = 0 event and will therefore be observed both in time-domain averaging (the ERP) and in time-frequency-domain averaging. Non-phase-locked activity (also sometimes called "induced") is time-locked but not phase-locked to the time = 0 event. Simulated examples of non-phase-locked activity were shown in figures 2.1 and 2.2. Non-phase-locked activity can be observed in time-frequency-domain averaging but not in time-domain averaging (thus, it has no representation in the ERP). It is not entirely clear what mixture of physiological dynamics would result in phase-locked versus non-phase-locked activity (David, Kilner, and Friston 2006), although in general, non-phase-locked activity is taken as stronger evidence for the presence of oscillations (Donner and Siegel 2011; Gray and Singer 1989; Tallon-Baudry and Bertrand 1999). Methods to compute phase-locked versus non-phase-locked activity are discussed in chapter 20.

Phase-locked and non-phase-locked activities are task related in that their time and/or frequency characteristics change as a function of engagement in task events. Background activity, in contrast, does not change as a function of the task (Freeman 2004). Background activity is used in resting-state studies; however, in most cognitive electrophysiology studies, background activity provides little useful information. Furthermore, background activity can be distracting if it has a large amplitude or is present in the same frequency band as the task-related effects. Because the background activity is unrelated to task events, applying a baseline normalization will remove most or all of the background activity, thus allowing you to focus on task-related dynamics.

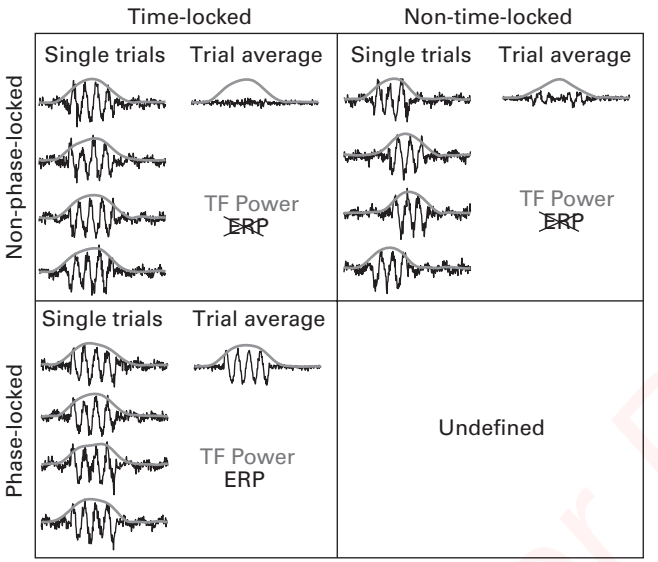

Figure 5.2
Illustration of whether time-frequency (TF) power and the ERP can measure phase-locked, non-phase-locked, time-locked, and non-time-locked activity. The left column of each cell shows four trials of simulated data, and the right column of each box shows the average of those four trials. Black lines show the raw time series, and gray lines show the time course of 10-Hz power. The ERP captures only phase-locked and time-locked activity. Time-frequency power can measure time-locked activity regardless of whether it is phase-locked or non-phase-locked. Activity that is not time-locked can be measured with time-frequency power, although the results will be smoothed and thus less temporally precise.

5.4 Neurophysiological Mechanisms of ERPs

Compared to oscillations, there is less empirical and theoretical work on the neurophysiological mechanisms that produce event-related potentials (ERPs)—that is, why there are positive and negative polarity peaks at somewhat regularly spaced intervals following experiment events. There are recent simulations that suggest mechanisms of ERPs through complex additive and nonlinear effects (David, Kilner, and Friston 2006) or rapid changes in frequencies (Burgess 2012), but there is less in vitro confirmation of ERP mechanisms compared to oscillation mechanisms. Other models about how ERPs can emerge from ongoing or oscillatory activity are described briefly below.

Additive This model proposes that the ERP reflects a signal that is elicited by an external stimulus such as a picture or a sound or by an internal event such as the initiation of a

manual response and is added to ongoing background oscillations. Because the oscillations are not related to the stimulus, they are attenuated in trial averaging. This model assumes that there is a distinction between the neurophysiological events that produce oscillations and the neurophysiological events that produce ERPs.

