Activation of the Maternal Immune System during Pregnancy Alters Behavioral Development of Rhesus Monkey Offspring

Supplemental Information

Table S1. Experimental Groups

Experimental Group	**Group Size (males, females)**
1st Trimester MIA (MIA1)	*n* = 6 (5m, 1f)
2nd Trimester MIA (MIA2)	*n* = 7 (4m, 3f)
Saline Controls	*n* = 8 1st Trimester (1m, 3f) 2nd Trimester (2m, 2f)
Untreated Controls	*n* = 3 (1m, 2f)

MIA, maternal immune activation.

Table S2. Behavioral Phenotyping Assays

Behavioral Assay	Brief Description	Relevance to Autism Spectrum Disorders (ASD) and Schizophrenia (SZ)
0-6 Months of Age		
Neonatal motor and reflex development†	At 1 week of age neuromotor reflexes, behavioral maturation and attention processes were evaluated with a standardized test battery modeled after the Neonatal Behavioral Assessment Scale commonly used in rhesus monkeys (1-3).	Measures of physical health, neurological reflexes, locomotion and muscle strength serve as control parameters to rule out global effects on development.
Biobehavioral assessment†	At 3 months of age infants were temporarily separated from their mothers for an assessment that includes assays of health, behavior, temperament and adrenal regulation (4, 5).	Measures of response to separation, adrenal regulation and temperament serve as control parameters.
Human intruder paradigm†	At 1, 3 and 6 months of age, response to threat was assessed with a modified human intruder paradigm. The human first presents an ambiguous threat (profile) followed by a direct threat (stare).	The human intruder paradigm is a standardized assay of emotional reactivity for rhesus monkeys (6-8). This paradigm can be used to identify animals with an anxious temperament (9).
Pre-weaning home cage observations*	Each infant was observed approximately twice daily while with their mothers in the home cage using a 1-0 scoring checklist.	Pre-weaning home cage measures provide a baseline of the animal's behavior alone with their mother. These data can be used to identify repetitive or stereotyped behaviors that are a common feature in laboratory animals and included in the diagnostic criteria for ASD (10).
Pre-wean social group observations**	Five-minute focal observations were collected twice weekly while the infants interacted with members of their social rearing group, consisting of four mother-infant pairs and one adult male.	Formal observations of the animals in their social groups provide a quantitative account of the emergence of social behavior within a familiar rearing group (11).
6-12 Months of Age		
Mother preference†	Following weaning, each infant was tested for four days to evaluate one aspect of mother-infant attachment, the infant's preference for its own mother vs. another familiar adult female (twelve 2min trials/subject).	Measures of attachment serve as control parameters for species-typical development and response to separation (12).
Solo observations*	At approximately 10 months of age the animals were observed alone in a large, unfamiliar cage for two 5min focal samples on two separate days to screen for abnormal behaviors such as motor stereotypies or self-directed behaviors.	Solo observations are conducted to screen for a wide array of stereotyped behaviors produced by rhesus monkeys (13-15).

Home cage observations*	Each infant was observed approximately twice daily while alone in their home cages using a 1-0 scoring checklist.	Baseline screen for the presence of repetitive or stereotyped behaviors that are a common feature in laboratory animals and included in the diagnostic criteria for ASD (10).
Post-weaning social group observations**	Five minute focal observations were collected twice weekly while the infants interacted with members of the social rearing group, consisting of four infants and one adult male.	Formal observations of the animals after weaning provide a quantitative account of the emergence of social behavior (12).
12-18 Months of Age		
Y-Maze	At approximately 18 months of age, animals were given visual access to a novel conspecific in one arm of a Y-maze test apparatus. Each animal was tested for six 2min trials on two separate days, meeting an opposite-sex conspecific on the first day and a same-sex conspecific on the second day.	Initial social assays with novel conspecifics were carried out using the Y-maze testing apparatus and later followed with the three-chambered social approach assay described below.
Solo observations*	At approximately 22 months of age the animals were observed alone in a large, unfamiliar cage for two 5min focal samples on two separate days to screen for abnormal behaviors such as motor stereotypies or self-directed behaviors.	Solo observations are conducted to screen for a wide array of stereotyped behaviors produced by rhesus monkeys (13-15).
Two-chambered social approach**	At approximately 24 months of age, social interactions with a novel conspecific were evaluated using a modified version of the mouse 3-chambered social approach assay (20 min/subject).	The high-throughput social approach assay used in mouse models (16) paired with the fine-grained focal observations utilized in our nonhuman primate studies (11, 12) provide a screen for sociability as indexed by the amount of time spent in a chamber with a constrained, novel conspecific.

* Behavioral assays targeting repetitive behaviors and restricted interests.
** Behavioral assays targeting social and communication domains.
† Assays used to control for changes in physical development, reflexes, fear response development, maternal attachment and activity levels that are not directly related to the core features of ASD and SZ.

