ABSTRACT

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The incidental discovery of mirror neurons (MN) has renewed interest in motor theories of development and has sparked considerable debate as to the existence and potential function of mirror neurons in humans. The use of invasive single-cell recordings, however, has precluded identification of single MNs in humans or developmental populations of non-human primates. Non-invasive techniques, such as the modulation of the mu rhythm in the electroencephalogram (EEG) of young infants and children, have demonstrated the existence of an action observation/execution matching system in humans. Moreover, the mu rhythm has become an effective tool for addressing questions of MN system ontogeny in other species.

The aim of this project is to address two questions that have thus far remained untested. The goal of study one is to address the question of whether or not we can identify activation of the human action observation/execution system under conditions in which the participants cannot see themselves executing a grasping action. Evidence from study one further validates our EEG measures as representing activation of the putative human MN system. The goal of study two is to examine the origins of MNs in 3-day-old mother- and nursery-reared infant rhesus macaques and the extent to which differential experience may contribute to the MN system during episodes of neonatal imitation.

The results of study one demonstrated activation of the putative human MN system to actions completed in the absence of visual feedback in both human adults and infants. The magnitude of mu rhythm activity in infants was significantly less than in the adults suggesting a role of experience in the formation of the putative human MN system. The results from study two further emphasized the role of early experience showing significantly greater modulation of the mu rhythm in the mother-reared compared to the nursery-reared infants to the observation of socio-affiliative facial gestures.

The evidence of studies one and two are discussed within a developmental framework of ongoing behavioral development and highlight the role experience plays, not in the foundation of, but rather the elaboration of the MN system.

DEVELOPMENT OF THE MU RHYTHM: UNDERSTANDING FUNCTION THROUGH TRANSLATIONAL RESEARCH

By

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Table of Contents

Tables

Figures

Chapter 1. Overview

The incidental discovery of mirror neurons (MN) by Rizzolatti and colleagues (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992) has renewed interest in motor theories of development and has sparked considerable debate as to the existence and potential function of mirror neurons in humans. The fundamental property of a MN is its activation for the execution of an action and observation of that action completed by another. This function underlies hypotheses that MNs are the neural mechanism for neonatal imitation, action understanding, theory of mind, language perception, and empathy. But the origins of MNs are, as of yet, unknown.

Much of the knowledge about MNs comes from single-cell recordings made in the rhesus macaque motor cortex (area F5). Testing each neuron under various conditions and controls, researchers have identified five key features of MNs. First, MNs are a subpopulation of neurons in area F5 that activate during action production in the absence of visual information (Gallese, Fadiga, Fogassi, & Rizzolatti 1996) and, under conditions of observation, are not involved in motor preparation (Rizzolatti, Fadiga, Gallese, & Fogassi, 1996). These neurons also fire for the goal of the action rather than the kinematics of the action (Caggiano, Fogassi, Rizzolatti, Pomper, Thier, Giese, et al., 2011; Ferrari, Rozzi, & Fogassi, 2005; Umilta, Kohler, Gallese, Fogassi, Fadiga, Keysers, et al., 2001). Moreover, these firing patterns are multi-modal and have the capacity to not only integrate visual information but also synthesize auditory information for actions (Kohler, Keysers, Umilta, Fogassi, Gallese, & Rizzolatti, 2002). Finally, work by Fogassi, Ferrari, Gesierich, Rozzi, Chersi, and Rizzolatti (2005) and Jellema, Baker, Wicker, and Perrett (2000) demonstrated that MNs are part of a broad fronto-parietal network that facilitates action understanding, including the inferior parietal lobe (IPL; important for the integration of sensory information and goal maintenance) and the superior temporal sulcus (STS; involved in the spatial characteristics of an action and representing the intentions of others).

The use of invasive single-cell recordings have precluded identification of single MNs in humans and the debate as to their existence continues. Despite this limitation, transcranial magnetic stimulation (TMS), functional imaging (fMRI), and electrophysiological studies (EEG/MEG) have provided converging evidence for an action observation/execution matching system at the network level. Moreover, these studies have identified patterns of activation that resemble many of the key features of MNs described above, including: (a) reactivity to observation of actions (e.g. Muthukumaraswamy & Johnson, 2004a,b); (b) coding of the goal of the action (Southgate, Johnson, El Karoui, & Csibra, 2010); (c) multi-modal sensory integration (Lepage, Tremblay, Nguyen, Champoux, Lassonde, & Theoret, 2010); and (d) activation of a similar fronto-parietal network (e.g. Buccino, Binkofski, Fink, Fadiga, Fogassi, Gallese, et al., 2001; Molenberghs, Cunnington, & Mattingley, 2012). Because all of studies with humans, thus far, have allowed the participant visual access to their own execution during the study, activation of the observation/execution matching system has not been identified during execution alone.

Evidence from MN studies in the rhesus macaque have shown that training macaques to produce a novel action can produce neurons that exhibit mirroring properties for that new action. This suggests that as an individual becomes motorically familiar with an action, the action observation/execution matching system becomes more tuned to that action. Experience related changes in humans have been studied extensively by examining the mu rhythm in the EEG of specialists and non-specialists. These studies showed that experts in producing an action (e.g. dancers or athletes) showed greater changes in relevant EEG markers of motor activity when observing actions associated with their expertise when compared to non-experts (Del Perchio, Infarinato, Iacoboni, Marzano, Soricelli, Aschieri, et al., 2010; Hadjidimitriou, Zacharakis, Doulgeris, Panoulas, Hadjileontiadis, & Panas, 2011; Holmes, Collins, & Calmels, 2006; Kim, Seo, Song, Yoo, Lee, Lee, et al., 2011; Orgs, Dombrowski, Heil, & Jansen-Osmann, 2008). This line of research suggests that experience affects the emergence of MNs in the monkey and the human action observation/execution system.

The aim of this project is to address two questions that have thus far remained untested. The goal of study one is to address the question of whether or not we can identify activation of the human action observation/execution system under conditions in which the participants cannot see themselves executing a grasping action. Evidence from study one will further validate our EEG measures as representing activation of the putative human MN system. The goal of study two is to examine the origins of MNs in 3-day-old infant rhesus macaques and the extent to which differential experience may contribute to the MN system during episodes of neonatal imitation. Using the neonatal imitation paradigm, described below, that elicits actions from very young infants will assess evidence for an action matching system operating early in the first week of life.

Chapter 2. Background

Since the discovery of mirror neurons (MN) by Rizzolatti and colleagues (di Pellegrino, et al., 1992), there has been a renewed interest in motor theories of social development (Gallese, Eagle, & Migone, 2007; Gallese & Goldman, 1998; Gallese, Keysers, & Rizzolatti, 2004; Rizzolatti, 2005; Rizzolatti, & Fabbri-Destro, 2008). Dating back to the earliest theories of development, researchers like Piaget recognized the importance of motor actions in the developing child (Piaget, 1952). These motor theories are dependent upon internalizing the motivations, goals, and desires of others' actions through one's own actions and interactions with the world (Gallese & Goldman, 1998; Meltzoff, 2006; Thelen, 2000; von Hofsten, 2004; 2007). These theories were based on behavioral observation; the neural mechanism through which information is internalized was poorly understood. Mirror neurons are a class of neurons in the monkey motor cortex that become active both for the execution of an action and observation of a similar goal-directed act completed by a conspecific (Gallese, et al., 1996); this simple feature has been led many scholars to suggest they may be the neural mechanism underlying early socio-cognitive development.

The functional properties of MNs are at the core of the mirror neuron hypothesis (Gallese, et al., 2004; Rizzolatti & Craighero, 2004). The proposal posited by this theory is that when an individual observes an action the same cortical network is activated as would have been if that individual had performed that same action. This matching system has been hypothesized to serve action recognition through the activation of an internal motor knowledge of an action via the visual or auditory description of the action.

Because the observer "knows" the outcome of the action based on his or her own motor representation, he/she gains a direct and embodied understanding of the actor’s goal.

It has been proposed then, based on the mirror neuron hypothesis, complex social behaviors such as action understanding, imitation, theory of mind, language perception, and empathy emerge as a result of MNs (Fabbri-Destro & Rizzolatti, 2008; Gallese et al., 2004; Rizzolatti & Craighero, 2004). The mirroring properties of these neurons make a clear connection to imitation and the neural circuits involved in the MN system have projections into regions associated with emotion processing, making them ideal candidates for the neural basis of socio-cognitive development. Moreover, it has been hypothesized that dysfunctional MN systems may be the underlying cause of disorders such as autism spectrum disorder (ASD), in which patients show marked deficits in imitation or processing and responding to social information (Rizzolatti, Fabbri-Destro, & Cattaneo 2009).

In this review, I first discuss the characteristics of MNs as evidenced by single-cell recordings made in adult rhesus macaques and highlight the hypotheses that have been proposed by this evidence. Because of the invasive nature of single-cell recording, there is limited evidence for MNs in the human motor cortex; however, there is converging evidence from functional imaging (fMRI), transcranial magnetic stimulation (TMS), and electroencephalogram (EEG) or magnetoencephalogram (MEG) studies to suggest that a human MN system exists and I will discuss this evidence in the second part of the review. Third, how MNs develop is still an open question and I will discuss the current evidence suggesting that MNs can be identified at birth, highlighting a recent translational study examining EEG during imitation in 1-week-old infant rhesus macaques. Finally, I will identify questions that still need to be addressed by future research.

2.1 Mirror Neurons in the Monkey

The incidental discovery of MNs was accomplished through the use of single-cell recordings. In trying to understand, at a neurological level, how monkeys perform simple grasping actions, di Pellegrino and colleagues (1992) noticed that the neurons they were recording from were activated when the monkey observed the experimenter preparing the stimuli. The researchers were focusing their study on area F5 of the monkey premotor cortex, a region dominated by neurons that are activated during goal-directed hand movements. Their discovery suggested that the premotor cortex was not solely involved in action planning and execution, but also contributed to visual processing of motor actions performed by others, suggesting an observation/execution matching system of motor processing.

Basic Properties of Mirror Neurons. The primary feature of MNs is their activation for the execution and observation of actions. There were two limitations of the initial study that did not clearly demonstrate MN activity exclusively in action execution and observation. The first problem was that while the monkeys performed the actions they had complete access to the visual information present in their own actions. Rizzolatti, et al. (1996) addressed this concern by testing the monkeys in the dark. Before each trial the monkey sat in complete darkness and the beginning of each trial began when a target object behind a transparent barrier was illuminated. The light was then turned off and the barrier was removed so the monkey was able to complete the grasp with no visual guidance. They found that 100% of the tested neurons that activated in the light also activated in the dark condition.

The second problem was that because the MNs were involved in the execution of actions, it was possible that they activated for motor preparation (in response to observing the action), an important behavior for monkeys who may be in competition for resources and need to perform an action on their goal quickly (Gallese, et al., 1996). During testing sessions, electromyogram (EMG) was recorded from arm, hand, and mouth muscles of the monkeys. When the monkeys were observing the actions of the experimenter, there were no changes in muscle activity, suggesting that no preparatory actions were occurring (Gallese et al., 1996).

By examining larger populations of neurons in the F5 region, two follow-up studies began to identify the key characteristics of MNs (Gallese et al., 1996; Rizzolatti et al., 1996). First, they noted that roughly 20% of the neurons tested behaved like those neurons described by di Pellegrino and colleagues (1992), dubbed mirror neurons, and another 10% were mirror-like canonical neurons, responding to observation, but not production, of actions. The majority of MNs were active during observation and execution of grasping behaviors and responded for one specific action (i.e. grasping for food versus grasping for manipulation). Moreover, these neurons were specific for the hand used or the directionality of the action. Finally, the magnitude of activity of the recorded cells was consistent throughout testing, showing no habituation upon repeated demonstrations.

Further studies have systematically explored the visual properties of MNs in the monkey premotor cortex. In the early studies, tools were used in control conditions during observation events and no activity in the neurons were reported. Ferrari and colleagues (2005), however, later identified a group of MNs that responded to goal-directed tool use in the monkeys. Two important aspects of this finding should be noted. First, while monkeys do not perform actions with tools, after considerable experience using tools (2 months of training in this study), MN activity to the observation of goal-directed actions completed with tools was identified. Second, other research suggests that MNs are present only for actions that are within the repertoire of the monkey. This suggests that there is some plasticity for learning and that MNs have some generalization capacities.

A number of MNs have been shown to represent broad characteristics regarding the target object. For example, different neurons within the monkey premotor cortex have been shown to selectively fire depending upon the orientation of the observed action (i.e. first person vs. third person perspective; Caggiano, et al., 2011), for the subjective value the individual monkey places on the effected object (Caggiano, Fogassi, Rizzolatti, Casile, Giese, & Thier, 2012), and for the location of the object in space with respect to the monkey's location (i.e. within or outside the monkey's peripersonal space; Caggiano, Fogassi, Rizzolatti, Thier, Casile, 2009). Taken together, these findings suggest that MNs may play a very specific role, not in understanding the actions, but in evaluating the actions of others and identifying appropriate behaviors to facilitate social interactions.

Proper evaluation of others' behaviors is also important when the presence or absence of an object is not observable. Umilta and colleagues (2001) demonstrated that while the goal of an action is important to activate a MN, the target object need not be visible. In their study, single-cell recordings were made while the monkey observed a series of grasps either directed at an object or pantomimed. After the action was completed, an opaque screen was slid to obstruct half the stage and the actions were preformed again. Umilta et al. found MN activity when the grasping was performed on the object but not when the actions were pantomimed. Moreover, when the target of the action was occluded, the actions still triggered MN activity, but again not in the occluded pantomimed condition. These findings suggest that MNs have the ability to represent the goals of actions even when the target of those actions is absent.

A continuous theme in these studies eliciting MN activity has been the specificity of the goal, and it seems that these neurons are not tuned for visual stimuli only. Examining neurons in F5, Kohler and colleagues (2002) identified neurons that responded both to the execution of an action and to the sound produced by that action (e.g. cracking a peanut). The researchers reported that about 13% of the neurons tested responded to auditory stimuli, and of those, a third also responded to the visual presentation (without the accompanying sound). The results reiterate the importance of the goal in the action for activating MNs rather than the modality through which the action is perceived.

While the majority of MNs that have been recorded were specific to hand actions, an important class of MNs is those activated for mouth movements. Ferrari, Gallese, Rizzolatti, & Fogassi (2003) identified MNs involved in a number of different mouth actions focused around ingestive actions. Interestingly, they identified a subset of the mouth MNs activated for observation of communicative gestures (e.g. lip smacking). This class of MNs has its greatest impact when considering their function in humans, specifically as it relates to their involvement in social learning and language development (Rizzolatti & Arbib, 1998).

Neurons exhibiting mirroring properties in the monkey premotor cortex have been studied extensively and individual neurons that demonstrate both ‘strict’ and ‘broad’ congruence are consistently observed. Strict congruence means that the observed and performed actions correspond both in terms of goals (e.g., grasping) and means to achieve the goal (e.g., whole hand grip). Broad congruence indicates correspondence in terms of action goals, rather than in the motor details adopted to achieve the goal (Gallese, et al., 2007). This pattern of firing activity has been interpreted as providing a functional mapping of the perceptual aspects of the actions of others onto the observer’s motor representation of that action (i.e., internal motor knowledge). As a consequence, neural mirroring allows the observer to have a direct, automatic, effortless understanding (an understanding from ‘within’) of the actions of others (Caggiano, et al., 2009; Caggiano, et al., 2011; Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010).

The Mirror Neuron Circuit. The MNs identified in area F5 all demonstrate action representational properties but do not encode the complex motor sequence that is required for complete action understanding. Neuronal populations outside of area F5 in the premotor cortex were studied in other motor regions, including the inferior parietal lobule (IPL; see Figure 1; Fogassi, et al., 2005) and the superior temporal sulcus (STS; Jellema, et al., 2000). These two regions combined with area F5 all support a more elaborated cortical circuit involved in action understanding (Craighero, Metta, Sandini, & Fadiga, 2007).

In a study by Jellema et al. (2000), single-cell recordings were made from cells in the STS. They identified subsets of cells that were active for different components of actions, including the gaze, the limb movements, and the overall intention of the action. Each of these subsets of cells is important for generating an enriched sensory description of the action and its components. For example, neurons that fire for the direction of gaze would have greater activity when the attention of the experimenter was directed towards the object but would not fire if the gaze of the experimenter was directed away from the object, thus playing a role in detecting the intention of the action, rather than the goal. While the neurons in the STS were not involved in the executions of actions, the output of the STS to a number of important regions of the monkey brain, including the premotor cortex, the IPL and the amygdala, make this a critical component of the MN system, both for the understanding of actions and as a possible contributor to higher order social behaviors such as emotion understanding and empathy.

The IPL, on the other hand, is a region that integrates sensory information but is also active during motor movements, containing mirror neurons similar to those identified in area F5. Fogassi et al. (2005) identified neurons in the IPL that became active to actions embedded in motor sequences and others that fired in preparation of actions. These neurons contribute to action planning and coordinating a series of actions in order to accomplish a goal. The addition of the STS, IPL, and F5 regions in the MN system enrich the subject's understanding of actions by combining the action representations, the intentions, and the motor sequence involved in action understanding.