Phase reset This model proposes that ERPs result at least partially from a sudden alignment of the phases of ongoing oscillations (Makeig et al. 2002). That is, when a stimulus appears, the ongoing oscillation at a particular frequency band is reset to a specific phase value, which may reflect a return to a specific neural network configuration. This model may not account for all ERP components, particularly the later "cognitive" ERP components (Fell et al. 2004).

Amplitude asymmetry or baseline shift Although the electrical currents generated by neurons are polarity balanced, it is possible that outward-going currents are less detectable from the scalp (Mazaheri and Jensen 2008). This would produce an asymmetry in the oscillations measured by scalp EEG electrodes such that peaks and troughs are not equally distributed. It might also produce a baseline shift, which would also effectively produce asymmetries between peaks and troughs of oscillations (Nikulin et al. 2010). Changes in overall power could thus produce asymmetries in ongoing oscillations, which, when averaged over trials, might appear as a slow ERP (de Munck and Bijma 2010; Jensen, van Dijk, and Mazaheri 2010; Nikulin et al. 2007).

Having more mathematically precisely specified models might help resolve this debate (de Munck and Bijma 2010), but it seems that these different models are difficult to disentangle empirically at the level of the scalp. Indeed, models and simulations do not seem to be able to produce unambiguous evidence for the veracity of any one of these models at the expense of the other models (Krieg et al. 2011; Yeung et al. 2007). Ultimately, this debate may never be fully resolved until the neurophysiological mechanisms of ERPs are better understood, regardless of the mathematical precision of the competing hypotheses. One issue that complicates matters is that different ERP components may have different neural origins, and so general explanations about the mechanisms of all ERPs may be doomed to fail to capture all ERPs. If you would like to read more about this debate, the following references, in addition to those listed above, will help you to get started (Burgess 2012; David, Harrison, and Friston 2005; Makeig et al. 2004; Makinen, Tiitinen, and May 2005; Mazaheri and Jensen 2006; Penny et al. 2002; Sauseng et al. 2007; Shah et al. 2004).

5.5 Are Electrical Fields Causally Involved in Cognition?

Anyone reading this book probably hopes the answer is "yes." However, at present there is limited causal evidence to state with certainty whether and how electrical fields are causally

Notes:
some evidence suggests brain rythms actually control cognition
1) Learning & memory depend on rythm timing

involved in cognitive processes. Nonetheless, several lines of evidence suggest that electrical fields are causally involved in neural computation and information transfer.

One line of evidence comes from in vitro studies on the relationship between local field potential oscillations and synaptic events that are thought to underlie learning and memory. For example, long-term potentiation in the hippocampus, which is thought to be a basis of Hebbian learning and thus memory formation, occurs preferentially at specific phases of theta-band (4–8 Hz) oscillations (see Axmacher et al. 2006, for a review).

Another line of evidence comes from studies on the relationship between the timing of action potentials and the phase of the local field potential oscillation. In general, these studies show that the timing of many but not all neurons is constrained by the local field potential, such that neurons are more likely to emit an action potential during some phases of the local field potential oscillation. The synchronization between action potential timing and field potential phase has led to theories of phase coding (Lisman and Otmakhova 2001; Yamaguchi et al. 2007).

Other theories suggest that interregional oscillatory synchronization is a mechanism underlying the transmission of information across neural networks and that this synchronization-mediated connectivity is crucial for perceptual and cognitive processes (Akam and Kullmann 2012; Fries 2005; Singer 1993). The idea is that spatially disparate neural networks can most efficiently cooperate and transfer information when they are phase synchronized. Partly for this reason, phase-based synchronization methods are the most widely used approaches for studying connectivity in electrophysiology data and are discussed in chapter 26.

Recent studies on ephatic coupling provide compelling evidence for a causal role of oscillations in brain function. Ephatic coupling refers to interactions among neurons that occur via the transmission of ions, which are transmitted via an electrical field through the extracellular space. Ephaptic coupling of individual neurons is impeded by the relatively small magnitude of individual neuron electrical fields compared to extracellular distances and insulation properties of myelin, but it is likely to occur if neurons are densely packed (Bokil et al. 2001) or if the field potential is relatively powerful. Local field potentials generated from networks of neurons produce larger fields and have been shown to entrain spike timing via ephaptic coupling in the cortex (Anastassiou et al. 2011), particularly in the delta and theta frequency bands.