Table S3. Behavioral Ethogram

	Behavior	Description
	States	*States must last for at least three seconds to score.*
	Breast Contact	Focal infant suckles mother.
	Ventral Contact	Ventral surface of the focal contacts ventral surface of another.
	Other Contact	Other physical contact (not breast contact or ventral contact) with another.
	Extended Contact	Any physical contact not described as ventral or breast contact.
	Extended Groom	One animal examines, picks, or licks at the other animal's fur or body part.
	Extended Proximity	Focal animal within arm's reach of another subject, but not in physical contact.
	Extended Play	Rough and tumble or chase play.
	Extended Aggression	Aggressive biting, slapping, or chasing.
	Extended Stereotypy	Focal is engaged in motor stereotypic and/or self-directed behavior.
	Nonsocial Activity	Focal remains out of proximity, contact, and social activity, showing active behavior.
	Nonsocial Inactivity	Focal remains out of proximity, contact, and social activity, showing passive behavior.
	Events	
Maternal	Maternal Restrain	Mother physically interferes with the infant's attempts to move away from her.
Maternal	Maternal Retrieve	Mother physically brings infant closer to her.
Maternal	Maternal Rejection	Mother physically prevents the focal infant from contact.
Maternal	Maternal Aggression	Mother slaps, bites, or grabs infant.
Maternal	Maternal Abuse	Mother drags, sits on, or aggressively throws infant.
Affiliative	Lipsmack	Rapid lip movements with pursed lips.
Affiliative	Coo	An affiliative contact call; moderate in pitch and intensity; usually sounds like a "whooooooo".
Affiliative	Grunt	Deep, muffled, low-intensity vocalization.
Affiliative	Girney	Narrow broadband used during affiliative social exchanges.
Affiliative	Wrestle Play	Contact play consisting of mounting, tumbling, and wrestling.
Affiliative	Chase Play	Active chasing between animals that is not agonistic.
Affiliative	Play Threat	Open mouth threats, ear flaps, lunges or head bobs occurring during play.
Affiliative	Approach	Animal moves within arm's reach of another subject.
Affiliative	Follow	Animal appears to be intentionally following another animal for at least one seconds.
Affiliative	Present Mount	Rigid posture with rump and tail elevated and oriented toward another.
Affiliative	Present Groom	One animal intentionally presents neck, belly, rump, limbs, back, of flank to another.
Affiliative	Groom	One animal examines, picks, or licks at the other animal's fur or body part.
Affiliative	Contact	Physical, non-aggressive contact.
Affiliative	Proximity	Focal animal moves within arm's reach of another subject, but not in physical contact.
Fearful	Avoid	Animal leaves the area due to the arrival of another animal.
Fearful	Flee	Animal rapidly moves away from another subject.
Fearful	Freeze	Animal sits or crouches rigidly and does not move for at least three seconds.
Fearful	Fear Grimace	Exaggerated grin with teeth showing.
Fearful	Crooktail*	Stiff-legged strut and tail held in stiff "?" shape (associated with dominance at later ages).
Fearful	Scream	High-pitched vocalization, with extreme high intensity; sounds like "eeeeeee".

Aggressive	Chase	Chasing lasting at least three seconds during an aggressive encounter.
	Displacement	Taking the place or an object from another animal.
	Threat	One of the following: open mouth stare, head bobbing, ear flaps or lunges.
	Aggressive Contact	Bite, slap, or grab.
	Aggressive Grunt	Deep, muffled, low-intensity vocalization occurring with a threat and/or aggression.
MISC	Alarm Bark	Short, sharp sounds given as an alarm call, usually to a non-social cue.
	Convulsive Jerk/Tantrum*	Violent shaking or spasms of the body.
	Cage Shake/ Display	Vigorous shaking of cage bars or body slams against the cage.
	Tooth Grind	Repetitive, audible rubbing of upper and lower teeth.
	Self-Sex	Anogenital exploration of self.
	Self-Groom	Grooming oneself.
	Self-Scratch	Scratches own body.
	Manual Explore	Specifically oriented manual exploration of the cage or other objects.
	Oral Explore	Specifically oriented oral exploration of the cage or other objects.
Sexual	Mount	Appropriate positioning of partner, hands on back, double foot clasp.
	Mount Refusal	Animal attempts to mount, but the animal who is about to be mounted moves away.
	Anogenital Explore	Visual, olfactory, or tactile manipulation of another’s genital region.
Stereotypic/Self-Directed Behavior	Salute	Animal covers hand over eye or holds hand over eyes.
	Spin	Repetitive twirling or spinning for at least 2 rotations.
	Self-Clasp*	Unusual holding of body part or limb with another body part.
	Self-Bite	Biting oneself.
	Pace	Repetitive pacing with the same path repeated at least three consecutive times.
	Bounce	Repetitive hopping or bouncing in the same place at least 2 consecutive times.
	Swinging	Repetitive swinging in the same place lasting at least 3 seconds to score.
	Head-Twist	Animal twists neck in a dramatic display.
	Backflip	Animal is observed back-flipping at least two times in a row.
	Rocking	Rocking back and forth.
	Other Stereotypy	Repetitive motor or abnormal behavior pattern not described by any of the above.

