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The Infant EEG Mu Rhythm: Methodological Considerations and Best Practices

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Abstract

The EEG mu rhythm, recorded from scalp regions overlying the sensorimotor cortex, appears to exhibit mirroring properties: It is reactive when performing an action and when observing another perform the same action. Recently, there has been an exponential increase in developmental mu rhythm research, partially due to the mu rhythm's potential role in our understanding of others' actions as well as a variety of other social and cognitive processes (e.g., imitation, theory of mind, language). Unfortunately, various methodological issues impede integrating these findings into a comprehensive theory of mu rhythm development. The present manuscript provides a review of the infant mu rhythm literature while focusing on current methodological problems that impede between study comparisons. By highlighting these issues and providing an in depth description and analysis we aim to heighten awareness and propose guidelines (when possible) that will promote rigorous infant mu rhythm research and facilitate between study comparisons. This paper is intended as a resource for developmental scientists, regardless of EEG expertise.

Keywords

EEG mu rhythm; infants; mirror neurons; action perception

What was once an electroencephalogram (EEG) rhythm noted primarily for its association with motor activity may now provide a window into our understanding of others' actions as well as a variety of other social and cognitive processes (e.g., imitation, theory of mind, language; for review, see Pineda, 2005). The EEG mu rhythm (adult 8–13 Hz), recorded from scalp regions overlying the sensorimotor cortex, appears to exhibit mirroring properties: It is reactive when performing an action and when observing another perform the

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same action. Converging neuroscience evidence using functional magnetic resonance imaging (fMRI; e.g., Buccino et al., 2004), transcranial magnetic stimulation (TMS; e.g., Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995), magnetoencephalography (MEG; e.g., Hari et al., 1998), and single-cell recording (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010) suggests that the human brain may have a neural mirroring system that is analogous to the mirror neuron system found in rhesus macaques (but see Gallese, Gernsbacher, Heyes, Hickok, & Iacoboni, 2011; Hickok, 2009; for reviews of human mirror neuron debate). Clearly, the potential implications for understanding the development of a neural mirroring system are widespread. There has been an exponential increase in developmental EEG mu rhythm research over the last few years, and the primary goal of this paper is to suggest guidelines that will facilitate cross laboratory comparisons toward a comprehensive understanding of the development of the EEG mu rhythm. To this end, we begin by providing a brief overview of the EEG mu rhythm with a focus on infant mu rhythm research. Next, we present a detailed analysis of methods used in infant mu rhythm research (from behavioral protocols to psychophysiology) and recommend steps that can be taken to facilitate between laboratory comparisons.

Initial evidence of mirror neurons was discovered in 1992 via single-cell recordings in area F5 in the ventral premotor cortex of rhesus macaques (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; see also Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). Specifically, these researchers reported that there were individual motor neurons that fired during both the execution of a goal-directed action and the observation of another completing the same action. Since their discovery, there has been much interest in determining whether the human brain exhibits similar neural mirroring properties. The adult 8–13 Hz mu rhythm, recorded over the sensorimotor cortex (e.g., scalp electrodes C3, C4), is prominent during periods of rest (i.e., quiet wakefulness) and is attenuated during the execution or observation of goal-directed actions (see Pineda, 2005 for a review). Recently, simultaneous EEG and fMRI recordings have suggested that activation of portions of the human neural mirroring system—inferior parietal lobe, dorsal premotor cortex, primary somatosensory cortex—are correlated with mu attenuation (Arnstein, Cui, Keyzers, Maurits, & Gazzola, 2011).

EEG is one of the most preferred neuroimaging techniques for developmental populations. As compared to other neuroimaging techniques, it has excellent temporal resolution, is noninvasive, and is relatively resistant to motor artifacts (Casey & de Haan, 2002). Marshall and Meltzoff (2011) provided an informative review of the infant mu rhythm literature, while also highlighting theoretical questions that are essential to this developing field. In the relatively brief time since their review, the infant mu rhythm literature has more than doubled. Unfortunately, various methodological issues impede integrating these findings into a comprehensive theory of mu rhythm development. Although Marshall and Meltzoff noted important methodological issues, the focus of their review was to suggest guidelines for tying mu suppression to action perception. The goal of the present manuscript is to outline methodological considerations for infant mu rhythm research and propose guidelines that will promote cross laboratory comparisons for infant mu rhythm work.

Infant EEG Mu Rhythm

A brief summary of infant mu rhythm studies during action observation only and action observation plus execution can be found in Tables 1 and 2, respectively. As can be seen, infant mu rhythm research has examined a variety of topics. The most common theme has been comparing mu reactivity (i.e., less mu power [a measure of neural activity] is associated with more reactivity of the mu rhythm) during the observation of goal-directed versus non-goal-directed actions. Evidence of greater mu reactivity during goal-directed

actions has been found with 8- to 9-month-olds (Nyström, Ljunghammar, Rosander, & von Hofsten, 2011; Southgate, Johnson, El Karoui, & Csibra, 2010), which parallels findings with 4- to 11-year-olds (Lepage & Theoret, 2006) and adults (e.g., Muthukumaraswamy, Johnson, & McNair, 2004; Nyström, 2008). Six-month-olds, on the other hand, failed to exhibit a difference in mu power between goal-directed and non-goal-directed actions (Nyström, 2008). The developmental trajectory may be complex, however, as a different pattern of results has been found in 18- to 36-month-olds—greater mu attenuation to mimicked actions than goal-directed actions (Ruysschaert, Warreyn, Wiersema, Metin, & Roeyers, 2013; Warreyn et al., 2013).

Another reoccurring theme in this literature is how experience is related to mu rhythm reactivity. There is evidence that 14- to 16-month-olds' crawling experience is related to their mu reactivity (i.e., a difference score of mu power during observation of walking and crawling videos; van Elk, van Schie, Hunnius, Vesper, & Bekkering, 2008), and that 14-month-olds display more mu reactivity to actions within their motor repertoire (during an interactive condition) than to actions not within their motor repertoire (during a non-interactive condition; Reid, Striano, & Iacoboni, 2011). These findings are analogous to EEG research with adults, which examines the role of experience on mu rhythm reactivity by comparing experts to non-experts. Professional dancers, for example, exhibit greater mu reactivity as compared to non-dancers when watching dance movements, and there are no group differences in mu reactivity in response to everyday movements (Orgs, Dombrowski, Heil, & Jansen-Osmann, 2008). Likewise, there is greater mu reactivity (both action observation and execution) for adults who are better at learning a novel motor skill (Nakano, Osumi, Ueta, Kodama, & Morioka, 2013). Other infant mu studies, however, have noted different patterns of mu reactivity in relation to experience. Stapel, Hunnius, van Elk, and Bekkering (2010) found that 12-month-olds exhibit more mu reactivity to extraordinary actions (and infants presumably have less experience with these actions) than ordinary actions. Furthermore, Virji-Babul, Rose, Moiseeva, and Makan (2012) found that 4- to 11-month-olds exhibited approximately the same amount of MRD to actions within and not within their motor repertoire (Figure 1; p. 239).

