Early social experience affects neural activity to affiliative facial gestures in newborn nonhuman primates

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Abstract

A fundamental issue in cognitive neuroscience is how the brain encodes others' actions and intentions. The discovery of an action-production-perception mechanism underpinning such a capacity advanced our knowledge of how these processes occur; however, no study has examined how the early postnatal environment may shape action-production-perception. Here we examined the effects of social experience on action-production-perception in 3-day-old rhesus macaques that were raised either with or without their biological mothers. We measured neonatal imitation skills and brain electrical activity responses while infants produced and observed facial gestures. We hypothesized that early social experiences may shape brain activity, as assessed via electroencephalogram suppression in the alpha band (5-7 Hz in infants, known as the mu rhythm) during action observation, and lead to more proficient imitation skills. Consistent with this hypothesis, infants reared by their mothers were more likely to imitate lipsmacking—a natural, affiliative gesture—and exhibited greater mu rhythm desynchronization while viewing lipsmacking gestures than nursery-reared infants. These effects were not found in response to tongue protrusion, a meaningless gesture, or a nonsocial control. These data suggest that socially enriched early experiences in the first days after birth increase brain sensitivity to socially relevant actions.

Keywords

mirror neuron; plasticity; sensitive period; action perception; mu rhythm; infant; communication; neonatal imitation; EEG; mother-infant interactions

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A fundamental issue in developmental psychology is how early social experience affects the developing brain, and thereby infant social, cognitive, and emotional development. In humans, the early postnatal period is characterized by heightened brain plasticity and in particular, sensitivity to social environmental influences [1]. Such social influences comprise a constellation of context and caretaking features—including face-to-face interactions and tactile contact—that are critical for optimal child cognitive and socio-emotional development [2,3]. Animal models demonstrate that the early maternal and social environment shapes an offspring's neural organization, thus determining structural and functional aspects of brain development and related behavior [4,5,6]. Experimental studies with human infants and other species have shown that key components of the neural network for socio-emotional processing (e.g. amygdala, temporal and frontal cortex) operate from very early in postnatal life at the time when perceptual-representation areas are attuned to relevant social signals, including faces, direct gaze, facial expressions, and social interactions contingencies [4,5,6]. Furthermore, research with both human adults and non-human primates indicates that action-perception brain networks including parietal and premotor cortical regions involve the activation of shared sensorimotor representations between individuals (e.g., [7,8]). Early postnatal influences on these action-perception networks, however, remain unexplored. Examining the plasticity of these networks is therefore important for determining the timing and nature of experiences that facilitate healthy social development [9].

Here we examined whether differential rearing experiences influence 3-day-old rhesus macaques' sensitivity to socially meaningful facial gestures. We assessed action-perception-production mechanisms of facial gestures in two ways: through imitative facial responses and functional brain activity (electroencephalogram; EEG) during facial gestures observation. We first measured variability in infants' production of imitative facial gestures [10,11]. Next, we examined EEG activity during infants' observation of facial gestures. Specifically, we measured desynchronization in the mu frequency band over central electrode sites. In humans, this EEG desynchronization is associated with both motor activation (i.e., producing actions) and perceiving biological movement [12,13,14,15,16], and appears to be generated in the sensorimotor cortex. Thus, the mu rhythm is thought to correlate with a putative human mirror neuron system [17,18], as also supported by simultaneous EEG and fMRI recordings [19]. Moreover, in adults, the magnitude of mu rhythm desynchronization during action observation appears modulated by execution expertise/competence [20], suggesting that mirror neurons may be affected by experiential factors. The mu rhythm has also been reported in rhesus macaques, and it desynchronizes during action execution and observation [5,21,22], thus making it an ideal measure for an action-perception mechanism in the current study.

We tested proposals with contrasting predictions about the role of infants' experience on their sensitivity to socially relevant actions (communicative facial gestures) that are likely underpinned by an action-production-perception mechanism. One possibility is that the plasticity of sensory-motor matching may depend on the socio-ecological relevance of the stimulus. Evolutionarily relevant stimuli may be particularly salient early in life, ensuring that infants attend to fitness-relevant signals, including social stimuli [23] and threats

[24,25]. We hypothesized that lipsmacking (LS), an ecologically meaningful, affiliative gesture for macaques [26,27], may be resilient in the face of diverse early experiences. This hypothesis predicts that all infants should be sensitive to LS, consistent with reports that nursery-reared macaques imitate [10,11] and exhibit mu suppression during LS observation [5] independent of their early experiences. That is, both mother-reared and nursery-reared infants should exhibit higher rates of imitation and show heighted event-related desynchronization (ERD) during the observation of evolutionary relevant (LS) compared to irrelevant (tongue protrusion; TP) gestures.