Summary. There are a number of important features of the MN system. The primary function of MNs is the matching of execution and observation within the motor cortex that provides the subject with an action representation system. This observation/execution system can: (a) represent actions directed towards a specific goal, even when that goal is occluded from the monkey's visual field (Umilta, et al., 2001); (b) differentiate between tools and hands used to achieve the same goal (Ferrari, et al., 2005); (c) represent goals across both visual and auditory inputs (Kohler, et al., 2002); and (d) generalize or even learn the goals of novel actions (Ferrari, et al., 2005). Moreover, MNs contribute to a broader circuit whose function is to combine the representations of action components to understand others' behavior (Rizzolatti & Craighero, 2004).

For a MN system to exist in humans, each of these properties must be identified and examined. As I will discuss in the next section, there is considerable evidence that suggests humans have a MN system that is structurally and functionally similar to that of the monkeys', however, many of the features of MNs have yet to be characterized in humans and there remains conflicting evidence in some of the domains.

2.2 Evidence for Mirror Neurons in Humans

The invasiveness of single-cell recordings has generally precluded its application to research with humans. Other methods such as functional neuroimaging (fMRI), transcranial magnetic stimulation (TMS) and changes in sensorimotor alpha (or mu) and rolandic beta rhythms of the electroencephalogram (EEG) and magnetoencephalogram (MEG) have been successful in identifying neural activity associated with action-perception. Moreover, by moving past single-cell recording we have begun to enrich our understanding of the MN system beyond what has been described in the monkeys.

Only one study has used single-cell recordings in humans to examine neural responses to observation and execution of motor actions. Mukamel and colleagues

(Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010) measured neural activity from electrodes implanted in patients with intractable epilepsy while the participant executed both gripping actions and facial gestures (e.g. smile or frown). They found a subpopulation of neurons in the supplementary motor area (SMA) that responded like typical MNs to grasping behaviors. This evidence is very exciting, but there are a number of limitations. The most significant limitation is that electrode placement was driven by clinical criteria and no electrodes were placed in regions analogous to those found to contain mirror neurons in the monkeys. Second, the specific properties of these neurons were not fully explored and their activation patterns may have been a result of proprioceptive processing.

Evidence from Transcranial Magnetic Stimulation. The earliest evidence of a MN system in humans was provided by transcranial magnetic stimulation (TMS). The first experiment, by Fadiga and colleagues (Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995), showed that by stimulating the motor cortex under threshold with TMS, the motor evoked potential (MEP) from the muscles of the distal extremity of the contralateral arm increased during the observation of hand actions but not during observation of spots of lights or 3-D objects. These data confirm that motor cortex excitability is increased during action observation, probably reflecting an activation of the mirror neuron system in premotor areas.

The findings from Fadiga et al. (1995) did not conclusively rule out the notion that activation of the MEP was merely driven by presentation of the goal of the biological motion. Their results were replicated by Enticott and colleagues (Enticott, Kennedy, Bradshaw, Rinehart, & Fitzgerald, 2010), who further demonstrated that MEPs were greater for transitive actions than for non-goal-directed actions or for the static image of a hand. Additionally, Keuken and colleagues (2011) collected EEG while participants observed or executed goal-directed actions before and after the application of TMS to either the left IFG or the vertex. Interestingly, TMS to the left IFG, but not the vertex, abolished mu rhythm desynchronization during the observation of goal-directed actions and attenuated the magnitude of desynchronization in the execute condition (Keuken, Hardie, Dorn, Dev, Paulus, Jonas, et al., 2011). These results highlight the activation of execution circuits during observation as well as the goal-directed nature of the MN system.

Evidence from Functional Imaging Studies. The major contribution of functional neuroimaging to identifying a human MN system is the high spatial resolution that can be achieved. During the observation and execution of actions a cortical network formed by the posterior part of the inferior frontal gyrus (IFG), the inferior parietal lobe (IPL), and the superior temporal sulcus (STS), is activated (Buccino, et al., 2001; Dinstein, Hasson, Rubin, & Heeger, 2007; Iacoboni, Woods, Brass, Bekkering, Mazziotta, & Rizzolatti, 1999; Manthey, Schubotz, & von Cramon, 2003). The cytoarchitecture of these regions have been proposed to be the human homologue of the monkey regions associated with the mirror neuron circuit (see Figure 1).

A recent meta-analysis on 125 fMRI studies that examined activation of the human brain whose results were attributed to the mirror system identified the IFG, premotor cortex (PM), and IPL as regions that consistently respond to action observation and execution. These areas are core areas in processing action observation and execution (Molenberghs, Cunnington, et al., 2012). The recruitment of other areas, such as the cerebellum and the limbic system, suggest that additional areas that are not strictly related to motor functions are probably involved in integrating the postural and affective components accompanying an action.

The IFG in the human most closely resembles area F5 in the monkey premotor cortex and has been a major focus of imaging studies. Dinstein and colleagues (Dinstein, Gardner, Jazayeri, & Heeger, 2008; Dinstein, et al., 2007) have used a repetition-suppression paradigm to better identify those regions that consistently respond to both observation and execution of hand gestures. In their study, participants played rock-paper-scissors with a videotaped opponent. The researchers were able to identify regions that were resistant to habituation after repeated execution or observation trials that suggest mirroring responses. This paradigm consistently activated both the IFG and IPL and both of these regions showed little or no habituation during the task.

Recall that the monkey IPL is involved in integrating sensory information during observed action sequences and plays a role in the extraction of the observed actor’s intentions (Fogassi et al., 2005). This holds true for the human IPL as well. Iacoboni and colleagues (2005) conducted an imaging study in which subjects observed a series of videos that showed the context of an action scene (Context), the action alone (Action), and the action within the context (Intention). They found greater activation of the IFG during Action and Intention videos, but greatest activation of the IPL in the Intention condition. This suggests that the IPL in humans plays a similar role to the homologous region in monkeys, integrating motor and contextual information in order to identify the intentions of an actor. Interestingly, half the participants in this study were given instructions to focus on the intentional aspects of the scene while the other half were instructed to passively watch each scene. The difference in instructions did not result in any differences in activation of IPL nor in the behavioral reports on the intention of the actions in either group, suggesting that the human mirror system is driven by bottom-up processes, rather than more cognitive mechanisms (Iacoboni, Molnar-Szakacs, Gallese, Buccino, Mazziotta, & Rizzolatti, 2005; Molenberghs, Hayward, Mattingley, & Cunnington, 2012).

The results of Iacoboni and colleagues (2005) have been replicated and extended to show that co-activation of the IFG and IPL are recruited during imitation. Ocampo, Kritikos, and Cunnington (2011) had participants observe a video of an individual performing a grasping gesture and asked them to perform imitative or complementary actions to the gesture they were observing. The IFG and IPL were active in both conditions. During imitative gestures, however, both regions showed greater activation than during complementary action production. The results suggest that two neural mechanisms may be involved in action selection. First, the human action observation/execution system may facilitate appropriate actions within contexts requiring similar actions; however, within contexts in which conflicting actions are required, the bottom-up process can be inhibited to allow for alternate actions (Ocampo, et al., 2011).

The majority of functional neuroimaging studies have focused on actions produced by hands because of the relative ease of obtaining execution data. Buccino and colleagues (2004) wanted to characterize the circuits involved in mouth gestures in humans. Participants observed biting and social communicative gestures produced by human, monkey, and dog models. During the biting condition, activation of the IPL and IFG were observed for all three models. During the communicative gestures, however, these regions were active when observing the human (silent speech movements) and slightly activated during the monkey (lip smacking) gestures but not during the barking dog. While the visual patterns of the gestures were considerably different in the biting and communicative gestures, the human MN system seemed to have an abstract representation of the ingestive actions, but seemed to detect the greater relevance of the human communicative gestures relative to those of other animals (Buccino, Lui, Canessa, Patteri, Lagravinese, Benuzzi, et al., 2004; Leslie, Johnson-Frey, & Grafton, 2004).

Evidence from EEG and MEG Studies. The spatial resolution of neuroimaging studies has contributed significantly to identifying the structures involved in the human MN system, but the sensitivity to movement and the cost has limited its use. Examining the reactivity of the sensorimotor alpha (or mu) and rolandic beta rhythms of the EEG and MEG has further contributed to our understanding of a number of the critical features of the human MN system.

The mu rhythm was initially believed to be an “idling” state for the motor system comparable to the idling visual system occipital alpha (Pfurtscheller, Neuper, Andrew, & Edlinger, 1997). Indeed, early studies examining the mu rhythm and rolandic beta focused on their role in action preparation and execution. Pfurtscheller and colleagues, for example, identified the characteristics of hand and foot mu rhythm desynchronization in adults performing repetitive movements. They noted a desynchronization in 10Hz and 20Hz frequencies prior to the onset of motion and then synchronization two seconds following the movement in lateral central electrodes (C3/4) for the hand and fingers and in the medial central electrode (Cz) for the foot (Babiloni, Carducci, Cincotti, Rossini, Neuper, Pfurtscheller, et al., 1999; Pfurtscheller, et al., 1997). The central electrodes are on scalp locations above the sensorimotor cortex and roughly correspond to the classical homunculus with activation patterns for foot movements identified medially and hand and mouth actions more laterally.

The mu rhythm's sensitivity to the observation of biological movement was first described by Gastaut and Bert (1952), who were interested in changes in the EEG as a function of viewing dynamic stimuli (e.g. videos of a bike race, boxing, or a funeral). They found that desynchronization of mu rhythm was associated with the onset of the videos. Interestingly, the magnitude of the desynchronization was related to the amount of identification the participant had with the actor on the screen. While this study was qualitative in nature and lacked control videos without biological motion, the specificity of mu rhythm desynchronization to the observation of human motions has been consistently replicated (Cochin, Barthelemy, Lejeune, Roux, Martineau 1998; Cochin, Barthelemy, Roux, & Martineau, 2001; Holtz, Doppelmayr, Klimesch, & Sauseng, 2008; Martineau & Cochin, 2003).

The desynchronization of the mu rhythm during action execution and observation of actions has made it a candidate signal for the human MN system. Indeed, a number of studies have shown equivalent desynchronization for execution and observation of hand movements (Babiloni, Babiloni, Carducci, Cincotti, Cocozza, Del Percio, et al., 2002; Kessler, Biermann-Ruben, Jonas, Siebner, Baumer, Munchau, et al., 2006; Lepage & Theoret, 2006; Muthukumaraswamy & Johnson, 2004a,b; Muthukumaraswamy, Johnson, & McNair, 2004). In addition, the studies by Muthukumaraswamy and colleagues included non-goal-directed hand movements as control conditions. They showed that mu desynchronization occurred when the participant observed a precision grip but not when they observed the grip form without a goal present, showing modulation of the mu rhythm to the goal, rather than the action itself (Muthukumaraswamy & Johnson, 2004a,b; Muthukumaraswamy, Johnson, Gaetz, & Cheyne, 2006).

The mu rhythm has been shown to respond to many of stimuli that MNs in the monkeys respond to and have been extended beyond simple observation/execution matching. Desynchronization of mu has been shown as a result of: (a) action related auditory stimuli (Hadjidimitriou, et al., 2011; Lepage, et al., 2010; Paulus, Hunnius, van Elk, & Bekkering, 2012); (b) oral ingestive and communicative gestures (Muthukumaraswamy, et al., 2006); and (c) abstract motion stimuli, including robotic actions (Oberman, McCleery, Ramachandran, & Pineda, 2007) and point-light biological motion (Perry, Troje, & Bentin, 2010; Marshall & Shipley, 2009; Ulloa & Pineda, 2007). Taken together, these data suggest that, in addition to basic observation/execution matching, mu rhythm desycnhronization is sensitive to the goals of actions across modalities and actions, and is also sensitive to the goals of abstract stimuli.

One of the important findings from the MN studies conducted with monkeys was the capacity of the MN system to learn new behaviors and actions. In humans this has been studied extensively by examining mu suppression during actions observed by specialists and non-specialists. Studies contrasting dancers versus non-dancers found greater mu desynchronization during dance observation in the dancers familiar with performing the dance movement (Orgs, et al., 2008). Similar results have been described in karate athletes and non-athletes performing karate arm extensions (Del Perchio, et al., 2010), elite air-rifle athletes (Holmes, et al., 2006), expert archers (Kim, et al., 2011), and musicians (with the use of auditory stimuli; Hadjidimitriou, et al., 2011). These findings demonstrate that mu rhythm activity is adaptive and moderated by the degree to which an individual relates to the action.

Although the studies described above examined adults who had years of experience with the actions, a number of researchers have exmained the role of more natural experience. In a study of 14- to 16-month-old infants, van Elk and colleagues (2008) recorded EEG while infants observed videos of other infants walking or crawling. They found greater desynchronization in the mu and beta frequencies during the observation of crawling than during the observation of walking. The desynchronization in these bands was significantly correlated with individual differences in the amount of crawling experience the infants had (van Elk, van Schie, Hunnius, Vesper, & Bekkering, 2008; Virji-Babul, Rose, Moiseeva, & Makan, 2012).

The natural experiments described above demonstrate a relation between experience and activation of the action observation/execution system, yet few studies have controlled the experience of the individual through training and examined subsequent changes in the mu rhythm response. In one example of this manipulation, Paulus, et al. (2012) provided 8-month-old infants with a rattle that made a unique sound when shaken. Parents were instructed to allow the infant to play with the rattle (sound-action pairing) for five minutes per day over the course of a week. After a week of training, Paulus and colleagues measured EEG while the infants heard novel and familiar sounds (not associated with their actions) and the sound produced by shaking the rattle. Mu rhythm desynchronization was greater for the sound associated with the rattle action than for the novel or familiar sounds. Taken together with the natural experiments, these data suggest that the mu rhythm is sensitive to the level of experience an infant has had with an action and its goals.

In many of the studies examining the mu rhythm, reseachers also noted modulation of the beta frequency (13-25Hz) over the rolandic cortex during observation of actions (Babiloni, et al., 2002; Lepage et al., 2010; McFarlan, Miner, Vaughn, & Wolpaw, 2000; Muthukumaraswamy & Johnson, 2004b; Muthukumaraswamy, et al., 2006). The sources of the mu rhythm and rolandic beta were examined by Ritter, Moosmann, & Villringer (2009), who recorded EEG while participants underwent fMRI. They found that the mu rhythm significantly correlated with activity in the somatosensory cortex, suggesting that the mu rhythm may be associated with the intentionality of the actor, while beta activity was correlated with activity in the motor cortex and may be related to premovement preparation and maintenance.

The functionality of the rolandic beta is more complex than simple action matching, and the data from Ritter et al. (2009) suggests that beta may be related to the preparation and maintenance of an action. Koelewijn and colleagues (Koelewijn, van Schie, Bekkering, Oostenveld, & Jensen, 2008), however, suggest that the beta rhythm has a broader function in maintaining the overaching goal of the action. They showed participants short video sequences of an individual making correct and erroneous actions. The observation of erroneous actions had the stronger effect on beta desynchronization. Their conclusions are not in direct conflict of the findings by Ritter and colleagues or by Serrien (who used simple repetitive movements as stimuli; Serrien, 2009), but these results suggest that the beta rhythm plays a larger role in action understanding.

Indeed, a recent study by Avanzini and colleagues (2012) suggests that the beta rhythm may index motor maintenance for sequences of actions. In their study, EEG was collected while participants observed videos of simple hand gestures that were either goal-directed or not and were either presented once or as a sequence of two movements. They observed mu rhythm desynchronization for the goal-directed actions regardless of whether the action was presented alone or as a sequence. The magnitude and time-course of the desynchronization in the beta rhythm, however, was correlated with the velocity profile of the action and particularly for the repetitive action videos, suggesting that the functional role of the beta rhythm may be in the maintainance of sequences of actions required for goal attainment (Avanzini, Fabbri-Destro, Dalla Volta, Daprati, Rizzolatti, et al., 2012).

Jarvelainen, Schurmann, & Hari (2004) further support the role of rolandic beta in the evaluation of actions. They collected MEG while they participants observed a series of actions. In the different actions, an experimenter used a tool (chopsticks) to complete a goal (Goal-Tool), completed the goal with his or her fingers (Goal-Hand) and engaged in the motion with the tool but without a goal-object present (NoGoal-Tool). They found desynchronization of the rolandic beta rhythm in the Goal-Tool and Goal-Hand conditions. Desynchronization was greatest in the Goal-Tool condition, suggesting that as more components were involved in achieving the goal (i.e. hand and tools together versus hand or tools alone) there was greater activation of the rolandic beta. Furthermore, the desynchronization in beta was correlated with the amount of experience the participant had had with chopsticks in the last year, analogous to the tool findings in the monkey (Ferrari et al., 2005).

