Designing MEG Experiments

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Abstract With well-designed experiments, the exquisite temporal resolution of MEG allows investigators to track the temporal progression of cortical activity throughout the brain during sensory and cognitive tasks and further allows investigators to capture the interplay between the nodes of the cortical network activity underlying brain function. Because of this high temporal resolution, a number of considerations must be considered to obtain good quality MEG data. These considerations include: recording parameters, participant considerations, stimulus equipment and timing reliability, stimulus parameters and temporal sensitivity of the response. This chapter reviews the common instrumentation parameters, peripheral equipment that provides the precise timing needed for MEG experiments, and participant-monitoring equipment that provides complementary information for data quality and data interpretation purposes. Modality-specific (auditory, visual, tactile and motor) factors to consider during data collection are also discussed.

Keywords Magnetoencephalography (MEG) · Experimental design · Visual · Auditory · Somatosensory · Motor · Timing parameters · Peripheral equipment

1 Introduction

The goal of this chapter is to provide an overview of the parameters that should be considered when setting up and conducting MEG experiments. MEG provides an incredibly rich dataset from which to study brain function and dysfunction. In particular, MEG provides high temporal resolution at the time resolution that brain

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activity occurs (Kandel et al. 2000). In addition, MEG signals are not distorted by the skull, providing improved spatial resolution relative to EEG (Flemming et al. 2005). Therefore, one can obtain exquisite sensitivity to cortical network oscillations and the interplay between different cortical areas. However, this richness comes with multiple challenges. One of the biggest challenges of MEG is to identify task related activity in the presence of background brain activity. Resting brain activity, including resting brain rhythms such as occipital alpha and sensorimotor mu rhythms are 10–100 times greater in amplitude than evoked responses (e.g. the magnetic field generated by the presentation of an auditory stimulus). That is, the signal to noise ratio for a single presentation of a stimulus is <1. Therefore, a common method to identify stimulus related activity is to present multiple trials of the same stimulus to allow for signal averaging in the time, frequency or time/frequency domain. Further challenges include minimizing magnetic artifact from both internal and external sources of magnetic fields and capturing complementary data that can better guide interpretation of the results. MEG experimental design is therefore focused on optimizing all parameters to ensure that the high temporal resolution is maintained and signal to noise is optimized despite the challenges of background brain activity and other artifacts.

2 Instrumentation

2.1 Recording Parameters

The magnetic fields that are generated by the brain oscillate with the onset and offset of local brain activity (Hamalainen et al. 1993). Based on in vivo and in vitro characterization of neuronal activity, we know that the temporal profile of brain activity that generates these magnetic fields changes on the order of milliseconds (Kandel et al. 2000). This suggests that in order to properly capture the rapid changes in magnetic field associated with brain activity; data must be sampled at or around one sample per ms or 1,000 Hz. Furthermore, to capture the ongoing network interactions, it is important to capture this activity synchronously from around the head to allow investigators to characterize the interplay of cortical activity during task performance or during rest.

Therefore, current MEG systems record data synchronously from hundreds of MEG channels at digitization rates of between 100–5,000 Hz. This provides a temporal resolution of between 10 and 0.2 ms, respectively. This high sampling rate and the rapid neuronal response underlie the high temporal resolution of MEG. Table 1 shows the parameters that one must choose before beginning data collection on a standard MEG system. The choice of sampling rate depends on the required temporal resolution and spectral content of the data of interest. There are trade-offs between high and low sampling rates. While a high sampling rate may always appear to be better, long experiments may lead to prohibitively large data sets (a 10 min continuous dataset including 306 sensors sampled at 1,000 Hz is

Table 1 Recording parameters

Channels to record	MEG, EEG, A/D channels, trigger channels
Digitization rate	100–5,000 Hz
Online filter settings	High pass filter, anti-aliasing filter $< \frac{sampling\,frequency}{2}$
Trigger settings	Choose triggers, averaging epoch for online averaging display

approximately 1 GB in size). The typical sampling rate for visual, auditory and cognitive studies is between 300–1,000 Hz. A sampling rate of 300–400 Hz is often sufficient for averaged evoked responses for cognitive studies, where most of the spectral content in an averaged response is less than 60 Hz. However, median nerve stimulation requires a sampling rate of at least 1,000 Hz to capture the temporal profile of the M20 response. Also, recent interest in high frequency activity, which has been found in the somatosensory modality (Curio et al. 1997), during cognitive tasks (Uhlhaas et al. 2011) and in patients with epilepsy (Engel et al. 2009), may require a sampling rate or $\geq$2,000 Hz. Some systems allow for higher data acquisition rates when subsets of channels are chosen.

In conjunction with the sampling rate, an online anti-aliasing filter must be applied to ensure that higher frequency signals do not appear as an aliased low frequency signal. The anti-aliasing filter should be set at a frequency less than the sampling frequency/2. That is, if your sampling frequency is 300 Hz the online low-pass filter should be less than 150 Hz. In addition to the anti-aliasing filter, one can also choose a high pass filter setting on most MEG systems. This choice is left to the discretion of the investigator. The relevant question is whether there is any low frequency activity that might be relevant to the study. If one is interested in delta wave activity, it is best to choose the lowest cutoff option (generally 0.01 or 0.03 Hz). On the other hand, if the system is located in an environment with considerable low frequency noise, it may be desirable to eliminate low-frequency noise at the point of data collection.

2.2 Other Recording Channels

MEG systems also have additional channels that are recorded simultaneously with the MEG data. This option for simultaneous recording is critical to ensure that peripheral devices are truly synchronized with the MEG data. Trigger channels are supplementary channels that allow one to simultaneously record the timing of stimulus presentations. These channels accept transistor-transistor logic (TTL) pulses, which are standard binary pulses denoting on/off status. The width of the TTL pulse should be brief to allow for multiple triggers in short periods of time and it must be long enough that the sampling rate can sufficiently capture the onset and offset of the TTL pulse. Within these constraints the normal duration is

Table 2 Other recording channels

Trigger channels	Collect TTL pulse triggers (5–10 ms) from stimulus computer/equipment
Referenced EEG	Collect 1–128 channels of referenced EEG
Bipolar EEG	Electro-oculogram (EOG), electromyogram (EMG), electrocardiogram (ECG)
A/D channels	Allows collection of miscellaneous ±10 V analog signal

between 5 and 10 ms. These TTL trigger pulses can be generated by stimulus delivery software (e.g. NBS Presentation, Neuroscan StimII, Eprime) or by custom-built equipment. Additionally, some MEG systems provide an option to set periodic internal triggers (independent of external stimuli) to allow for epoching of the data (breaking the data into equal sized bins) if no stimulus triggers are present. These are often used to generate averaged spectra for noise runs or spectral analysis of resting-state MEG data.

Current MEG systems offer at least 64-channel referenced EEG capabilities allowing for simultaneous MEG/EEG recordings. In addition, at least 4 bipolar EEG channels are available for recording eye blinks and muscle movement. Our standard adult studies use two bipolar EEG channels to capture horizontal and vertical eye movements, respectively and one bipolar channel to collect ECG.

Finally, A/D channels accept any type of analog data generally within a ±10 V range. This allows one to collect any type of supplementary continuous data that is within the appropriate amplitude range. Examples of analog data that we have collected in MEG studies include: pressure transducer amplitude from a squeeze device to evaluate the strength of the squeeze (Berchicci et al. 2011), eye position and pupilometry data obtained from an MEG compatible eye-tracking system (Coffman et al. 2013), and voice recordings during task completion. A BNC connector is generally required to interface with the MEG electronics (Table 2).