Alternatively, socio-ecologically-relevant stimuli are typically the most common environmental stimuli [28]; therefore, an action-perception mechanism may develop through experiential canalization, a developmental process that ensures the reliable emergence of important features of the organism, across diverse environments [29]. That is, the early postnatal period may be a sensitive period during which the social environment alters the neural organization of action observation and production. Such a system may exist for face perception [30,31] and auditory-visual perception [32], but it remains unknown how early experiences influence the action-observation mechanism. If experiences impact action-perception from birth, then this hypothesis predicts that mother-reared macaque infants, but not nursery-reared macaque infants, should exhibit higher rates of imitation and show the greatest ERD during the observation of familiar (LS) compared to unfamiliar (TP) gestures.

Methods

Facial Gesture Production

While infants' facial gestures were measured in two experiments, outlined below, the purpose of tracking these behaviors was different in each experiment. The purpose of tracking infants' facial gestures in Experiment 1 was to assess their neonatal imitation skill. Previous work has shown that macaque infants demonstrate low rates of imitation during an EEG task (Experiment 2) compared to a purely behavioral task (Experiment 1) [5]; thus, gesture production during EEG may not be an accurate reflection of an infant's imitation ability. Differences in the frequency of gesture production between mother-reared (MR) and nursery-reared (NR) animals during the EEG task are also important to measure, as we needed to assess EEG activity during both the observation and the production of facial gestures. Therefore, the measurement of gesture production was important in both tasks.

Experiment 1: Neonatal imitation tests

Subjects—Our final sample included 39 mother-reared (MR) and 46 nursery-reared (NR) infant rhesus macaques (*Macaca mulatta*), tested on their third or fourth day of life (MR: age M = 3.45 days, SD = .55; NR: age M = 3.65 days, SD = 0.48). MR infants (19 males and 20 females; birth weight M = 489.63g, SD = 66.83) were reared by their biological mothers and housed in social groups. NR infants (22 males and 24 females; birth weight M = 514.85g, SD = 94.98) were separated from their mothers on day 1 post-partum and individually housed in incubators. For rearing details, see Supplementary Materials. 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 the University of Maryland and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

Procedure—NR and MR infants were tested in slightly different contexts in order to make each infant as calm and comfortable as possible, thus maximizing their likelihood of imitating [33]. To accomplish this, both groups of infants were tested while in contact with their respective caregivers—the mothers for the MR infants and the human caregiver for the NR infants. For testing the MR group, each mother-infant pair was separated from their social group, and mothers were lightly sedated with ketamine (3-10 mg/kg IM). One experimenter gently restrained mothers in a sitting or lying position; infants remained alert and clinging to their mothers' front (Figure 1). A second experimenter served as the model and directed facial gestures and control disk movements at the infants. A third experimenter filmed the infant (either a Canon ZR600 MiniDV or Sony Digital Video HDR-CX560V) with only the infant in view, thereby allowing scoring whilst being blind to the model. For NR testing, an experimenter held each infant in her lap for testing.

The imitation paradigm has been described in detail in previous studies (e.g., [33,34]), and is outlined in the Supplementary Materials. Briefly, all infants received live presentation of stimuli for three conditions: a) Tongue Protrusion (TP) with maximal extension and retraction of the tongue; b) Lipsmack (LS) a rapid opening and closing of the lips; and a 15-cm diameter plastic Disk (DK) with a red and black cross painted on it that was rotated 180°. The order of these conditions was randomized between subjects; for MR infants, there was an interval of ca. 1 minute between conditions whereas for NR infants, there was an interval of ca. 2h between conditions. By spacing the stimulus conditions by ca. 2h for NR infants we intended to decrease the likelihood of carry-over effects (such as continuing lipsmacking or tongue protrusion responses between conditions that can interfere with imitation responses); however, such spacing was not possible with MR infants, whose mothers had to be sedated for the test.

Each testing session began with a 40-second static baseline in which the monkey was presented with a still face (SF) in TP and LS conditions, and a still disk in the DK condition. SF was followed by a 100-second stimulus period consisting of a 20-second dynamic stimulus presentation and a 20-second static period (still stimulus), repeated 3 times: dynamic-static-dynamic-static-dynamic. Finally there was another 40 sec still stimulus. Total test time was 3 minutes per each of the three conditions (LS, TP, DK). This method allowed us to rule out arousal effects (see Supplementary Materials).