* Behaviors included in the “reactivity” composite score for the mother-preference test following weaning.

Pregnant Dams Methods and Results

Subject Selection. Twenty-four pregnant rhesus macaques were selected from the colony timed-mating program, which provides accurate estimates of conception within +/- 2 days. Candidate females were between six and eighteen years of age (mean age = 11 years), had been reared in a naturalistic social group, demonstrated species-typical behaviors and had a successful history of raising offspring.

Prenatal time line. Pregnancy was confirmed at approximately 20 days of gestation and was followed by blood assays to detect fetal DNA for sex determination (17). Pregnancies were monitored via ultrasound on gestational days 40, 100 and 150. Rhesus monkey gestation is approximately 165 days, and MIA was targeted at the end of the first trimester (MIA^1 injections on gestational days 43, 44, 46) or the end of the second trimester (MIA^2 injections on gestational day 100, 101, 103).

Maternal response to poly ICLC. Behavioral observations, temperature and blood cytokine profiles of the dams were monitored before, during and after poly ICLC injections. Pregnant dams in the MIA and CON^{saline} groups received three injections over a 72 hour period: Day 1 – 8 am injection, Day 2 – 8 am injection, Day 3 – no injection, Day 4 – 8 am injection. Temperature, behavior and cytokine profiles of the pregnant rhesus monkeys were monitored before, during and after injections (Table S2). To minimize stress to the animals, blood draws were only collected after the 2nd and 3rd injections and temperature was only collected for the 4 days spanning the poly ICLC treatment.

Table S4. Maternal Response to Poly ICLC

Gestational Day (GD)	Poly ICLC Injection	Temperature	Behavior	Cytokine
Baseline (minimum 24 hours before first injection)	---	Baseline Temperature	12-2pm Behavior checklist (two days – combined for "before" composite score)	2pm Baseline blood draw
MIA^1 GD 43 MIA^2 GD 100	8am Injection #1	8:30am 2:00pm 4:00pm	---	---
MIA^1 GD 44 MIA^2 GD 101	8am Injection #2	8:30am 2:00pm 4:00pm	12-2pm Behavior checklist (day 1 of composite "during" score)	2pm Blood drawn 6 hours after injection #2
MIA^1 GD 45 MIA^2 GD 102	No injection	8:30am 2:00pm 4:00pm	---	---
MIA^1 GD 46 MIA^2 GD 103	8am Injection #3	8:30am 2:00pm 4:00pm	12-2pm Behavior checklist (day 2 of composite "during" score)	2pm Blood drawn 6 hours after injection #3
Baseline (minimum 96 hours after last injection)	---	---	12-2pm Behavior checklist (two days – combined for "after" composite score)	2pm Baseline blood draw

Temperature. Microchip temperature data (BioMedic Data Systems Inc., Seaford, DE) were collected on poly ICLC injection days at 8 am (within 30 minutes of poly ICLC injections), 2 pm and 4 pm (Table S3, Figure S1).

Table S5. Pregnancy data. Descriptive statistics for temperature

	Average Group Behavior		
	Poly 1	Poly 2	Control
Temperature	Mean (SD)	Mean (SD)	Mean (SD)
Day 1			
8:30 am	98.6 (2.1)	98.7 (0.6)	97.8 (1.4)
2:00 pm	100.5 (1.0)	100.0 (0.8)	97.5 (1.1)
4:00 pm	99.7 (1.2)	99.2 (1.1)	98.0 (1.3)
Day 2			
8:30 am	98.9 (0.8)	98.6 (0.3)	98.0 (1.6)
2:00 pm	100.9 (1.5)	101.2 (1.2)	97.9 (1.2)
4:00 pm	99.8 (1.2)	99.0 (2.2)	97.8 (0.8)
Day 3			
8:30 am	98.9 (1.2)	97.7 (1.1)	98.2 (1.0)
2:00 pm	100.8 (1.3)	101.8 (1.4)	97.6 (1.0)
4:00 pm	100.3 (1.3)	100.7 (1.0)	97.8 (0.9)

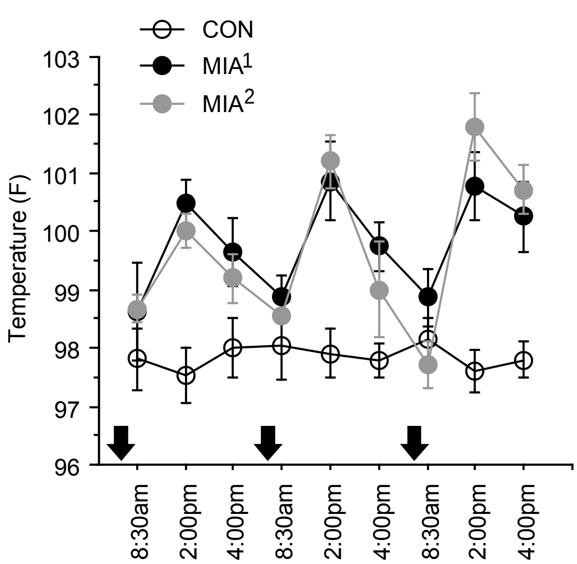

Figure S1. Temperature following poly ICLC injections. There were no group differences in baseline temperature collected via axillary measures (data not shown). Poly ICLC injections were associated with a transient increase in temperature peaking approximately six hours after the injection. Black arrows represent poly ICLC injections time points.