2.3 Peripheral Devices

Since the high temporal resolution (∼1 ms) of MEG is one of its strengths, it is critical that temporal resolution is not compromised due to peripheral equipment. Most off-the-shelf equipment (e.g. computer sound cards, visual projectors or computer screens) is not tested for millisecond timing accuracy. Therefore, when choosing new equipment it is recommended to contact other MEG labs or the MEG manufacturer to obtain information about recommended devices. While MRI-compatible equipment available for fMRI studies is useful to control magnetic artifacts from peripheral devices, these devices are not always tested for high temporal resolution due to the lower temporal resolution of fMRI. In addition, it is recommended that you work with a representative of the company who has sufficient technical expertise of the peripheral equipment to determine the temporal

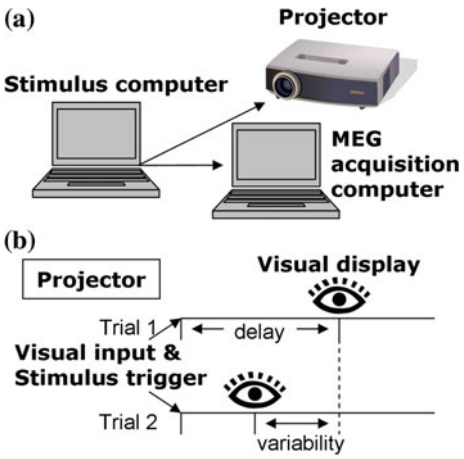

Fig. 1 **a** Basic visual setup. **b** Schematic of different timing parameters for evaluating an MEG visual setup

characteristics. In some cases, the companies are willing and able to allow on-site demonstration of the equipment. In this case it is recommended that you measure the temporal characteristics directly. Finally, it is important to test the timing of the final setup to ensure that the timing of the complete setup is accounted for (e.g. stimulus computer, amplifiers, peripheral equipment).

In accounting for timing, it is important to understand what factors may or may not introduce delays. Any signal that is transmitted at the speed of light is effectively transmitted instantaneously over the distances considered for MEG data collection. That is, signal is transferred along a 5 m long cable in $\sim$0.00001 ms at the speed of light leading to no measurable delay. However, electronic equipment (sound cards, electronic circuits, etc.) can introduce delays in the transfer of signal and should be tested. Furthermore, the speed of sound is considerably slower than the speed of light and any distance from the generation of the sound wave to the participants' ears should be accounted for in the delay calculation. The delay can be calculated based on the speed of sound in air ($\sim$0.344 m/ms). So for every 1/3 of a meter traveled in air, sound is delayed by 1 ms. All other signals need to be tested empirically.

Generally, the trigger is sent from the stimulus computer to the MEG electronics at the same time that the signal is sent to the peripheral equipment (see Fig. 1a). Therefore, the parameters to be tested are the *delay* of the peripheral device (defined as the time from when the signal was sent to the peripheral device to the time the stimulus reaches the participant) and the variability in this transfer time (*jitter*). If there is variability in the presentation time of the peripheral device, meaning that one presentation may occur 5 ms after the projector received the signal and a second presentation may occur 50 ms after the projector received the signal, this will not be captured by the trigger sent in parallel to the MEG acquisition computer. A delay in the peripheral equipment can be measured and accounted for in post-processing steps, however, jitter cannot easily be addressed based on triggers alone. The variability in the onset times can be large depending

on the equipment. This introduces a significant shift in latencies across trials thereby blurring the temporal resolution of the measured cortical response (leading to peak broadening and/or reduced amplitude due to cancellation across trials). Therefore, the optimal jitter is <1 ms. In some cases, one can still account for jitter (described in more detail below). However, experiments that require precise timing between stimuli (e.g. testing the ability to predict the next stimulus) or experiments that require multiple stimuli to be presented synchronously (e.g. multisensory integration studies) require consistent timing (jitter <1 ms) across trials to provide the required timing between stimuli.

The other significant challenge with peripheral equipment is identifying equipment that does not introduce artifact (strong magnetic fields) during data collection. This is often addressed by placing electrical equipment outside of the magnetically shielded room (MSR) and passing the signal/stimulus into the room through non-magnetic stimulus delivery systems. These can include shielded and properly grounded wires and fiber optic cables. Fiber optic cables are ideal for two primary reasons. First the signal travels at the speed of light, introducing no measurable delay in transfer of the signal. Second, the fiber optic cables are made of non-ferromagnetic materials (plastic sheathing and glass), thereby introducing no magnetic artifacts into the MSR. All other peripheral equipment including screens, response buttons, etc. should be built with non-ferromagnetic materials which include plastic, wood and brass. The prevalence of fMRI has made acquisition of non-ferromagnetic stimulus equipment more readily available. However, as mentioned throughout this chapter, not all MRI-safe equipment is suitable for MEG.

2.3.1 Bipolar EEG Channels

Bipolar EEG channels are used to monitor muscle activity. The most common use is to monitor eye blinks. It is important to have a set policy for eye blinks when providing your participant with instructions prior to beginning data collection for the MEG study. This however, can be difficult. If too much emphasis is placed on not blinking, the participants will almost invariably blink more (e.g., their eyes become dry which causes involuntary blinking). It is generally recommended that you tell the participants when they can blink rather than informing them that they cannot blink. “When you need to blink please blink after you’ve responded or blink between the stimuli.” Some studies (e.g. Tesche and Karhu 2000) have explicitly set aside a blink period between stimuli.

Regardless, it is important to use eye blink detection channels in most if not all MEG experiments. The magnetic fields generated by the muscles around the eyes are significantly larger than the magnetic fields of interest. This leads to two problems. First, eye blinks can completely swamp any signal that you are interested in measuring. Second, eye blinks are large amplitude events with a consistent field pattern so that there is very little chance that they will average out across trials. It is also the case that many subjects will blink in response to a stimulus (partially time-synched), making it even more likely that you will obtain a large

amplitude eye blink artifact. There are a number of different configurations that can be used to monitor eye blinks and eye movement. It is generally best to incorporate a setup that can monitor both vertical and horizontal eye movements. With two sets of electrooculogram (EOG) electrodes it is best to place one set of electrodes on the superior and inferior orbital ridges of one eye to monitor eye blinks and vertical eye movement and the second set of electrodes on the left and right outer canthi to monitor horizontal eye movements. With one set of EOG electrodes, one electrode can be placed on the superior orbital ridge of one eye and the other on the outer canthi of the other eye to incorporate both horizontal and vertical eye movements into one EOG channel.

Bipolar EEG channels are also useful for monitoring heartbeat. While it is highly recommended to monitor heart beat in clinical cases, it is not as critical to monitor in basic research studies. However, there are some subjects that exhibit significant heart beat artifact in their MEG. By recording the electrocardiogram (ECG), it is much easier to confirm and eliminate heart beat artifact from the MEG signal than if the data are simply not acquired. A standard placement of two EEG leads just below the left and right clavicle generally provides a good ECG recording. Heart beat artifact can be removed from the signal using projection methods described in Sect. 4.1.

Another common use of bipolar EEG channels is to monitor other muscle movement. These can be used with standard electromyogram (EMG) placements to monitor specific muscle activity to confirm or disprove mirror movements that may occur in cases of brain injury such as Cerebral Palsy or Stroke (Grosse et al. 2002). EMG channels have now been widely used to quantify coherence of brain oscillations with oscillations measured in the EMG to better understand the mechanisms associated with Parkinson's Disease (Timmermann et al. 2003, 2004).