Data analysis—An experimenter blind to the experimental condition coded all occurrences of LS and TP within each phase and each condition. LS was defined as any unobstructed opening and closing of the mouth; yawns, rooting for their mothers' nipple, or mouthing of other body parts were not counted as LS. TP was scored if the infant's tongue was thrust beyond the outer edges of the lips and retracted back into the mouth. For inter-observer agreement, see Supplemental Materials.

Experiment 2: EEG measurements during gesture observation

Subjects—We tested a separate sample of 30 3-day-old MR (11 males and 19 females; birth weight M = 489.00g, SD = 63.77) and 26 NR (19 males and 7 females; birth weight M = 500.35g, SD = 53.73) infant rhesus macaques. Six MR and three NR infants were excluded from analyses due to insufficient epochs (n = 2 MR), statistical outliers (n = 1 MR; n = 2 NR), or technical difficulties at the time of testing (n = 3 MR; n = 1 NR), leaving a final sample of 24 MR and 23 NR infants with usable data. We tested a separate population of infants in this task who did not participate in the first task (Experiment 1) due to test duration limitations and to prevent infants from habituating to the stimuli (same stimuli in both tasks).

Procedure—Each mother-infant pair was separated from their social group, and mothers were lightly sedated with ketamine (3-10 mg/kg IM). Infants were then separated from the mother and brought to a separate testing room, where a human experimenter held them. NR infants were removed from their incubator and tested in the way as MR infants. During each testing period, the infant was presented with all three conditions of the imitation paradigm presented in a random order (described in Experiment 1).

EEG was collected during the imitation paradigm. A custom Lycra cap was made for the acquisition of EEG data in infant rhesus macaques with 8 tin electrodes (see [5] Figure 1). Two anterior electrodes were placed on scalp locations above the motor cortex 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. Infants' heads 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. Testing sessions were recorded on DVD synchronized with the EEG for behavioral coding. Coders recorded the direction of the infant's gaze and LS or TP gestures throughout the session to identify the onset and offset times of epochs when the infant was still gazing toward the stimulus (baseline and observe) or while the infant was producing a LS or TP (execution); we only analyzed epochs that were at least one second in duration during baseline and observe. Epochs in which a gesture occurred lasting for less than one second were lengthened to a one-second epoch centered on the gesture period. There were no differences between rearing groups in the number of epochs for baseline and observation analyzed between DK, LS, or TP or for gesturing in LS and TP (see Supplementary Materials for details).

Artifact free epochs were 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 20Hz. 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 [35] and mu rhythms [5]. All data processing was performed using EEG Analysis System software, James Long Company (Caroga Lake, NY).

Event-related desynchronization was computed as $[(S - B) / B] \times 100$, 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 [14]). Therefore, negative values are interpreted as a decrease from baseline or event-related desynchronization (ERD) and positive values as an increase from baseline or event-related synchronization (ERS).

Results and Discussion

Experiment 1: Neonatal Imitation Tests

Our data were not normally distributed; therefore, for each rearing group, we carried out Wilcoxon Signed Ranks Tests (two-tailed) to examine whether there was a difference in lipsmack (LS) gestures from the baseline period to the stimulus period in each condition. We found that MR infants, but not NR infants, demonstrated an increase in LS gestures from the baseline to the stimulus period in the LS condition (baseline MR: $M = 2.03$, $SD = 2.71$; NR: $M = 1.96$, $SD = 2.57$; stimulus MR: $M = 3.44$, $SD = 3.26$; NR: $M = 2.72$, $SD = 2.74$; MR: $z = 2.81$, $p = .005$; NR: $z = 1.77$, $p = .077$), but not the DK condition (baseline MR: $M = 1.62$, $SD = 2.27$; NR: $M = 1.46$, $SD = 1.50$; stimulus MR: $M = 2.02$, $SD = 2.35$; NR: $M = 2.04$, $SD = 1.95$; MR: $z = .92$, $p = .360$; NR: $z = 1.94$, $p = .052$), suggesting that as a group, MR infants were LS imitators but NR infants were not (Figure 2). Neither group showed significant increases in tongue protrusion (TP) in the TP condition (baseline MR: $M = 1.90$, $SD = 1.97$; NR: $M = 3.43$, $SD = 3.15$; stimulus MR: $M = 2.57$, $SD = 2.15$; NR: $M = 1.51$, $SD = 1.51$; MR: $z = 1.40$, $p = .160$; NR: significant decrease, $z = 4.17$, $p < .001$). We also found, using Mann-Whitney U tests, that there were no differences in baseline rates of gesturing (LS and TP combined) between MR and NR infants, when infants were first presented with either the still-face (LPS condition, $p = .773$) or still-disk (DK condition, $p = .993$), suggesting our findings are unlikely to be exclusively due to rearing differences in arousal (see Supplemental Materials for further discussion).