Summary. The evidence of a MN system in humans thus far is highly suggestive but remains inconclusive. While regions typically associated with the monkey MN system are active during execution and observation in humans, there is, as of yet, no evidence that the activity originates from the same single unit as in monkeys. However, results from neuroimaging studies demonstrate that, in humans, homologous regions of the monkey MN system are activated for the observation and execution of goal-directed actions. Like the F5 area of the premotor cortex in the monkey, the inferior frontal gyrus (IFG) is involved in action representation of both reaching and mouth gestures in humans (Buccino, et al., 2004; Dinstein, et al., 2007) and the IPL has been implicated in intention understanding (Iacoboni, et al., 2005).

A number of studies have replicated findings from the single-cell literature in the monkey that identify changes in the mu rhythm of the EEG and MEG in humans to: (a) tool-use (Jarvelainen, et al., 2004); (b) audition (Lepage, et al., 2010; Paulus, et al., 2012), (c) oral ingestive and communicative gesture (Muthukumaraswamy, et al., 2006); and d) abstract motion stimuli (Oberman, et al., 2007a; Marshall & Shipley, 2009; Perry, et al., 2010; Ulloa & Pineda, 2007).

The use of these noninvasive methods has provided converging evidence for a human MN system. Moreover, these techniques have provided opportunities to further explore hypothesized features of the putative MN system. These alternative methods provide an opportunity to measure observation/execution matching activity in different populations and under different conditions to begin to better understand the role of MNs in typical and atypical development as well as more complex social behaviors such as imitation and empathy.

2.3 Evidence for the Mirror Neuron Hypothesis in Development

Development of the MN System. For MNs to play a significant role in social development, their functional properties should be evident at birth and continue through early childhood and adolescence. The evidence for this assumption has only recently begun to emerge but has been heavily supported by the behavioral and electrophysiological studies described below.

The alpha rhythm, in which the mu rhythm is embedded, has been shown to increase in frequency over infancy and early childhood. Marshall, Bar-Haim, & Fox (2002) first described a central alpha rhythm that peaked at 6 or 7Hz at 5 months of age and increased to 8Hz by the second year and 9Hz by 4 years of age. In adults, the mu rhythm is typically found in 9-11Hz and; the data by Marshall and colleagues suggest that the mu rhythm matures by 4 years. Borrowing the procedures developed by Muthukumaraswamy et al. (2004a), Lepage and Theoret (2006) examined mu rhythm activity during the execution and observation of a precision grip in 4- to 11-year-old children. They found desynchronization to both execution and observation condition in frequencies similar to that reported in the adult literature. The peak mu rhythm frequency in this work was no different than those reported in adults, confirming the finding by Marshall et al.

The majority of research examining mu rhythm desynchronization has employed reaching behaviors as stimuli and young infants are still developing reaching behaviors across the first year of life (Berthier & Keen, 2006; Fagard, 2000; McCarty, Clifton, Ashmead, Lee, & Goubet, 2001). Behavioral studies examining the onset of reaching and successful grasping of objects have shown the ability to emerge around 4 months of age

(Clifton, Muir, Ashmead, & Clarkson, 1993; von Hofsten, 1991), however, most of the actions are ballistic, meaning once the action sequence is triggered it continues through completion without the use of visual feedback to update the motor plan. Over the course of her career, Rachel Keen (formerly Rachel Clifton) and her colleagues (Clifton, et al., 1993; Clifton, Rochat, Litovsky, & Perris, 1991; Clifton, Rochat, Robin, & Berthier, 1994; Goubet & Clifton, 1998; McCarty, et al., 2001; Perris & Clifton, 1988) have shown that infants rely on proprioceptive cues in the 5 to 6 months following grasping onset. In these studies, the researchers had infants perform grasps on objects in the dark effectively removing the influence of any visual feedback on the action performed. They found that infants' ability to grasp in the dark develops concurrently with the onset (Clifton, et al., 1993), kinematics (Clifton, et al., 1994), selection of an appropriate hand orientation for the grasp (McCarty, et al., 2001), the selection of uni-manual or bimanual strategies (Clifton et al., 1991) as they do in the light suggesting an early reliance on proprioceptive information for completing these tasks.

After roughly 9 months of age, infants begin to better incorporate visual feedback information into their actions and this coincides with a decrease in ballistic grasping actions and an increase in arm, wrist, and hand coordination that resembles the grasping kinematics of adults (Berthier & Keen, 2006; Berthier, Clifton, Gullapalli, McCall, & Robin, 1996; Churchill, Hopkins, Ronnqvist, Vogt, 2000; Savelsberg, von Hosten, & Jonsson, 1997; von Hofsten & Ronnqvist, 1988). Moreover, the motor plans of the grasping actions begin to show prospective characteristics and the linking of action sequences (Chen, Keen, Rosander, von Hofsten, 2010; Claxton, Keen, McCarty, 2003; McCarty, Clifton, & Collard, 1999). Because of this protracted development of grasping behaviors, it is unlikely that we would see mu rhythm desynchronization to grasps before 9 months in infants.

The developmental literature on the emergence of the mu rhythm shows just that. In a study of 6-month-old infants with limited grasping experience, no suppression of the mu rhythm during observation of grasps was found (Nystrom, 2008). However, a number of studies have examined the observation and execution of grasping behaviors in infants as young as 8 months (Nystrom, Ljunghammar, Rosander, & von Hofsten, 2011; Southgate, et al., 2010; Southgate, Johnson, Osborne, & Csibra, 2009). Nystrom et al. found desynchronization during the observation of goal-directed grasps in 8 month olds in the 6-8 Hz frequency band but did not collect data on the execution of grasps.

Southgate and her colleagues replicated the findings of Nystrom et al. (2011) in 9 month olds. They also collected EEG while the infants completed grasps and thus were able to fully characterize the mu rhythm during action execution (Southgate, et al., 2010; Southgate, et al., 2009). In their task, similar to Umilta et al (2001), infants were presented with goal-directed grasps and non-goal directed actions in visible and occluded conditions. Southgate and colleagues found mu desynchronization for both the occluded and visible goal-directed grasp (Southgate, et al. 2010).

If the mu rhythm is an index of MN activity in development, the desynchronization to the mere observation and execution of actions is insufficient, the extraction of the goal has the greatest importance to this link. Luckily, behavioral evidence in 9-month-olds suggests that infants are able to extract the goal of grasping actions completed by a human (Cannon & Woodward, 2012; Woodward, 1998, 2005).

The lack of finding mu rhythm desynchronization before 9 months of age likely reflects the reliance on grasps as the eliciting stimuli.

Between birth and 9 months, infants recruit the motor system to make sense of everyday actions they observe in their environment. In fact, eye gaze has been an informative measure in understanding infants' processing of action events. Older infants and adults will make predictive eye-movements when observing action sequences within their motor repertoire (Cannon & Woodward, 2012; Cannon, Woodward, Gredeback, von Hofsten, & Turek, 2012; Falk-Ytter, Gredeback, & von Hofsten, 2006). Falk-Ytter and colleagues showed adults, 12-, and 6-month-old infants videos of an object being grasped and moved to another location while the participants' eyes were tracked. They found that the adults and 12-month-old infants made predictive gaze shifts to the location where the object was placed while 6-month-old infants tracked the action reactively. In a similar design, Cannon and colleagues (Cannon, et al. 2012) showed 12-month-old infants videos of objects being pickup and placed in a pail. They found that predictive gaze shifts were found in infants who spontaneously placed objects in a pail in a free play session. These results suggest that infants rely heavily on their own experience when observing actions. Indeed, 6-month-old infants rarely make grasping actions for displacing an object to alternate location, but will routinely grasp and object and bring it to their mouths. In a follow-up eye-tracking study of 6-month-old infants observing grasping movements that result in the object being brought to the mouth, Kochukhova and Gredeback (2010) found that infants made predictive gaze shifts to the mouth but not when the object moved by itself without the presence of a hand. Taken together, these studies demonstrate a reliance on the motor system in making sense of everyday actions. Furthermore, these studies demonstrate the role of experience with actions in order to activate the motor system and provide one possible method for assessing early mu rhythm development.

Future research on the mu rhythm should harness the experimental designs of behavioral studies in younger infants in order to address questions on the ontogeny of the MN system. Neonatal imitation is a behavior that is present at birth and has significant importance in the social development of infants (Meltzoff, 2002, 2006; Meltzoff & Moore, 1977). Indeed, the use of imitation has become a valuable tool for eliciting execution behaviors from infants and provides a natural observation condition as well. Marshall, Young, and Meltzoff (2011) exploited this methodology in order to examine the development of the mu rhythm in 14-month-old infants. Using a button-box that played a short melody when pressed, Marshall and colleagues showed mu rhythm desynchronization in central electrodes for both action execution and observation. In the observation portion, in contrast to in the execution portion, desynchronization was identified over a broader surface of the scalp. Although desynchronization is isolated to the central electrodes for observation conditions in adults, multiple studies now confirm that this desynchronization has a broader distribution including frontal and parietal electrodes in infants (Marshall et al., 2011; Saby, Marshall, & Meltzoff, 2012; Stapel, Hunnius, van Elk, & Bekkering, 2010).

Recently, it was discovered that infant rhesus macaques imitate their mothers and other nonconspecifics' tongue protrusion and lip smacking gestures and that these behaviors can be observed on the first postnatal day (Ferrari, Paukner, Ionica, & Suomi, 2009; Ferrari, Visalberghi, Paukner, Fogassi, Ruggiero, & Suomi, 2006). These imitative behaviors offer an execution paradigm that can be used with infant macaques shortly after birth. Recently, Ferrari and colleagues (2012) used this paradigm while collecting EEG from rhesus infants in the first week of life. We found suppression in the 5-6 Hz frequency band in electrodes placed approximately over area F5 during the execution and observation of lip smacking and tongue protrusion, compared to a non-biological control condition (see Figure 2). These findings are the first to show that the motor system is activated equally to action production and observation in early imitative behaviors over the first week of life (Ferrari, Vanderwert, Paukner, Bower, Suomi, & Fox, 2012).

The Mirror Neuron System in Social Behaviors. The MN hypothesis suggests a role for the motor system in the development of imitation and social interactions, theory of mind, and language processing. Moreover, the theory suggests that disorders marked by deficits in these domains may stem from a dysfunctional MN system. These hypotheses have driven researchers to explore the role of the motor system in each of these domains.

In an early study examining mu rhythm suppression to social interactions, Oberman and colleagues (2007b) collected EEG while adults passively watched videos of individuals playing a game of catch. For some of the videos, the ball was tossed toward the camera as if the participant was included in the game. Oberman et al. found that the social nature of the actions directly impacted mu desynchronization. When the participant was “included” in the game, he or she had greater mu desynchronization as compared to observing the game without involvement (Oberman, Pineda, & Ramachandran, 2007b; Perry, Stein, & Bentin, 2011). This suggests that the motor system is activated in joint activities and is directly involved in social behaviors.

In fact, the effect described by Oberman and colleagues (2007b) has been replicated in 3-year-olds (Meyer, Hunnius, van Elk, van Ede, & Bekkering, 2011) and in 14-month-olds (Reid, Striano, & Iacoboni, 2011) interacting with an adult. In both of these studies, the children observed the adult performing actions independently or as part of a joint action with the child. The joint action condition caused greater mu rhythm desynchronization in both age groups; additionally, in the 3-year-olds, there was a significant desynchronization in the sensorimotor beta rhythm during the joint action condition (Meyer, et al.). These studies demonstrate that the motor system is more activated during social interactions in which the participant benefits from coordinating his or her actions with another.

This effect has been shown in a more direct fashion with adults. Dumas, Nadel, Soussignan, Martinerie, & Garnero (2010) simultaneously collected EEG from adult dyads while the participants took turns modeling or imitating hand gestures. Remarkably, they found synchronous activity between participants in both the mu rhythm and beta rhythm over comparable scalp locations during periods of interactional synchrony. This is the first study to simultaneously record EEG from two interacting partners and the findings from this study demonstrate a strong congruence between mu and beta rhythms during ongoing social interactions.

Meltzoff (2002, 2006) has proposed that social interactions emerge through imitative processes. The study described above examining imitation in infant monkeys demonstrated that the MN system is involved in neonatal imitation (Ferrari, et al., 2012), but social imitation continues to be an important behavior throughout the lifespan, facilitating the acquisition of new skills. Using imitation tasks in human infants and adults, Marshall and colleagues demonstrated that the observation/execution matching system continues to be recruited for imitation across development (Marshall, Bouquet, Shipley, & Young, 2009; Marshall, et al., 2011; Saby, et al., 2012). Interestingly, in the studies with adults, the amount of motor activation measured during observation was related to the fidelity of the imitated action, suggesting that affiliation may have a greater impact on the activation of the MN system (Marshall et al., 2009).

Both the social interaction and imitation data described above involved an explicit motor component. Thus, activation of the motor system should not be surprising. It has been hypothesized that imitation facilitates the acquisition of theory of mind (Meltzoff, 2002), a more complex social-cognitive behavior. One study examined the relation of mu desynchronization to mental attribution to cartoon stimuli (Pineda & Hect, 2009). The results of this research were inconclusive and only demonstrated a trend in mu suppression during the mental attributions condition. The lack of findings may have been an effect of task difficulty as participants had lower accuracy scores for emotions and mental attribution tasks compared to their control conditions.

Evidence for the role of MNs in understanding others' emotions have also begun to emerge. Using fMRI, Saarela, Hlushchuk, Williams, Schurmann, Kalso, & Hari (2007) examined neural responses to faces displaying differing intensities of pain. Consistent with the MN hypothesis, there were significant activations of the IFG, SMA, and IPL in response to the pain faces. Additionally, participants rated their own distress in response to viewing each of the faces and their own distress was significantly correlated with activation in the IFG. This study has been replicated using both EEG and MEG and again, the magnitude of suppression was related to the participant's own ratings of personal distress and perspective taking (Cheng, Yang, Lin, Lee, & Decety, 2008; Woodruff, Martin, & Bilyk, 2011; Yang, Decety, Lee, Chen, & Cheng, 2009).

The links between MNs and language acquisition are tied directly to the cytoarchitecture of the MN system. Broca's region, an area associated with language production, is embedded within the IFG and the isomorphism between Broca's and area F5 suggests that MNs may play a direct role in the evolution of human speech (for a review see Rizzolatti & Arbib, 1998). In addition to the communicative gesture data presented above (Buccino, et al., 2004; Ferrari, et al., 2003; Muthukumaraswamy, et al., 2006), the funtional role of MNs in communicative gestures was further explored by Schippers, Roebroeck, Renken, Nanetti, and Keysers (2010). They scanned couples while the pair engaged in a game of charades. Scanning occurred both while participants were the guesser and the gesturer in the game. The design of the study allowed the researchers to measure the brain activity for the guesser and gesturer simultaneously, revealing a temporal correspondence in activity in the observation-execution circuit and in regions involved in assessing the mental state of others. This is the first study to examine neural activation during social interactions and the results demonstrate neural resonance between social partners, a key component of the MN hypothesis.

Finally, if MNs play a significant role in facilitating imitation, theory of mind, empathy, and language in typically developing individuals, then individuals with dysfunctional MN systems would show marked deficits in these domains. Autism spectrum disorder (ASD) is a disorder marked by such deficits in these domains (American Psychiatric Association, 2000).

The evidence from studies with children and adults diagnosed with ASD is conflicting. For as many studies that report deficits in MN responses to observation of goal-directed actions, there are just as many that show a typical response. In the earliest studies, mu desynchronization was observed during the execution of repeated releasing and clenching of a fist, but in children and adults with ASD, no desynchronization occurred during observation (Oberman, Hubbard, McCleery, Altschuler, Ramachandran, & Pineda, 2005). A similar effect was observed in children with ASD watching videos of a woman stretching (Martineau, Cochin, Magne, & Barthelemy, 2008) and for images of faces expressing the five basic emotions (Dapretto, Davies, Pfeifer, Scott, Sigman, Bookheimer, & Iacoboni, 2005). These studies concluded that children and adults diagnosed with ASD had impairments in the MN system for the observation of actions.

Conversely, Raymaekers and colleagues used the same paradigm as Oberman, et al. (2005) and found that children with ASD had similar patterns of mu desynchronization as typically developing children during the observation condition (Raymaekers, Wiersema, & Roeyers, 2009). Using the repetition-suppression design described above, Dinstein, Thomas, Humphreys, et al. (2010) showed that adults with ASD did not differ from typical adults in activation of the MN circuit during a game of rock-paper-scissors. These findings, in contrast to those described above, suggest that children and adults with ASD do not have impairments in their MN system.

There are a number of reasons these studies could have found contradictory results. First, there were no assessments of the participants' imitation ability and this may play a role in the degree to which there is MN activation. Bernier, Dawson, Webb, & Murias (2007) tested typical adults and adults with ASD on a number of different hand gestures, facial expressions, meaningless hand movements, and actions on objects and the fidelity of imitation was scored. They then collected EEG with these subjects using the commonly used precision grip paradigm (Lepage & Theoret, 2006; Muthukumaraswamy, et al., 2004a,b). Compared to the typical adults, adults with ASD had significantly less mu desynchronization during the observe condition. Importantly, this suppression was related to their fidelity scores in the imitation task.