Behavior. Behavior was observed between 12-2 pm using a health checklist (Table S4). Composite scores of behaviors recorded on two occasions before, during (data collected after 2nd and 3rd injections) and after poly ICLC injections were collected for each animal. The behavior “lethargy” was evaluated with 1-0 scoring (i.e., present versus absent), appetite was evaluated using the following scale: 3 = Good (ate 7-9 Purina Monkey Chow biscuits), 2 = Fair (ate 4-6 biscuits) and 1 = Poor (ate 1-3 biscuits).

Table S6. Pregnancy data. Descriptive statistics for appetite and lethargy

	Average Group Behavior		
	Poly 1	**Poly 2**	**Control**
Behavior	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Appetite			
Before	2.9 (0.2)	2.9 (0.4)	2.4 (0.4)
During	2.0 (0.6)	1.6 (0.5)	2.5 (0.6)
After	2.3 (0.9)	2.9 (0.2)	2.4 (0.5)
Lethargic			
Before	0.0 (0.0)	0.0 (0.0)	0.1 (0.2)
During	0.7 (0.5)	0.8 (0.4)	0.0 (0.0)
After	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Average group behaviors were based on observed behaviors over two observations 24-48 hrs prior to injections, two observations during poly ICLC injections and two observations approximately 96 hours after the final injection.

Cytokine profiles. Baseline blood samples were collected 24-48 hours before poly ICLC injections. Additional blood samples were collected 6 hours after the second and third poly ICLC injections. A final sample was collected approximately 5 days after poly ICLC injections to serve as a second baseline measure. Baseline blood samples were collected during ultrasounds (animals sedated with ketamine 5-30 mg/kg), injection day samples were collected without ketamine. Four ml of whole blood was collected and serum was frozen in 2 ml Nalgene tubes. Serum was diluted with PBS/0.2%BSA to fall into the linear range of a primate-specific IL-6 ELISA assay (Cell Sciences, Canton, MA), and the assay was performed according to the manufacturer’s instructions (Table S5, Figure S2).

Table S7. Pregnancy data. Descriptive statistics for immune response

	Average Group Behavior		
	Poly 1	Poly 2	Control
Il-6 (pg/ml)	Mean (SD)	Mean (SD)	Mean (SD)
Baseline	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
6 hr after 2nd infusion	1245.9 (847.1)	2700.9 (2092.8)	1.5 (3.0)
6 hr after 3rd infusion	831.6 (431.9)	1094.4 (928.0)	4.8 (7.5)
Baseline	0.0 (0.0)	0.0 (0.0)	2.8 (6.3)

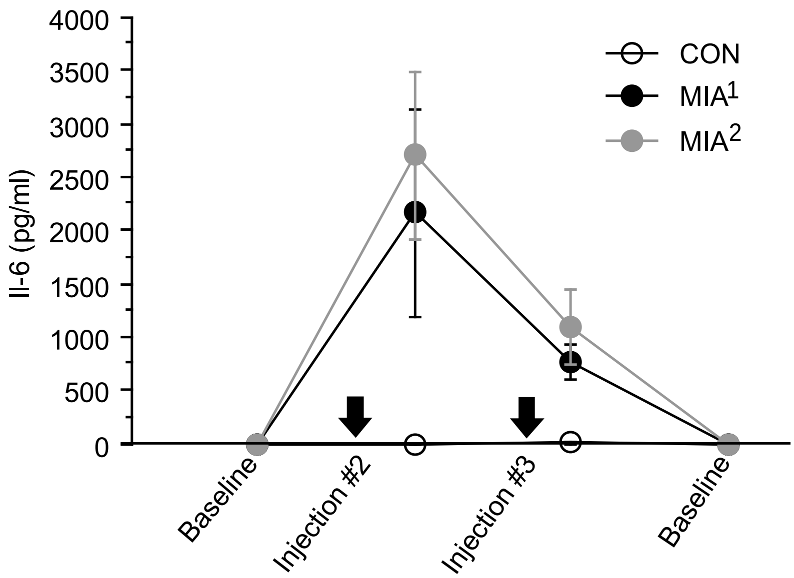

Figure S2. IL-6 response following poly ICLC injections. Poly ICLC was associated with a transient increase in IL-6 after the 2nd and 3rd injections. Black arrows represent the 2nd and 3rd poly ICLC injections time points (blood samples were not collected after the first poly ICLC injection to minimize stress to the dam). The 2nd poly ICLC injection was associated with a more pronounced IL-6 response compared to the 3rd injection. IL, interleukin.