2.3.2 Visual Equipment

Currently, projectors are the standard equipment used to present visual stimuli (often with the projector located outside the MSR such that it can project onto a rear-projection screen located within the MSR). Most off-the-shelf projectors do not provide reliable timing. The timing profile of a projector can be tested by collecting MEG data with the visual stimulus trigger and a photosensor attached to the screen. The photosensor signal should be routed to one of the analog-to-digital (A/D) channels and timing of the photosensor signal relative to the visual triggers can then be measured (see Fig. 2). Depending on the type of projector, timing may also vary across the screen (e.g. cathode ray tube (CRT) monitors) so timing parameters should be tested at the location of the visual stimuli. To test the timing parameters, a separate visual stimulus at the desired screen location should be used such that the stimulus changes from black to white (or vice versa) at the onset of the stimulus to provide a clear change in photo luminance for the photosensor. Collect approximately 30 trials to determine the variability in this timing measurement. If the maximum variability of this timing is low ($\sim$3 ms or less), then

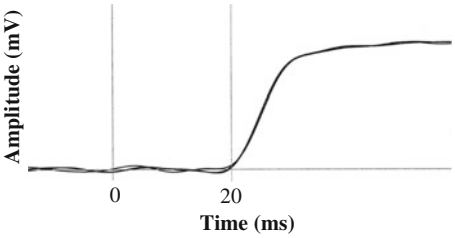

Fig. 2 Photo diode recording on an A/D channel. Time 0 is the time the trigger pulse reached the MEG data acquisition system. Time 20 ms is the onset of the photodiode response. This 20 ms delay denotes the delay from when the projector was signaled to present the stimulus to the time the stimulus was actually presented. Three trials are overlaid showing no difference in timing and represents <1 ms jitter. When jitter is present the onset of the individual trials is variable relative to time 0

one can record the absolute timing difference (delay) and use this as a correction factor for the timing of the visual response after data processing. If the variability is high, then one should incorporate the photo-diode in your studies and use the photo-diode signal as the visual stimulus onset trigger for averaging across trials. Variability in visual stimulus presentation can also be minimized by optimizing the timing of stimulus presentation relative to the projector refresh rate. The stimulus onset for visual studies should be a multiple of the refresh rate of your projector so that the signal is received by the projector at the same phase in the refresh cycle (e.g. a 60 Hz refresh rate means stimuli should be presented at multiples of 16 ms). This is also relevant if you are trying to present carefully timed stimuli such as characterizing the frequency response of the visual system. Again, it is best to confirm the actual projector oscillation rate with a photo-diode.

There are two primary types of projectors that are currently being used for MEG studies, liquid-crystal display (LCD) and digital light processing (DLP) projectors. DLP projectors have the best temporal characteristics for MEG studies (low variability (<1 ms), and synchronous color presentation for 3-chip DLP projectors). However, the price of these projectors is often prohibitive. Some LCD projectors also have low variability in stimulus onset from trial-to-trial. Both of these projectors often have a 20–40 ms delay from the time the projector receives the signal to the time the stimulus is presented. A few MEG systems are compatible with using monitors for displaying visual stimuli directly. However, LCD monitors have not been well characterized in terms of timing parameters. Some measurements from our lab suggest that timing jitter can be high in LCD monitors and should be carefully tested.

Another important projector variable to consider is brightness. Many commercially available projectors are designed to project tens to hundreds of feet. The path length from the projector to the screen is ~ 3 m for MEG rooms. This leads to intense lighting for visual studies which can produce significant eye strain. The projector menu may allow for brightness control. An additional option is to buy a neutral density filter that reduces the brightness across all projector settings. While one of the

motivations behind reducing eye strain is to make the experience comfortable for the participants, reducing eye strain also reduces eye movement artifacts and tearing during task performance.

2.3.3 Auditory Equipment

Ear Inserts: MEG labs often use foam ear inserts connected by tubing to Etymotic sound transducers placed between 0.5 and 3 m from the MEG helmet. These sound transducers can be placed within the MSR and generate minimal noise. One advantage of these devices is that the signal is transferred at the speed of light until it reaches the sound transducer. The slower speed of sound ($\sim$0.344 m/ms) will introduce delays in the auditory signal, which need to be accounted for based on the distance to the participant once the signal is converted into a sound wave (length of the tubing from transducer to participant). Other delays and jitter in the auditory stimulus timing can arise from the stimulus computer sound card or speaker electronics. Another consideration with presenting sounds via tubing is that the manufacturer characterizes the sound quality for a specified tube length (the sound will be attenuated with longer tube lengths). Tubing also acts as a filter, thereby limiting the frequency range of the stimuli that can be presented through this setup. Etymotic sound transducers are supplied with a frequency response curve that is calibrated to a recommended tube length and tube characteristic. If different lengths, diameter or rigidity of the tubing are employed additional sound characteristic testing would be required. Unfortunately, MRI-compatible headphones are not feasible for MEG systems because headphones generally do not fit within the MEG helmet.

Speakers: Standard speakers are used in some MEG studies, e.g. (Stephen et al. 2012). However, sound is generated from standard speakers through movement of magnets, therefore, they are not artifact free. Some flat panel speakers generate minimal artifact relative to traditional speakers and maximizing the distance between the speakers and the MEG helmet also reduces the amplitude of the noise. With significant artifact it is important to recognize that speakers are active for the full duration of the auditory stimulus, therefore, it is important to ensure that one can eliminate speaker-generated artifact from MEG data through data processing if the stimuli will be longer than $\sim$50 ms. Finally, speakers within a closed room do not provide the same characteristics as open field sound sources. Sound dampening material on the walls can improve sound characteristics within the confined space.

2.3.4 Somatosensory Equipment

Electrical Stimulation: Direct electrical stimulation of a nerve (e.g. median or tibial nerves) provides temporally precise somatosensory stimulation. Timing of the system can be tested by recording the electrical output used to stimulate the nerves relative to the stimulus trigger. However, electrical stimulation can introduce artifacts. Twisting the wires that travel from the stimulator to the nerve helps to minimize artifact from signal traveling through the wires. Despite these artifacts,

stimulation of nerves provides a reliable stimulus and a very short duration pulse (0.5 ms) can be used to obtain a robust cortical response. Therefore, artifact is limited to a brief period before cortical activation. Finally it is important to recognize that the distance traveled along the peripheral nerve (from the location that the nerve is stimulated to the brain) will induce delays in cortical activation. Unlike auditory and visual systems where differences in the length of the peripheral nerves are, negligible, there is considerable variation in height across participants with systematic differences in height by gender leading to potential group differences. Therefore, recording height from study participants is useful to ensure height differences do not account for group latency differences.

Vibration Stimuli: Tactile devices can be driven with an oscillatory electrical signal to generate a vibration stimulus when placed directly on the skin. This stimulus can provide precise timing for the somatosensory stimulus since the electrical signal is converted directly to vibratory motion. However, these devices generally require that the electrical motor be located close to the skin, again causing varying levels of artifact from the device.

Pneumatic Stimuli: Pneumatic stimuli are often generated by an air puff presented directly to the skin to activate hair sensory receptors or a puff of air filling a balloon to generate a pressure stimulus. The pneumatic stimulus provides a non-threatening somatosensory stimulus for pediatric populations and is artifact-free, if the air regulating device is located outside of the MSR. However a pressure stimulus introduces a significant time delay based on the time that it takes for a pressure stimulus to travel along the plastic tubing from the external air regulator to the participant (approximately the speed of sound). This requires that a pressure transducer be available to assess the time delay of the stimulus relative to the trigger. Also, rigid tubing is essential to preserve the pressure profile across the 3–5 m distance.

2.3.5 Motor Equipment

Equipment used to assess motor function is primarily designed to capture the onset of motor activation. The different types of equipment used in motor paradigms are described below.

Finger lift device (Fig. 3): A finger lift device is often comprised of fiber optic tubing connected to a light source at one end and a photo diode at the other with a break in the middle. Both the light source and the photo diode are located outside the MSR. The trigger is generated either when the light beam is broken or when the light beam is allowed to pass to the photo diode. In any case, breaking or connecting the light beam provides a rapid transition that the photodiode registers and is then converted to a TTL pulse acting as a stimulus trigger. Many systems are designed to trigger either at the time the light beam is interrupted or at the time the light beam passes through unimpeded.

