Supplementary Materials

Methods

**Experiment 1: Neonatal imitation tests**

Neonatal imitation refers to neonates’ ability to reproduce modeled actions, such as facial gestures. While this method is not without its critics (e.g., [1,2]), neonatal imitation is a widely replicated phenomenon and has been proposed to be a useful measure of individual differences in infancy (for reviews, see [2,3,4,5]).

Subjects

 We tested 39 mother-reared (MR) and 46 nursery-reared (NR) infant rhesus macaques (*Macaca mulatta*) 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 containing 8-10 adult females and several infants of the same age as well as 1-2 adult males. Monkeys were housed in indoor-outdoor enclosures measuring 2.44 × 3.05 × 2.21 m (indoor), and 2.44 × 3.0 × 2.44 m (outdoor). NR infants (22 males and 24 females; birth weight *M* = 514.85g, *SD* = 94.98) were involved in an ongoing experimental protocol that required separating them from their mothers on day 1 post-partum. Infants were individually housed in incubators (51 × 38 × 43 cm), maintained at 24-28 degrees Celsius, containing one soft toy, two hard toys, three fleece blankets, and a surrogate mother made from an upright, flexible polypropylene cylinder covered in fleece (for further rearing details, see [6]). Attached to the surrogate mother was a two-ounce bottle containing Similac formula. Toys and fleece blankets were replaced daily. NR infants could see and hear other infants, but had no physical contact with them. No face-to-face interactions among NR infants were observed in the first 3 days of life. The final sample of 85 infants does not include an additional 8 MR infants who were tested but were excluded because of inattention due to rooting or nursing, and an additional 9 infants (3 MR and 6 NR) who were tested but excluded for being outliers (> 2.5 *SD* from pooled mean). 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).

Procedure

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 infants’ reactions with a video camera (either a Canon ZR600 MiniDV or Sony Digital Video HDR-CX560V) with only the infant in view, thereby allowing blind scoring (i.e., blind to the model presented to the infant). After testing, mothers and infants were transferred to a single cage and mothers were allowed to recover completely before being released back into their social groups. NR infants were removed from their incubator and transferred to a test room. An experimenter held each infant in her lap for testing; otherwise the procedure was identical to MR testing. After testing, infants were returned to their incubator.

The imitation paradigm has been described in detail in previous studies (e.g., [7,8]). Briefly, all infants received live presentation of stimuli from a human experimenter 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 without sound production; and a 15-cm diameter plastic Disk (DK) with a red and black cross painted on it that was rotated 180° clockwise and counterclockwise. 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. Each testing session began with a 40-second baseline in which the monkey was presented with a still face in TP and LS conditions, and a still disk in the DK condition. The baseline was followed by 20-seconds of dynamic stimulus presentation and then a 20-second static period of the still stimuli. The 20-second dynamic stimulus presentation and still period were repeated 3 times in order to obtain 60-seconds of data for each infant within each condition. After the last stimulus phase, the final still face phase was 40 sec, rendering total test time for each condition 3 minutes. Two different experimenters served as models for the LS and TP condition so that for infants, each gesture was uniquely associated with one particular human face.

Data analysis

An experimenter blind to the experimental condition (model) coded (30 frames per second) 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. A TP was scored if the infant’s tongue was thrust beyond the outer edges of the lips and retracted back into the mouth. The average inter-observer agreement for gesture frequencies was calculated for 13 infants (15% of sample) and was high for LS (*r* = .936, *p* < .001) and TP (*r* = .951, *p* < .001). Non-parametric tests were performed in cases in which data were not normally distributed.

**Experiment 2: EEG measurements during gesture observation**

Subjects

We tested a separate sample of 30 healthy 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 (*Macaca mulatta*). 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. An additional five MR and four NR infants did not complete all three conditions and so their data was excluded from 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.

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 (described in Experiment 1) presented in a random order. Following completion of testing, MR infants were reunited with their mothers and returned to their social group; NR infants were returned to their incubators.

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 [9], 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. 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. Testing sessions were recorded on DVD for behavioral coding and synchronized with the EEG. 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 (i.e., no gross motor movements) and the infant’s gaze was directed toward the stimulus (baseline and observe) or while the infant was producing a lipsmack or tongue protrusion (execution); only those epochs that were at least one second in duration during baseline and observe were analyzed. Epochs in which a gesture occurred lasting for less than one second were lengthened to a one-second epoch centered on the gesture period.