In the following sections, we highlight methodological considerations essential to future infant mu rhythm research while noting “best practices” that will enhance the interpretability of these findings.

Methodological Considerations of Infant EEG Mu Rhythm Research: Best Practices

A challenge for both behavioral and psychophysiological developmental research is adapting adult-designed procedures for use with a developmental population. Developmental EEG research must also take into account that infant EEG is at a lower frequency than adult EEG, and that the mu frequency band will gradually increase to the adult 8–13 Hz range throughout early childhood. In this section, we provide a systematic analysis of methodological considerations for current and future infant mu rhythm research and highlight “best practices” (see Table 3). To this end, we separate this section into four different aspects of the mu methodology: (1) considerations regarding baseline; (2) considerations for action observation and execution trials; (3) considerations regarding stimulus duration, outliers, and minimal amount of usable data; and (4) considerations regarding frequency bands, reference type, and scalp distribution. Although some of these topics have been addressed in behavioral or other developmental EEG papers (for reviews, see Bell & Cuevas, 2012; Fox, Schmidt, Henderson, & Marshall, 2007), our review of the infant mu literature revealed that these topics were the most salient to infant mu researchers for enhancing between study comparisons.

Considerations for Regarding Baseline

In order to quantify mu rhythm “suppression,” “attenuation” or “desynchronization,” “best practice” is to collect a baseline measure of EEG power at central sites for comparison to the EEG power during the experimental conditions of interest. Typically, mu rhythm desynchronization (MRD) is calculated in terms of percentage of change between an experimental event and a baseline, e.g., $(\text{Event-Baseline}/\text{Baseline}) \times 100\%$, as described by Pfurtscheller and colleagues (e.g., Pfurtscheller & Lopes da Silva, 1999), or calculated as ratio, e.g., $\log(\text{Condition}/\text{Baseline})$ as described by Pineda and others (e.g., Pineda & Hecht, 2009; Oberman, Ramachandran, & Pineda, 2008; Oberman et al., 2005). In these calculations, MRD is reflected by negative values, mu rhythm synchronization (MRS) as positive values, and zero represents no changes in power. In the following sections, we outline the importance of having a baseline measure, choosing a baseline, timing of the baseline presentation, and the importance of statistically measuring all conditions with respect to the baseline against zero.

Inclusion of baseline, even when multiple conditions are present

One problem for interpreting the results of infant mu studies is that some lack a baseline measure entirely. These studies take the approach of comparing power in one condition to power in another (e.g., Paulus, Hunnius, & Bekkering, 2013; Paulus, Hunnius, van Elk, & Bekkering, 2012; Stapel et al., 2010). Potential problems in the interpretation of results arise when there is no baseline. For instance, van Elk et al. (2008) compared mu activity between two conditions: observation of an infant crawling and an infant walking. Comparisons of multiple conditions does not control for differences in reactivity to different objects or perspectives (e.g., seeing an infant in a horizontal versus vertical position), regardless of the type of movement or event seen (e.g., crawling versus walking). It also overlooks the possibility that both conditions may (or may not) be desynchronized with respect to a resting baseline. And while report of less mu power in one condition versus another may important, it cannot provide information on whether either or both of these conditions show suppression relative to no stimulation. It is entirely possible that two conditions could be synchronized (i.e., show increased power with respect to a proper resting baseline condition), but one condition is less synchronized than the other. The absence of a baseline measure could result in misleading interpretations of desynchronization when in fact it is a condition difference that may be attributed to increased EEG power (synchronization) in one condition rather than desynchronization of another.

Inclusion of a baseline, even when comparing amongst different conditions, will enhance the interpretability of the data, facilitate between study comparisons, and heighten our understanding of the mu rhythm’s properties early in development. Some of the infant mu studies that use the comparison of conditions approach do use the term “desynchronization” (Paulus et al., 2012; Stapel et al., 2010; van Elk et al., 2008). We suggest this terminology be reserved for studies that analyze the experimental conditions with respect to a baseline, thus reducing the potential for readers misinterpreting the findings. For all other comparisons, we recommend referring to “changes in mu power” with less mu power being associated with more reactivity of the mu rhythm.

Including statistical tests for magnitude of MRD (both central and other sites)

The MRD calculation discussed at the beginning of this section normalizes the data with respect to zero. The presence of a baseline would allow for tests of significant desynchronization against a null hypothesis of no change in power (represented by zero) during the event of interest. Examples of infant mu studies that did this test include Marshall, Young, and Meltzoff (2011) and Virji-Babul et al. (2012).

This distinction, to test specifically for mu desynchronization is an important one for reasons alluded to above in studies that only compare power across conditions. Additionally, there are also some studies that have calculated MRD with respect to baseline, then made cross condition comparisons of those values, without having initial tests of the magnitude of MRD or MRS within each condition (Marshall, Saby, & Meltzoff, 2013; Saby, Marshall, & Meltzoff, 2012). While condition effects reported are informative, they should also be examined with respect to a baseline to understand whether desynchronization is actually present at all. Reporting significance tests of MRD/MRS for all conditions is a simple way to greatly inform our understanding of the characteristics and development of mu desynchronization, and will allow for cross-study comparisons. Furthermore, these calculations should be done at sites other than central, to help inform issues of whether MRD is specific to central sites or if desynchronization in this frequency band is more widespread (see *Reporting EEG changes beyond the central sites* section).

Issues of baseline type: Including multiple types of baseline

An important consideration when designing an experiment with the aim of measuring mu rhythm is what the ideal baseline measure is, and when should it be presented. Both adult and infant literature include a wide variety of baseline measures: the absence of a stimulus, static images, moving objects, and moving body parts. It is possible that the choice of this resting baseline may influence whether MRD is found in a particular study (see Tangwirisakul, Verhagen, van Putten & Rutten, 2013 for discussion in adult literature). The range of baseline measures may be problematic because a finding of MRD or the lack of MRD is likely to be interpreted in terms of qualitative aspects of the test event rather than the baseline measure. EEG power during both the resting baseline and the action/event of interest can influence whether MRD during the event is found. However, because mu-related research questions tend to be focused on the EEG response during an action or event, it is easy to overlook the influence of baseline choice and whether it was truly a representative measure of resting EEG.