Offspring Methods and Results

Offspring and Rearing Conditions. Behavioral data collected between 0-24 month of age include first trimester polyI:C exposed (*n* = 6), second trimester polyI:C exposed (*n* = 7), saline controls (*n* = 8) and untreated controls (*n* = 3). The three offspring of untreated mothers were added to the control group to achieve equal numbers in rearing groups. Social group interactions took place in a large chain link enclosure (2.13 m W x 3.35 m D x 2.44 m H) equipped with perches and enrichment devices. The six socialization groups were each composed of one mother/infant pair from both MIA and control groups. The age range between the youngest and oldest infant within each group was approximately one month. At least one female and one male offspring were included in each social group. The dams demonstrated species-typical behavior during the formation of the rearing groups and quickly established a dominance hierarchy. Dominance assessments of the dams within each socialization cohort indicated that the mean ranking within each rearing group did not significantly differ across the experimental groups. When the youngest subject within a rearing group reached six months of age, the infants were permanently separated from their mothers, but otherwise continued to experience the same housing and group socialization in the absence of their mothers. At this time, a new adult female was added to each socialization cohort to provide continued exemplars of adult female social behavior.

Behavioral Data Collection Overview. All data were collected using Observer software (Noldus, Sterling, VA) by trained observers demonstrating an inter-observer reliability > 85% (agreements/ [agreements + disagreements] X 100). Each infant received a dye mark, allowing the observers to score behaviors while remaining blind to their experimental condition. Our behavioral assays are designed to detect changes in macaque social and communication development and screen for the presence of repetitive behaviors and restricted interests (2-5). We also include a broad array of additional biobehavioral assays to quantify other aspects of development (i.e., neonatal maturation, endocrine responses, etc.) that may contribute to behavioral pathologies.

1 Week Neonatal Motor/Reflex Development. Neuromotor reflexes, behavioral maturation and attention processes were evaluated at 1 week of age with a standardized test battery modified from published macaque neonatal evaluations (6, 7). All animals were rated on a three point scale (2 = reflex well developed, 1 = reflex partially developed, 0 = reflex absent) for measures of: 1) Visual orientation (i.e., focusing on and following a pen light), 2) Motor maturity (i.e., head posture in prone and supine positions, coordination, maturity spontaneous locomotor pattern), 3) Reflexes (i.e., placing, moro, righting and rooting reflexes), and 4) State control (i.e., agitation, consolability).

3 Month Biobehavioral Assessment (BBA). Infants were temporarily separated from their mothers for a 25 hour biobehavioral assessment between the ages of 90 and 115 days. The BBA is a colony wide assessment of infant macaques at the California National Primate Research Center that includes assays of health, behavior, temperament, adrenal regulation, and responses to threatening (i.e., human intruder), social (i.e. videos and pictures of conspecifics) and nonsocial (i.e., novel objects) stimuli. Detailed descriptions of the complete BBA assessment battery are available in previous publications (8-11). Following relocation to a temporary holding cage, blood samples were drawn via femoral venipuncture at four time points during the testing period, in order to assess the infants' response to relocation and separation from peers and to evaluate HPA axis regulation. Infants were awake and not sedated during venipuncture. Sample 1 (1.0 ml) was drawn at approximately 11:00 am (2 hours after removal from the mother). Sample 2 (0.5 ml) was drawn after completion of the Human Intruder test (see below) at 16:00 hr. Immediately after the Sample 2 venipuncture, each monkey was given an injection of 500 μg/kg dexamethasone intramuscularly. Sample 3 (0.5 ml) was taken following completion of Holding Cage observations on Day 2 at 08:30 hr, immediately after which subjects were administered 2.5 IU ACTH. The final sample (0.5 ml) was drawn 30 min after ACTH was given. Blood was drawn into unheparinized syringes and was immediately transferred to EDTA tubes, which were subsequently centrifuged at 1,000 g for 10 min at 4°C. Plasma was decanted into microtubes for storage at −80°C until assayed for cortisol concentration by RIA (Diagnostic Products Corp., Los Angeles, CA).

Human Intruder Paradigm. At 1, 3 and 6 months of age response to threat was evaluated using a modified version of the human intruder paradigm (12). Infants were tested in a room in which they could not hear or see conspecifics. Testing took place in a standard macaque indoor housing cage. Infants were left alone in the test cage for one-minute before testing commenced. After the one-minute baseline period had expired, two experimenters (behavioral observer and the human intruder) entered the room. The human intruder (an adult female) stood in each presentation condition for one minute. For the first trial, the human intruder positioned herself three feet in front of the test cage, and presented her left profile to the infant (profile-far). At the end of the first minute, the human intruder took one step sideways toward the test cage and positioned herself approximately one foot from the front of the test cage, while still holding the profile position (profile-near). Next, the human intruder returned to the position three feet away and turned to face the infant and established direct eye contact with the infant (stare-far). Finally, the human intruder took one step forward while maintaining eye contact (stare-near). Behaviors commonly elicited by rhesus monkeys during this paradigm were assessed (Table S1). Data was collected live during each one-minute posture presentation via one-zero sampling at 10-second intervals. The maximum value per behavior per trial was six and the minimum value zero.