Squeeze ball: A squeeze ball has been used to obtain a larger motor response than the finger lift task and it allows certain patients to perform a motor task who may not have sufficient manual dexterity to perform the finger lift task (e.g. patients

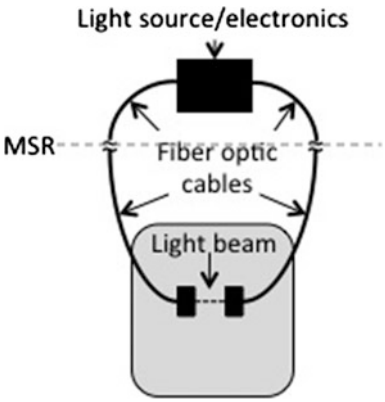

Fig. 3 Example Fiber Optic Motor Apparatus. The light source and electronics that identify triggers are located outside the MSR. The light source is connected to one side of the fiber optic cable loop and the light is delivered back to the electronics through the other side. The hand rests on the motor pad (*grey* platform) and the finger is aligned such that it interrupts the light beam when it is lowered to the motor pad. The electronics can be set to trigger based on the interruption or completion of the light beam across the space on the motor pad

who have suffered a stroke). Onset of motor function in this case, is registered when the ball is squeezed. Release of air or water from the squeeze ball can push an object that in turn breaks a beam of light (e.g. fiber optic cable) or through a sudden change in pressure registered by a pressure transducer (generally located outside the MSR). However, the delay in registering the squeeze can be quite long if the signal is measured by a pressure transducer at the end of the tube located outside the MSR due to the slow speed of a pressure stimulus traveling along a tube. Furthermore, the pressure profile can be quite variable depending on the strength of the squeeze, thereby making it challenging to define a trigger with low jitter.

EMG signal: As mentioned above, bipolar EEG channels can be used to collect EMG signals by placing them on the muscle group of interest (with an appropriate reference location for the second electrode) to capture onset of muscle movement. EMG signal that is recorded simultaneously with the MEG data provides signal with no equipment induced delay or jitter. However, EMG signal can be contaminated by muscle activity that is not of primary interest to the specified task, if the electrodes are not placed correctly or if the participant cannot isolate the movement for task purposes only. Furthermore, the EMG signal needs to be converted to a trigger signal using post-processing methods to indicate movement onset. Varying levels of movement quality (slow vs. fast onset) may also lead to ambiguous movement onset for trigger creation.

Response Devices: MEG systems are generally equipped with artifact-free response devices that record the participant's response during cognitive tasks to collect behavioral reaction times and accuracy. These devices can also be used to signify onset of motion in a finger lift task. See Sect. 2.3.6.

2.3.6 Behavioral Response Devices

It is important to have some type of behavioral response device which is compatible with the MEG system. This allows one to not only obtain behavioral information about how individual participants performed the task, but also provides some confidence that the participants are performing the task, as instructed. While many of the MEG manufacturers provide four button response pads, it is often useful to develop a reaction time device that allows for responses from all fingers. One example of this type of device has been developed by Michael Doty at the Mind Research Network (http://www.mrn.org/collaborate/imaging-equipment/). This is a fully optical system with non-metallic buttons and is also fully compatible with MRI. One particular challenge in developing a noise free response device is finding reliable response buttons that do not have ferromagnetic springs. Yet, it is critically important to ensure that response pads do not generate any noise due to the variability in responses that can and will generate artifacts throughout much of your data set. Also, there should be no significant delay between when the response button is pressed and when the information is registered to the stimulus or acquisition computer. It is also useful to have an ergonomically comfortable device to ensure that participants do not tense their shoulders or become uncomfortable, leading to potential muscle artifacts in the MEG data.

3 Experimental Design Considerations

3.1 Interstimulus Interval (ISI)

One of the important factors to consider when designing an MEG study is determining the rate at which stimuli will be presented. The interstimulus interval (ISI) defines the time between stimuli. This timing parameter must be balanced between keeping the interval between stimuli short to decrease overall task duration and minimize participant fatigue, while optimizing the cortical response for the proposed task. Numerous studies have described the impact of different ISIs on brain function. Rapid ISIs tend to decrease secondary and higher order brain activity and emphasize primary sensory activity (Wikstrom et al. 1996). However, primary sensory activity also decreases with rapid presentation of repetitive stimuli (Hari et al. 1982). In contrast, designing experiments with long ISIs will increase the overall duration of data collection, thereby contributing to participant fatigue. Therefore, a number of factors should be considered when choosing ISI.

1. It is important that stimuli are sufficiently separated in time such that the cortical processing associated with the previous stimulus has ended prior to the presentation of the next trial. For example, the cortical response to median nerve stimulation is complete by $\sim$400 ms after stimulus onset (see Fig. 4).

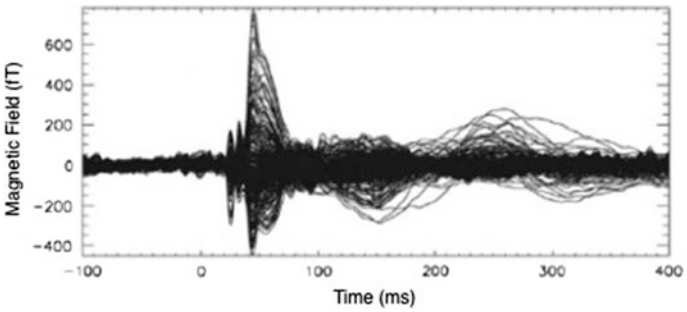

Fig. 4 Somatosensory response to median nerve stimulation. The median nerve stimulation was presented at time (t = 0 ms). The MEG channels are overlaid to show the response across the MEG array. A baseline time interval (−100, 0) is shown prior to stimulus presentation. The response has returned to baseline levels by 400 ms post-stimulus

Therefore, stimuli can be presented every 0.5 s. On the other hand, language stimuli for example evoke a more protracted cortical response (Aine et al. 2005) requiring that the time between stimuli be longer. Therefore, ISI should be determined based on the previous literature or empirical testing of the response across a range of ISIs.

2. The ISI must also include sufficient time to provide a baseline time interval between the offset of the cortical response to the previous stimulus and the onset of the stimulus for the following trial. Due to the natural drift in MEG channel amplitude over time, most MEG studies employ baseline correction during data processing. Therefore, the ISI should be chosen such that the interstimulus interval is greater than the (baseline time interval) + (duration of the cortical response). The duration of the baseline time interval varies depending on the paradigm and the analysis to be performed. Following the example provided in Fig. 4, the baseline time interval chosen for median nerve stimulation is often 100 ms.
3. The duration of stimuli is an important consideration when determining ISI. If a visual stimulus is presented for 1 second, the onset of subsequent visual stimuli must be separated by approximately 1.5 s. This provides sufficient time for the visual off-response and a baseline time interval between stimuli prior to the onset of the next visual stimulus.
4. Varying ISI across trials also helps eliminate anticipatory responses such as the contingent negative variation (CNV) response first identified in EEG studies (Rohrbaugh et al. 1986). Furthermore, introducing variability in the ISI also helps to limit anticipatory behavioral responses during repetitive tasks (participants may respond with a button press prior to stimulus presentation). However, some paradigms require a constant ISI (e.g. studies that specifically focus on understanding the ability to predict stimulus timing). Finally, by varying the ISI, one may help reduce habituation of responses (i.e., a reduction in amplitude across time to a repetitive stimulus presented at a constant ISI).

5. During cognitive tasks it is also important to take reaction times into consideration when determining ISI. It is important to provide sufficient time for the participant to respond prior to the onset of the next trial so that brain activity in the following trial is not contaminated by motor responses from the previous trial. Slower reaction times often associated with patient populations should also be considered. One approach is to allow for dynamic changes in ISI by initiating the next trial as soon as a response is made. However, this may introduce systematic group differences in ISI if a patient group is consistently slower than the control group, leading to an experimental confound as described above.
6. Finally, the number of trials per condition is also a consideration when determining ISI. As described in the signal averaging section below, most stimuli in MEG studies are presented 10–100 s of times to allow for noise reduction through signal averaging. However, the number of trials per condition and the ISI interact to determine the duration of the task. For example, a study with 2 conditions with 100 trials per condition and an average ISI of 1 s will take 3.3 min. If the ISI is doubled, the data collection time will also double (6.6 min). Balancing the number of trials with the ISI helps to optimize signal quality and task duration to ensure participants can provide good quality data and attentive responses throughout data collection.