These results suggest that, as a group at 3 days of age, MR infants imitated LS gestures, a natural and affiliative gesture in macaques [26,27]. This is consistent with previous reports that newborn macaque infants imitate LS gestures across the first week of life [10,11,34], and extends this finding, demonstrating that early experiences impact this skill [33]. We found no evidence, however, of TP imitation, nor did we find any increases in facial gesturing in the non-biological control condition. Together, these results suggest that social experiences in the first few days of life may improve imitative skills, particularly for socially meaningful actions.

Experiment 2: EEG measurements during gesture observation

We focused specifically on the 5-7 Hz frequency band, previously identified as mu [5], and verified desynchronization during execution (average of LS and TP conditions) with a one-sample t-test ($M = -13.44$, $SD = 24.66$; $t(20) = 2.50$, $p = .011$, $d = 1.12$). 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 (MR/NR) as a between-subjects factor. Greenhouse-Geisser correction of degrees of freedom was used when sphericity was violated, denoted by epsilon (ε). The analysis revealed main effects of Condition ($F(2, 72)$ = 5.52, p = .014, ε = .69) and Region ($F(1, 36)$ = 8.51, p = .006) qualified by Condition × Group ($F(2, 72)$ = 3.93, p = .040, ε = .69), Condition x Hemisphere ($F(2, 72)$ = 3.10, p = . 051), and Condition × Region × Hemisphere x Rearing Group ($F(2, 72)$ = 3.45, p = .042) 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 = .029, η_p^2 = .12) main effect qualified by a Region × Hemisphere × Rearing Group interaction ($F(1, 38)$ = 4.75, p = .036). Examination of the means of the main effect revealed greater synchronization (ERS) in the left hemisphere compared to the right. Exploration of the interaction revealed that in the MR 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, d = 1.35, Bonferroni corrected).

Analysis of the LS condition revealed main effects for Region ($F(1, 38)$ = 7.48, p = .009, η_p^2 = .16) and for Rearing Group ($F(1, 38)$ = 9.23, p = .004, η_p^2 = .20). Examination of the means of the Region main effect revealed greater event-related desynchronization (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 MR infants (M = −27.08, SD = 23.18), however, had greater overall ERD compared to the NR 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 < .002, η_p^2 = .23), in which there was greater desynchronization 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 3.

To analyze rearing group differences in mu rhythm desynchronization during gesture production, we used a 2 Condition (LS/TP) × 2 Region (Anterior/Posterior) × 2 Hemisphere (Left/Right) repeated-measures ANOVA with Rearing Group (MR/NR) as a between subjects factor. For this analysis we combined LS and TP gesture production within the conditions, as there were not enough data to analyze each specific gesture. As we hypothesized, there were no main effects or interactions with rearing condition, suggesting that mu rhythm desynchronization is comparable between the two populations of infants during gesture production (see Figure 3).

Any observed differences in the magnitude of mu rhythm desynchronization between the rearing groups 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 stimuli. Independent samples t-tests revealed the NR infants' gaze was directed towards the disk longer than MR infants during the baseline (M = 4.49 seconds, SD = 3.39 and M = 2.50, SD = 1.25, respectively; $t(45)$ = 2.69, p = .013, d = 0.80) and

stimulus ($M = 6.98$, $SD = 6.75$ and $M = 3.41$, $SD = 1.98$, respectively; $t(45) = 2.48$, $p = .022$, $d = 0.74$) 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.

In summary, differential rearing appeared to impact neural activity during the observation of a species-typical affiliative facial gesture in 3-day-old infant macaques. Infants reared with their biological mothers, compared to nursery-reared infants, showed greater mu rhythm desynchronization during LS observation. These findings are consistent with the hypothesis that an action-production-observation mechanism emerges early in ontogeny, and is affected by early social experience.