The historical view of mu rhythm is that high amplitude activity at central sites reflects periods of being motorically idle, i.e., in a resting state (see Pineda, 2005). Thus, an appropriate baseline measure of mu from this view may be an abstract (non-meaningful) image, or the presentation of a blank screen, etc. In terms of the infant literature, this type of baseline has been employed by Marshall and colleagues, who used static shapes presented on a flash card (Marshall et al., 2011, 2013; Saby et al., 2012). On the other hand, those that study mu as a reflection of the MNS have used any number of these conditions, static or non-goal-directed movements, as appropriate measures of baseline. Reid et al. (2011), Ruyschaert et al. (2013), and Warreyn et al. (2013) are examples of infant studies that used moving shapes or objects, respectively, as baseline conditions. A potentially informative aspect of Ruyschaert et al.'s (2013) and Warreyn et al.'s (2013) baseline procedures is that the same objects are subsequently presented during the goal-directed action observation and execution trials, which controls for the possibility that simply a change in stimuli between baseline and "test" could result in changes in the infant mu rhythm (as noted in the adult literature by Muthukumaraswamy & Johnson, 2004). This is in line with the perspective that an "optimal" baseline condition is identical to the experimental condition except for variable of interest (i.e., goal-directed movement).

It is important to note, however, that both static and moving baseline measures present methodological challenges when testing infants: for static images, or periods of stillness, keeping the infant attentive is not easy, and likely to result in a large amount of data loss due to movement artifact. Moving stimuli are more likely to capture quiet attentiveness in infants, but moving stimuli themselves may elicit desynchronization thus making a

comparison with a test condition biased by baseline activity. In general, the use of different baseline measures across studies contributes to the difficulty in comparing these findings, especially in developmental populations.

Perhaps the inclusion of multiple comparison conditions including static stimuli and non-biological movement stimuli should be considered for future infant MRD procedures. Ferrari et al. (2012), for instance, examined anterior EEG reactivity in infant rhesus macaques to facial gestures by including both (a) a nonmoving baseline of the same stimulus presented during action observation (i.e., nonmoving face, nonmoving object) and (b) a control condition of nonbiological movement (i.e., moving object). This procedure has the same stimuli present during baseline and “test;” permits for comparison of whether similar baseline-to-test changes in mu power occur for biological and non-biological movement; and provides multiple potential baselines (e.g., non-biological motion; static face) for comparison with the measure of interest (i.e., mu power during facial gestures). To our knowledge, this rigorous methodology has not been used with human infants, but we recommend this as a “best practice” because it will be informative in terms of appropriate baselines and controls.

Issues of baseline timing: Implementation of true event-related designs

EEG is dynamic, with its properties changing over the course of the experimental session. Many infant studies measure a baseline relatively close to each test period, and thus are spread across the session. For example, Marshall et al. (2011) and Southgate and colleagues (Southgate, Johnson, Osborne, & Csibra, 2009; Southgate et al., 2010) preceded each trial with a baseline segment. Others precede small blocks of trials, e.g., baseline, execute, observe (Saby et al., 2012) or baseline, condition 1, condition 2, condition 3, then repeat (e.g., Ruyschaert et al., 2013; Warreyn et al., 2013). In contrast, the procedure used by Reid et al. (2011) included a single 3-min baseline block, a 3-min condition 1 block, and a 3-min condition 2 block, which were counterbalanced. We urge caution when considering this type of design because (a) temporal separation between baseline and condition(s) of interest is not ideal; and (b) assessing baseline after conditions 1 and/or 2 could be influenced by carryover effects.

At this time, we do not know enough about the timing or characteristics of MRD in infants, including the potential for motor preparation responses (also noted by Marshall & Meltzoff, 2011). The two points discussed above, namely that the choice of baseline used and that baselines preceding test periods, are design issues that can influence what we learn about the timing of MRD onset. To illustrate, Nyström et al. (2011) collected one second of baseline while a person sat still in front of a graspable object, just prior to acting on it. Although this would be considered an “optimal” baseline from an experimental perspective, it is possible that the presence of a person in front of a graspable toy elicited mu desynchronization. Such a baseline has been used as a “rest” state measure in adult studies testing assumptions of neural mirroring function (e.g., Muthukumaraswamy et al., 2004). Although the neural mirroring hypothesis would predict greatest reactivity at the period of a grasp action rather than before, there is reason to believe that when measuring MRD, the presence of an agent and an object may elicit earlier anticipatory reactivity. The work of Southgate and colleagues (2009, 2010) highlights this issue, as they found, over the course of trials, mu attenuation during periods prior to the target action.

Perhaps the best method for addressing issues of MRD timing is through implementation of a true event-related design (e.g., Pfurtscheller & da Silva, 1999), where one would ideally collect a baseline period just prior to the period of interest, the onset of action, and compare power in that baseline interval directly to the preceding test period. This approach is common in the adult literature, perhaps because of easy implementation and participant

cooperation in remaining still during the procedure. It is best achieved using analysis techniques such as wavelet analyses, or those described by Pfurtscheller and colleagues (Pfurtscheller, 2003; Pfurtscheller & Lopes da Silva, 1999). True event-related designs pose unique challenges however to infant work: live presentation settings are difficult to create seamless procedural transitions, and, inclusion of trials becomes dependent on artifact-free baseline and action segments that are continuous (or very close) in time. However, they serve as informative measures worth striving for in the future, as they allow one to plot the mu signal over the entire time-course of the event.

Considerations for Action Observation and Execution Trials

Importance of having action execution and observation trials

Marshall and Meltzoff (2011) detail the importance of having both action execution and observation trials. Tables 1 and 2 can be used to classify the infant mu rhythm studies to date. By definition, the neural mirroring system is active during both the observation and execution of a particular action. In the absence of an execution condition, the most parsimonious explanation is that a particular frequency band is sensitive to motion (perhaps biological, if non-biological controls are included) at a specific point in development. In other words, in the aforementioned scenario, it is unknown whether mirroring properties are in fact present. This point is precisely demonstrated by Lepage and Theoret's (2006) examination of the mu rhythm in 4- to 11-year-olds. Although there was suppression (i.e., decreases in power as compared to rest) during the observation of hand movements in multiple frequency bands (theta 1: 3.5–5.5 Hz; theta 2: 5.5–7.5 Hz; mu: average 9–11 Hz), the only band that was suppressed during action execution was the mu frequency band. Without an action execution condition, Lepage and Theoret would have been unable to make this important distinction.

The inclusion of action execution trials will be critical to defining the frequency of the infant mu rhythm at different points in development. Furthermore, there is some evidence of variability in infant mu reactivity (e.g., Marshall et al., 2011), and analysis of mu reactivity in both execution and observation conditions will likely be essential to understanding these differences as well as interpreting their potential association with other cognitive and social skills (e.g., Warreyn et al., 2013). In sum, even if the action presented during observation trials is outside of the infant's motor repertoire, including action execution trials that are within the infant's motor repertoire will be helpful in understanding the infant mu rhythm in general and could also be useful in defining individual mu frequency bands (see *Frequency Band* section).