In summary, it is important to balance timing parameters with other considerations such as participant fatigue and task complexity to obtain high quality MEG data based on the constraints of the experimental paradigm.

3.2 Training the Participant

It is important to allow time for the participant to practice the task for a number of reasons. Once data collection has begun, it is important that the participant feel comfortable with task instructions to minimize the likelihood that data collection needs to be stopped due to confusion over the task. Starting and stopping data collection is problematic and can lead to participant fatigue and frustration as well as introducing variability in data acquisition time across participants. Therefore, it is best to get the participant comfortable with the setup and the stimuli and the required responses prior to data collection. If the experiment is incorporating a behavioral task, one might set a percent correct criterion during the practice session to decide how long the subject practices the task. Depending on access to the machine, practice can occur in the MSR or at a practice computer.

3.3 Habituation

It is also useful to randomize different conditions within an experiment for a number of reasons. First, cortical responses are largest in response to changing stimuli. Using visual stimuli for demonstration purposes, if a participant is expected to look at the exact same visual stimulus over a long period of time, the salience of the stimulus will fade due to the physiology of the visual system. Therefore, if you are testing both left and right visual fields it is best to randomize the left and right stimuli within blocks. This randomization also helps to prevent a shift in gaze away from the fixation point. While it is most common to place a small cross-hair at the location that the participant is supposed to maintain visual fixation, if all of the stimuli are below the visual fixation, for example, participant gaze will tend to shift below the intended fixation point. Randomizing stimuli, such that the average location is at the fixation point, helps to minimize fixation drift. If the experimental design does not allow for full randomization of the location of the stimuli, then it is best to block the stimuli in relatively small blocks and present different locations in blocks of ~ 30 stimuli per location, while presenting as many blocks in a randomized fashion to allow for the desired number of averages. Randomizing the conditions across the entire data collection period also helps to ensure that differences in responses between conditions are not simply due to changes in attention across time. Similar habituation considerations are important for auditory, somatosensory, motor and cognitive paradigms.

3.4 Subject Positioning

It is important to consider the primary areas of interest when positioning the participant in the MEG dewar. For participants with large heads, placement within the dewar will not be a consideration. However, a large number of subjects have significant room to move their head both front and back and side-to-side in the current MEG helmets. It is generally best to try to center the head as much as possible from left to right, unless your hypothesis focuses specifically on a well-documented lateralized response. However, for a basic visual study, you should encourage the participant to move their head back as far as possible and perhaps tilt the head forward a bit to provide additional coverage below the occipital cortex. On the other hand, if you want to focus on orbital frontal cortex, moving the head forward and tilting the head back would be most ideal for optimal coverage of the area of interest.

Furthermore, when the subject has sufficient room in the helmet to move their head around, it is important to provide some mechanism to help maintain head position within the dewar. Placing covered foam pieces on either side of the head near the cheekbones generally works well both in providing the subject with tactile feedback while also maintaining head position. Another alternative for head

stabilization sometimes provided by the MEG manufacturer is an inflatable bladder placed around the head where different sections may be independently inflated. These systems sometimes make the participant hot or uncomfortable.

3.5 Artifact Prevention

Artifacts are one of the most challenging aspects of collecting good quality MEG data. The sources of artifacts include both external and internal factors. External factors include any large ferromagnetic object that moves close (up to 0.5 km away) to the MEG system. That is, elevators, cars, gurneys, chairs, etc. can all generate noise in the MEG system. Fortunately, the noise generated by these examples is very low frequency. This type of noise is problematic if the MEG amplifiers become saturated and leads to data loss. Identification of these artifacts is generally performed by working as a team to monitor MEG activity while another individual observes external activity.

There are also a large number of artifacts that can be associated with the participant. Clearly, it is important that the participant remove all electronic devices before entering the MSR, including cell phones, pagers, watches, etc. The largest problem is with dental work. Permanent bridges are almost invariably too noisy for good quality data. Unfortunately, the frequency range of noise generated from dental work directly overlaps with physiological signal. Therefore, it is challenging to eliminate this noise from the signal without also losing signal of interest. It is also heterogeneous across data collection, making projection techniques such as that used for eyeblinks and heart beat artifact unusable. It is important to ask the participant to take out all removable dental work. Sometimes de-gaussing will work in removing magnetization from permanent dental work. If the participant is a member of a difficult-to-recruit study group, it is important to attempt de-gaussing at least a couple of times. While participants with removable dentures may seem to be ideal subjects, the absence of dentures may lead to more mouth movement and muscle artifact.

Muscle artifact is the next largest contaminant to MEG data. Both eye and mouth movements affect the MEG signal. In general, the magnetic fields generated by muscle movement are much larger than the magnetic fields generated by brain activity. Therefore, necessary muscle movements, such as eye blinks, present a constant problem for MEG. The participant may also have habits that lead to artifacts that include muscle movement such as tensing the jaw or shoulders. Mouth movements can be particularly difficult for MEG since the jaw muscles extend posteriorly across much of the head. This artifact is best identified by asking the subject to consciously tense their jaw or shoulders and then asking the subject to consciously relax while one is observing the continuous MEG signal. Some subjects are tense when they first start a study, but relax once the study begins. If this is a possibility, it is useful to let the subject practice the task to help them settle into the environment.

The other main source of artifacts originates from participant clothing and other accessories. All piercings should be removed prior to data collection unless it is known that the piercing is non-ferromagnetic. Some mascara, makeup, hair dye and finger nail polish can have metallic ingredients. Mascara can generate amplified eye blink artifacts. Breathing artifacts can be seen from a number of different sources. (1) T-shirts with metallic ink in the silk screen; (2) underwire bras; (3) clothes with metallic dyes; and (4) belts. While it is best to encourage participants to come dressed in plain metal-free clothes, an alternative is to provide metal free clothes (e.g. medical scrubs) to participants.

4 Data Preprocessing

4.1 Artifact Removal

The first priority with MEG artifacts is to minimize the contribution of artifacts that contaminate MEG data. As mentioned above, a number of sources of artifacts can be eliminated prior to data collection. However, there are a number of artifacts that cannot be eliminated entirely (e.g. flux jumps, eye blinks, movement artifact, etc.). For the artifacts that remain, there are two competing goals when removing artifacts from data. If the artifact is a large amplitude, rare event, then it is necessary to eliminate it from the signal by removing the trial, since it is very unlikely to be reduced by signal averaging. On the other hand, it is important to maintain as many trials for each condition so that one gains the advantage of signal averaging for low amplitude noise.

The most reliable method for eliminating artifacts (i.e., guarantees that the artifact will be removed without removing any signal of interest) is to eliminate any trials that contain artifacts. If you are able to collect more trials than needed, then trial removal can be performed either using automated or manual methods. For example, eye-blink rejection is often performed by eliminating any trials that contain a signal that exceeds 75 μV in the EOG channel. Additional criteria may be included which only eliminate blinks in the eye channel within a certain time range relative to the stimulus trigger (e.g. eye blinks that occur after the signal of interest). This approach can also be used for large movement artifacts (e.g. cough or shifting position). Often these trials are identified by setting an upper bound on the magnetic field strength ($\sim$2,000 fT) and eliminating trials that exceed that value. However, if one channel is noisy throughout the entire recording, then it is recommended that the channel not be used (turned off/marked bad) for the analysis rather than eliminating bad trials based on this channel.