General Discussion

The goal of the present study was to examine the plasticity of sensorimotor development; specifically, whether differential experiences in the first three postnatal days affect imitative skill and functional brain activity. We compared facial gesture imitation and EEG activity of infant macaques separated from their mothers at birth to infants who remained with their mothers, using a neonatal imitation task to measure the neural underpinnings of action-perception-production [5,10,11]. We tested whether the plasticity of action-perception-links early in development depend on a stimulus's socio-ecological relevance.

At 3 days of age, MR infants, but not NR infants, imitated LS gestures. In the first days of life, macaque mothers naturally engage in face-to-face exchanges with their infants and often direct LS gestures at infants [36]. Thus, mothers provide infants with an important source of visual stimulation, including facial emotional communication. This finding is also consistent with a behavioral study in a separate sample of NR macaques, in which 1-week-old infants who received daily face-to-face LS gestures, compared to a standard-reared nursery control group, demonstrated enhanced LS imitation, but not TP imitation ([33]; similar findings in humans: [37]). Together these findings suggest that social experiences in the first few days of life can improve imitative skills, especially for socially meaningful actions.

Next, we confirmed that MR infants exhibit desynchronization of the mu rhythm (5-7Hz) in anterior electrodes during facial gesture observation, and that during their own gesture production, MR infants show desynchronization in both anterior and posterior regions. All infants, therefore, exhibited mu rhythm desynchronization during observation and production of facial gestures, but not during non-biological motion, replicating and extending findings in NR infants [5].

Finally, and most interestingly, we found that, early in development, rearing influenced neural activity during the observation of a species-typical affiliative facial gesture. Specifically, 3-day-old MR infant macaques, compared to NR infants, showed greater mu rhythm desynchronization during LS observation, a natural and affiliative gesture in macaques [26,27] and one that infants readily imitate [10,11,34]. Mu-desynchronization may be generated in the sensorimotor cortex [38] and involves a network that includes the parietal and motor regions [39], perhaps reflecting mirror neuron system (MNS) activity

[17,40]. The proposal that the mu rhythm reflects MNS activity was further supported with simultaneous EEG and functional MRI [19], which showed that during action observation mu desynchronization correlated with activity in MNS areas.

Our results support the hypothesis that an action-production-observation mechanism emerges early in infancy, particularly for facial gesture matching, and that it is affected by early social experience. Based on our previous study [5] and recent findings in adult monkeys [21], we suggest that that there is mirroring of action production and perception in early infancy and that it is influenced by experience. These results also demonstrate that the type of facial gestures to which infants are exposed can selectively influence their perception of those actions. While MR infants demonstrated stronger desynchronization to LS gestures, there were no differences between MR and NR infants' desynchronization to TP gestures. In sum, early experiences with certain actions influence newborns' imitation of those actions and their neural processing during action observation.

The present findings are also consistent with reports that the mu rhythm is highly sensitive to experience with particular movements. For example, adult elite athletes, compared to non-athletes, have greater mu rhythm desynchronization during observation of activity-related actions (e.g., [41,42,43]). Even short amounts of experience using a novel tool can modulate adults' mu rhythm desynchronization during action observation [44]. Likewise, in infancy, for infants' emerging actions, the mu rhythm desynchronization increases as an individual's own action experience or skill increases [20,45]. In our sample, infants who, as a group, received greater experience with LS gestures (produced by their mothers or other conspecifics) exhibited greater mu rhythm desynchronization while observing LS. This desynchronization was nearly three times as large in MR compared to NR infants.

The presence of such large differences in desynchronization after such a short period of differential experience suggest that the neural networks that supports sensory-motor integration, at least for these affiliative facial gestures, are highly sensitive early in life. It is hypothesized that the hand and mouth neural mirror systems may have separate developmental trajectories, with the former developing slowly through experiences observing own and other's actions, and the latter being present at birth to facilitate early social interaction [46]. Early face preferences [47] and imitation in the first postnatal days [48] suggest the early emergence of a face mirroring system. Moreover, this system is further modified through contingent social interactions [46]. The present results further support this hypothesis, specifically for the mouth or face matching system, and reveal the speed with which experience can modify the system.