Home Cage Checklist. Offspring were observed in their home cage on a daily basis both in the morning and the afternoon. Mothers were present during pre-wean home cage observations that took place from 0-6 months of age. During the post-weaning period (6-24 months of age) the animals were observed while alone in their home cage. At that time, checklists continued once per day. On average, animals were observed on more than 100 occasions for both pre-wean and post-wean periods. Trained observers who were blind to the assigned experimental conditions conducted observations in a pre-determined pseudo-random order for six ten-second periods. At the onset of each observation, the observer approached to one meter in front of the home cage and recorded behaviors using a one-zero sampling method (13). Any behavior occurring within the ten-second observation received a score of "1" (even if the behavior was repeated), whereas behaviors that were not observed during the trial received a score of "0". Prior to weaning behaviors initiated by the infant or received by the infant from the mother were scored, including physical contact with mother, nursing, grooming, sleep, no contact, maternal behaviors (i.e., restrain, retrieve, rejection, aggression), fear grimace, lipsmack (initiated or received), threat, scream, tantrum, toy play, manual/oral explore and motor stereotypies. The list was modified after weaning to include vocalizations and present groom/mount.

Rearing Group Observations. Each cohort was assigned to one of two identical, large chain link cages where daily group socialization occurred for approximately three hours per day, five days per week. Rearing group observations were divided into two phases: 1) Pre-weaning observations (approximately 0-6 months of age), 2) Post-weaning observations (approximately 6-12 months of age). During the pre-weaning phase, the infants were observed twice a week while freely interacting with members of the social rearing group, consisting of four mother-infant pairs and one adult male. Five-minute focal observations were conducted on each infant in a pre-determined pseudo-random order, with no more than two observations per individual per week. Following removal of the mothers, the infants continued daily socialization with their original group members (three other infants plus one adult male). A novel adult female was introduced during the post-weaning period to provide continuous exposure to species-typical adult macaque behavior. The same group observation strategy continued into the post-weaning period (6-12 months).

Mother Preference Test. Permanent separation from the mother (weaning) took place when the youngest infant of the socialization cohort reached six months of age. Starting on the day after weaning, each infant was observed in a test designed to evaluate one aspect of mother-infant attachment, the infant's preference for its own mother vs. another familiar adult female (3). Three daily trials were conducted for four consecutive days, with each two-minute trial consisting of a choice between the infant's mother and one of the three other adult females from the infant's socialization cohort (the stimulus female). A different stimulus female was used for each trial in a pre-determined pseudo-random order. The infant's mother was placed in one of two holding cages, located at either end of the testing enclosure, and the stimulus female was placed in the opposite holding cage (right and left holding cage assignments were balanced across trials). The holding cages were separated from the testing enclosure by metal bars and clear plastic panels prevented physical contact between the infant and the adults. Opaque plastic panels in front of the holding cages prevented the adults from seeing the infant release box prior to testing. At the onset of the trial, the infant's release box and the opaque plastic panels in front of the holding cages were raised simultaneously, allowing the infant to freely move around the center cage and to see both its mother and the stimulus female. The following observations were recorded during each two-minute trial: 1) The first adult approached by the infant (scored when the infant moved within a 1 m half-circle in front of the adult holding cage within the first 15 sec of the trial), 2) The number of midline crosses (an index of activity level), and 3) Focal animal samples using the behavioral ethogram (Table S2).

Solo Observations. At approximately 10 months of age the animals were observed alone in a large, unfamiliar cage for two 5 min focal samples on two separate days to screen for abnormal behaviors such as motor stereotypies or self-directed behaviors. Solo observations were repeated during the juvenile observation period (at approximately 22 months of age). MIA animals displayed an array of abnormal, repetitive behaviors (i.e., whole body, self-directed etc.), thus we utilized a composite score of all repetitive behaviors, rather than sub-categories for these analyses (15).

Y-Maze Social Interest. At approximately 18 months of age, we conducted an exploratory assay designed to evaluate social interest with an unfamiliar conspecific using a Y shaped testing chamber in which the animal, once released, has access to two chutes. A novel stimulus animal was housed in a holding cage at the end of one chute, while the other arm led to an empty cage. Following 10 seconds of visual access to each arm, the experiential subject was released from the start box and allowed to explore each arm freely for two minutes. This was repeated 6 times over two days. Each animal met a stimulus animal of the opposite sex on the first day, and a same-sex partner the next day. Animals were acclimated to the Y-Maze test chamber for a minimum of two ten minute sessions, and were not allowed to begin testing until they exhibited low levels of fearfulness or vigilance in the testing chamber.