A second issue concerns the role of stimulus familiarity in the activation of the MN system in children with ASD. Oberman, Ramachandran, and Pineda (2008) varied the degree to which the hand performing the action was familiar to the participant. When children observed their own hand or a hand of a relative performing the grasp, the magnitude of mu suppression was no different than typically developing children; only in the condition in which the hand belonged to a stranger did the children with ASD show no mu suppression. Taken together, the results of Oberman, et al. and Bernier, et al. (2007) suggest that features of the stimulus or individual differences may impact the degree to which the MN system is activated in children and adults with ASD.

Summary. Research using noninvasive methodologies has revealed a number of interesting findings that support the MN hypothesis. The MN hypothesis posits that the observation/execution matching system plays an important role in the formation of action understanding, imitation, theory of mind, and language acquisition and processing. In order to claim that MNs facilitate this learning, it is crucial to understand their development. There is emerging evidence that MNs activate to the observation of goal-directed actions in human infants, children, and adults (Lepage & Theoret, 2006; Muthukumaraswamy & Johnson, 2004a,b; Nystrom, et al., 2011; Southgate, et al. 2009).

Moreover, there is evidence to suggest that MNs are present at birth and are prepared to respond to imitative gestures (Ferrari, et al., 2012).

Furthermore, there is convincing evidence that MNs: (a) facilitate imitation and provide a system for the refinement of imitative actions (Marshall, et al. 2009); (b) are involved in social interactions (Dumas, et al., 2010; Meyer, et al., 2011; Oberman, et al., 2007b; Reid, et al., 2011); (c) play a role in language processing and communicative gestures (Schippers, et al., 2010); (d) activate during feelings of empathy (Cheng, et al., 2008; Saarela, et al., 2007; Yang, et al., 2009); and (e) may be a primary deficit in children and adults with ASD (Bernier, et al., 2007; Oberman, et al., 2008).

2.4 Open Questions

As is the case with any new discovery or theory, there are more questions asked than answered. In order to validate many of the assumptions generated by the MN hypothesis, a number of big questions will need to be addressed. First, the validation of the mu and rolandic beta rhythms as indexes of MN activity needs further exploration. Simultaneously recording single-cell and scalp EEG from an adult monkey will provide considerable evidence linking the mu and beta rhythms to their underlying mirror system. Moreover, this data would provide important information dissociating the functionality of each frequency band.

The origins of MNs are poorly understood. There is evidence suggesting that MNs emerge through experience (Ferrari, et al., 2005) and are only present for motor actions within the repertoire of the individual (van Elk, et al., 2008). A recent theory questions the innateness of MNs. Heyes (2010) suggests that MNs evolve through a process of associative learning. Through the natural process of pruning, neurons that are originally wired for one function, but occasionally fire for the other, become selected for both observation and execution. Conversely, others have suggested that MNs are present at birth and are the underlying mechanism for neonatal imitation (Lepage & Theoret, 2007; Casile, Caggiano, & Ferrari, 2011). This debate is an exciting area for continued research.

The methodical testing of MNs in the monkey has demonstrated key features of MNs that distinguish them from other neurons. This level of control has not yet been integrated into research in humans. First, activation of MNs during repeated testing and their failure to habituate suggests that the MN system is involved in bottom-up processing of motor stimuli (Gallese et al., 1996; Rizzolatti et al., 1996). This function has been adapted to some extent in the neuroimaging literature (Dinstein, et al., 2008; Dinstein, et al., 2007), but these techniques have not been used in EEG or MEG studies. Second, MNs in the monkey are activated during both the execution (without observation) and observation (without execution) of goal-directed actions. Every study with humans that involved an execute condition allowed the participant to visually guide their action. This contaminates the neural response and does not allow us to categorically say that human MNs activate for execution alone.

It has been hypothesized that MNs are important for developing social behaviors such as empathy. While evidence is beginning to emerge implicating the motor system in the observation of pain (Cheng, et al., 2008; Saarela, et al., 2007; Woodruff, et al., 2011; Yang, et al., 2009), characterization of the neural circuits in the monkey have not been established. Exploration in this domain will enhance our understanding of the MN system’s role, if any, in empathy and social interactions. Additionally, identifying the cytoarchitecture of empathy in the human will point neurophysiologists to regions of interest for empathic MNs.

Finally, understanding what mechanisms inhibit the action sequence activated by action observation will provide us with a more complete picture of learning within the MN system. Opponents of the MN hypothesis argue that a motor resonance system may be maladaptive if it always resulted in imitation (Jacob & Jeannerod, 2005); the mechanisms that provide an individual the opportunity to select appropriate actions in social interactions must be explored further.

2.5 Conclusions

The incidental discovery of MNs in the monkey premotor cortex has sparked a renewed interest in motor theories of development with the MN hypothesis at the forefront. The MN hypothesis emphasizes the link between observation and execution within the motor system as the fundamental mechanism for internalizing the goals and intentions of others. Evidence from single-cell recordings in monkeys has described neurons and circuits that represent the specific goal of actions whether the target of the action is occluded, being retrieved by a hand or a tool, or outside of the visual field. These neurons can extract the intentions of actions and maintain the goal throughout the dynamics of action sequences.

In humans, single-cell recordings are highly suggestive, but inconclusive regarding the existence of MNs. Neuroimaging studies have identified homologous regions of the monkey MN system that are activated during the observation and execution of goal-directed actions in humans. EEG and MEG have been useful tools for better understanding the key features of the human MN system. Data has demonstrated that the human MN system is active in conditions analogous to those in the monkey. In fact, the evidence suggests that the human MN system has greater flexibility and can extract the goals from abstract stimuli such as point-light displays or the actions of nonconspecifics.

Finally, the noninvasive measurement of MN activity has extended our understanding of the development of the MN system in humans. Studies examining MNs in infancy demonstrate their involvement in behaviors believed to seed the learning systems for complex social behaviors such as theory of mind, empathy, and language acquisition. Imitation is an important behavior that will allow researchers to examine MN activity in human infants at younger ages to better characterize the role MNs play in human development and to weigh in on the debate over the origins of MNs.

Chapter 3. The Current Studies

3.1 Statement of the Problem

The identification of MNs in the rhesus macaque has fueled a number of theories concerning the role of these cells in imitation, action understanding, and the learning of complex social behaviors such as theory of mind, language, and empathy (Fabbri-Destro & Rizzolatti, 2008; Gallese et al., 2004; Rizzolatti & Craighero, 2004). Although converging evidence from noninvasive techniques, such as fMRI, EEG, and TMS, suggests that humans have a MN system, these methods have very low resolution for identifying activity in single cells. It is, therefore, likely that what is actually being measured in these studies are larger networks associated with social information processing, motor preparation, and action plan maintenance. Because we cannot rule out all of these explanations for the patterns of activation observed in each technique, we are required to manipulate our stimuli to tease apart the specific activations patterns and their functional role.

One of the primary characteristics of MNs in the rhesus macaque is that they fire for observation alone, execution alone, and for the observation of one's own executed actions (Rizzolatti et al., 1996). As of yet, the literature in humans has neglected to measure activity in the execution alone condition. Study one addressed this gap by measuring EEG while human adults and 9-month-old infants executed grasps of small toys in the dark.

The earliest reports of identification of mu rhythm desynchronization to grasping actions are in 8 to 9 month olds (Nystrom, et al., 2011; Southgate, et al., 2009; Southgate, et al., 2010). At this age, behaviorally, infants rely heavily on proprioceptive cues to execute grasps in both the light and dark (Clifton, et al., 1993; McCarty, et al., 2001; von Hofsten, 1991). In contrast to the infants, adults employ different motor strategies while executing grasps in the light versus grasps in the dark. In the dark, adults are similar to infants relying on proprioceptive cues to complete their actions, however, when completing actions in the light, adults incorporate visual feedback to monitor and guide their action online (Berthier & Keen, 2006; Berthier, et al., 1996; Churchill, et al., 2000). This difference of motor strategies has the potential to provide a richer interpretation of the functional role of desynchronization of the mu rhythm during light and dark grasps.

A second fundamental question concerns the origins of MNs. For MNs to play a significant role in the acquisition of complex social behaviors, their functional properties should be evident at birth and continue to be identified across the lifespan. Recent evidence suggests that imitation activates the human observation/execution matching system and that this activation continues into children and adults (Marshall et al., 2009; Marshall et al., 2011). The same is true for infant rhesus macaque infants over the first week of life (Ferrari et al., 2012). Study two examines how the pattern of motor activation (as measured through mu suppression) differs between a sample of mother- and nursery-reared rhesus macaques on day 3 post-partum. This study further contributes to our understanding of the origins of the MN system by measuring EEG changes during the observation and execution of imitative gestures in a population of rhesus infants reared in more naturalistic settings than those in Ferrari et al. (2012). Furthermore, the study examines the role of early experience on the developing MN system.

3.2 Previous Evidence

Study two was an extension of work previously completed in collaboration with Dr. Ferrari, Dr. Suomi, Dr. Paukner, and Dr. Fox. This work examined changes in the EEG signal during the observation and execution of facial gestures of infant rhesus macaques reared in a nursery setting. EEG was collected from infants on days 1, 3, 5, and 7 postpartum in the imitation paradigm.

We found that desynchronization was greatest in the 5-6Hz frequency band of the EEG during gesture production across the scalp ($t(14) = 5.01$, $p < 0.001$) and that during observation this desynchronization was most prominent for lip smacking and tongue protrusion over the anterior scalp locations (A3/4; $t(20) = 3.27$, $p < 0.005$; $t(20) = 2.26$, $p < 0.05$ respectively) but not posterior locations (P3/4; $t(20) = 1.22$, *n.s.;* $t(20) = 1.57$, *n.s.* respectively; see Figure 2). The findings from this study suggest that there is an action observation/execution system present in the first week of life and that it is active during the observation and execution of facial gestures.

3.3 Overview of the Project

This project consisted of two studies designed to examine the neural correlates of action perception in (1) human infants and adults and (2) a cross-species sample of mother- and nursery-reared rhesus macaque infants. These two studies addressed four major issues in the literature. First, in highlighting the role of mirror neurons in execution alone, the first study addressed a major gap in the literature examining desynchronization of mu rhythm during the execution of actions without visual information. Using the procedure outlined below, the findings from study one contribute to our understanding of the functional role of the mu rhythm, either as an index of mirror neuron activity or as an attention signal modulated by motor events. In this study, I also examined the timing of mu rhythm desynchronization in relation to the execution of simple grasping behaviors.

The second study contributes to our understanding of the origins of the MN system. There is considerable debate as to the origins of MNs in the monkey cortex. One theory suggests that they are operational at birth highlighting their role in early social learning and neonatal imitation (Lepage & Theoret, 2007; Casile, et al., 2011) while others suggest that MNs develop through processes of Hebbian learning (Heyes, 2010). Identification of MNs was first done in rhesus macaques and study two measured scalp EEG from a sample of mother- and nursery-reared rhesus infants in the first week of life. Identification of a mu rhythm in infants in the first week of life provided evidence for the origins of MNs; furthermore, using the neonatal imitation paradigm (described below) I was able to demonstrate recruitment of the motor system during observation and execution of affiliative gestures. Moreover, by using noninvasive techniques to measure motor activation during observation in macaques, this study provided converging evidence for the mu rhythm as an index of MN activity in humans.

Our previous work (described above) demonstrated changes in EEG during the production and observation of facial gestures in infant nursery-reared macaques. Study two extended these findings by examining changes in the EEG of mother-reared infants during the observation and execution of the same facial gestures. Mother- and nursery-reared infants were raised with differing experience with the neonatal imitation gestures used in this study; by examining rearing effects on the mu rhythm, I was able to identify the effects of early experience, in as little as three days, on the formation of the MN system.

3.4 Research Questions and Hypotheses

1. *Does the mu rhythm desynchronize for reaching execution in the dark in human adults and infants?*

I hypothesized that there would be desynchronization in the mu rhythm specific to the central electrodes during the execution of a reach in the dark in both adults and infants. This would provide further evidence that the mu rhythm is an index of activity of the putative human mirror neuron system.

2. *Is the time course of the mu rhythm different between human adults and infants when reaching in the dark compared to reaching in the light?*

This question was exploratory and I had no hypotheses about the time course of mu rhythm desynchronization or any group differences that might exist. Previous evidence examining the time course of mu rhythm desynchronization in infants (Southgate, et al 2009) was inconclusive and differed greatly from the evidence from adults (Pfurtscheller, et al. 1997).

3. *Is the mu rhythm observable in three–day-old mother-reared infant macaques during both action execution and observation?*

I hypothesized that mu desynchronization would be evident in the anterior electrodes of three-day-old mother-reared macaques during the observation of facial gestures and in anterior and posterior electrodes during gesture production of facial gestures. Evidence from my previous work suggested that mu rhythm is present within the first week of life in the rhesus infant and that desynchronization during the observation of facial gestures is greatest over anterior electrodes. During gesture production, however, desynchronization was observed across the scalp (see Previous Evidence; Ferrari et al., 2012).

4. *Is there a difference between nursery-reared and mother-reared infant rhesus macaques in the magnitude of mu rhythm desynchronization during action execution and observation?*

I hypothesized that there would be greater mu rhythm desynchronization during action execution and observation in the mother-reared sample compared to the sample of nursery-reared infants. The data from previous studies on the imitative behaviors of nursery-reared and mother-reared macaque infants suggested that mother-reared infants receive significantly more experience with lip smacking than nursery-reared infants and this might result in differences in the magnitude of electrophysiological signals in response to the observation and execution of facial gestures.

Chapter 4. Study 1: Mu Development in Human Infants and Adults

4.1 Methods

Participants. Thirty-five 9-month-old infants (*M* = 9.25 months, *SD* = 0.43; 13 males and 22 females) and 22 adults (*M* = 19.25 years, *SD* = 1.52; 9 males and 13 females) participated in this study. Infants were recruited through bulk mailing soliciting contact information from parents interested in having their child participate in the study. All infants were typically developing with no obvious genetic anomalies, were not born premature, and had no significant medical or motor problems. The adults in this sample were undergraduate students at the University of Maryland recruited through the university's online participant database. Demographic breakdown for the infant and adult samples can bee seen in Table 1. Seventeen infants and 8 adults were excluded from the final sample due to technical difficulties (n_{infants} = 6; n_{adults} = 3), excessive fussiness (n_{infants} = 5), insufficient number of grasping trials (n_{infants} = 5; n_{adults} = 3), or because their data were statistical outliers (n_{infants} = 1; n_{adults} = 2). Informed consent was obtained from the parents of the infant sample and directly from the adult participants; the University of Maryland Institutional Review Board approved all procedures.

Procedure. After the EEG net was applied, infant participants were seated on their parent's laps and adult participants in a chair placed in front of a small stage and curtain. Participants completed two tasks: (a) Resting EEG and (b) a Grasping Task. In the resting EEG task, participants sat quietly while EEG was recorded for 2 minutes of alternating 15-second periods of lights on and lights off. While the lights were on, participants observed a bingo wheel filled with colorful ping-pong balls placed on the stage and manually rotated by an experimenter out of the participant's view. The bingo wheel had been previously used in our laboratory to standardize behavior and attention states in awake infants (Marshall, et al., 2002).

Following the collection of the resting EEG, the grasping task began. The grasping task consisted of three trial types: (a) Observe, (b) Light Execute, and (c) Dark Execute. Observe trials began when the curtain of the stage rose and a toy was seen at the center of the stage; the experimenter made eye contact with the participant before shifting her gaze towards the toy. Then the experimenter completed a reach and grasp of the toy. The trial finished once the experimenter had given the toy a shake to produce a rattle sound and the curtain closed.

Light Execute and Dark Execute trials were completed using the same procedure, except that the Dark Execute trials were completed in complete darkness. The trials began when the curtain rose to reveal a toy placed on the center of the stage. The experimenter then lit up the toy with a small light that emitted a sound to orient the participant's attention for approximately 2 seconds before extending the platform of the stage out to within the participant's reach. If the infant did not complete a grasp on the toy within 4 seconds, the toy was relit for 1 second.[1] The relighting of the toy was repeated as many times as needed to get the infant to grasp the toy. The trial ended once the participant grasped the toy and the curtain closed.

Participants completed up to 10 blocks of the task. A block of trials consisted of two Observe trials and two Execute trials randomized in their presentation order followed by two Dark Execute trials. The number of trials each group completed on average can be seen in Table 1. The procedure used for the Dark Execute trials was a modification of a paradigm developed by Clifton and colleagues which has been shown to reliably elicit dark grasps from 9-month-old infants (Clifton, et al., 1991; Perris & Clifton, 1988). Of the 20 possible trials in each condition, infants completed $M = 14.84$ ($SD = 4.06$) Observe trials, $M = 14.05$ ($SD = 4.45$) Light Execute trials, and $M = 12.32$ ($SD = 4.92$) Dark Execute trials. Adults completed all possible trials.