Does seeing the whole person matter?

In some studies, the infant sees an entire person completing an action during action observation trials (e.g., Marshall et al., 2011, 2013; Nyström et al., 2011; Reid et al., 2011). In other studies, the face of the model is hidden (e.g., Nyström, 2008; Virji-Babul et al., 2012) or there is a stage that only permits a hand to be seen (e.g., Southgate et al., 2009, 2010). Although the "whole person" design is the most ecologically valid scenario (i.e., more similar to situations that the infant encounters in his/her natural environment), experimenters must maintain a neutral expression as to not confound the EEG data with other (e.g., emotion) processes. To our knowledge, no mu rhythm study has directly compared these different forms of action observation. It is possible that seeing the "whole person" might be particularly important to mu attenuation early in development (see Nyström, 2008 and Nyström et al., 2011; but see Virji-Babul et al., 2012).

Does the type of action observation/execution design matter?

In line with the previous section, it is unknown whether mu reactivity is affected by the ordering of the action observation and execution trials. Some studies have used a blocked design during which infants are presented with only one type of stimulus (e.g., action observation trials; e.g., Southgate et al., 2009, 2010). As with any repeated-presentation protocol, keeping the infant engaged in the task (especially when the same stimulus is used repeatedly) is a potential challenge for researchers when using blocked designs. In contrast, other studies have used intermixed and/or dyadic presentations (e.g., Marshall et al., 2011, 2013; Warreyn et al., 2013) in which action execution and observation trials are intermixed. Although intermixed presentations are often more engaging, and thus, likely to keep the infant in the experimental session longer, researchers have to consider carryover effects¹ from one trial type to another (see Saby et al., 2012). Direct comparisons are necessary to determine if one design is more effective at eliciting mu attenuation than the other during infancy, but ultimately the design choice will also be influenced by the empirical question of interest with different fields often using different design types.

Live observations trials are more effective than video

The manner in which action observation trials are presented (i.e., live versus video) is especially salient during early development because in a variety of behavioral paradigms (e.g., habituation, object search, imitation), infants and young children exhibit a video deficit effect (see Barr, 2010 for a review). Specifically, learning from televised media is poor (e.g., lower imitation scores) as compared to learning from live situations. Although this issue is not unique to mu studies, it is yet another factor that may contribute to difficulty in cross study comparisons. Further, based on behavioral evidence alone, developmental EEG research should be cautious about using video observation trials.

Recent evidence that 19- to 36-month-olds fail to exhibit mu attenuation during video observations trials (Ruysschaert et al., 2013) is consistent with the video deficit effect. Prior to Ruysschaert et al.'s direct comparison of live and televised actions, a handful of studies have had variable success when using video presentations for action observation trials. Whereas 6-month-olds in Nyström's (2008) video protocol failed to exhibit clear evidence of mu reactivity, 4- to 11-month-olds in Virji-Babul et al.'s (2012) video protocol did exhibit mu reactivity. Two additional studies (Stapel et al., 2010; van Elk et al., 2008) found differences in 12- to 16-month-olds' mu power to different types of video-presented action; however, the lack of baseline/resting state comparisons prohibits concluding whether the mu rhythm was attenuated (see *Baseline* section).

An examination of the broader neuroscience literature reveals that the adult motor cortex is 15–19% more responsive when observing live actions as compared to video-presented actions (Järveläinen, Schürmann, Avikainen, & Hari, 2001). Likewise, mirror neurons in rhesus macaques that were active during “live” action presentations showed little or no response during the observation of recorded actions (Ferrari, Gallese, Rizzolatti, & Fogassi, 2003). Together, findings from a variety of methodologies suggest that developmental mu researchers are most likely to find evidence of mu attenuation when action observation trials are presented “in person” as compared to via video.

¹ Using a counterbalanced experimental design is one key way to identify and control for potential carryover effects. From the trial-by-trial perspective, EEG researchers often ensure a minimum of 2 s (e.g., Reid et al., 2010), with conservative estimates of 5–7 s (e.g., Milston et al., 2013), between trials or when accounting for motor artifacts.

Including adult comparison groups tested with infant-modified procedures

Currently, it is unknown whether the same stimuli will result in mu reactivity throughout development. For instance, if a particular stimulus elicits MRD in adults will the same stimulus elicit MRD in infants? The answer to such questions will enhance our understanding of the properties of mu reactivity as a function of age. To our knowledge, only a single infant mu rhythm study, Nyström (2008), has included an adult comparison group using infant-modified procedures. Adult comparison groups (a) are relatively easy for researchers to include (e.g., low attrition, high availability); (b) ensure that infants and adults are tested under the same conditions² (i.e., eliminating procedural differences); and (c) permit direct comparison of adult and infant data. Adult comparison groups will also facilitate the integration of developmental mu findings with the substantial literature on the adult mu rhythm.

Enhancing interpretability with complete experimental designs

As developmental researchers, we attempt to obtain as much data as possible before our participants lose interest or change states (e.g., become hungry, tired, fussy, etc.). In addition, EEG researchers are faced with the challenge of obtaining sufficient artifact-free data (see next section), which requires numerous trials for each condition (i.e., experimental question) of interest. Thus, this limits the number of conditions presented to individual infants, which can be a challenge for complete experimental designs. One way that researchers have addressed this problem is to use between-subjects comparisons. For instance, Ruyschaert et al. (2013) had two groups of infants observe and execute hand movements, except one group observed an “in person” model and the other observed a televised model. Although within-subjects comparisons would be ideal, there were four conditions per model type and it would have been highly unlikely that individual infants would stay engaged if the session length were doubled. Thus, between-subjects designs can offer a reasonable tradeoff to lengthy within-subjects designs by providing initial information about a novel area of infant mu research. Ideally, these findings will be confirmed via future within-subjects designs that are targeted to the key variable(s) of interest.

The alternative of having an incomplete experimental design often leads to inconclusive findings. For instance, Reid et al. (2011) included both interactive and non-interactive conditions, but these two conditions also differed as a function of whether or not an action was within the infant’s motor repertoire. Thus, it is unknown which aspects of the design (i.e., social vs. non-social; within vs. not within motor repertoire) are linked with the between condition differences in mu reactivity. Either a simpler design (i.e., only examining one of these factors) or additional/alternative conditions, such as a non-interactive condition with actions within the infant’s motor repertoire, would be essential to determining the underlying cause of these differences. We encourage infant mu researchers to consider which of the above methods would permit complete experimental design in conjunction with their research question of interest.

²Although some infant-modified action execution procedures might not be feasible for use with adults, most infant-modified action observation procedures (i.e., a primary measure of interest) should be feasible to use with adults.