Additional methods for artifact rejection provide mathematical solutions to artifact rejection. However, these techniques run the risk of eliminating signal as well as noise in the artifact removal process. For example, eye blinks can be identified by using an eye blink template. Whenever a sufficient match is made with the template the magnetic field associated with the template eye blink is

projected out of the data (Uusitalo and Ilmoniemi 1997). This technique can be very useful when eye blinks are relatively homogeneous to maximize the number of trials retained in the average.

Independent components analysis (ICA) has also been used to eliminate artifacts from MEG data. The advantage of ICA is that artifacts should be independent of the brain signal of interest. Therefore the underlying assumption of the method is valid. This technique has been used by many MEG groups (e.g. Vigario et al. 2000; Iwaki et al. 2004; Mantini et al. 2007). However, there are a number of different forms of ICA. Some of the ICA programs separate the data into many components as decided upon by the user. Others separate the data into the same number of components as number of input channels. Either way the actual assignment to any particular independent component is random. Therefore, it is necessary for the investigator to determine a method that identifies artifact versus signal components. Depending on the artifact, this may or may not be obvious.

4.2 Removal of Bad Channels

The choice to remove bad channels is based on two factors. If the channel is bad because of technical difficulties with the SQUID, the noise is clearly not physiological with multiple square wave jumps throughout the dataset. These channels should be eliminated since they do not provide any useful information regarding brain activity and yet can dramatically bias source modeling. The other factor is physiologic noise. Sometimes eye blinks can be found throughout the entire dataset. If none of the above artifact removal options appear to solve the problem, it may be more useful to delete channels that are largely affected by eye blinks. This is done, for example, if you are not interested in activity in brain areas near the eyes. Most, if not all, MEG analysis programs allow you to toggle bad channels on and off. So the data is not deleted, it is just not marked for display and analysis purposes. Again, it is important to balance the two factors of retaining as much information as possible, while also eliminating as much noise from the signal as possible.

4.3 Filtering

The choice of filter settings should be carefully considered. Historically, ERP recording equipment limited the dynamic range of the signal leading to narrow filter settings. Some MEG studies have followed these filter settings since this facilitates direct comparisons with previous ERP work. However, the acquisition equipment for both EEG and MEG is far advanced at this time. Filter settings can be adjusted during post-processing steps and it is recommended that acquisition filters be set as wide as possible. Due to these early filtering restrictions, both slow wave activity and high frequency gamma were not initially reported in ERPs (filtering was often

set with a bandpass of 5–30 Hz). Our results have described the importance of slow wave activity in cognitive tasks (Aine et al. 2003, 2005). Recent EEG and MEG studies have also identified the role of high gamma oscillations in cognitive tasks (Engel et al. 2009; Uhlhaas et al. 2011).

4.4 Averaging

Signal averaging is still the norm for obtaining reliable evoked responses in MEG studies. This requires a trigger from which to average the signals. As described above, these triggers can either be generated by a program that delivers the stimuli to the subject (e.g. trigger pulse sent from Presentation program) or by a device that measures when the stimulus is presented to the subject (e.g. photo-diode). After eliminating any noise sources from individual trials, the trials for each condition are then averaged together. This allows for an increase in signal to noise ratio (SNR) that is approximately equal to $\sqrt{N}$ where N is the number of trials. This relationship is exact in the case of truly Gaussian white noise. It is only approximate in cases where the noise is not truly random as is the case with brain noise. Therefore, if there is a consistent noise source that is time-locked to the stimulus (e.g. the participant always blinks with the presentation of a visual stimulus or artifact from a stimulation device), the signal will not average out.

It is important to check various factors when performing signal averaging. For example, it is useful to compare the averages between the 1st and 2nd half of the recording session or the average of the even versus odd trials. This can be easily automated. It ensures that the average is not biased by the presentation of the first few trials (as in the case of habituation) or by a random noise event that was not eliminated using other artifact removal techniques. It is also important to define a unique trigger for each stimulus condition. It is easy to automate averaging across conditions. However, it is not easy to separate out different conditions after data acquisition, if one does not provide unique triggers for these conditions at the outset. The generally accepted number of averages that are needed to obtain good SNR in most MEG studies is a minimum of 100 trials/condition. This number may be larger or smaller based on the amplitude of the signal of interest. For example the high frequency activity reported by Curio et al. (1997) required thousands of trials to obtain the necessary SNR. On the other hand, inter-ictal epileptic spike activity provides sufficient SNR for single trial analysis in many cases.

Signal averaging has some disadvantages because it assumes that the signal of interest is exactly time-locked to the stimulus and identical on each trial. If these assumptions are not true, the variability from trial to trial will be lost in the averaging process. Time-frequency analysis has provided an additional means to look at activity that is related to the signal and yet not perfectly time-locked with the stimulus (Tallon-Baudry et al. 1996). This method of analysis is especially relevant for high frequency signals such as gamma activity (>30 Hz), since without perfect time-locking this activity will average out based on the rapid oscillation rate.

5 Visual Experiments

5.1 Stimulus Parameters

Stimulus parameters for visual experiments are discussed in more detail in the chapter describing visual studies (Aine et al. this volume). These parameters include but are not limited to: visual stimulus characteristics such as visual contrast, luminance, spatial frequency, size and timing. Below we describe the parameters that one must consider with respect to designing a visual study to provide consistent visual stimulus presentation across participants.

5.2 Ambient Lighting

During visual experiments it is important to maintain similar ambient lighting conditions across participants. Most MSRs include a dialed light switch that allows one to choose a consistent setting across participants for each experiment. The difference in ambient light is important since it changes perceived contrast levels. Differences in contrast cause differences in onset latencies with higher contrast visual stimuli leading to shorter onset latencies (Robson 1966; Campbell and Kulikowski 1972; Okada et al. 1982). It is also important to consider ambient light with regards to stimulus brightness. If the background lighting is turned down, then the perceived brightness will be greater.

5.3 Calculating the Visual Angle

The visual angle of a stimulus can be calculated by measuring the size of the stimulus (size) and the distance from the stimulus to the participant's eyes (dist). Generally, one can use the distance from the stimulus to the participant's nasion as a good approximation. It is important to use identical units when measuring size and distance as well as being aware of whether the output of the inverse tangent function is reported in radians or degrees. Use the following equation for the calculation:

$$\theta = 2 \cdot \tan^{-1}\left(\frac{size}{2 \cdot dist}\right)$$

5.4 Calculating the Cortical Magnification Factor

In order to activate similar amounts of primary visual cortex across different eccentricities, it is important to apply the cortical magnification correction factor. More cortical cells are devoted to the central visual field than to the peripheral visual field. Therefore, to activate equivalent patches of cortex, the peripheral visual stimuli need to be larger than the central visual stimuli. The human cortical magnification factor was most precisely mapped out by Rovamo and Virsu (1979). They provided a cortical magnification factor for stimuli in peripheral field in the nasal, superior, temporal, and inferior directions. They suggest linear interpolation between these four equations when trying to equate activation along other meridians. Horton and Hoyt (1991) derived an equation based on fMRI and occipital lesion studies in humans that provides an approximation for all directions:

$$M_{\mathbf{linear}} = \frac{17.3}{E + 0.75},$$

where, E is the eccentricity in degrees and M is the linear correction factor in mm/degree. This equation agrees well with the dimensions determined for non-human primates while accounting for the larger size of the visual cortex in humans. Horton and Hoyt also provide an areal correction with the assumption that the cortical magnification is isotropic. While this deviates from the results of Rovamo and Virsu, it is perhaps a reasonable approximation for neuroimaging studies as suggested by the agreement of these results with PET and phosphene mapping.

5.5 Measuring Luminance

Matching luminance of the stimuli and background is important to ensure that differences in responses are not generated based on simple luminance changes throughout the experiment. Luminance measures are performed using a light meter and are a measure of the total light output for a part of a stimulus for a given period of time. A full description of how one measures luminance and mean luminance for complex stimuli such as visual gratings is described in detail by Brigell et al. (1998).