Video Recording and Reliability. During testing sessions, a video was recorded on a computer time-locked to the EEG data for later behavioral coding. The camera was equipped with a night vision setting and two infrared lights were placed in the room so that the behaviors of subjects could be observed during Dark Execute trials. Coders identified the time at which the experimenter (Observe trials) or participant (Execute and Dark Execute trials) touched the toy that resulted in a successful grasp and the hand that was used. Two independent coders coded the videos with 20% overlap for reliability purposes. Three frames (or 100ms) variability was tolerated and percentage of correct grasp judgments was computed. Reliability between the two coders for Time of Touch to Grasp was 95.2% for adults and 92.9% for infants. During the Dark Execute trials, coders noted if the light was on within 500ms of the grasp and trials in which this was the case were excluded from analyses.

EEG Acquisition and Analysis. EEG was recorded during the task using a 64-channel Electro Geodesic Inc. (EGI) system. EGI sensor nets that were the appropriate size for the head circumference of subjects were fitted on the participant. EEG data was digitized with a sampling rate of 500Hz and recorded on a computer using EGI's NetStation software with respect to the vertex reference and a sensor placed on the midline just posterior to the reference sensor as ground. Vertical eye movements were recorded from electrodes placed above and below the left and right eyes in adult participants; the infant sensor nets do not include sensors below the eyes. Impedances were kept below 50kΩ for adults and 80kΩ for infants.

EEG processing was completed offline using the EEG Analysis System from the James Long Company. EEG was re-referenced using an average mastoid montage. In adults, eye-blink artifact was regressed from the EEG and artifactual signals exceeding +/-150μV were identified and excluded from subsequent analyses. In infants, blinks and artifactual signals exceeding +/-250μV were excluded from subsequent analyses. I then computed event-related synchronization/desynchronization (ERS/ERD) using the methods described by Pfurtscheller (2003): EEG was band-pass filtered for the alpha rhythm (9 to 13 Hz in adults and 4 to 8 Hz in infants) and then squared to produce power values (μV^2). I then averaged the filtered and squared data and time-locked it to one-second windows beginning 500ms before the Time of touch to Grasp. I computed the average power in 125ms bins across the one-second window. Finally, I computed the natural log of the ratio of the event power divided by the baseline power (Pineda & Oberman, 2006). Because ratio power is inherently non-normal (values cannot be less than 1), the log transform better approximates a normal distribution. Values less than zero indicate desynchronization (ERD) and values greater than zero indicate synchronization (ERS).

Data Analysis. The between condition analyses were examined using a repeated-measures Analysis of Variance (ANOVA) with Condition (Observe/Light Execute/Dark Execute), Region (Frontal/Central/Parietal/Occipital), and Hemisphere (Left/Right) as within-subjects factors and age group as the between-subjects factor and Greenhouse-Geisser correction of degrees of freedom for violations of sphericity. Post-hoc paired t-tests were used to follow-up on any significant main effects or interactions and all p-values were Bonferroni corrected for multiple corrections. Examination of mu rhythm desynchronization employed one-tailed one-sample t-tests to identify significant desynchronization from zero.

To examine group differences in the timing of mu rhythm desynchronization between adults and infants, an 8 Time (-500 to 500ms in 125ms bins) x 2 Condition (Light Execute/Dark Execute) x 2 Group repeated-measures ANOVA for each condition was used with polynomial trend analyses to explore changes in mu rhythm desynchronization over the Time factor. Preliminary analyses revealed no significant hemisphere effects for the timing variables. Therefore, the ERD values were averaged across hemisphere in central electrodes and used in the analyses for the timing tests.

4.2 Results

Does the mu rhythm desynchronize for execution in the dark in human adults and infants? To examine whether there were differences between conditions in mu rhythm desynchronization, a 3 Condition (Observe/Light Execute/Dark Execute) x 4 Region (Frontal/Central/Parietal/Occipital) x 2 Hemisphere (Left/Right) x 2 Age Group repeated-measures ANOVA was employed. The analysis revealed main effects of Condition ($F(2, 60) = 19.30$, $p < .001$, $\varepsilon = .70$), Region ($F(3, 90) = 24.02$, $p < .001$, $\varepsilon = .69$) and Hemisphere ($F(1, 30) = 5.74$, $p < .05$). These main effects were qualified by a Region x Age Group interaction ($F(3, 90) = 5.35$, $p < .01$, $\varepsilon = .69$) and a Condition x Region interaction ($F(6, 180) = 3.58$, $p < .05$, $\varepsilon = .54$; see Figure 3). Examination of the means for the main effect of Condition indicated greater desynchronization in the Dark Execute condition compared to the Observe and Light Execute conditions but no differences between Observe and Light Execute. The Region effect revealed ERS in the Occipital region compared to ERD in the Frontal, Central, and Parietal regions. The main effect of Hemisphere revealed greater desynchronization in the right compared to the left hemisphere.

Post-hoc analyses of the Region x Age Group interaction revealed that the Occipital electrodes showed ERS that was significantly different than the ERD in Frontal ($t(30) = 3.37$, $p < .05$), Central ($t(30) = 4.94$, $p < .001$), and Parietal ($t(30) = 5.34$, $p < .001$) regions in adults. No differences were observed between the Frontal, Central, and Parietal regions for this group (all *p*s > .05). In the infant group, there was greater ERD in Frontal and Central regions compared to ERD in Parietal ($t(30) = 2.94$, $p < .05$ and $t(30) = 4.12$, $p < .001$, respectively) and the ERS in Occipital ($t(30) = 4.43$, $p < .001$ and $t(30) = 3.93$, $p < .001$, respectively) regions. The ERD in the Parietal region did not differ from the ERS in the Occipital region ($t(30) = 2.10$, *ns*; see Figure 3B).

Follow-up analyses of the Condition x Region interaction showed that there was significantly more ERD in the Dark Execute condition compared to the Observe and Light Execute conditions in the Frontal (vs. Observe: $t(30) = 4.23$, $p < .001$; vs. Light Execute: $t(30) = 4.43$, $p < .001$), Central (vs. Observe: $t(30) = 4.09$, $p < .001$; vs. Light Execute: $t(30) = 3.89$, $p < .001$), and Parietal (vs. Observe: $t(30) = 3.93$, $p < .001$; vs. Light Execute: $t(30) = 3.44$, $p < .005$) regions. The interaction was driven by the Occipital region where there was ERS in Observe and Light Execute conditions that was significantly different than the ERD in the Dark Execute (vs. Observe: $t(30) = 3.58$, $p < .01$; vs. Light Execute: $t(30) = 5.95$, $p < .001$) condition. The Observe and Light Execute conditions did not differ in any region (all *p*s > 0.15). Figure 3C displays a summary of these results.

Examination of Mu Rhythm Desynchronization. The results of the between-condition analyses revealed no significant interactions between Condition or Region with Hemisphere; therefore, an average score was computed for each region and each condition to examine significant desynchronization in the mu rhythm.[2] A series of one-tailed (desynchronization) one-sample t-tests were computed for each region and each condition for both adult and infant groups. Table 2 summarizes the findings from these analyses. In brief, desynchronization in mu rhythm was observed in both Central and Parietal regions of the adult group in all three conditions with significant desynchronization also occurring in the Frontal region in the Dark Execute condition. The infants, however, only showed significant desynchronization during Dark Execute trials, and this desynchronization was observed across the scalp.

Is the time course of the mu rhythm different between human adults and infants when reaching in the dark compared to reaching in the light? Computing the event-related synchronization/desynchronization allowed for a more detailed analysis of the changes in mu rhythm over 125ms segments. Comparison of the timing effects between Adults and Infants was analyzed using an 8 Time (-500 to 500ms in 125ms bins) x 2 Condition (Light Execute/Dark Execute) x 2 Age Group repeated-measures ANOVA examining the average of power between left and right hemispheres in the central electrodes. The results of this analysis revealed a main effect of Time ($F(7, 210) = 3.48$, $p < .05$, $\varepsilon = .41$) and Condition ($F(1, 30) = 14.61$, $p < .001$), qualified by a marginal Condition x Group ($F(1, 30) = 3.62$, $p = .067$) interaction. Examination of the Condition means revealed greater desynchronization in the Dark Execute compared to the Light Execute across time. The trend analysis of the Time effect suggested a linear decrease in ERD across the one second window ($F(1, 30) = 6.12$, $p < .05$).

The Condition x Group interaction was followed-up by running separate 8 Time x 2 Age Group repeated-measures ANOVAs for Light and Dark Execute conditions. In the Light Execute condition, there was only a main effect of Age Group ($F(1, 32) = 4.77$, $p < .05$), indicating that adults had greater mu rhythm desynchronization than infants. The analysis in the Dark Execute revealed a significant main effect of Time ($F(7, 210) = 3.88$, $p < .05$). There were no main effects or interactions with Age Group. The follow-up trend analysis revealed a significant linear effect ($F(1, 30) = 5.81$, $p < .05$), suggesting that ERD steadily decreased over time. The changes over time in mu rhythm for each of the conditions are summarized in Figure 4.

4.3 Discussion

EEG data were collected while infants and adults completed goal-directed grasps in the dark in order to examine the sensitivity of the mu rhythm to conditions in which there was no visual information. The results confirmed my hypothesis that the mu rhythm does desynchronize to motor actions performed in the dark in both adults and infant suggesting that actions completed without guidance from the visual system recruit comparable neural networks to those used for executing actions with visual feedback and the observation of similar actions. These results further refute the suggestion that mu rhythm activity is not an index of the human MN system.

In adults, we observed significant desynchronization in the mu rhythm in the central and parietal regions during the observation of goal-directed grasps and while the participant completed goal-directed grasps when the target was both visible and not (i.e. in the dark). This pattern of activity is consistent with the body of evidence examining the mu rhythm during the observation and execution of goal-directed actions in adult populations (Avanzini, et al., 2012; Dumas, et al., 2010; McFarland, et al., 2000; Muthukumaraswamy, et al., 2004; Muthukumaraswamy & Johnson 2004a,b). Previous research has demonstrated that mu rhythm activity within the central and parietal regions are likely the result of activity within the somatosensory cortex and the inferior parietal lobe which are key regions in the putative human mirror neuron system (Arnstein, Cui, Keysers, Maurits, & Gazzola, 2011; Ritter, et al., 2009). Importantly, the desynchronization in all three conditions cannot be attributed to activation of visual attention networks as we observed synchronization in the occipital region during the observation and light execute conditions and in the dark execute condition the desynchronization in the occipital region was not statistically significant from zero (with a one-tailed t-test).

The pattern of mu rhythm activity in the infants during the observation and execution of actions in the light resembled previously published data in older infants (Marshall et al., 2011; Saby et al., 2012; Stapel et al., 2010). Consistent with those experiments, the magnitude of mu rhythm desynchronization was greater in frontal and central regions as compared to parietal and occipital regions. This pattern of activation held for grasps completed in the dark demonstrating some consistency across conditions. It should be noted, however, that when analyses were conducted to identify any significant changes in power between the action conditions and the baseline, only the dark grasping condition reached statistical significance. Taken together, these data suggest that, in infants, the mu rhythm has a distinct pattern of scalp activity that is different from adults and may represent ongoing developmental processes in the motor system between infants and adults.

Evidence from experiments measuring the progression of grasping kinematics beginning from grasping onset in infancy through the second year of life may offer some insight into these neural differences. Grasping onset occurs around 4 to 5 months of age and is typically guided by proprioceptive cues rather than visual feedback (Clifton, et al., 1993; von Hofsten, 1991). In the following 5 to 6 months, infants continue to use proprioceptive information to guide their actions incorporating more information into their motor plans, such that by roughly 9 months of age, infants' grasps kinematically resemble adult grasps in the coordination of the joints of the arm and hand (Savelsberg, et al., 1997), the shaping of the hand prior to contact with the object (Clifton, et al., 1991; McCarty, et al., 2001), and the speed of approach (Berthier & Keen, 2006; vonHofsten, 1991); however, infants' grasps are not as complex or demonstrate any sequential linking with future actions as the grasps of adults show (Chen, et al., 2010; Claxton, et al 2003; McCarty, et al., 1999).

The age range between 9 and 11 months is a transition point in the development of grasping as infants move from ballistic actions guided by proprioceptive cues to visually guided actions (Keen, 2011). By 12 months, infants rely on visual information to update their actions online and their actions become more sequenced and efficient for problem solving (Chen, et al., 2010; Claxton, et al., 2003; von Hofsten & Ronnqvist, 1988). Because the time around 9 months marks this behavioral transition, there is a large variability in grasping skill at this age. There have been no studies that have examined the effect of skill level on mu rhythm activity; however, we do know that experience plays a significant role in the magnitude of mu rhythm desynchronization (see Study 2; Orgs, et al., 2008; Paulus, et al., 2012; van Elk, et al., 2008). Taken together, these two factors, the highly variable skill level in grasping actions at 9 months and as a result limited experience with grasps may partially explain the lack of significant mu rhythm desynchronization.

Interestingly, the magnitude of mu rhythm desynchronization for actions completed in the dark was greater than for actions completed in the light. In the dark, adults change their reaching and grasping strategy by slowing the entire reach and adopting a larger hand aperture relying on proprioceptive information to guide their grasp similar to infant grasping strategies (Berthier, et al., 1996). The magnitude differences observed for dark execution may reflect activation of the proprioceptive motor system in conjunction with the MN system. The data, however, are insufficient to draw any conclusions to this effect and a number of control conditions will be required to fully understand these neural signatures.

Research with infants is limited by the number of conditions you can run with any one infant and the decision to run the current conditions without additional control conditions was made to maximize the number of trials we could successfully record for each condition. This study relied on previous research that has identified mu rhythm desynchronization to the observation of goal-directed actions and not for non-goal directed actions (Southgate, et al., 2010), however, this study would have benefited by inclusion of an observation of a non-goal directed reach condition. This condition would have provided an additional comparison to control for any differences in effects due to our resting baseline. In the same vein, presentation of the object without any actions produced on it would have controlled for the effects of a goal on mu rhythm activity.

The procedure for eliciting dark grasps from infants differed from the procedures used by previous research using dark grasping procedures. While many of the studies focused on auditory localization of objects (Berthier, Clifton, McCall & Robin, 1999; Clifton, et al., 1991; Clifton, et al., 1993; Clifton, et al., 1994; Goubet & Clifton, 1998; McCarty, et al., 2001; Perris & Clifton, 1988), a select few used glow-in-the-dark objects to make the target visible to the infants. Incorporating glow-in-the-dark stimuli would provide addition control conditions for both the light and the dark procedures, in that we could: (a) measure activity of presentation of the object in the dark without having an action performed on it and (b) have the goal of the action visible to measure the additional contribution of a visible goal on mu rhythm desynchronization.

Finally, the addition of a condition in which the participants were asked to performed a non-goal-directed action in both the light and the dark. While this would not be feasible in the infants, the addition of this control condition in the adults would provide an opportunity to identify the motor contribution of mu rhythm desynchronization. This procedure has been accomplished in previous studies in adults (Muthukumaraswamy & Johnson, 2004a,b; Muthukumaraswamy, et al., 2004), however, the non-goal-directed action condition was not contrasted with the goal-directed action condition. By contrasting these different control conditions we would begin to tease apart the individual contributions of goals, action plans, and visual feedback on mu rhythm activity.

The event-related design of this experiment provided an opportunity to explore changes in the mu rhythm over time. Interestingly, there were no differences between adults or infants in the time course of mu rhythm activity during either the light or dark execute conditions. In adults, previous research showed that maximal mu rhythm desynchronization occurred synchronously with the action (Pfurtscheller, et al., 1997) and a similar effect was observed in this sample of adults. The time course of mu rhythm desynchronization in infants has not been studied as thoroughly as in adults. One study, by Southgate and colleagues (2010), used wavelet analyses to examine mu rhythm desynchronization during the execution of grasps of 9-month-old infants. Their results suggested a pattern of desynchronization comparable to that reported in adults, with maximal desynchronization simultaneous with the action onset. While we did not directly assess action onset, we did analyze the period from 500 milliseconds prior to contact with the toy to 500ms following the grasp and the lack of any group interactions with timing suggest that the time course of mu rhythm desynchronization is comparable in adults and infants over this brief window.

The adult human MN system, like that of the monkey, is a network of cortical regions, including the inferior frontal gyrus (IFG), inferior parietal lobule (IPL), and the superior temporal sulcus (STS) that contribute to the processing of actions (Dinstein, et al., 2007; Iacoboni, et al., 1999). While the IFG activates for the representational aspects of the action, including the goal, the IPL is involved in goal maintenance over a sequence of actions (Fogassi, et al., 2005; Iacoboni, et al., 2005). While the developmental trajectory of these regions has not been examined, the behavioral evidence suggests that the IPL may not be functionally active until later in the first year in humans and may partially explain the regional differences observed between adults and infants in this study. While further research will be needed to address this question, this experiment is the first to directly compare the scalp distribution of mu rhythm activity between adults and infants.