Considerations Regarding Stimulus Duration, Outliers, and Minimal Amount of Usable Data

Stimulus duration: Using multiple short intervals in close temporal proximity to a specific action

Another methodological concern when evaluating the infant and the adult mu rhythm literature pertains to the duration of the stimulus presented. Puzzo and colleagues (2011) addressed whether mu rhythm desynchronization is best evoked using multiple short repetitions (3 seconds) or long stimulus presentations (80 seconds). They found that presenting short repetitions of the stimuli was a more efficient procedure, which resulted in a consistent and a robust pattern of results in comparison to the 80-second protocol. The inconsistencies of findings in the infant mu rhythm literature may also be attributed to the variations of the duration of stimulus presentation. Much of the existing infant studies utilize multiple short stimuli lasting around 1000 milliseconds (e.g., Nyström, 2008; Marshall et al., 2011; Stapel et al., 2010; Virji-Babul et al., 2012) similar to the adult mu rhythm studies (e.g., Cooper et al., 2012; Moore, Gorodnitsky, & Pineda, 2012). However, there are differences in where the stimulus interval falls around the target action. Some studies include the interval preceding the target action (e.g., Marshall et al., 2011, 2013; Saby et al., 2012), whereas in other studies this interval falls after the target action (Nyström et al., 2011; Paulus et al., 2012, 2013; Stapel et al., 2010). On the other hand, longer stimulus durations are utilized that are in the tens of seconds (Reid et al., 2011; Ruyschaert et al., 2013; Warreyn et al., 2013). These studies raise some concerns for two reasons: (1) no specific time precision is provided for the onset of the various movements occurring in the stimulus, thus, it is not clear to what particular motor act(s) is mu reactive; (2) stimuli with longer durations are divided into shorter epochs for artifact editing, so it becomes difficult to deduce exactly what motor acts were included in the analyses. Providing these details in future studies will be crucial for guiding researchers in designing their experiments effectively. Because of the aforementioned concerns, we encourage researchers to use multiple short intervals (e.g., 1–3 seconds) that are in close temporal proximity to a specific action.

Reporting outliers and minimal amount of usable data

Based on our review of the literature, adult mu rhythm studies generally do not report outliers (i.e., participants being excluded based on outlier mu reactivity values); however, participants without a reactive mu rhythm may be excluded (e.g., Hari et al., 1998). Due to the unique challenges of infant EEG data collection (e.g., infant fussiness, excessive movement artifact), it is pertinent to address the exclusion of outliers and how these are computed. Marshall and colleagues (2011, 2013; Saby et al., 2012) compute mean EEG desynchronization scores across trials within execution and observation conditions for each scalp region (e.g., central, frontal, parietal, and occipital). Infants who exhibit desynchronization values that are more than 1.5 times the interquartile range from the median at one or more scalp regions for a particular condition (e.g., execution) are excluded from corresponding analyses (e.g., execution). This is a conservative approach because it considers all data to be questionable if even a single region's value is an outlier. A slightly different criterion of three standard deviations (plus or minus) away from the group mean has been used by other investigators, but it is not clear whether non-central regions were examined (Warreyn et al., 2013). However, most other infant mu rhythm studies do not report statistical outliers, and it is unclear whether outliers were considered and/or excluded (e.g., Paulus et al., 2012, 2013; Southgate et al., 2010; van Elk et al., 2008; Virji-Babul et al., 2012). Because even a single extreme mu power value can significantly alter findings at a group level (e.g., whether infants exhibit MRD), we recommend that mu researchers

explicitly state their outlier criterion, how outliers were handled, and the number of infant subjects who were outliers.

In the infant mu literature, the minimum number of artifact-free trials required for infants to be included in the analyses varies across studies. To provide more reliable estimates of spectral power (i.e., reduce effects of random noise), it is crucial to maximize the number of trials that are averaged to increase the signal-to-noise ratio as much as possible (Davidson, Larson, & Jackson, 2000). To this end, most protocols include a large number of trials (e.g., 30 trials or more) or as many trials as possible until the infant is no longer interested because of the large amount of data that is lost due to artifacts. For the majority of infant mu studies, the minimum number of artifact-free trials stands at nine or ten for each experimental condition (e.g., Nyström, 2008; Nyström et al., 2011; Paulus et al., 2012; Stapel, et al., 2010). This number is even smaller for other studies (e.g., Marshall et al., 2011, 2013), and some studies do not report this information (e.g., Southgate et al., 2010; Virji-Babul et al., 2012). Studies that use stimuli with longer durations (in the tens of seconds) implement a minimum amount (e.g., 40 seconds) of artifact-free EEG data, however, it is difficult to deduce to how many “trials” this translates or how many repetitions of the same motor act were presented (Reid et al., 2011; Ruysschaert et al., 2013; Warreyn et al., 2013). In line with standard EEG guidelines (e.g., Davidson et al., 2000), we encourage all researchers to report the mean, range, and minimum number of artifact-free trials per condition in addition to the mean and range for the total number of trials per condition.

It is especially important for infant mu researchers to consider outliers and minimum amount of usable data because infant studies inherently have higher attrition rates compared to adult studies (see Tables 1 & 2 for details). As can be seen in Tables 1 and 2, there are large differences in the amount of usable data within the infant mu rhythm literature. These differences are likely related to multiple factors, such as the participant’s age (i.e., age-related changes in compliance), the number of different conditions (i.e., increases session length), and the inclusion of execution trials (i.e., increases motor artifacts). A discussion of procedures to maximize the amount of usable EEG data with developmental populations can be found elsewhere (e.g., Bell & Cuevas, 2012; DeBoer, Scott, & Nelson, 2006; Pivik et al., 1993).

Considerations Regarding Frequency Bands, Reference Type, and Scalp Distribution

Frequency bands: Reporting multiple bands and considering age-related changes in the mu rhythm

As can be seen in Tables 1 and 2, researchers have examined different frequency bands when analyzing the infant mu rhythm. As in the adult mu literature, some infant EEG mu research use predefined bands with the most common band being the 6- to 9-Hz frequency band (e.g., Marshall et al., 2011, 2013; Paulus et al., 2013; Virji-Babul et al., 2012) or a close derivative (e.g., 5- to 9-Hz; Nyström et al., 2011). Similar to the adult 8- to 13-Hz central rhythm, the infant 6- to 9-Hz central rhythm is dominant during quiet wakefulness and is functionally distinct from the occipital alpha rhythm (Marshall, Bar-Haim, & Fox, 2002; Stroganova, Orekhova, & Posikera, 1999). There is longitudinal evidence of age-related increases in the peak mu frequency from 5 months ($\approx 6-7$ Hz) to 51 months (≈ 9 Hz), and the 6- to 9-Hz band captures peaks in the mu rhythm throughout this developmental span (Marshall et al., 2002).