Two promising methodological approaches to determining whether oscillations are causally involved in brain computation are optogenetics in mice and rats and transcranial alternating-current stimulation in humans. Optogenetics involves shining a light at a

particular wavelength into the brain of a mouse. The mice (rats are also sometimes used) are from a genetic line that allows specific channels on specific types of neurons to be activated with millisecond precision when those channels are exposed to a light of a particular wavelength (Kravitz and Kreitzer 2011; LaLumiere 2011). By the use of optogenetics, oscillatory activity at a desired frequency can be exogenously introduced into a specific region of the brain. For example, optogenetics studies have demonstrated that gamma-band oscillations can be exogenously enhanced, and this enhancement facilitates signal transmission and noise suppression (Sohal et al. 2009).

In humans, transcranial alternating-current stimulation (TACS) involves passing an electrical current between two electrodes placed on the scalp. The frequency of stimulation can be specified, typically between 0.1 and 100 Hz. Thus, a frequency-band-specific electrical current can be exogenously introduced into the human brain. This is advantageous over transcranial magnetic stimulation, which can evoke oscillations but in a transient manner that depends on the brain region to which the stimulation is applied (Rosanova et al. 2009; Thut et al. 2011). Using TACS, researchers have demonstrated, for example, that 20-Hz stimulation over motor cortex increases motor evoked potential magnitude, whereas stimulation at a range of other frequencies does not (Feurra et al. 2011). Another study showed that stimulation at an individual subject's alpha peak increased alpha power (Zaehle, Rach, and Herrmann 2010). Furthermore, large-scale frontal-parietal network entrainment by theta-band TACS facilitates cognitive processing (Polania et al. 2012). TACS is not often used in cognitive electrophysiology but may become an important tool for testing hypotheses about the role of specific oscillation frequencies in cognition (see also chapter 38).

5.6 What If Electrical Fields Are Not Causally Involved in Cognition?

Perhaps all of the empirical work on the causal role of neural oscillations is misguided or flawed. Perhaps all of the theories on the role of oscillations in cognition are wrong, and perhaps the computational models providing putative mechanisms of oscillations in cognition rely on incorrect assumptions. If electrical fields were not causally involved in cognition, would this be the end of cognitive electrophysiology?

Not at all. Electrical fields produced by neural populations are undeniably powerful and insightful indices of brain function. If they are not causally involved in cognition but rather are epiphenomenal curiosities that result from the mechanisms that truly underlie cognition, the use of field potential oscillations in the study of brain organization is still valid. Along a similar vein, it is not widely believed that the blood oxygenation-level-dependent (BOLD)

signal measured by fMRI is a causal mechanism of neural information processing, but it is widely believed that the BOLD response is a powerful and useful indirect index of brain function. The lack of causality does not stop thousands of scientists around the world from using the BOLD signal to understand brain functional organization. Similarly, conclusive evidence against a causal role of oscillations in cognition would not stop cognitive electrophysiologists from using EEG to make important discoveries about the functional organization of cognition and the brain.

6 Practicalities of EEG Measurement and Experiment Design

This chapter covers some details about how to design experiments so they are appropriate for time-frequency-based analyses. It also contains some suggestions for additional equipment that might facilitate your research. General information about setting up an EEG lab and additional advice for designing EEG and ERP experiments can be found in the books by Luck and Handy (Handy 2004; Luck 2005).

6.1 Designing Experiments: Discuss, Pilot, Discuss, Pilot

Do not underestimate the importance of good experiment design. Beautiful, clean data and clever, properly done analyses combined with a poorly designed task will give results that are difficult to interpret and may see rejections at peer-reviewed journals. On the other hand, a well-designed experiment is likely to produce meaningful and interpretable results that have implications for theories and may inspire new research, even if the data are noisy and only basic analyses are performed.

Before collecting data for your experiment, discuss your experiment design with colleagues and have them do the task (if there is a task) to give you feedback on its design. Pilot test your experiment behaviorally to make sure you can obtain the predicted behavioral effect before proceeding to collect brain data. You should then collect EEG data from one or two subjects and analyze those datasets completely, including the analyses corresponding to the main hypotheses. You may find flaws, suboptimal design features, extraneous conditions, or the need for additional conditions only after analyzing these pilot datasets. It is better to discover suboptimal and fixable design features during piloting than after collecting all of the data. If you are satisfied with the design and do not plan on changing any experiment parameters, you can keep those datasets for the final group-level analyses. If you change the task after piloting and cannot use the data you have already collected in the final results, then at least you had to throw out only one or two datasets rather than 20 datasets.