5.6 Vision Correction

It is important to have a method to correct for differences in visual acuity across participants since blurred images tend to produce lower amplitude responses and differences in the ability to see the stimuli will lead to differences in task difficulty.

Although vision correction is generally only considered when performing visual studies, it is also advisable to offer vision correction during a nonvisual MEG scan since some individuals get a headache without their glasses. Vision correction can be a challenge in MEG because in adults eyeglass frames do not fit in the MEG dewar and most eyeglasses contain ferrous screws, including glasses with titanium frames. Unless an individual has MRI-safe glasses, wearing glasses will likely cause artifacts. If the participant needs vision correction there are three standard options.

Contact lenses. One option is for the individual to wear contact lenses. However, many individuals blink more frequently with their contact lenses in place. Therefore, it is advisable to have other vision correction options.

Pinhole glasses. A simple option for vision correction is pinhole glasses. If the individual only needs to fixate on a chosen point throughout the task, a single pinhole, in a piece of paper for each eye can be created. This approach addresses difficulties with nearsightedness, farsightedness and astigmatisms. Despite its wide-ranging use, the challenge of attaching the pieces of paper to the participant in such a way that the pinhole remains in place throughout the experiment remains. Often tape is the best option. The drawbacks of this approach are that it can be annoying to participants since it severely limits their field of view and it may be viewed by participants as a low-tech approach to vision correction.

Optical lenses. A complete set of optical corrective lenses can be purchased. These sets include lenses to help account for myopia, hyperopia and astigmatism. The lenses can either be taped to the subject or a device compatible with the MEG system can be designed to hold the lenses in front of the subject. These corrective lenses are also compatible with MRI systems. MRI compatible glasses with interchangeable lenses are also an option; however, they should be tested prior to purchase due to the space limitations of the MEG dewar. The clear advantage of these lenses is that one can match the individual's eyeglass prescription.

5.7 Eye-Tracking

MEG compatible eye-tracking systems are now available commercially. These systems can be an important complement to MEG data collection by providing confirmation of experimental compliance (participant fixates as instructed), testing emotional responses to stimuli by capturing the pupillary diameter, analyzing the participant's eye-movements throughout a task (e.g. quantifying eye-position during a face processing task), or for understanding the eye-control network (saccades). It is important to acquire an MEG-compatible eye-tracker since standard eye-tracking systems use a head-mounted device that does not fit within the MEG helmet. The MEG-compatible systems perform eye-tracking through a remote camera. A couple of factors to consider while designing a study with an eye-tracking system are:

(1) These systems currently require that head position relative to the eye-tracker camera remain constant. These systems require highly restricted head movement similar to MEG systems that do not have head movement compensation.
(2) Vision correction options (e.g. contact lenses) generally eliminate the ability to perform eye-tracking experiments since the corneal reflection is used to quantify the eye-movements and additional reflections interfer with capturing the corneal reflection.
(3) Eye-tracking will fail in a certain number of participants due to a number of factors that inhibit the ability to capture the corneal reflection (e.g. droopy eyelid, amblyopia, etc.).

Therefore, careful selection of participant group and task design is important prior to requiring eye-tracking for a study.

6 Auditory Experiments

6.1 Stimulus Parameters

All auditory parameters can be manipulated using currently available software. In light of the fast temporal processing that occurs in the auditory system including at the cochlear, brainstem and cortical levels, it is important to understand the characteristics of the stimuli that are being presented. Simple tones represent one frequency and can easily be generated in Matlab. However, any sudden onset of a sound represents a square-wave transition and thereby activates frequencies across the frequency spectrum. Therefore, when testing tonotopy or simply reporting that a simple tone was presented, it is important to increase the volume gradually over a short period of time to reduce the 'click' associated with a sudden onset/offset of a sound. This is commonly performed by applying a 10–20 ms amplitude taper to the onset and offset of the tone (e.g. Hanning window). More complex auditory stimuli can also be characterized through a spectrogram to characterize the contribution of an array of frequencies to the sound. To ensure good matching of stimuli across conditions, it is good to match stimuli on the basis of duration, mean amplitude and frequency content.

6.2 Auditory Threshold Testing

Auditory threshold testing should be performed to account for differential hearing loss across participants. This can vary widely in participants at all ages. The testing should be performed at frequencies that characterize the auditory stimuli in the study. If you are using auditory inserts for presenting auditory stimuli, these should be inserted just prior to data collection and auditory thresholding should be performed with the ear inserts in place. The placement of the ear inserts influences the

perceived volume and auditory threshold testing is sensitive to minor adjustments to this placement. If there is a large difference in auditory threshold between ears, it may be related to poor placement in one of the ears. Repositioning and retesting of the auditory threshold is recommended in this case. With a speaker setup, auditory threshold testing can occur at a prior visit, assuming that the volume can be carefully controlled from one visit to the next. The general approach for auditory threshold testing is to present volumes that are well above and well below threshold and have the participant respond to every sound they hear. This requires an adaptive program that continually decreases the interval between the above and below threshold sounds. Randomly presenting tones of different volumes and randomizing the time between stimuli, while working toward the ultimate goal of identifying the threshold helps to eliminate the possibility of false reports.

6.3 Volume Assessment

Volume can be measured using a sound meter. Volume should also be tested with the stimulus program and any sound equipment used, to determine if the actual sound volume is consistent with the expected volume output. For example, the volume increases/decreases by a specified dB level based on programming parameters in the Neurobehavioral systems Presentation software. We have found our system to track well with the expected increases and decreases in sound volume, although the absolute volume is larger than reported. Furthermore, the length of the tubing from the sound transducers/distance from speakers will change the volume level accordingly. The volume should be measured to emulate the conditions of the stimulus. Therefore, if sounds are being presented through ear inserts, the ear inserts should be connected to the sound meter with a piece of tubing at a distance approximately equivalent to the distance to the tympanic membrane. The volume from speakers should be measured with open air access to the sound meter sensor at the approximate location of the participant.

7 Somatosensory Experiments

7.1 Stimulus Parameters

There are three different types of somatosensory stimulation that have been employed in MEG studies: direct nerve stimulation with electric pulse, pressure stimulus generated by a balloon, and vibration stimuli. There are six different tactile receptors in the skin and each of them responds to different types of tactile stimuli (Kandel et al., 2000). Vibration stimuli primarily activate Pacinian corpuscles, whereas multiple receptors likely respond to a pressure stimulus such as a

balloon inflating next to the skin, e.g. Ruffini corpuscles and Merkel receptors, which respond to skin stretch and pressure, respectively.

7.1.1 Direct Nerve Stimulation

Direct nerve stimulation requires that one ensure that the nerve is properly activated by the electrical pulse. Due to differences in skin conductance and other factors, the most common method to ensure proper electrode placement is to position the electrodes and increase the voltage until a known reflex to nerve stimulation occurs (e.g. median nerve stimulation evokes a natural thumb twitch). Some median nerve studies choose a voltage setting relative to the onset of the thumb twitch, whereas other studies simply increase the voltage until the current is first perceived by the participant. The interstimulus interval can be very brief with median nerve stimulation (down to 0.5 s) although shorter ISIs decrease the strength of the later components and longer ISIs lead to a larger contribution from secondary somatosensory cortex (Wikstrom et al. 1996).

7.1.2 Tactile Stimulation

Tactile stimulation is most commonly performed with an air puff achieved by filling an air bladder that is placed directly on the skin. The compressed air must be connected to a device that can control the duration and pressure of the stimulus. There are two parameters that must be considered when designing a tactile experiment: pressure and duration. The pressure is often set around 40 PSI with duration of 20–50 ms to provide time for the balloon to inflate, provide a pressure stimulus, and deflate again (Lauronen et al. 2006). Activation of the somatosensory system through a pressure stimulus takes longer than direct nerve stimulation. Therefore, longer ISIs are recommended ($\geq$1 s).