The primary aim of this study was to identify whether or not the mu rhythm could be identified while individuals performed actions without visually guiding their actions as a corollary to the early single-cell recordings of MNs made in the monkey (Gallese, et al., 1996; Rizzolatti, et al. 1996). The original criticisms against the existence of mirror neurons in the monkey were (a) that these neurons were providing a motor preparation command, an adaptive ability in social groups that may be highly competitive for resources; or (b) that their activity reflected the observation of their own hand performing that grasp. The lack of any EMG activity in the arms or hands of the monkeys while they observed actions demonstrated that MNs were not activating a preparatory motor command but that the neurons were activating purely for the observation of the action (Gallese et al., 1996). Rizzolatti and colleagues (1996) further demonstrated that it was not the observation of the monkey's own hand that activated MNs, but that the neurons also fired for the execution of grasps in the dark, in which the monkey could not see their hand. This experiment is the first to show mu rhythm desynchronization to the execution of grasps in the absence of visual feedback providing further evidence that the mu rhythm is an index of the putative human MN system.

Conclusions. The present findings demonstrate that activation of the cortical networks involved in the observation and execution of actions are also recruited during the execution of goal-directed actions in the absence of visual information. The period late in the first year marks a transition from ballistic actions to more mature, visually guided actions and evidence from this study suggests that grasping skill plays an important role in mu rhythm activity. Future research will be required to better tease apart the role of maturity of skill on mu rhythm activity. Previous research has suggested that the mu rhythm is an index of the human MN system and evidence from this study suggests that, in addition to activation for observation and execution of goal-directed actions and no activation for observation of meaningless actions, the mu rhythm is also sensitive to execution of actions in the absence of visual feedback.

Chapter 5. Study 2: Mu Development in Infant Rhesus Macaques

5.1 Methods

Subjects. Thirty mother-reared and 26 nursery-reared infant rhesus macaques from a population of rhesus macaques maintained at the National Institutes of Health Animal Facility, Poolesville, MD. Mother-reared infants (11 males and 19 females; birth weight M = 489.00g, SD = 63.77) were all being reared by their biological mothers and housed in social groups containing eight to ten adult females and their offspring. Nursery-reared infants (19 males and 7 females; birth weight M = 500.35g, SD = 53.73) were involved in an ongoing experimental protocol that required separating the infants from their mother on day 1 post-partum. Nursery-reared infants were handfed with Similac Infant formula (Ross Laboratories, Columbus, Ohio, USA) ad libitum and housed individually in plastic incubators (51 x 38 x 43 cm) that contained a 25cm inanimate "surrogate mother" composed of a 16.5cm cylinder wrapped in fleece fabric. The incubator was maintained at a temperature of approximately 27°C and 50% humidity.

Both groups of infants were tested on day 3 post-partum, taking advantage of a period in which mother-reared infants were temporarily removed from their mothers for a routine post-partum examination. During each testing period, the infant was presented with all three conditions of the imitation paradigm (described below) presented in a random order. All animal care and testing was conducted in accordance with regulations governing the care and use of laboratory animals and had prior approval by the Institutional Animal Care and Use Committee of both the University of Maryland and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).

Six mother-reared and three nursery-reared infants were excluded from analyses due to insufficient epochs (*n* = 2 mother-reared), statistical outliers (*n* = 1 mother-reared; *n* = 2 nursery-reared), or technical difficulties at the time of testing (*n* = 3 mother-reared; *n* = 1 nursery-reared). An additional five mother-reared and four nursery-reared infants did not complete all three conditions and so their data was excluded in the omnibus tests. The effects did not change when those infants were excluded for any follow-up within-task analyses so they were included in the final analyses.

Imitation Paradigm. The imitation paradigm was modified from the behavioral paradigm developed by Ferrari and colleagues (2006) for assessing imitation in infant rhesus macaques. The imitation paradigm had three conditions in which the infants received live presentation of stimuli from a human experimenter: a) Tongue Protrusion (TP) with maximal extension and retraction; b) Lip Smack (LS) a rapid opening and closing of the lips without sound production; and a 15-cm diameter plastic Disk (DK) with a red and black cross painted on it and rotated 90° clockwise and counterclockwise. Each testing session began with a 40-second baseline in which the monkey was presented with a still face in TP and LS and a still disk in the DK conditions. The baseline was followed by 20-seconds of stimuli presentation and then a 20-second period of the still stimuli. The 20-second stimuli presentation and still period were repeated 3 times in order to obtain 60-seconds of data for each infant within each condition (Figure 5A).

EEG Acquisition and Analysis. EEG was collected during the imitation paradigm. Custom Lycra caps were made and fitted with 8 tin electrodes. A custom cap was made for the acquisition of EEG data in infant rhesus macaques (Figure 5B). The cap size was based on a plaster mold of a 1-week-old rhesus skull. Electrode placement was based on x-rays made of a typical infant rhesus macaque. Two anterior electrodes were placed on scalp locations above the motor cortex, two central electrodes were roughly over the temporal lobes, and two posterior electrodes were placed approximately over the occipital lobes. The zenith served as reference and an electrode on the forehead served as ground. The heads of the infants were shaved and a mild abrading gel was used to improve impedances with special care to keep them below 20kΩ. During acquisition, EEG data was band pass filtered from 0.1 to 100Hz, digitized with a 16bit A/D converter (+/- 5V input range) at a 1000Hz sampling rate, and recorded on a separate acquisition computer. Signals exceeding +/- 250μV were automatically removed from analysis. Epochs of clean EEG were then submitted to a Fast Fourier Transform (FFT) using a 1 second Hanning window with 50% overlap and spectral power (μV^2) was computed for single hertz bins from 2 to 15Hz. Single hertz bins were then summed to compute 2 – 4Hz, 5 – 7Hz, and 8 – 10Hz frequency bands. Previous research had shown that the 5 – 7Hz band shares many of the characteristics of the human alpha (Vanderwert, Ferrari, P. F., Paukner, A., Bower, S. B., Fox, N. A., Suomi, S. J., 2012) and mu rhythms (Ferrari, et al., 2012). All data processing was performed using EEG Analysis System software, James Long Company (Caroga Lake, NY).

Video Recording. Testing sessions were recorded on DVD for behavioral coding and synchronization to EEG. Video signal was time-stamped with a vertical interval time code (VITC) synchronized with the EEG acquisition time-base. The VITC was read using the Video Coding System (VCS), James Long Company, while coders identified the infant's gaze and movements throughout the session. The output of the VCS was then read into the EEG analysis software to identify epochs of data when the infant was still and his or her gaze was directed toward the stimuli. Coding of infant movements was also done in order analyze EEG activity while the infant was imitating the presented stimuli.

Event-related desynchronization was computed as [(S – B) / B] × 100, where S is the absolute power in a particular frequency band while the monkey observed the stimulus presentation (for observation analyses) or produced a facial gesture (for execution analyses) and B is the power in the same frequency band during periods of EEG in which the stimulus was still and the monkey's gaze was directed towards the experimenter (see Marshall, et al., 2011). Therefore, negative values are interpreted as a decrease from baseline or desynchronization (ERD) and positive values as an increase from baseline or synchronization (ERS).

Data Analysis. To examine the EEG data during observation, I conducted a repeated-measures ANOVA with Condition (TP/LS/DK), Region (Anterior/Posterior), and Hemisphere (Left vs. Right) as within-subjects factors and rearing group as the between-subjects factor. For analysis of gesture production a repeated-measures ANOVA with Condition (LS/TP), Region, and Hemisphere as within-subjects factors and rearing group as the between-subjects factor was used. Any significant main effects and interactions were followed up using 2-tailed paired t-tests with Bonferroni correction for multiple comparisons. The Greenhouse-Geisser correction for degrees of freedom was used for violations of sphericity.

5.2 Results

Is the mu rhythm observable in three–day-old mother-reared infant macaques during action execution? Based on previous work, newborn rhesus macaques that display imitative motor actions show desynchronization of the mu rhythm in response to the observation of an experimenter in the 5 – 7 Hz frequency band. To verify this we averaged the ERD scores across the scalp for the frequency bands of 2 – 4Hz, 5 – 7Hz, and 8 – 10Hz while the infants produced an imitative facial gesture. The independent one-sample t-test revealed statistically significant desynchronization in the 5 – 7Hz band (*M* = -13.44, *SD* = 24.66; *t*(20) = 2.50, *p* < .05) but not in the 2 – 4Hz (*M* = 3.17, *SD* = 48.50; *t*(20) = 0.30, *n.s.*) or the 8 – 10Hz (*M* = 0.64, *SD* = 41.57; *t*(39) = 0.87, *n.s.*) bands. Because the 5 – 7Hz band displayed the greatest desynchronization during gesture production, we used this frequency band to examine group differences in ERD for the observation and execution of facial gestures.

Is the mu rhythm observable in three–day-old mother-reared infant macaques during action observation? Based on previous work, desynchronization of the 5 – 7Hz band during the observation of biological stimuli occurs in the anterior regions. To verify that the mu rhythm desynchronizes for the observation of biological stimuli in the mother-reared infants, an average of the left and right anterior electrodes for both LS and TP conditions was computed for the 5 – 7Hz band and independent one-sample t-tests were run. The results revealed significant desynchronization for the observation of biological stimuli in the anterior region (*M* = -25.75, *SD* = 20.14; *t*(21) = 6.00, *p* < .001) but not for the posterior region (*M* = -9.57, *SD* = 28.02; *t*(21) = 1.60, *n.s.*).

Is there a difference between nursery-reared and mother-reared infant rhesus macaques in the magnitude of mu rhythm desynchronization during action observation? To examine group differences during the observation of facial gestures, we employed a repeated-measures ANOVA with Condition (DK/LS/TP), Region (Anterior/Posterior), and Hemisphere (Left/Right) as within-subjects factors and rearing group as a between-subjects factor. The analysis revealed main effects of Condition ($F(2, 72) = 5.52$, $p < .05$, $\varepsilon = .69$) and Region ($F(1, 36) = 8.51$, $p < .05$) qualified by Condition x Group ($F(2, 72) = 3.93$, $p < .05$, $\varepsilon = .69$), Condition x Hemisphere ($F(2, 72) = 3.10$, $p = .051$), and Condition x Region x Hemisphere x Group ($F(2, 72) = 3.45$, $p < .05$) interactions. To follow-up on the four-way interaction, separate repeated-measures ANOVAs were run for each condition.

Analysis for the DK condition revealed a significant Hemisphere ($F(1, 38) = 5.14$, $p < .05$) main effect qualified by a Region x Hemisphere x Group interaction ($F(1, 38) = 3.97$, $p = .054$). Examination of the means of the main effect revealed greater ERS in the left hemisphere compared to the right. Exploration of the interaction revealed that in the Mother-Reared group, there was greater ERS in the left posterior region ($M = 33.97$, $SD = 89.10$ compared to the right posterior region ($M = 6.98$, $SD = 64.27$; $t(20) = 3.01$, $p < .005$).

Analysis of the LS condition revealed main effects for Region ($F(1, 38) = 7.48$, $p < .01$) and for Rearing Group ($F(1, 38) = 9.23$, $p < .005$). Examination of the means of the Region main effect revealed greater ERD in the anterior region ($M = -22.89$, $SD = 28.59$) compared to the posterior region ($M = -9.57$, $SD = 31.81$) in both groups. The mother-reared infants ($M = -27.08$, $SD = 23.18$), however, had greater ERD across the scalp when compared to the nursery-reared infants ($M = -4.23$, $SD = 24.36$).

Analysis of the TP condition revealed only a significant main effect of Region ($F(1, 39) = 11.41$, $p < .05$). Examination of the means revealed greater ERD in the anterior region ($M = -15.43$, $SD = 35.84$) compared to posterior region ($M = 6.04$, $SD = 37.19$). A summary of these results is displayed in Figure 6.

Is there a difference between nursery-reared and mother-reared infant rhesus macaques in the magnitude of mu rhythm desynchronization during action execution? In order to analyze any group differences in mu rhythm desynchronization during gesture production, a 2 Condition (LS/TP) x 2 Region (Anterior/Posterior) x 2 Hemisphere (Left/Right) repeated-measures ANOVA with rearing group as a between subjects factor was used. The results revealed no significant main effects and no interactions with rearing condition, suggesting that mu rhythm desynchronization is comparable between the two populations of infants during gesture production (see Figure 6).

Is there a difference between nursery-reared and mother-reared infant rhesus macaques in their behavior during the tasks? The observed differences in the magnitude of mu rhythm desynchronization may have been a result of behavioral differences between the rearing groups during each task; therefore, the behavioral coding was examined to ensure that the rearing groups did not differ in the number of gestures produced or on the amount of time their gaze was directed toward the stimulus. Independent samples *t*-tests on the behaviors revealed the nursery-reared infants' gaze was directed towards the disk longer than mother-reared infants during the baseline (M = 4.68, SD = 3.70 and M = 2.51, SD = 1.28, respectively; $t(36) = 2.41$, $p < .05$) and stimulus (M = 7.33, SD = 7.34 and M = 3.66, SD = 2.06, respectively; $t(36) = 2.12$, $p < .05$) blocks. There were no other significant differences observed between the rearing groups during LS or TP tasks in average duration of observation of the stimulus or in the number or duration of gestures produced. Table 3 is a summary of the means and standard deviations of the behaviors examined for each group.

5.3 Discussion

Differential rearing had a significant impact on mu rhythm desynchronization to the observation of species-specific affiliative facial gestures and those differences emerged very early in development. Indeed, 3-day-old infant macaques who were reared with their biological mothers showed greater mu rhythm desynchronization to the observation of lip smacking, a socio-affiliative gesture in rhesus macaques and one that is readily imitated by infants. These results confirm my hypotheses that the action observation/execution matching system emerges early in infancy, particularly for facial gesture matching, and that it is dependent upon early social experience.

I hypothesized that the mu rhythm would be evident in mother-reared infants on day 3 postpartum and in fact, the EEG of mother-reared infants share similar patterns of scalp EEG activity during the observation and execution of facial gestures with previous research examining activity in nursery-reared infants tested over the first week (Ferrari, et al., 2012). The results confirm that mother-reared infants show desynchronization of the mu rhythm (5 – 7Hz) in anterior electrodes to the observation of facial gestures and, that during gesture production, they show desynchronization in both anterior and posterior regions.

We further replicated previous research examining mu rhythm activity in a new sample of nursery-reared monkeys. Desynchronization in the 5 – 7Hz band was identified for the observation of both lip smacking and tongue protrusion in the anterior but not posterior region compared to the disk control. In fact, in the current sample of nursery-reared infants, the magnitude of desynchronization was comparable to that observed in our previous work (see Figures 2 and 6).

Previous research has shown that the mu rhythm is highly sensitive to experience with particular movements. Studies in adult elite athletes and dancers have shown greater mu rhythm desynchronization to activity-related actions in the expert compared to non-experts (Del Perchio, et al., 2010; Kim, et al., 2011; Orgs, et al., 2008). Even in infancy, the mu rhythm shows greater desynchronization to emerging actions and the magnitude is dependent upon the individual's own experience with those actions (Paulus, et al., 2012; van Elk, et al., 2008). In our sample, infants who, as a group, received greater experience with lip smacking gestures produced by their mother or other conspecifics showed greater mu rhythm desynchronization while observing that gesture. Amazingly, this difference was nearly triple in the mother-reared infants compared to the nursery-reared infants and was evident within three days of life.

The short period in which the mother-reared infants received suggest that the action observation/execution matching mechanism for these affiliative gestures is operating very early in life. Casile and colleagues (2011) hypothesize two separate developmental trajectories for the mouth and hand mirror areas. They propose hand mirroring areas develop slowly through experience observing own and other's actions while the mouth mirroring areas are present at birth and facilitate early social interaction. Evidence demonstrating facial preference and neonatal imitation in the first days after birth suggest the early emergence of this system may be the case. Moreover, Casile et al suggest that this system is further modified through social interactions in which feedback regarding the infant's facial movements is provided. The evidence from this study suggest this hypothesis, specifically for the mouth or face matching system, may be correct and demonstrates how quickly the system is modified through experience.

Unfortunately, we did have a measure of the amount and quality of experience that the mother- and nursery-reared infants received in their first three days. The evidence of differential experience comes from behavioral studies of monther-infant interaction (Ferrari, et al., 2009) and an interesting question for future research would be how individual differences in experience modulate mu rhythm desynchronization to the observation of lip smacking.

The use of a neonatal imitation paradigm during the acquisition of EEG data provides a unique methodology for eliciting both observation and execution of actions in very young infants. By identifying periods in the EEG in which the infant was still and observing the stimuli or when they produced a lip smacking or tongue protrusion gesture themselves, we were able to identify the mu rhythm in these infants. Unfortunately, in order to have enough data to analyze execution we had to collapse across gestures and coud not analyze imitation of lip smacking and tongue protrusion separately.

My hypotheses regarding early social rearing experiences were partially confirmed. Based on our previous work, I expected to find greater desynchronization for both lip smacking and tongue protrusion gestures in the mother-reared infants compared to nursery-reared infants. While mu rhythm desynchronization differences were most pronounced in the the lip smacking condition there were no differences between mother- and nursery-reared infant while observing tongue protrusion. Research examining rates of neonatal imitation in mother-reared infant rhesus macaques over the first month has shown that mothers incease in their lip smacking gesturing towards their infant over the first week of the infant's life (Ferrari, et al., 2009). Mother tongue protrusion is rarely directed at the infant, and is typically only seen within the context of a lip smack. In contrast, nursery-reared infants only receive experience with simple lip smacking and tongue protrusion gestures within the context of neontal imitation experiments. The results of this experiment likely represent the difference in experience with these gestures and the context in which they are viewed.