6.2 Event Markers

Experiment event markers, or triggers, are square-wave pulses that are sent from the stimulus-delivering computer to the EEG amplifier and are usually recorded as a separate channel in the raw data file (or sometimes, multiple channels). The amplitude of the pulse is used to encode specific events such as stimulus onset or response. Most systems have eight-pin cables that allow up to 255 unique codes (zero is the no-marker default). During data importing, the markers are converted to labeled time stamps that indicate when different events in the experiments occurred. An example may be seen in figure 6.1.

Event markers are critical because they are used to time-lock the EEG data offline. They are also used to reconstruct different conditions and responses. Therefore, it is better to have markers encode too much rather than too little information. It is easy to collapse multiple markers during the analyses but can be difficult to reconstruct specific trial events when they were not encoded in the data file. Combining data from a text file with the EEG data becomes even more difficult after preprocessing and trial rejection. Although most systems allow only 255 possible marker values, there is no practical limit to how much information can be encoded in the EEG data using 255 markers. For example, a pair of markers spaced 10 ms apart provides 65,025 possible unique markers (255 times 255).

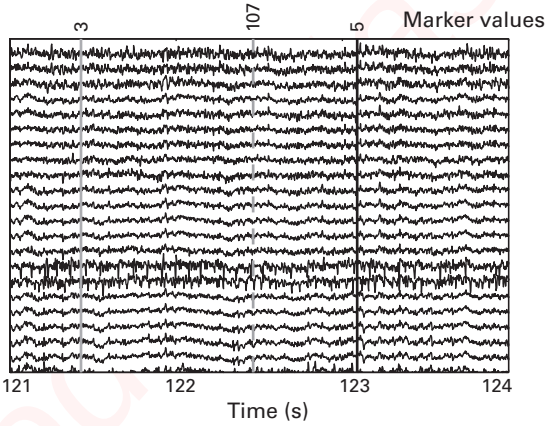

Figure 6.1
Example EEG data showing 3 s of data and three experiment markers. The experiment markers are represented as vertical lines, and the numbers on top of the vertical lines correspond to particular events. In this case the numbers 3 and 5 refer to two response buttons being pressed by the subject, and the number 107 corresponds to a particular stimulus. This picture was made using the eeglab lab function `eegplot`.

Many data acquisition systems do not allow multiple markers to be sent simultaneously, and when there are overlapping markers, only one is registered. Dropped markers can be detrimental because those trials may be lost. Simultaneous markers occur occasionally, for example if the subject accidentally presses a button at the exact same time a stimulus marker is sent. This may happen once every few thousand trials and therefore is unlikely to have a significant negative impact on the analyses or on the results. If there are many simultaneous and blocked markers, there is a problem with your experiment, and you should investigate and resolve the issue before collecting additional data.

The temporal duration of each marker (this refers to the length of time in which the marker channel has a nonzero value) should be at least a few samples; a 0.5-ms marker might not be recorded if the sampling rate is 500 Hz. On the other hand, if the markers have a duration of 200 ms, they are likely to overlap with other markers. In general, 5 ms should be a sufficient duration.

There are many software packages that deliver stimuli to the subject and event markers to the EEG acquisition system. Some software programs are commercial (e.g., Presentation or E-Prime), and others are toolboxes for Matlab (e.g., Psychophysics toolbox or Cogent). Make sure the software you use can integrate with the computer hardware to maximize timing precision and stimulus delivery.

Because the timing and the values of the event markers are so critical to the experiment, if the timing is off or if the values are not properly encoded, there is little you can do with the data. Therefore, when using new software or hardware, it is a good idea to confirm the timing and event marker values. You can test the event marker values by sending codes 1–256 with 10 ms spaces between markers. You should be able to reconstruct these markers from the EEG data. If not, there is a problem somewhere, perhaps with the computer output port or the cable.