Two-Chambered Social Approach. At approximately 24 months of age, we conducted a modified version of the mouse three-chambered social approach assay used to assess sociability in mouse models of autism (14). We utilized two large adjacent chain link enclosures (2.13 m W x 3.35 m D x 2.44 m H) and the chute that runs along the back to reproduce the center, left and right cages used in the mouse three-chambered social approach task. Opaque panels separated the left and right chambers, though animals could freely enter and exit each of the chambers through small retractable doorways (42 cm W X 56 cm H) connecting the chambers. Once the animal was released from the chute (center chamber) access to the chute was restricted and the animal could only move freely between the left to right chambers, which each contained a small holding cage (61 cm W x 75 cm D x 79 cm H) centered on the floor. The holding cage in the "social chamber" contained an unfamiliar 3-year-old female conspecific while the holding cage in the "nonsocial chamber" was empty. Right-left location of the stimulus animal was alternated so that each experimental animal had one session with the right chamber serving as the social chamber and one session with the left chamber serving as the social chamber. Primary measures included time spent in each of the chambers (i.e., social, nonsocial) and the frequency of entrances into each chamber. To capitalize on the rich repertoire of macaque social behavior we also collected focal observations on the interactions between the experimental animal and the stimulus monkey using a comprehensive ethogram.

Summary of Infant Development. There were no group differences in overall health and weight gain (Table S6). Although MIA[1] received lower scores on righting reflex and motor coordination at the 1 week neonatal assessment, they received higher scores on other reflex development measures (i.e., visual orientation). At three months of age, there were no differences in activity levels and preferential looking paradigms (data not shown), suggesting that these early differences were transient. The MIA[1] offspring also produced fewer defensive behaviors in the “profile condition” of the human intruder assay at three months of age. While blunted reactivity was also observed in this group during the mother preference test, it is important to note that there were no group differences in the more challenging “stare condition” at three months and no group differences in either profile or stare conditions when the test was repeated at six months of age (data not shown). MIA[1] offspring were also more frequently observed nursing their mothers during observations in the home cage between 0-6 months of age, but not during observations during their daily rearing groups (data not shown). No other significant group differences were detected when the animals were observed with their mothers in their home cage or when interacting with familiar peers in their rearing group between 0-6 months of age. Likewise, there were no group differences detected when the animals were observed alone in their home cage or when interacting with familiar peers in their rearing group between 6-24 months of age (data not shown).

Table S8. Summary of Infant Development

Measure	Control Mean ± SE	MIA[1] Mean ± SE	MIA[2] Mean ± SE	MIA[1] vs CON *p**	MIA[2] vs CON *p**
Weight					
1 Week Weight (kg)	58 ± 3 (m) 52 ± 2 (f)	54 ± 3 (m) 46 ± 0 (f)	55 ± 6 (m) 50 ± 5 (f)	.35 .28	.56 .73
1 Month Weight (kg)	71 ± 3 (m) 62 ± 3 (f)	65 ± 3 (m) 52 ± 0 (f)	65 ± 8 (m) 61 ± 5 (f)	.26 .12	.25 .73
3 Month Weight (kg)	108 ± 8 (m) 95 ± 3 (f)	99 ± 2 (m) 74 ± 0 (f)	110 ± 8 (m) 102 ± 3 (f)	.39 .13	.99 .21
1 Year Weight (kg)	246 ± 11 (m) 207 ± 8 (f)	229 ± 10 (m) 221 ± 0 (f)	233 ± 25 (m) 225 ± 17 (f)	.32 .27	.23 .17
2 Year Weight (kg)	367 ± 14 (m) 331 ± 19 (f)	368 ± 19 (m) 335 ± 0 (f)	362 ± 19 (m) 356 ± 24 (f)	.90 .66	.77 .36
1 Week Neonatal Assessment[1]					
Visual orientation	.35 ± .15	.83 ± .17	.57 ± .20	.06	.37
Visual follow	.60 ± .26	.67 ± .30	.79 ± .34	.91	.68
Head posture prone	1.9 ± .09	2.00 ± .00	2.00 ± .00	.44	.40
Head posture supine	1.70 ± .21	2.00 ± .00	1.86 ± .14	.26	.71
Coordination	1.25 ± .23	.50 ± .34	.64 ± .24	.07	.11
Motor locomotion	1.4 ± .19	.41 ± .27	.85 ± .32	.02*	.16
Placing reflex	1.9 ± .07	1.83 ± .17	2.0 ± 0	>.99	.22
Moro reflex	1.85 ± .12	1.5 ± .34	1.86 ± .09	.43	.79
Righting reflex	2.0 ± .00	1.5 ± .22	2.0 ± .00	.02*	>.99
Rooting reflex	1.0 ±.24	.67 ± .33	.29 ± .18	.37	.04*
Predominant state	1.25 ± .15	.83 ± .28	1.10 ± .23	.13	.59
Consolobility	.20 ± .13	.42 ± .20	.50 ± .24	.29	.27
3 Month Biobehavioral Assessment					
Plasma Cortisol µg/dL (initial separation)	90 ± 8	92 ± 10	101 ± 10	>.99	.68
Plasma Cortisol µg/dL(afternoon)	91 ± 11	81 ± 6	90 ± 10	.62	.89
Plasma Cortisol µg/dL(Dex. supression)	58 ± 5	46 ± 9	49 ± 7	.14	.50
Plasma Cortisol µg/dL(ACTH stimulation)	95 ± 6	85 ± 10	89 ± 7	.37	.56
3 Month Human Intruder					
Threat/ tooth grind (response to profile)	4.73 ± 1.15	.50 ± .34	.30 ± 1.30	.02*	.58
Threat/tooth grind (response to stare)	6.18 ± 1.14	3.5 ± 1.65	5.57 ± 1.45	.13	.70
0-6 Month Home Cage Checklist[2]					
Breast contact with mother	3.16 ± .11	3.91 ± .17	3.6 ± .29	.01*	.12
Other contact with mother	3.33 ± .15	2.86 ± .22	2.95 ± .21	.76	.56
Stereotypies	.011 ± .004	.017 ± .015	.023 ± .007	.51	.12
6-12 Month Home Cage Checklist[2]					
Stereotypies	1.30 ± .32	1.02 ± .33	1.02 ± .32	.67	.62