Another method of defining the infant mu rhythm has been to create individualized frequency bands (e.g., Ruysschaert et al., 2013; Southgate et al., 2009, 2010; Warreyn et al., 2013); a procedure that is also used in some of the mu rhythm literature with adults and

older children (e.g., Lepage & Theoret, 2006; Muthukumaraswamy & Johnson, 2004). The basic idea is that there are individual differences in the particular mu frequency that is most reactive, and by determining that peak frequency (as well as adjacent frequencies) during action execution, we can then compare functionally analogous frequency bands between participants during action observation. This method might be particularly important to developmental EEG researchers who also have to consider individual differences in the rate of maturation of the EEG signal.

It should be noted, however, that adult mu research using both predefined (e.g., Frenkel-Toledo, Bentin, Perry, Liebermann, & Soroker, 2013; Marshall, Bouquet, Shipley, & Young, 2009) as well as individualized (e.g. Babiloni et al., 2009), frequency bands has noted functional dissociations in the upper and lower mu frequency band. For instance, Frenkel-Toledo et al. (2013) found that although the higher mu band (10–12 Hz) exhibited maximal MRD during action execution (see also Babiloni et al., 1999), only the lower mu band (8–10 Hz) exhibited MRD during both action observation and action execution. Other researchers have found that the lower mu band is associated with attention processing and is not as topographically specific in its reactivity to different actions as the upper mu band (e.g., Pfurtscheller, Neuper, & Krausz, 2000). It is unknown if similar dissociations occur in the mu rhythm during development, but such dissociations could potentially obscure findings when using wide frequency bands.

In an emerging field, such as the infant mu rhythm, we recommend what will be most informative over the long-term—presenting findings using multiple methods. We realize that this is a more time consuming approach, but it is essential to providing a foundation to future research. As highlighted in this section, there are numerous methodological decisions that infant mu researchers have to make based on their research interests, and having one standard in infant mu research could be quiet informative. We recommend that regardless of whether researchers are using a narrow band or individualized band approach, to also include analysis of the 6- to 9-Hz band. Individual differences in reactivity of the standardized band are potentially meaningful and obscured by the individualized and/or narrow band methods. At the same time, researchers interested in the 6- to 9-Hz band, should also consider analyzing their findings based on individualized and/or narrow frequency bands. To our knowledge, no infant mu rhythm study has published both sets of findings. However, information provided in supplementary material, such as Southgate et al. (2010), reveals the number of infants for each individual band of interest and is a step in this direction.

In the adult mu rhythm literature, there is also evidence of mirroring properties within the beta frequency band (\approx 14–30 Hz; Milston, Vanman, & Cunnington, 2013; Nakano et al., 2013). Although a few infant mu rhythm studies have included the beta band (Nystrom, 2008; van Elk et al., 2008, Virji-Babul et al., 2012), the findings have been mixed. The functional significance of mirroring in the beta band is even less clear than mu in the adult literature (see Avanzini, Fabbri-Destro, Dalla Volta, Daprati, Rizzolatti, & Cantalup, 2012, for a potential function). Thus, we still have much to learn about the properties and functional significance of the beta band, and we encourage researchers to report data from the beta frequency band in addition to the mu frequency band.

Finally, because of developmental changes in the peak frequency of the mu rhythm and potential age-related changes in mu reactivity (plus cognitive and motor skills); caution must be taken when handling data from infants of different ages. To this end, the majority of infant EEG mu research has focused on a specific developmental span (e.g., ± 2 weeks, ± 1 month; see Tables 1 and 2). Another potential approach, especially in later infancy and early childhood (i.e., when age-related changes in EEG frequency are not as substantial as during

the first year), would be an individualized frequency band approach (e.g., Ruyschaert et al., 2013; Warreyn et al., 2013). This approach would presumably ensure that peak mu reactivity is captured for each infant, regardless of age, allowing researchers to combine data from a large developmental span. To be conservative, we would also encourage additional analyses controlling for age in order to account for other developmental differences (e.g., cognitive, motor skills).

We provide the following example to illustrate that inconclusive nature of combining data from a large developmental span when using a standard frequency band approach. Virji-Babul et al. (2012) found that 4- to 11-month-olds exhibited MRD (6–9 Hz) in response to object motion (i.e., ball rolling); actions within the motor repertoire (i.e., hand reaching for objects); and actions not within the motor repertoire (i.e., torso and legs of human walking). Can we conclude that young infants exhibit MRD regardless of experience and early MRD fails to differentiate between biological and non-biological motion? These findings are difficult to interpret because (a) none of the analyses took age into consideration, and it is unknown whether peak MRD is captured by the same frequency band during this 7-month span of development; (b) even within the “action within motor repertoire” condition, there are vast differences in infants’ reaching and grasping experience in this 7-month span; and (c) different patterns of findings have been found with slightly older infants (Reid et al., 2011; van Elk et al., 2008). Unfortunately, the sample size was too small ($n = 10$) to separate infants into more specific developmental spans. Thus, we urge infant mu researchers to take developmental changes in the EEG frequency bands (in addition to other changing abilities) into consideration when determining the age range of interest.

Reporting EEG changes beyond the central sites

Reporting of power changes in “alpha” or the 6–9 Hz rhythm for multiple locations across the scalp topography is an important issue, particularly for the developmental community. Adult work suggests the mu rhythm distinct from the classic occipital alpha rhythm, with an independent source generated by motor areas (Formaggio et al., 2008; Hari & Salmelin, 1997; Ritter, Moosmann, & Villringer, 2009; see also Pineda, 2005 for review). Specificity of mu in central regions is supported developmentally in infant studies-- EEG power at central sites exhibits a power increase in the alpha range during resting states in the first year, that appears to be unaffected by changes in occipital alpha, i.e., lights on versus lights off (Marshall et al., 2002; Stroganova et al., 1999). However, less is known developmentally about the topographical specificity of the mu signal during periods of execution and observation, and particularly, how the central region relates to occipital alpha during this time.

Although the developmental community has been largely responsive to Marshall and Meltzoff’s (2011) suggestion to report changes in EEG power beyond solely the central sites, studies differ in which non-central regions they report. For example, Marshall et al. (2011) reported MRD values for frontal (comprised of F3/Fz/F4/F7/F8), central (C3/Cz/C4), parietal (P3/Pz/P4/P7/P8), and occipital (O1/O2) regions. In a later paper by this group, Saby et al. (2012) broke these groupings down further into medial and lateral clusters within the region which, as noted by the authors, may have contributed to less topographical specificity reported for similar conditions at 14 months (Marshall et al., 2011). This discrepancy in findings brings to light a need for the reporting of similar electrodes across the scalp. This is particularly important to allow for cross-study comparisons. Paulus et al. (2012) reported statistics from Fp1 and Fp2 to conclude no effects at “frontal” regions, but due to their proximity to the eyes, those sites may be less representative of frontal activity than F3/F4 or F7/F8 which were not reported. Virji-Babul et al. (2012) report MRD at central, parietal, and temporal sites, without reference to whether they used single electrodes

or clustered grouping, and without reference to electrode placement with respect to a 10–10 or 10–20 system.