6.3 Intra- and Intertrial Timing

Partly because of some temporal smoothing introduced by time-frequency decomposition, and partly because time-frequency responses may linger for hundreds of milliseconds after an experiment event has ended, it is ideal to have experiment events within a trial separated by at least several hundred milliseconds (note that stimulus offsets also produce EP perturbations). This will allow the brain response to one event to subside before the response to the next event begins. For some kinds of experiments this can be difficult to achieve, for example, if a button press quickly follows a stimulus, or if the stimuli need to appear closely temporally spaced to each other, as in the attentional blink task. If

the approximate duration of the electrophysiological response to a stimulus is not known, you could pilot the task using long interevent durations to see how long the electrophysiological response to each event lasts. How long the brain response to an event will last depends on the kind of task and on the cognitive process that is elicited. The time-frequency dynamics elicited by a simple and neutral visual stimulus, such as a Gabor patch, will likely decay quickly after stimulus offset. But the time-frequency dynamics elicited by a picture of a sick child in a poor country might take longer to subside because of lingering reactions to that picture.

Concerning the duration of time between the end of one trial and the start of the next trial (also called the *intertrial interval*), consider carefully what period of time you will use for baseline normalization of task-related data and what frequencies you want to analyze. Keep in mind that baseline time period for time-frequency decomposition should end before trial onset, for example –500 to –200 ms. This is different than ERPs, for which the baseline period typically ends at the time = 0 event. The reason for the earlier baseline period with time-frequency decomposition is that temporal filtering may cause some early poststimulus activity to "leak" into the prestimulus baseline period, and this temporal leakage can be worse at lower frequencies. You will learn more about why this is the case in chapters 10–14, and chapter 18 contains deeper discussions on baseline normalization and the choice of the pretrial baseline period. For most cognitive electrophysiology experiment designs, intertrial intervals of at least 1000 ms should be sufficient.

Intertrial intervals can be constant (e.g., 1000 ms on each trial) or variable (e.g., randomly selected on each trial from an interval between 800 ms and 1200 ms). There are advantages and disadvantages of each of these choices. On the one hand, if subjects can predict the temporal onset of the upcoming trial, they can prepare for the trial, and this preparation may be reflected in the EEG, for example, as changes in posterior alpha-band activity (Rohenkohl and Nobre 2011). On the other hand, with variable intertrial intervals, subjects may try to guess the next trial onset, which can introduce temporal expectations, surprise, and variable task preparation. These processes may vary in nonlinear ways as a function of the duration of the intertrial interval (e.g., as a hazard function). Indeed, both behavioral performance and EEG measures are affected by the duration and variability of intertrial intervals (Appelbaum et al. 2012; Egner, Ely, and Grinband 2010; Gonsalvez and Polich 2002). Subjects will nearly always generate temporal expectations about when the next trial will occur, and these expectations are likely to affect EEG and behavior. The question is whether to constrain these expectations in the experiment or leave them unconstrained. Note that the presence of pretrial activity does not hinder baseline normalization or the ability to examine condition differences in pretrial activity; this is discussed in chapter 18.

6.4 How Many Trials You Will Need

The number of trials you need for each condition depends on the signal-to-noise characteristics of the data (that is, how clean vs. how noisy the data are), how big the effect is, and the type of analysis you will perform. There is no magic number of trials that will guarantee good results or sufficient signal-to-noise ratios. That said, for many analyses a minimum of 50 trials per condition per subject is a reasonable number of trials that should lead to a sufficient level of signal-to-noise ratio, but this is not a strict rule. For example, the error-related negativity—an ERP component elicited by response errors—seems to be statistically robust and with reasonable reliability with as few as 14 trials (Larson et al. 2010). The reliabilities of other EEG dynamics such as band-specific power, measures of connectivity, and cross-frequency coupling are understudied or unknown (although this would be useful practical information). Further issues related to the number of trials necessary for analyses are presented in some analysis-specific chapters (for example, chapters 18 and 19).

6.5 How Many Electrodes You Will Need

The number of EEG electrodes or MEG sensors you need depends on what analyses you plan on performing and what inferences you plan on drawing. If you want to perform brain source reconstruction analyses and make inferences about brain localization, more electrodes (>100) are useful to increase the signal-to-noise ratio and accuracy of the spatial filters. On the other hand, if all you will do with the data is measure the P3 amplitude, you technically need only three electrodes (one placed over central parietal cortex to record the P3, one for a reference, and one for a ground). Unless you have compelling reasons otherwise (e.g., if you are testing a special clinical population that cannot tolerate many electrodes), use at least 64 electrodes if possible. This will allow you to apply spatial filters, perform connectivity analyses, and examine topographical distributions of the results. Sixty-four electrodes should be sufficient for nearly all analyses without significantly adding to data analysis time or hard disk data storage.