In the present study, however, the precise cause of the rearing difference in mu suppression —namely, whether it is due to differences in exposure to the LS facial gesture, or to differences in infants' practice or production of that gesture (or both), or some other environmental difference (e.g., cage type, diet, physical contact)—remains to be determined. Future work closely tracking infants' early production and observation of gestures can address this question. However, evidence of differential experience comes from behavioral studies of mother-infant interaction (e.g., [36]), which reveal that, macaque mothers engage in complex emotional exchanges in which infants actively participate, which include

sustained mutual gaze and exaggerated LS [40]. Similar to human infants, macaque infants may benefit from exaggerated infant-directed communicative expressions (e.g., "motherese"), which help infants learn to communicate (e.g., [49]). How the timing, duration, and quality of these early face-to-face interactions impact the action-perception system, is a question for future research.

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 young infants. By identifying periods in which the infants were still and observing the stimuli or when they produced a LS or TP gesture, we identified the mu rhythm. Unfortunately, to have enough data to analyze execution we had to collapse across gestures and could not separately analyze periods of LS and TP production.

Finally, the differences in mu rhythm desynchronization between MR and NR infants observed in this experiment are not due to differences in the behaviors of the infants during the testing sessions, nor in the background EEG activity. Behaviorally, during EEG testing the two groups of infants were similar in their gesture frequency and in the time they fixated on the face. The only behavioral difference we observed was that the NR infants spent more time fixating on the disk than the MR infants. This difference may have been partly due to the NR infants' greater experience with objects. The results of the behavioral analyses suggest that the effects observed in the EEG were not effects of behavioral differences during testing, but reflect early experience on sensory-motor cortical sensitivity to facial gestures.

Some of our behavioral results may, at first, appear inconsistent. For instance, MR infants imitated LS more than NR infants in the behavioral task, but not in the EEG task. This is likely due to differences in the testing context. First, ideal conditions for eliciting imitation include ensuring infants are comfortable, calm, and alert. Anecdotally, infants appear less calm in the EEG testing context, perhaps because they are wearing a novel EEG cap, which is somewhat restricting, but also because infants were held by a human experimenter rather than their mothers, as MR infants were in the neonatal imitation assessment. The presence of the mother likely had a calming effect on the infants, making them more comfortable engaging in a social interaction. In fact, these methodological differences are in line with our goals for each task, with the behavioral task aimed at assessing rearing effects on imitation and the EEG task aimed at assessing rearing effects on sensorimotor activity during action observation.

It may also seem puzzling that we did not observe significant imitative responses at the population level in our 3-day-old NR infants, as reported previously [10,11]. Such differences from previous reports at the population level are likely due to large individual differences in imitative skill (for a review, see [33]). Briefly, not all infants imitate; only about half of neonates consistently imitate (e.g., humans: [50]; macaques: [51]). Our examination of only effects at the level of the entire sample based on only a one-day assessment may therefore portray a misleading picture of imitation, making it falsely appear uncommon [52]. Clearly further examination is warranted to understand such variability.

One limitation of the present design was that behavioral testing of neonatal imitation necessitated a procedural difference between MR and NR infants: the time interval between conditions was 1 minute for MR infants and 2 hours for NR infants. It is unlikely that this difference can explain our findings, however. Infants tend to get disinterested during longer test session [53], and so in order to maximize imitative performance, we spaced individual test sessions throughout the day for NR infants, but due to the sedation of the mother, this was not possible for MR infants. Thus, NR infants appear predisposed to perform better at the imitation task than MR infants. However, our results suggest MR infants show higher levels of imitative skills than NR infants, at least for lipsmacking. In addition, in the neonatal imitation task, MR infants were tested while clinging to their mothers, while NR infants were tested while being held by a human experimenter. Removing MR infants from their mothers for neonatal imitation testing commonly leads to increased anxiety levels in the infant as evidenced by frequent separation calls (personal observation), which can potentially interfere with imitative performance. Thus to make MR infants more comfortable and therefore more likely to engage in a social interaction, we tested them while clinging to their mothers. However, it is worth emphasizing that in the EEG task, there were no procedural differences in testing between MR and NR infants; therefore, the rearing effect differences found in the EEG data are likely not due to procedural differences.