Perhaps the most important non-central region for mu researchers to report is from the occipital region because this information is useful in separating the 6–9 Hz occipital rhythm from the 6–9 Hz central mu rhythm. Some studies make no report of analyses conducted in the occipital region (e.g., Southgate et al., 2009, 2010; Paulus et al., 2012). Other studies provide topographic maps of power distributions across the scalp (e.g., Paulus et al., 2013; Stapel et al., 2010; van Elk et al., 2008), which is helpful. Ironically, all of these topographic maps tend to suggest EEG reactivity in the occipital region. One method for examining the 6–9 Hz occipital rhythm with respect to mu, has been to determine whether there is significant desynchronization at occipital regions during action observation and/or execution. Using this technique, Marshall et al. (2011) reported that 14-month-olds exhibited significant desynchronization at central regions, but not occipital regions, during action observation and execution. Another, perhaps more conservative, technique involves comparisons of the magnitude of desynchronization at occipital versus central (mu) regions (i.e., if MRD at central sites is the dominant rhythm, it is expected that it would be greater in magnitude than desynchronization of the occipital rhythm.). See Warreyn et al. (2013) and Ruyschaert et al. (2013) for examples of studies with 18- to 36-month-olds that made these comparisons, though ultimately with different outcomes. Using a variant of this technique, Saby et al. (2012) plotted the time course of 14-month-olds' desynchronization for central and occipital regions during action observation, which revealed these rhythms were independent. Thus, it is imperative that infant mu researchers consider the potential contribution of the occipital alpha rhythm to MRD findings, which will permit others to interpret findings of occipital overlap with caution (e.g., Warreyn et al., 2013).

Finally, adding to the confusion is the clustering of multiple electrodes that may be used in high density systems (e.g., the averaged signal from five channels on a 128 channel net may be represented as “C4”). Moreover, some may collapse over many electrodes to compute a “region” – such as Marshall and colleagues' use of many frontal electrodes (described above) comprising the frontal region. With regard to the clustering, we suggest a good start would be to identify and report exactly which electrodes went into each calculation entered into the analysis. To address variability in terms of which sites are included in analyses, we further suggest some standardization for the reporting of analogs of the 10/20 system, such as the medial sites across the scalp (F3/F4, C3/C4, P3/P4, and O1/O2). Additionally, as discussed in the section *Statistical tests for magnitude of MRD*, it would be useful to understand which of these regions show desynchronization with respect to a common baseline. This is arguably more meaningful for cross-study comparisons than reporting a lack of condition differences across the scalp (e.g., Marshall et al., 2013).

Reporting results with multiple types of references

A methodological issue that may covertly hinder cross-study comparisons is the use of different offline re-referencing montages. Two approaches have been taken in the infant mu literature to-date: averaged (or “linked”) mastoid sites (e.g., Marshall et al., 2011, Stapel et al., 2010, van Elk et al., 2008) or use of an average reference (e.g., Nyström et al., 2011; Paulus et al., 2012, Southgate et al., 2009, 2010). To our knowledge, no research has examined the effect of reference type on MRD, particularly in development. However it is a general consensus in the EEG literature to consider reporting multiple references, although this is not often followed, we suggest it as a “best practice” for this emerging field.

One referencing method that should seriously be explored, and suggested by Marshall and Meltzoff (2011), is the Laplacian reference. This reference was used in an adult study that indicated topographical specificity to the central region during action execution and

observation (Muthukumaraswamy & Johnson, 2004). It could be particularly useful for studying the development of topographical specificity of MRD, as it controls for volume conduction. Developmental studies have yet to implement this reference, likely due to the easy access of mastoid and average referencing algorithms readily available in existing software programs. However we believe implementation of a Laplacian reference will make a substantial contribution to our understanding of the development of the central mu rhythm.

Summary and future research

As can be seen above, there are numerous methodological variations that potentially influence the characterization of the mu rhythm early in development, and warrant future investigation. For instance, future research aimed at examining the timing of MRD over development will be extremely helpful in moving the field forward in terms of understanding when during an action event the mu rhythm becomes reactive. Furthermore, in order to determine whether there are developmental shifts in mu reactivity in relation to experience or goal-directedness (as compared to protocol-dependent findings), systematic research using the same experimental procedures at different points in development is necessary.

Despite the current state of the literature, we identified specific aspects of infant mu rhythm methodology that will facilitate the interpretation and integration of future findings (see Table 3). In general, it appears that short “live” (as compared to video) presentations will be most likely to produce robust mu reactivity. We recommend that future infant mu rhythm research (a) include a baseline period to determine if the mu rhythm does, in fact, desynchronize; (b) include both action observation and action execution trials to confirm that the mu rhythm exhibits mirroring properties at a specific point in development; (c) include adults with infant-modified procedures to examine age-related changes in the properties of mu reactivity; (d) report findings at more than central sites to aid in determining regional specificity of infant mu rhythm as well as potential contributions of occipital rhythm within the same frequency range; and (e) report findings for 6–9 Hz in addition to any other bands of interest to enhance between-study comparisons. Furthermore, given the unique challenges of infant EEG studies, it will also be informative for researchers to report specific details related to (a) the presence of any outliers and how these outliers were computed; and (b) the minimum number (or amount of EEG data) of artifact-free trials required for participants to be included in further analyses as well as a justification for the specified minimum. These considerations are particularly important for infant studies where the sample size can already be small and can potentially affect the interpretations of the data.

A future direction for the infant mu rhythm research will be to also consider measures of functional connectivity (e.g., EEG coherence) during action observation and execution. This information will be essential to providing a comprehensive understanding of the properties of the neural mirroring system early in development. Recent adult mu rhythm research has revealed increases fronto-parietal coherence during action observation (van der Helden, van Schie, & Rombouts, 2010). Further, individual differences in fronto-parietal functional connectivity were related to imitation performance.

Conclusion

The infant mu rhythm is a burgeoning area of research, with potential links to the understanding of others' actions as well as a variety of other social and cognitive processes (e.g., imitation, theory of mind, language; see Marshall & Meltzoff, 2011; Pineda, 2005). Although we are in the initial stages of understanding the properties of the infant mu rhythm, the diversity of empirical interests is already apparent. By highlighting both methodological and empirical discrepancies in the literature and providing in depth

description and analysis, we aim to heighten awareness and establish guidelines (when possible) that will promote rigorous infant mu rhythm methodology and facilitate between study comparisons. This resource recommends initial steps that will be critical to forming a comprehensive understanding of the infant mu rhythm and is intended to be useful for developmental scientists interested in the infant mu rhythm, regardless of EEG expertise.