In addition to analysis considerations there are also practical considerations for the number of electrodes you will need, including preparation time and data storage. Most EEG electrode caps require electroconductive gel placed into each electrode to form a physical electroconductive bridge with the scalp. This procedure takes time and may take longer if the subject requires extra scalp preparation (asking your subjects to wash their hair and avoid using hairspray or gel will help provide a clean EEG signal). The preparation time increases with the number of electrodes. EEG caps with 64 gel-filled electrodes can be prepared in less

than 30 min by two trained workers, but 256 gel-filled electrodes may take considerably longer. There are also gel-free EEG caps that use sponges that are soaked in a salt-water solution; these sponge-based caps can decrease preparation time considerably.

Having more electrodes also has the practical implication of increasing data storage and processing time. Whether having more than 64 electrodes is worth the extra cost of data storage and computation time depends on the cost of data storage and backup and the computer systems available for analyses. Writing efficient Matlab code will help decrease computation time.

6.6 Which Sampling Rate to Use When Recording Data

Sampling rate refers to the number of times per second that data are acquired from all electrodes. This defines the temporal resolution of the data. The choice of sampling rate depends on several factors, including the kinds of analyses you plan on performing, the frequencies you plan on analyzing, and the available disk space and processor speed/type.

Technically, you need to sample at least twice the highest frequency of interest. This means that you need to use a sampling rate of at least 100 Hz if you want to test for 50-Hz activity. This is because of the Nyquist theorem, which, applied to time-frequency dynamics, states that only frequencies below half the sampling rate can be recovered (you will learn about why this is the case in chapter 11).

In practice, however, you should sample more than twice the highest frequency you will analyze. Having more data points per oscillation cycle increases signal-to-noise ratio and therefore allows for better estimation of high-frequency activity, as shown in figure 6.2. This is particularly the case if your analyses will involve using the phase-angle time series because the phase-angle time series is more susceptible to loss of temporal information compared to the power time series. Also keep in mind that there is an upper limit beyond which little additional information can be obtained. For example, sampling EEG data at 20,000 Hz is unlikely to provide any information not available in 1000-Hz-sampled data. In practice, sampling rates between 500 Hz and 2000 Hz are likely to be sufficient for all analyses. (The data provided with this book were originally recorded at 2048 Hz and were downsampled to 256 Hz to minimize download and analysis times.) Sampling rates higher than 2000 Hz are useful only in some situations where large artifacts must be precisely measured, such as during simultaneous EEG and fMRI recording.

Higher-sampling-rate data will take up more disk space, increase analysis time, and increase file read/write times. These issues may or may not be a concern depending on whether and

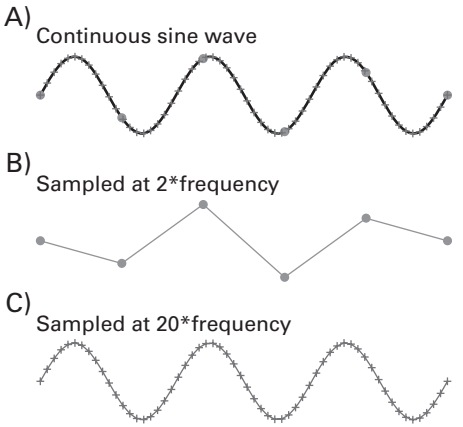

Figure 6.2
A continuous sine wave (panel A) and an illustration of the effect of subsampling that sine wave. Panel B shows that sampling the sine wave at twice its frequency (see gray dots along the sine wave in panel A) can reconstruct some features of the sine wave but fails to reconstruct the finer features, in particular the precise peak and trough times and the ongoing phases. Panel C shows that sampling at 20 times the frequency (see gray plus signs in panel A) can reconstruct the time-varying features of the sine wave with much higher accuracy.

how much you pay for data storage and backup and what types of computers you use for analyses. Less powerful computers running 32-bit Matlab may underperform or may crash due to insufficient memory if the sampling rate is too high. You can downsample the data after recording them, so it is better to record the data using a high sampling rate (e.g., 1000–2000 Hz) and then downsample the data prior to analyses if necessary. This is better than recording the data at 128 Hz and then having a poor signal-to-noise ratio for power and phase estimates in the beta and gamma frequency ranges.