7.1.3 Vibration Stimulation

Vibration stimuli require a longer duration stimulus and are often used in a pseudo-steady-state design. This is related to the natural oscillatory nature of the stimulus requiring that a sufficient number of cycles are presented to provide a robust response. Rate of oscillation is another variable to consider to ensure that the stimulus is comfortable for the participant.

7.2 Paradigms

Most somatosensory paradigms include simple sensory designs. However, it is good to alternate left and right median nerve stimulation to reduce habituation effects. Additional studies have explored the utility of MEG for further understanding somatosensory processing including: mapping somatotopy (Inoue et al. 2013; Jamali and Ross 2013), understanding the interaction between sensory and motor functioning (Cheyne 2013; Piitulainen et al. 2013), linking pain perception with somatosensory processing (May et al. 2012; Rossiter et al. 2013) and exploring cognitive aspects to somatosensory processing (Moseley et al. 2013; Sun et al. 2013).

8 Motor Assessment

8.1 Stimulus Parameters

An important consideration when designing motor experiments is minimizing motor related artifact. Tasks as simple as pressing a button with an index finger activate a complex set of muscles that can introduce significant stimulus-locked muscle artifact in the MEG dataset. Furthermore, muscle tension from holding the hand or arm in position for movement can lead to muscle tension related artifact. It is advisable to achieve ergonomic positions for the participant to reduce muscle tension during data collection. It is also advisable to ask the participant to remain relaxed throughout data collection. A common approach to identify shoulder tension is to ask the participant to raise their shoulders into a shrug and then relax.

8.2 Paradigms

Motor paradigms focus on capturing the onset of motion with the goal of capturing the activity that initiates the movement. In many cases, it is advisable to cue the participant to initiate movement (e.g. every time the circle appears on the screen, lift your right index finger). Without pacing provided by external stimuli, participants tend to decrease the ISI over time and may decrease it to the point that the motor activity is not easily distinguishable across trials. It is also important to provide concise instructions and allow the participant to practice. Better synchronization across trials is obtained with a precise and rapid finger lift as opposed to slowly lifting the finger. However, other motor tasks may introduce too much muscle artifact and head motion with rapid onset movement. Pilot testing helps to provide guidance on developing novel motor paradigms.

9 Cognitive Paradigms

Due to the large number of cognitive paradigms employed in MEG studies, specific paradigms are not discussed here. However, there are common considerations to keep in mind when developing cognitive paradigms that are described below.

First, it is important to match sensory properties across cognitive conditions to allow one to properly assess cognitive function independent of stimulus parameter differences (as discussed in the chapter by Aine et al. in this volume). For example, in Aine et al. (2006) we performed a passive viewing task and a spatial working memory task using Walsh stimuli. Although the visual stimuli were complex and changed in complexity across trials, the presentation of these stimuli during a passive viewing task allowed us to identify the visual processing components that were independent of the spatial working memory task. Maintaining stimulus characteristics ensures that contrasts between the control and the cognitive condition are not simply related to sensory differences.

Second, cognitive tasks generally require confirmation that the participant is performing the task to a specified accuracy level. Therefore, it is important to find a way to assess whether the participant is performing the task, as instructed. Many investigators require some type of response using a button press, for example. This provides a behavioral correlate (reaction time and percent correct) to the neurophysiological response as well as allowing the investigator to assess whether the participant understands the task and is performing the task throughout data collection. If a behavioral response confounds the task, one strategy is to perform a pre-scan training session and a post-scan questionnaire to determine task compliance. Another strategy is to require the participant to count the number of target stimuli (rare stimuli designed to test compliance).

Third, the timing of the stimuli and the likely variability of the response must be considered, to determine if the cognitive process that one is most interested in studying can be assessed using an MEG study. For example, sentence comprehension occurs over a prolonged time window and comprehension may not occur at the same time relative to the onset of the sentence. One strategy that has been employed is to complete the sentence with a coherent or nonsense word and trigger off of the final word of the sentence (e.g. Maess et al. 2006). This helps to minimize the variability of the cortical response across time, trial and participants.

Finally, a number of strategies have been employed to reduce artifacts that may contaminate the brain response of interest. For example, Tesche and Karhu (2000) employed a fixed temporal pattern during a working memory task. Included in the experimental design was a ‘blink’ command to ensure participant did not contaminate the remainder of the trial with eye blinks. Other strategies include imposing a delayed response to ensure that motor responses do not contaminate cognitive responses to different stimuli. In that case, it is also important to recognize that imposing a delayed response (respond when you hear the ‘beep’ cue) also introduces additional cognitive load into the experiment.

In summary, high temporal resolution provides an exquisite view into the cortical dynamics underlying brain function. However, the variability in cortical response during cognitive tasks can inhibit interpretation. Careful design of the experiment is important to capitalize on the strengths of MEG.

10 Good Practices

There are a number of good practices outlined below that will facilitate good quality data collection. Before beginning a study it is important to pilot test the paradigm to ensure that the behavioral results are as expected. Behavioral testing in a small group of participants is inexpensive and increases the likelihood that the MEG results will be meaningful. The question to be answered is whether the patient group or age group can perform the task to the desired accuracy level. Once the paradigm is established and the stimulus computer has been programmed to present the desired task, stimulus timing evaluation should be performed. Empty room MEG data collection can be performed to test the relative timing of triggers, to verify the number of triggers/condition is correct and to establish the timing of all peripheral devices. One should also check that data is being collected for all relevant channels (including MEG, EEG, bipolar EEG, trigger, and A/D channels), the correct sampling rate is being used, and the correct filter settings are chosen. This is a necessary step that will help prevent the loss of data due to incorrect settings. Finally, it is important to run one or a few pilot test participants to ensure that the expected evoked responses are attained with the paradigm (e.g. auditory M100 is observed when an auditory stimulus is presented, etc.). Once the paradigm is established, it is important to maintain identical stimulus parameters across participants to ensure that sufficiently powered statistical comparisons can be performed at the end of the study. It is also recommended that a naming convention be established at the beginning of the study to ensure consistency across subjects. Our current naming convention includes the SubjectNumber_studyName_Run#_visit#_cont/ave, where studyName is a descriptive name of the paradigm (e.g. audMMN, visP300, spatwm), Run# is the number of a series of runs with the same stimulus conditions if the study population requires breaks during data collection, visit# accounts for longitudinal studies where the same paradigm is collected over multiple time points and cont/ave refers to either a continuous data file or the online average data file. Consistency facilitates auto-analysis pipelines and compilation of data across studies. Finally, record all stimulus settings and data acquisition parameters to ensure that the same conditions can be replicated across participants. This is particularly important in labs where multiple study teams use the same equipment.

Prior to each data collection session it is important to perform a simple test to ensure that the equipment is in the same state as recorded above. For example, confirm stimuli are being presented as expected (you can hear the sound through the auditory inserts, the visual system is functional, etc.). Also, test triggers in the

MEG data to ensure the program is sending triggers through to the data acquisition system. Finally, check the participant response device and confirm that the signals are being received by the stimulus presentation computer and the MEG data acquisition computer.

11 Summary

There are a number of critical factors to consider in properly designing and implementing MEG studies to produce high quality data and to eliminate artifacts that can mislead the interpretation of the results or mask the signal(s) of interest. Identifying sources of artifact and confounding factors prior to data collection can simplify post-processing thereby reducing the number of processing steps needed to obtain good SNR. Being able to reliably identify when stimuli are presented or when events of interest occurred and characterizing confounding activity provides the best means to understand the cortical networks involved in brain function. Finally, establishing good data acquisition procedures to ensure reliable and consistent data collection across participants is imperative to developing generalizable knowledge. With proper experimental design and participant monitoring novel MEG analysis techniques will continue to be developed to capitalize on the rich spatio-temporal datasets obtained with MEG.

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