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Research Highlights

- The EEG mu rhythm is reactive when performing and observing a specific action.
- Currently, methodological issues impede integrating infant mu rhythm findings.
- We outline methodological considerations for infant mu rhythm research.
- We propose guidelines to promote cross laboratory infant mu rhythm comparisons.
- Methodological topics include baseline, frequency bands, and experimental design.

Table 1

Summary of Infant Mu Rhythm Studies: Mu Only During Action Observation.

Paper	# Infants with Usable Data (Total)	Age (Range)	Mu band	Task	Results
Nyström (2008)	19 (34)	6 months (24–26 weeks)	3–8 Hz (Mu Peak $M=5.4$ Hz; $SD=.8$)	Observe videos: static dot, goal-directed grasping, non-goal-directed hand movement	No difference between goal-directed action and any condition except static dot.
Nyström et al. (2011)	32 (36)	8 months (not reported)	5–9 Hz	Observe goal-directed grasping & non-goal-directed hand movement	Less mu power for goal-directed actions than non-goal-directed hand movements.
van Elk et al. (2008)	12 (23)	14–16 months $M=483$ days (436–489)	7–9 Hz	Observe videos: infant crawling & walking	Less mu power for crawling than walking; mu power difference score (walking-crawling) related to crawling experience.
Stapel et al. (2010)	12 (36)	12 months ($M=12$ mo., 5 d.; $SD=10$ d.)	7.5–8.3 Hz	Observe videos: adult grasping object & bringing it to either mouth or ear.	Less mu power for the unusual action than the ordinary action.
Paulus et al. (2012)	15 (19)	7–8 months $M=250$ days (7 mo. 8 d. – 8 mo. 30 d.)	6–8 Hz	Listen to action sounds (AS); non-action sounds (NAS); & control sounds (Pretraining NAS & grasping with AS)	Less mu power for action sound than other sounds.
Paulus et al. (2013)	11 (18)	9 months $M=287$ days (8 mo. 25 d. – 9 mo. 24 d.)	6–9 Hz	Listen to AS, NAS, & control sounds (Pretraining NAS & observing grasp with AS)	Less mu power for actions sound than other sounds.
Reid et al. (2011)	10 (32)	14 months (14 months \pm 12 days)	6–9 Hz	Observe actions not within motor repertoire (non-interactive); experimenter imitate infants' actions (interactive); & non-biological motion	Less mu power during interactive condition than non-interactive & non-biological motion conditions, which did not differ.
Virji-Babul et al. (2012)	10 (14)	4–11 months ($M=7.08$)	6–9 Hz	Observe videos: actions within/not within motor repertoire & object motion	Mu suppression for all conditions.

Table 2
 Summary of Infant Mu Rhythm Studies: Mu During Action Execution and Action Observation.

Paper	# Infants with Usable Data (Total)	Age (Range)	Mu band	Task	Results
Southgate et al. (2009)	15 (25)	9 months $M = 270$ days (254–285 days)	individual 3- Hz bands (6–13 Hz)	Execution: grasp toy reaches, grasps, & retrieves toy	Execution: Bilateral mu attenuation Observation: Lateralized mu attenuation (C3)
Southgate et al. (2010)	22 (49)	9 months $M = 273$ days (256–292 days)	individual 3- Hz bands (6–13 Hz)	Execution: grasp toy grasping hand or back of hand (within groups) either disappear behind occluder or rest on stage (mimed; between groups)	Execution: Mu attenuation (Occluder group-C4; Mimed group-C3/C4) Observation: Mu attenuation (Occlusion group Grasp-C3). Mu enhancement (Mimed group Back of Hand-C3/C4)
Marshall et al. (2011)	Observation 27 (58) Execution 29 (58)	14 months ($M = 62$ weeks, $SD = 1.3$)	6–9 Hz	Execution & Observation: button press	Execution: Mu attenuation Observation: Mu attenuation
Saby et al. (2012)	Observation 16 (44) Execution 13 (44)	14 months ($M = 62$ weeks, $SD = 1.6$)	6–9 Hz	Execution: button press or toy grasp Observation: button press following execution of the same or different action	Execution: Mu attenuation did not differ based on action type. Observation: Greater mu attenuation during observation of same action (vs. different action) during 500-ms pretouch epoch.
Marshall et al. (2013)	16 (35)	14 months ($M = 61.7$ weeks; $SD = 1.4$)	6–9 Hz	Execution & Observation: reach & grasp light and heavy objects	Execution: Reaching larger mu attenuation light object (C4); Grasping larger mu attenuation heavy object (C3) Observation: Grasping larger mu attenuation heavy object (C4)
Warreyn et al. (2013)	35 (54)	18–30 months ($M = 24.54$ months; $SD = 3.96$)	individual 3- Hz bands ($M = 7.84$ Hz; $SD = 1.13$; range = 5.37–9.77)	Execution: object-directed actions Observation: object movement (baseline), object-directed actions, & mimicked actions	Execution & Observation: Mu attenuation for all conditions. Greater mu attenuation for observation of mimicked actions than object-directed actions.
Ruysschaert et al. (2013)	34 (68)	19–36 months ($M = 26.44$ months; $SD = 3.96$)	individual 3- Hz bands ($M_s = 8.1$ Hz; $SD = 0.75$ live, $SD = 0.60$ video)	Execution: object-directed actions Observation: object movement (baseline), object-directed actions, & mimicked actions	Execution: Live & video groups exhibit mu attenuation. Observation: Mu attenuation for object-directed & mimicked actions only for live group.

Table 3**Summary of Methodological Considerations and Best Practices for Infant EEG Mu Rhythm Research**

Baseline
<i>Inclusion of baseline, even when multiple conditions are present.</i>
<i>Including statistical tests for magnitude of mu rhythm desynchronization (both central and other sites).</i>
<i>Including multiple types of baseline.</i>
<i>Issues of baseline timing: Implementation of true event-related designs.</i>
Action observation and execution trials
<i>Importance of having action execution and observation trials.</i>
<i>Does seeing the whole person matter?</i>
<i>Does the type of action observation/execution design matter?</i>
<i>Live observations trials are more effective than video.</i>
<i>Including adult comparison groups tested with infant-modified procedures.</i>
<i>Enhancing interpretability with complete experimental designs.</i>
Stimulus duration, outliers, and minimal amount of usable data
<i>Stimulus duration: Using multiple short intervals in close temporal proximity to a specific action.</i>
<i>Reporting outliers and minimal amount of usable data.</i>
Frequency bands, reference type, and scalp distribution
<i>Reporting multiple frequency bands (including 6–9 Hz) and considering age-related changes in the mu rhythm.</i>
<i>Reporting EEG changes beyond the central sites.</i>
<i>Reporting results with multiple types of references.</i>