From a convenience-of-analysis perspective, 1000 Hz is the optimal sampling rate. At 1000 Hz, there is a one-to-one conversion between time in milliseconds and time in samples. That is, 14 ms is also 14 samples. 500 and 2000 Hz are the next-most convenient sampling rates (14 ms is, respectively, 7 and 28 samples). After that, converting time to samples becomes difficult to do in your head (e.g., how many samples at 256 Hz fit into 350 ms?). This should not be a major guiding force behind your data acquisition because it is very easy to convert between time in samples and time in milliseconds. But given the choice between sampling rates of 1024 Hz and 1000 Hz, I would choose the latter.

6.7 Other Optional Equipment to Consider

Obviously, the EEG electrodes are the most important equipment components of an EEG lab. But there is more than just brain activity to consider; monitoring other behavioral and physiological events may improve the quality of your research. These improvements could come from removing trials that contain artifacts that are difficult to identify in the EEG data, or the improvements could come from providing additional insights into neurocognitive processes such as interactions between the brain and the body. Following is a list of possible add-ons that can be integrated with the EEG setup.

1. *Response EMG or force grips* There are several reasons why precise information about the motor actions of the subject during the task might be useful. Recording simultaneous EMG from the muscles used to indicate responses will allow you to examine cortical-muscular connectivity (Lattari et al. 2010) and will allow you to identify trials in which subjects twitched the muscles of the incorrect hand and then pressed the button of the correct hand (more on these "partial errors" in section 8.6). Force grips can also be used and provide largely consistent findings compared to EMG (Hoozemans and van Dieen 2005).

2. *Eye tracker* An eye tracker can be used for several purposes. It will facilitate preprocessing and cleaning the dataset by allowing you to remove trials in which the subject looked away from a fixation spot. It will allow you to use saccades and looking times as dependent measures, which can facilitate comparison with nonhuman primate research that uses saccades, and which are arguably underutilized dependent measures in cognitive neuroscience (Hannula et al. 2010). EEG data during the saccade will contain oculomotor artifacts that must be carefully removed or avoided before the data can be interpreted (Keren, Yuval-Greenberg, and Deouell 2010). Eye trackers can also be used to measure changes in pupil dilation, which can provide insight into cognitive processes (Granholm and Steinhauer 2004). Although the pupil has a relatively sluggish time course, there have been recent deconvolution methods that improve the temporal precision of the pupil response (Wierda et al. 2012). Finally, information from the eye tracker can be used to improve the quality of oculomotor artifact removal (Plochl, Ossandon, and Konig 2012).

3. *Electrode localization equipment* It is common in MEG research to record the precise location of the head with respect to the sensors, but this is infrequently done in EEG research. Most EEG research relies on standard templates of electrode positions. With careful EEG cap placement, the template locations are likely to be accurate to within 1–2 cm. Considering the spatial smoothing resulting from volume conduction, uncertainties of electrode positions within 2 cm are unlikely to have significant negative consequences for the results. However,

these uncertainties decrease the spatial precision of EEG, which may be detrimental when some spatial filters are applied such as the surface Laplacian or beamforming. If you plan on performing source localization analyses, and precise localization is important for the conclusions you hope to make from the data, using electrode localization equipment will be beneficial.

4. *A comfortable chair for the subject to sit in* This is more important than it may initially seem. If you have an uncomfortable chair, your subjects may shift around as they try to find a more comfortable position. This will cause movement artifacts in the EEG data. If the subjects are very uncomfortable they may become distracted from the task. If the chair promotes bad posture subjects might strain their back, shoulder, and neck muscles, which could introduce EMG artifacts in the EEG data.

5. *A good response device* Choose a response device that has good timing and is comfortable and intuitive to use. The response device should be easy to hold with an intuitive layout of the buttons. You do not want subjects looking down at their hands to figure out where the correct button is, and you do not want subjects to press the wrong button accidentally. Standard computer keyboards are poor choices for response devices: subjects may have to look down to confirm that they are pressing the correct button, and most keyboards have timing uncertainties on the order of several tens of milliseconds (some keyboards that are specially designed for gaming have millisecond-precision responses). The response buttons should not be too easy to press, otherwise subjects might not know whether their response was registered. On the other hand, if the buttons are too difficult to press, subjects may get tired. Some response devices make soft clicks when the buttons are pressed or when the circuit is closed. These clicks can be useful for subjects to know that their response was registered, although this may introduce confounds in auditory experiments.