The present findings provide new insight into how an execution-observation mechanism may emerge ontogenetically. First, the data from naïve NR infants suggest that the mu rhythm is evident in infants prior to exposure to contingent and congruent actions (i.e., NR infants had no previous experience with turn-taking face-to-face interactions), making it unlikely that this activity is a consequence of associative learning. Second, the observed differences in EEG between MR and NR infants suggest that the mu rhythm is plastic in the first three days of life and can be enhanced with enriched social experiences, perhaps aiding infants' in encoding socially relevant stimuli. Such a system might be advantageous in helping infants navigate their complex social worlds. In addition, rather than characterizing mirror neurons as a uniform and stable neural mirroring mechanism, there is a need for a revised account which acknowledges their development, variability, and plasticity (e.g., [54]). Future work exploring the timing and nature of early sensitive periods in development for the action-perception system will be particularly helpful in this regard.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Fig 1.
Mother-reared (MR) rhesus macaque newborns during neonatal imitation. The infant on the left is imitating lipsmacking (LS) and the infant on the right is imitating tongue protrusion (TP).
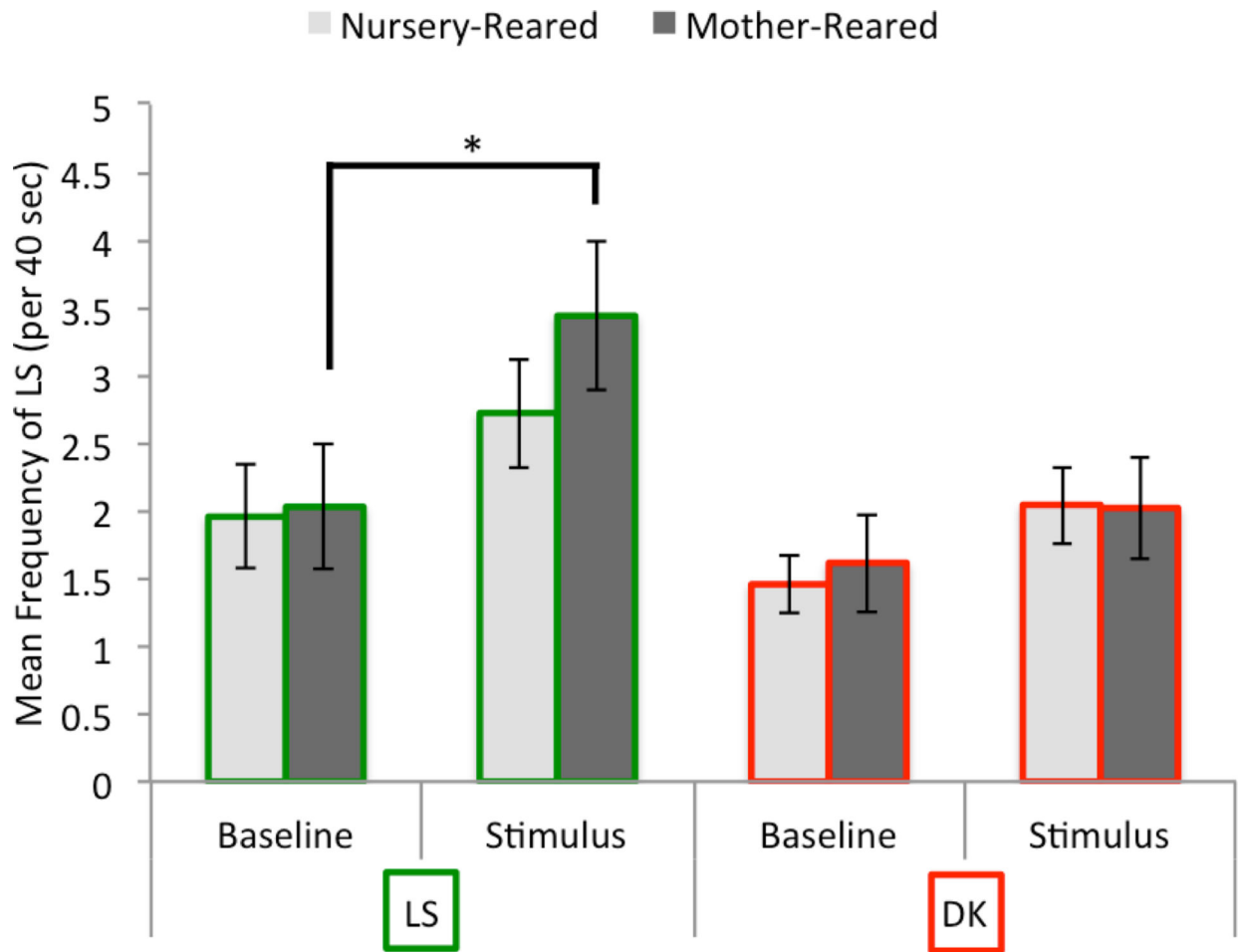
Fig 2.
Neonatal imitation of lipsmacking in 3-day-old in mother-reared (MR) and nursery-reared (NR) infant rhesus macaques. Light grey represents nursery-reared ($n = 46$) and dark grey represent mother-reared ($n = 39$) groups. Infants viewed two conditions: lipsmack (LS) gestures and a control disk (DK), including both baseline (static; still face or still disk) and stimulus (dynamic; gesture or disk rotating) phases. Error bars reflect standard error of the mean. *$p = .005$.
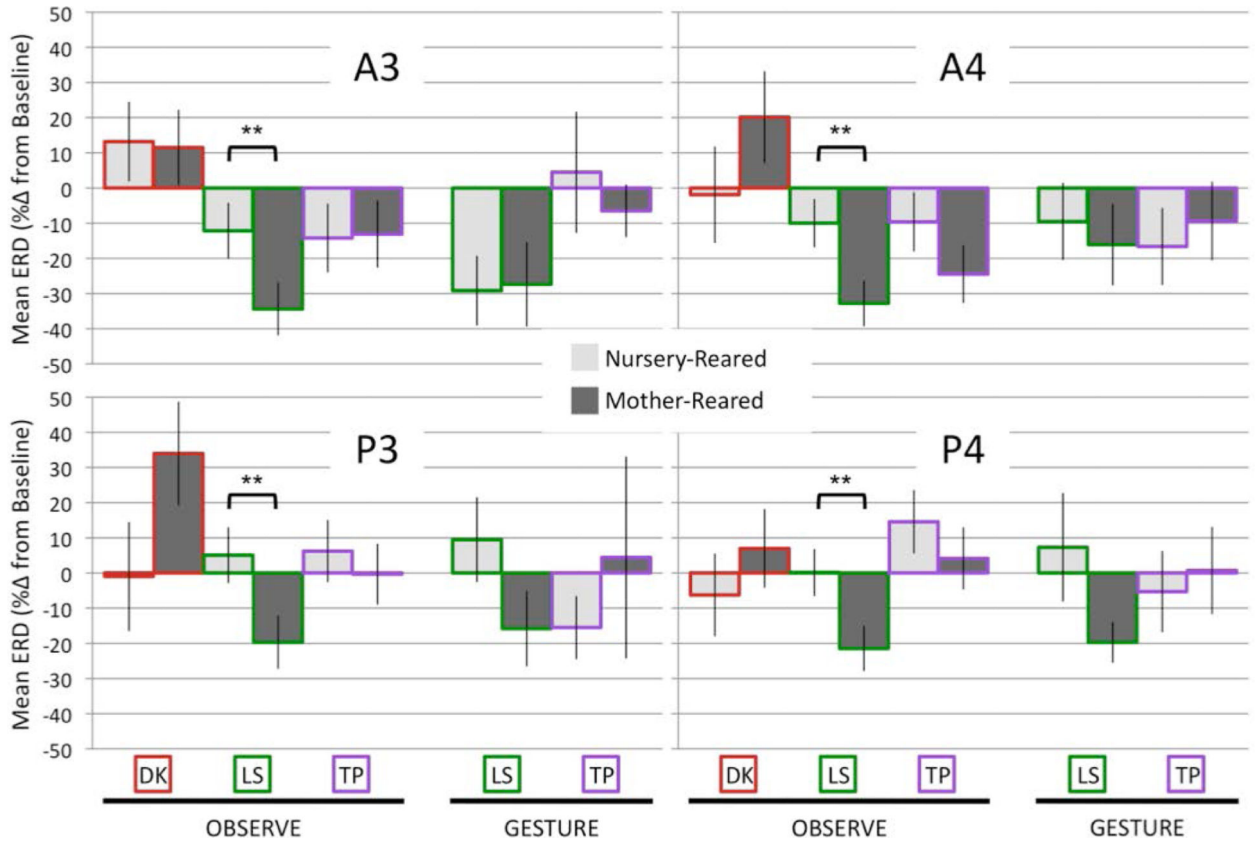
Fig 3.
Mu rhythm desynchronization in mother-reared (MR) and nursery-reared (NR) infant rhesus macaques. Light grey represent nursery-reared ($n = 23$) and dark grey represent mother-reared ($n = 24$) groups. Red outlines are for the disk (DK), green outlines are for lipsmacking (LS), and purple outlines are for tongue protrusion (TP) conditions. Error bars reflect standard error of the mean. ** $p < .005$.