Finally, the differences in mu rhythm desynchronization between mother-reared and nursery-reared infants observed in this experiment are not due to differences in the behaviors of the infants during the testing sessions. Behaviorally, the two groups of infants were quite similar in the numbers of imitative gestures that were produced and in the amount of time that they fixated on the face. The only behavioral difference observed between the two groups was that the nursery-reared infants spend more time fixating on disk than the mother-reared infants. This may have been partly due to the nursery-reared infants' previous experience with the disk. The results of the behavioral analyses suggest that the effects observed in the EEG were not an effect of behavioral differences during testing, but reflect an effect of early experience on the neural activity of the action observation/execution matching system to facial gestures.

Conclusions. The evidence from this study suggests that the neural networks linking action observation and execution of facial gestures develop rapidly over the first week of life and are highly dependent upon individual differences in experience with those gestures. Consistent with previous research showing that the action observation/execution matching system can be identified early in infancy, these data extend this to show that greater experience with socio-affiliative gestures greatly enhances activity in the neural networks involved in processing those actions.

Chapter 6. General Discussion

The results presented in studies one and two extend two very important aspects of what we know about the development of the mu rhythm. First, the mu rhythm is an index of the action observation/execution matching system that responds to both the observation and execution of actions and strengthens the connection between human EEG studies and the evidence from invasive single-cell studies.

Study one demonstrated that during execution, the magnitude of desynchronization is not driven by the visual guidance of the action or the first person observation of the action, but through the kinematics and the goal-directed nature of the action. Differentiating between whether the activity in the mu rhythm is driven by the kinematics or the goal of the action is one limitation of study one and future work will need additional control conditions. Early studies of the mu rhythm demonstrated that it did not desynchronize to the observation of non-goal-directed actions, suggesting that the goal was necessary for desynchronizing the mu rhythm (Muthukumaraswamy, et al., 2004), however, there has been no direct measurement of the mu rhythm during the execution of non-goal-directed movements. Further studies will need to have participants complete non-goal-directed actions while the action is visible or occluded from the performer.

Study two continued a line of inquiry into the role of the action observation/execution matching system in neonatal imitation and more specifically how that system matches the observation with the execution of facial movements. Both human and monkey infants imitate facial gestures of adult conspecifics (Ferrari, et al., 2006; Ferrari, et al., 2009; Meltzoff & Moore, 1977) and the evidence from study two suggested that this early gesture matching system is online early in development. Indeed, it has been suggested that the coordination of visual and facial movements occurs prenatally in preparation for early social interactions such as neonatal imitation (Casile, et al., 2011).

Together, studies one and two provide additional evidence for the mu rhythm as an index of mirror neuron activity. Mirror neurons were first discovered in rhesus macaques as a mechanism for mapping the observation of actions onto a preexisting motor repertoire (di Pellegrino, et al., 1992; Gallese, et al., 1996; Rizzolatti, et al., 1996). Over the past two decades a number of discoveries surrounding the functionality of MNs have been made using single-neuron recordings, however, the invasiveness of this technique has precluded its use in humans. There has been a recent push to adapt the task designs from single-neuron studies for use with non-invasive techniques in both humans and monkeys to bridge the gap between species (Nelissen, Borra, Gerbella, Rozzi, Luppino, Vanduffel, et al., 2011; Nelissen, Luppino, Vanduffel, Rizzolatti, & Orban, 2005; Nelissen & Vanduffel, 2011). Study one adapted the dark grasping task to identify mu rhythm activity during an "execution-only" condition as a corollary to the study by Rizzolatti et al. (1996) and study two used non-invasive EEG to measure activity in infant rhesus macaques, establishing an EEG metric of action observation and execution in the infant monkey. Future developmental work between the species will continue to link these differing methodologies and build a better understanding of the functional role of MNs in development.

Second, studies one and two enhance our understanding of the mu rhythm by demonstrating how important the role of experience is in tuning the action observation/execution matching system. Study one found a link between experience with goal-directed grasps and the magnitude of mu rhythm desynchronization. In the inexperienced graspers (infants), the scalp activity for the observation and execution of actions was different from the adults suggesting that greater experience with an action drives developmental change in the action observation/execution matching system. Behavioral evidence suggests that 9-month-old infants understand the goal of reaching actions (Woodward, 1998, 2005); however, they are still in a period in which grasping skill is ever improving. Around this age, infants are incorporating more visual information into their actions and variability in maturity of grasping skill may play a significant role in mu rhythm activity. Indeed, in her studies with 9-month-old infants, Southgate and colleagues (Southgate, et al., 2009; Southgate, et al., 2010) found that at least 20% of the infants in her samples either could not perform the task or had no mu rhythm response.

In addition to the proposal that the neural mechanism underlying the matching of facial gestures may be present at birth, Casile and colleagues (2011) hypothesized a different developmental trajectory for the neural mechanism for matching hand or body actions. Based on evidence of the protracted development of coordinating hand actions in human infants, Casile et al suggest this separate system is tuned through both the infant's experience observing their own hand movements and through social interactions (e.g. joint attention). Our results are consistent with this hypothesis, in that the infants did not demonstrate strong mu rhythm desynchronization during the observation or execution of goal-directed grasps, an emerging behavior in the infant's repertoire.

In study two, the experiential effects of enriched face-to-face interactions were compared to experience with simple face-to-face interactions. The findings from study 2 demonstrate that qualitatively different experiences are reflected in the activity of the infant action observation/execution matching system. The importance of this early social experience has been highlighted as a fundamental mechanism for the development of complex social behaviors such as joint attention, theory of mind, and empathy (Meltzoff, 2002; 2006). The short period in which the infant macaques received this experience suggests that the neural mechanisms underlying the observation of this gesture are highly sensitive to these early social interactions (Casile, et al., 2011).

It has been proposed that the MN system is involved in action understanding, by mapping the actions of others onto the observer's motor command, the observer has access to understanding the underlying goals or intentions of the actor. In fact, there are a number of examples in the literature comparing experts and non-experts neural responses to the observation of actions within the domain of their expertise (Abreu, Macaluso, Azevedo, Cesari, Urgesi, & Aglioti, 2012; Del Perchio, et al., 2010; Orgs, et al., 2008; Tomeo, Cesari, Aglioti, & Ugesi, in press), and the evidence suggests that through activating the action observation/execution matching system, these athletes are better able to understand the outcome or meaning of the action sooner and more accurately than their non-expert comparison samples. Together, studies one and two highlight the sensitivity of the mu rhythm to experiential differences with actions.

Mirror neurons are a neural structure within the motor system and the evidence from studies one and two provide evidence to suggest that the development of the MN system parallels and may even contribute to the development of the motor system. Studies one and two provide neurophysiological evidence for something that researchers studying the development of motor systems have known behaviorally for some time;

experience drives change. The structure of the neural networks involved in action execution and perception may be based on a predetermined set of genetic commands but it is through the experience individuals gain through repeated interactions with themselves, objects, and their environment that constructs how the individual perceives the world, approaches problems, and develops social cognition (Gallese & Goldman, 1998; Meltzoff, 2006; Piaget, 1952; Thelen, 2000; von Hofsten, 2004; 2007).

Conclusions. The evidence from the study of human adults and infants reaching in the dark and in the differential rearing effects on mu rhythm of 3-day-old infant rhesus macaques contribute to three major issues concerning the development of the mu rhythm and the action observation/execution matching system. First, both studies use non-invasive methods to measure neural activity during the observation and execution of actions, finding consistent evidence with the activity of MNs measured by single-cell recordings. Second, they provide evidence supporting the hypothesis that facial gestures and hand grasping actions follow two separate developmental trajectories that benefit from different sources of experience. And third, both studies demonstrate the importance of experience in the activation of the action observation/execution matching system and suggest that the development of the mirror neuron system, much like the development of the broader motor system, is shaped by the interactions between the individual and their environment.

Footnotes

[1] Adults always completed a grasp within 4 seconds.

[2] Desynchronization analyses were run for each hemisphere separately but the effects were no different than the averaged data presented in the text.

Table 1.

Study 1: Sample Demographics.

	Infants	Adults
Total	35	22
Mean Age (*SD*)	9.20 months (0.43)	19.25 years (1.52)
Gender		
Male	13	9
Female	22	13
Ethnicity		
White	18	9
Black	5	6
Asian	1	4
Hispanic	5	2
Pacific Islander	1	1
Other/Not Reported	5	0
Handedness		
Right	-	6
Left	-	16
Mean Trials Completed	41.21 (12.88)	60 (0.0)
Observe	14.84 (4.06)	20 (0.0)
Execute	14.05 (4.45)	20 (0.0)
Dark Execute	12.32 (4.92)	20 (0.0)

Note: All values are the total *N* in each cell unless otherwise noted. Handedness was not assessed in the infants.

Table 2.

Study 1. Changes in Mu Rhythm Desynchronization between the Age Groups for each Condition

		Adults				Infants			
Condition	Region	*M*	*SD*	*t*(13)	*p*	*M*	*SD*	*t*(17)	*p*
Observe	Frontal	0.012	0.410	0.107	.54	-0.050	0.438	0.48	.32
	Central	-0.144	0.304	**1.776**	**.05**	-0.011	0.358	0.131	.45
	Parietal	-0.120	0.275	**1.634**	**.06**	0.017	0.342	0.205	.58
	Occipital	0.259	0.430	2.259	.98	0.137	0.580	1.004	.84
Execute	Frontal	-0.053	0.379	0.525	.30	-0.045	0.308	0.612	.27
	Central	-0.248	0.436	**2.123**	**.03**	-0.048	0.269	0.756	.23
	Parietal	-0.275	0.453	**2.275**	**.02**	0.032	0.347	0.385	.65
	Occipital	0.368	0.469	2.936	.99	0.445	0.439	4.301	.99
Dark Execute	Frontal	-0.433	0.863	**1.877**	**.04**	-0.754	0.556	**5.761**	**< .001**
	Central	-0.537	0.889	**2.261**	**.02**	-0.730	0.529	**5.859**	**< .001**
	Parietal	-0.638	0.860	**2.776**	**.008**	-0.418	0.297	**5.979**	**< .001**
	Occipital	-0.283	0.883	1.198	.13	-0.474	0.630	**3.191**	**.002**

Note: All *p*-values are 1-tailed testing for desynchronization from zero.

Table 3.

Study 2. Infant Behaviors During Each Task

			Nursery-Reared		Mother-Reared	
Task	Behavior	Block	*M*	*SD*	*M*	*SD*
DK	Gaze-Directed	Baseline	4.68*	3.70	2.51*	1.28
		Stimulus	7.33*	7.24	3.66*	2.06
LS	Gaze-Directed	Baseline	3.08	2.71	4.56	5.06
		Stimulus	4.54	4.10	2.78	1.06
	Gesture	Stimulus	.69	.68	.45	.34
	Total Gestures[a]	Stimulus	7.74	7.05	11.42	10.57
TP	Gaze-Directed	Baseline	6.96	9.43	3.03	1.25
		Stimulus	3.73	3.53	4.07	3.04
	Gesture	Stimulus	.71	.51	.48	.41
	Total Gestures[a]	Stimulus	7.37	4.65	12.47	12.54

Note: Task abbreviations are as follows: DK = Disk Control; LS = Lip Smacking; TP = Tongue Protrusion. Gaze-Directed are periods in which the infants' gaze was directed towards the still stimulus (Baseline block) or the moving stimulus (Stimulus block).

[a] All values in the table are the mean duration (in seconds) of the coded behaviors except for those behaviors denoted by the superscript. Total Gestures are the total number of discrete instances in which a gesture was coded.

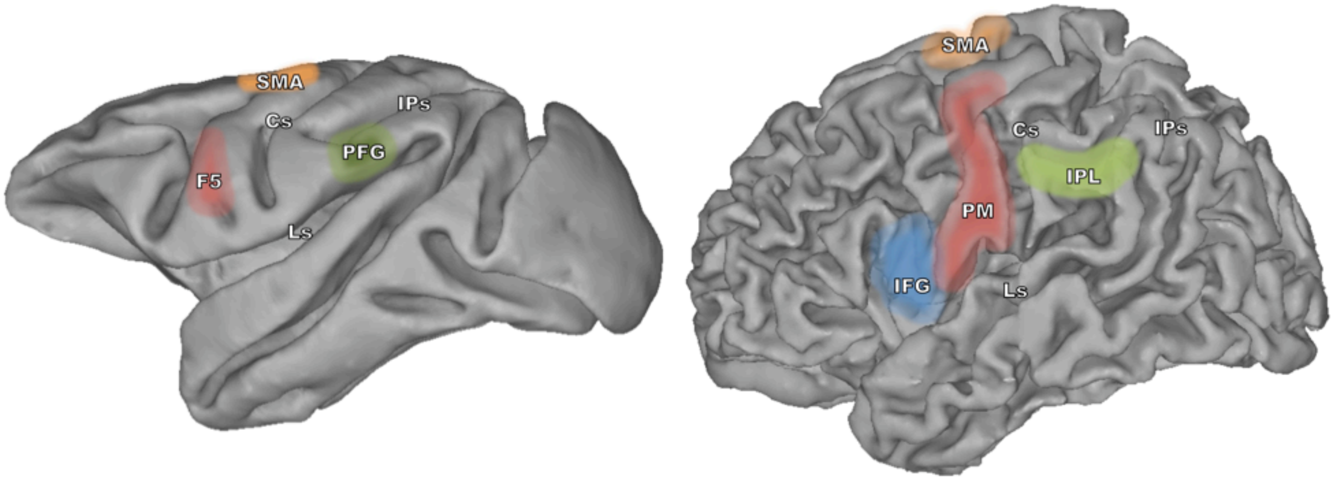

Figure 1. Schematic view of brain regions considered to part of the MN system of monkeys (left) and humans (right). Cortical regions in red and blue identify the frontal nodes of the MN system in the cerebral cortex of humans and monkeys. The regions in green constitute the parietal node of the MN system. IFG, inferior frontal gyrus; PM, premotor cortex; SMA, supplementary motor area; IPL, inferior parietal lobule; Cs, central sulcus; Ls, lateral sulcus; IPs, intraparietal sulcus.

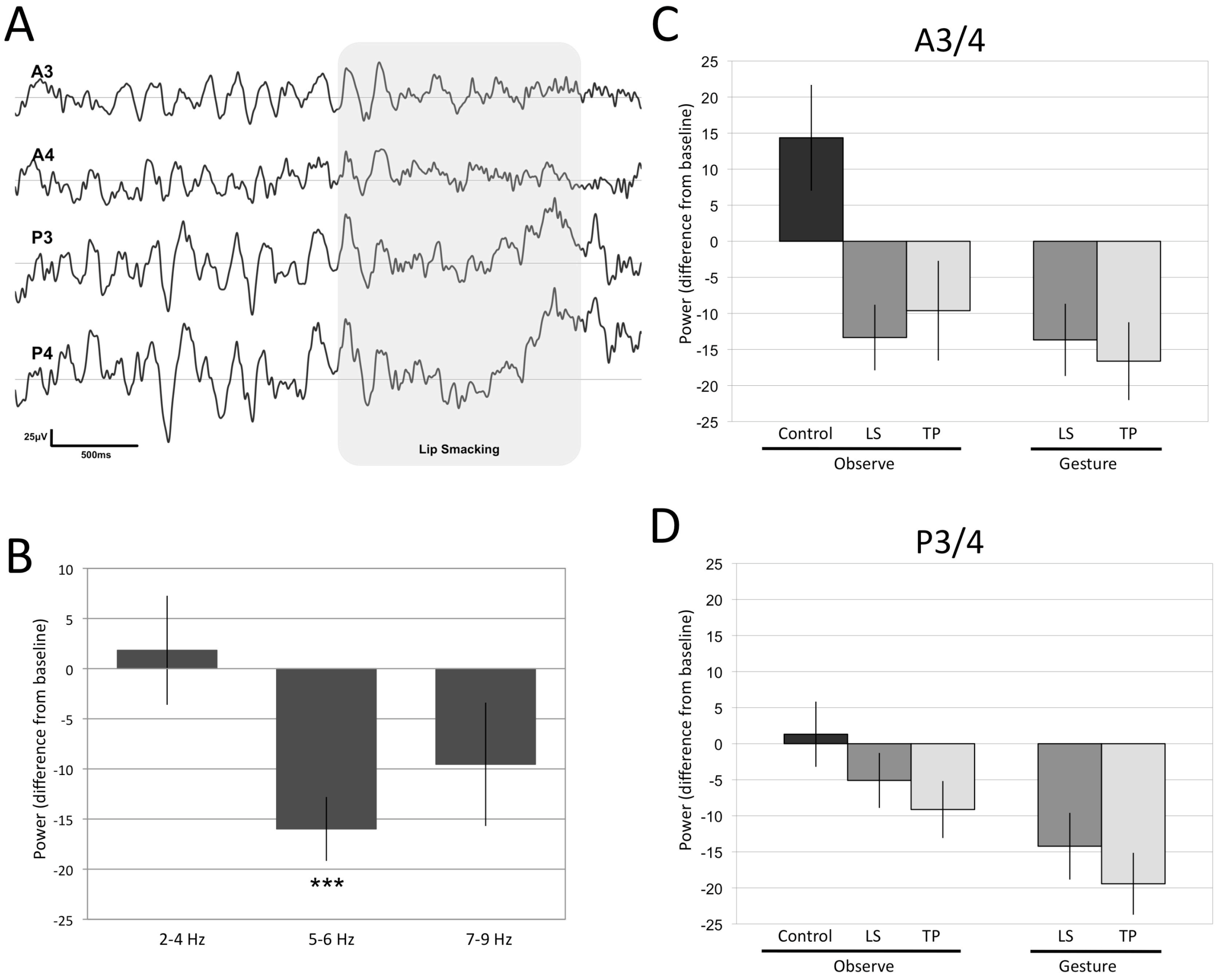

Figure 2. Mu suppression in week-old rhesus macaques to the production and observation of lip smacking (LS) and tongue protrusion (TP). (A) An example of the EEG from a 3-day-old infant rhesus with suppression in the 5-6 Hz frequency band during lip smack gesturing (shaded area). (B) Changes in EEG frequencies between baseline and execution of facial gestures for 3 frequency bands; ***$p < .001$; Average suppression during the observation and gesture conditions versus the control in the 5-6Hz frequency band for (C) anterior and (D) posterior regions.