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 (μV2) 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 [10] and mu rhythms [9]. All data processing was performed using EEG Analysis System software, James Long Company (Caroga Lake, NY). There were no differences in the number of epochs analyzed between the two groups of infants; on average, infants provided 34.58 (*SD* = 28.10) baseline and 28.26 (*SD* = 22.47) observe epochs in the DK condition; 81.71 (*SD* = 35.35) baseline and 24.11 (*SD* = 19.69) observe epochs in the LS condition; 84.53 (*SD* = 38.92) baseline and 23.47 (*SD* = 17.38) observe epochs in the TP condition; and 10.39 (*SD* = 9.27) and 10.62 (*SD* = 10.89) gesture epochs in LS and TP, respectively. There were significantly fewer epochs in the baseline of DK compared to the baselines of LS and TP conditions (*t*(37) = 8.80, *p* < .001 and *t*(37) = 7.10, *p* < .001, respectively) but the number of observation or gesture epochs did not differ between any of the conditions (*p*s > .15).

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 [11]. 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).

Discussion

*Neonatal imitation reflects more than arousal*

The neonatal imitation assessment used in the present study followed “best practices” guidelines, ensuring control for baseline arousal (for details, see [8]). This method takes into account that infants may produce more actions in an aroused state, and may be differentially aroused by different actions.

Briefly, the neonatal imitation response in infant macaques, like humans, is characterized by response specificity, with increased LS when infants view a LS model and increased TP when viewing a TP model [12]. If the LPS response reflected a more general arousal response then the specific body part and action of that part would not be expected to match that which is modeled, as they did in the present study. While it is impossible to entirely rule out arousal differences between MR and NR infants, we found no evidence that this variable can account for the EEG differences detected in the current study.

In addition, imitative responses are not automatic, as would be expected if they were simply a reflection of arousal. Rather, LPS imitative responses can be controlled, as evidenced by deferred imitation (e.g., delayed imitation; [7]) and infants’ ability to selectively directed LPS to familiar social partners with whom they previously imitated [13]. Finally, previous work measured infants’ arousal (e.g., states, arm and finger movements), which could not account for neonatal imitation performance [14]. For these reasons, LPS imitation observed in the present study is unlikely to be simply due to arousal.

References

1. Cook R, Bird G, Catmur C., Press C, Heyes C: Mirror neurons: from origin to function. Behavioral and Brain Sciences 2014; 37:177-192.
2. Oostenbroek J, Slaughter V, Nielsen M, Suddendorf T: Why the confusion around neonatal imitation? A review, J Reproductive and Infant Psychol 2013; 31:328-341.
3. Meltzoff AN, Moore MK: Explaining facial imitation: A theoretical model. Early Development and Parenting 1997; 6:79-192.
4. Nagy E, Pilling K, Orvos H, Molnar P: Imitation of tongue protrusion in human neonates: Specificity of the response in a large sample. Dev Psychol 2012; 49:1628-1638.
5. Simpson EA, Paukner A, Suomi SJ, Ferrari PF: Neonatal imitation and its sensory-motor mechanism. Mirror Neurons. In press; Oxford University Press.
6. Shannon C, Champoux M, Suomi SJ: Rearing condition and plasma cortisol in rhesus monkey infants. Am J Primatol 1998; 46:311-321.
7. Paukner A, Ferrari PF, Suomi SJ: Delayed imitation of lipsmacking gestures by infant rhesus macaques (*Macaca mulatta*). PLoS ONE 2011; 6:1-7.
8. Simpson EA, Murray L, Paukner A, Ferrari PF: The mirror neuron system as revealed through neonatal imitation: Presence from birth, predictive power, and evidence of plasticity. Philos Trans R Soc Lond B Bio Sci 2014; 69:1-12.
9. Ferrari PF, Vanderwert RE, Paukner A, Bower S, Suomi SJ, Fox NA: Distinct EEG amplitude suppression to facial gestures as evidence for a mirror mechanism in newborn monkeys. J Cogn Neurosci 2012; 24:1165-1172.
10. Vanderwert RE, Ferrari PF, Paukner A, Bower SB, Fox NA, Suomi SJ: Spectral characteristics of the newborn rhesus macaque EEG reflect functional cortical activity. Physiol Behav 2012; 107:787-791.
11. Marshall PJ, Meltzoff AN: Neural mirroring systems: Exploring the EEG mu rhythm in human infancy. Dev Cogn Neurosci 2011; 1:110-123.
12. Ferrari PF, Visalberghi E, Paukner A, Fogassi L, Ruggiero A, Suomi, SJ: Neonatal imitation in rhesus macaques. PLoS Biol 2006; 4:e302.
13. Simpson EA, Paukner A, Sclafani V, Suomi SJ, Ferrari PF: Lipsmacking imitation skill in newborn macaques is predictive of social partner discrimination. PloS ONE 2013; 8:e82921.
14. Nagy E, Pilling K, Orvos H, Molnar P: Imitation of tongue protrusion in human neonates: Specificity of the response in a large sample. Dev Psychol, 2013; 49:1628-1638.