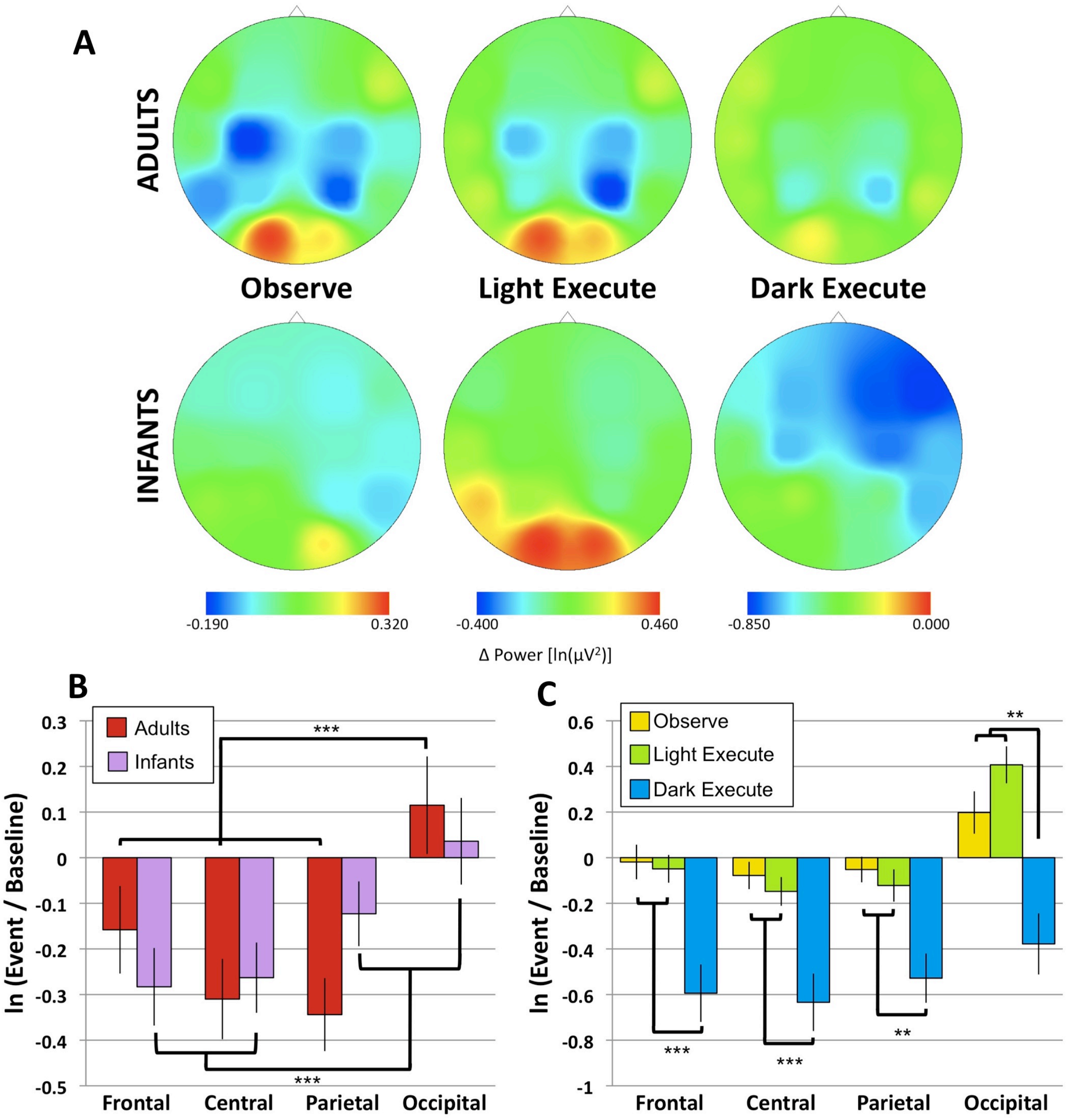

Figure 3. Scalp Distribution of the mu rhythm in human adults and infants. (A) Heat maps of mu rhythm desynchronization for each group in each condition. Note the scales are different for each condition. (B) Display of the Region x Age Group interaction. (C) Display of the Condition x Region interaction; *** $p < .001$; ** $p < .01$.

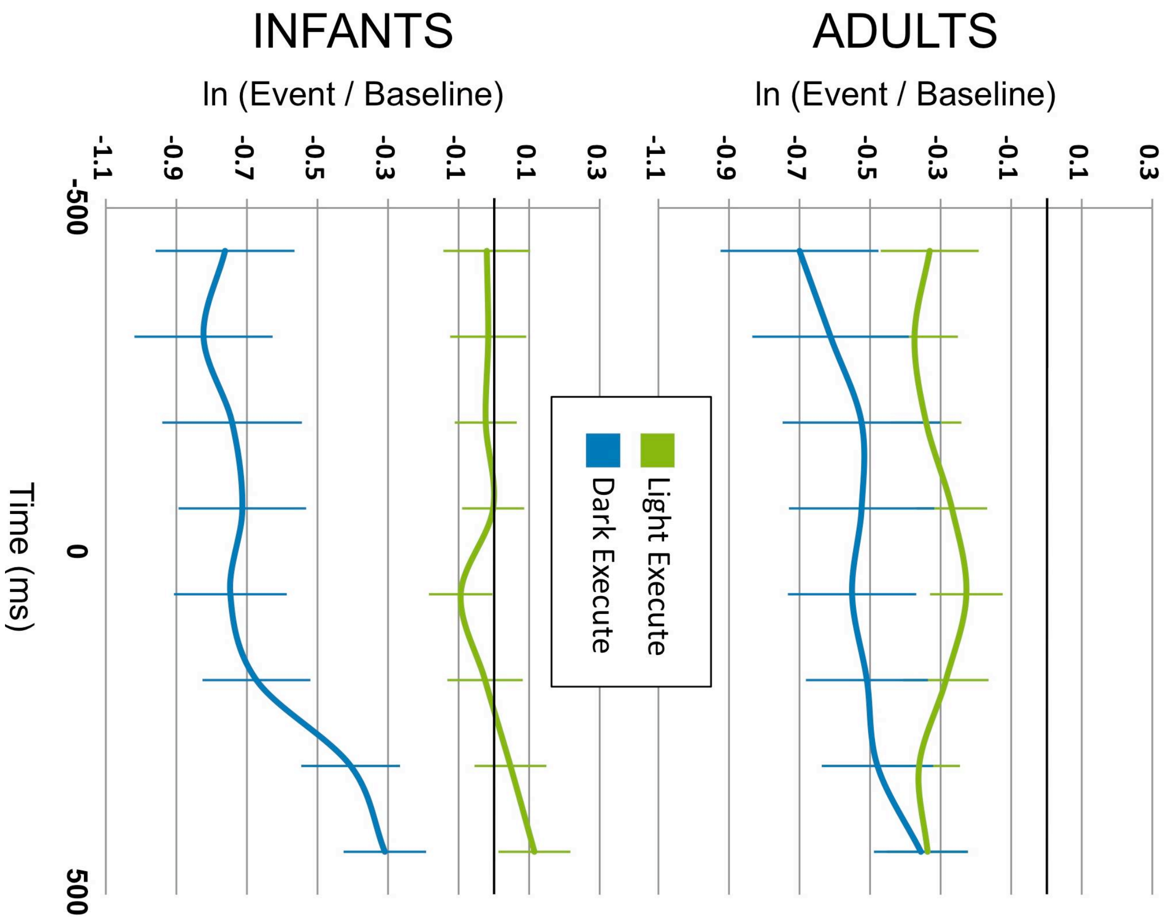

Figure 4. Timing of mu desynchronization in human adults and infants. Lines represent the change in mu rhythm power over time in the average of left and right central electrodes during the execution of a grasp. Time zero is the time the participant made contact with the toy during their grasp. Analyses revealed a significant difference between Adults and Infants during Light Execute ($F(1, 32) = 4.773$, $p < .05$) in overall power change; and a linear decrease in ERD across both Adults and Infants during Dark Execute ($F(1, 30) = 5.807$, $p < .05$). Negative values represent event-related desynchronization and positive values represent event-related synchronization.

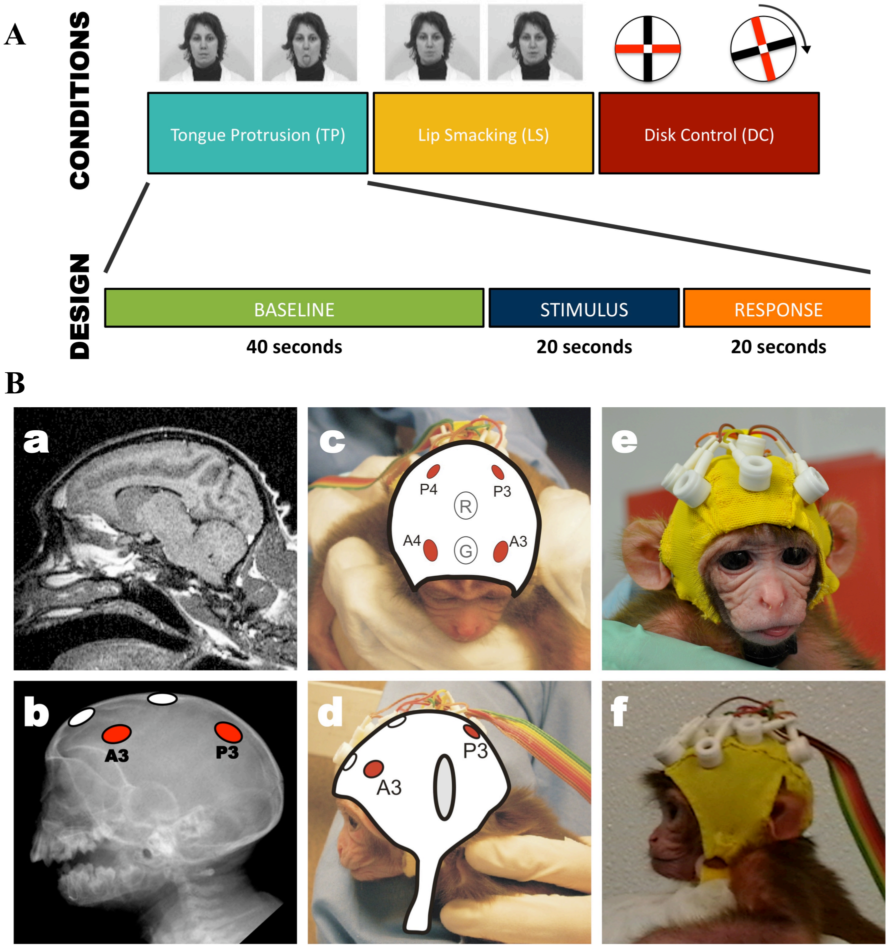

Figure 5. Example of the task design and schematic of the cap design for acquiring EEG for study 2. (A) The task consists of three conditions: 1) tongue protrusion (TP); 2) lip smacking (LS); and 3) a disk control. For each condition a 40 second baseline period of the still stimulus will be followed by three repetitions of 20-second stimulus and 20-second response periods. (B) Development of the infant primate EEG cap. Structural MRI (a) and X-rays (b) of a 1-week-old infant macaque were used to detect approximate locations of major sulci and lobes of the cerebral cortex for electrode placement. (c, d) The anterior electrodes (A3/4) were placed approximately above the motor cortex and the posterior electrodes (P3/4) were placed above the parietal lobes; R: reference and G: ground. (e, f) Close-up view of the cap placed on a 5-day-old infant.

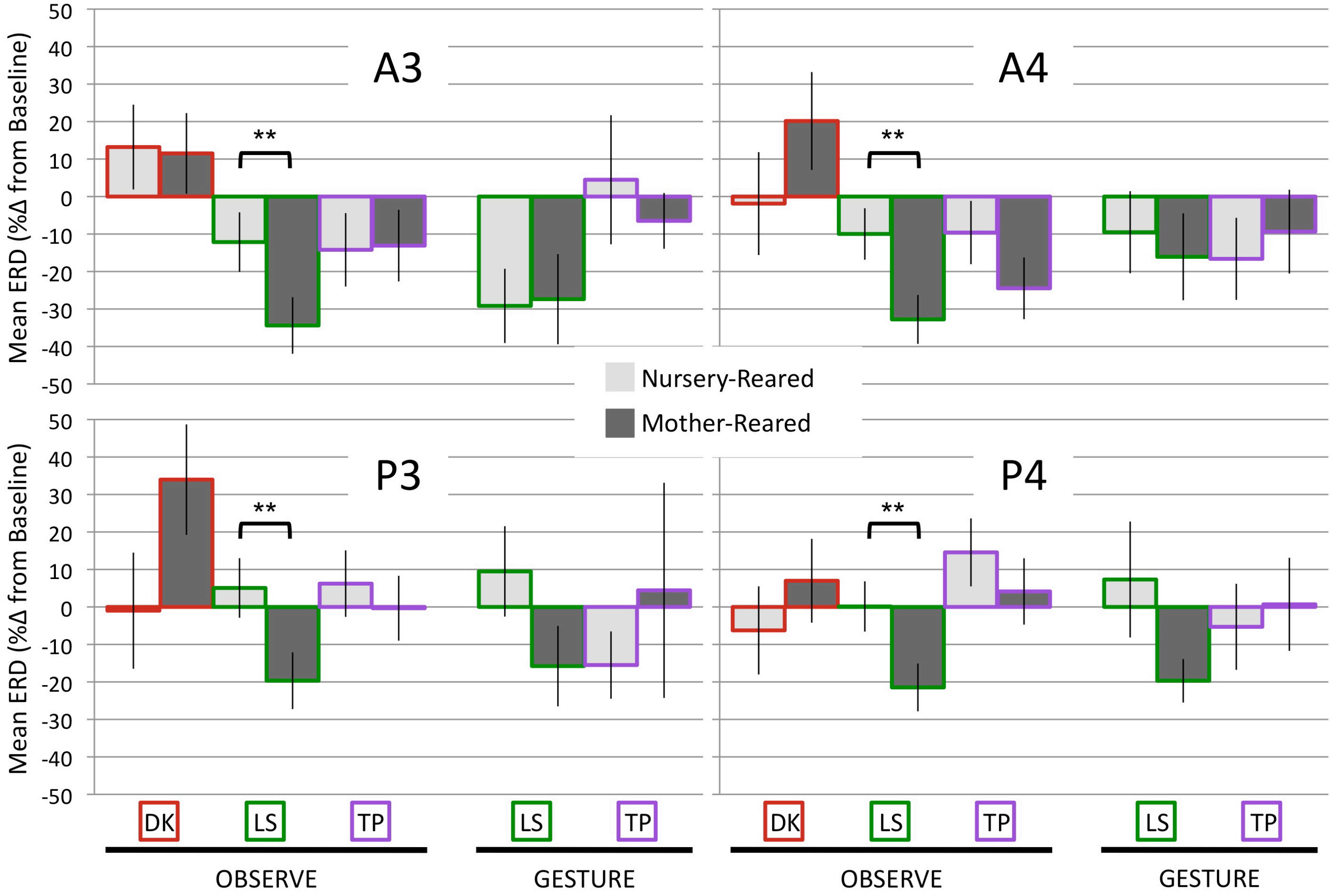

Figure 6. Mu Rhythm Desynchronization in Mother- and Nursery-Reared Infant Rhesus Macaques. Light grey represents nursery-reared and dark grey represent mother-reared groups. Red outlines are for the disk (DK), green outlines are for lip smacking (LS), and purple outlines are for tongue protrusion (TP) conditions. ** *p* < .005.

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