* Mann-Whitney tests with a 0.05 alpha level were used to determine between-groups differences.

[1] All animals were rated on a three point scale (2 = reflex well developed, 1 = reflex partially developed, 0 = reflex absent).
[2] Each animal was observed once daily for six ten-second periods. Behaviors observed within the 10-second observation bins were scored as “1”.

Table S9. Behaviors during mother preference test. Descriptive statistics

	Average Group Behavior		
	MIA[1]	MIA[2]	Control
Behavior	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Distress/self-soothing	0.1 (0.1)	2.9 (1.9)	0.5 (0.6)

Average group behaviors are based on observed frequency of behaviors, averaged first within each monkey over 4 days.

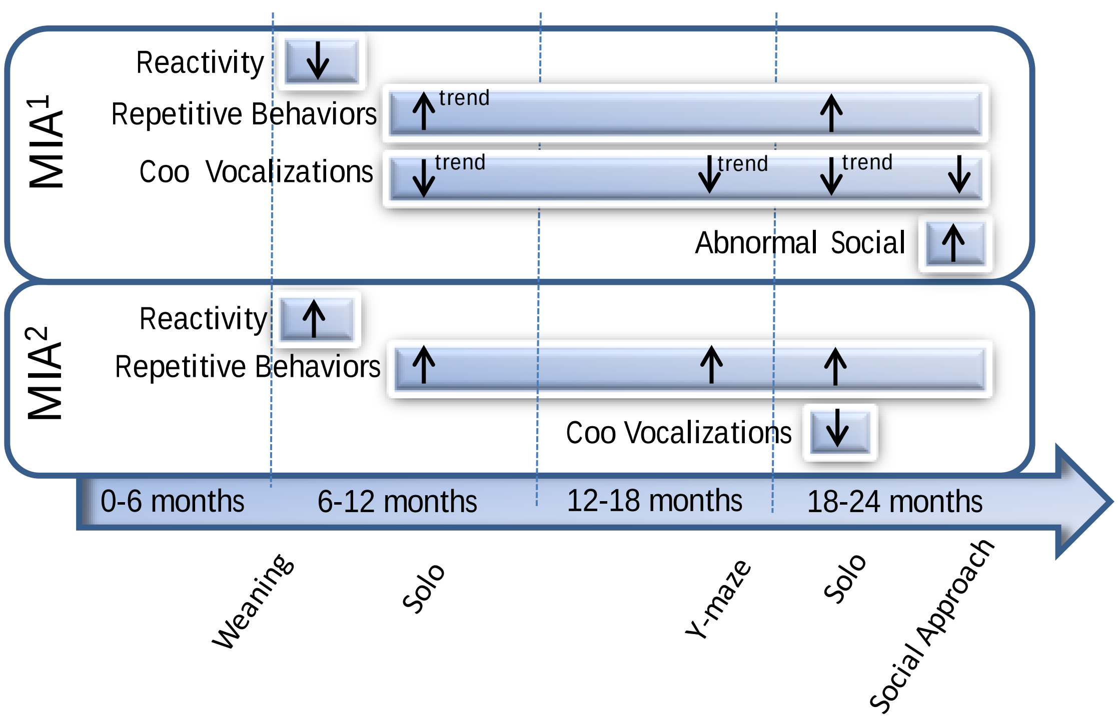

Figure S3. MIA exposure during either the first or second trimester in a nonhuman primate model yields offspring with overlapping phenotypes (decreased affiliative vocalizations and increased repetitive behaviors observed in both MIA groups), as well as divergent phenotypes (increased reactivity in MIA[2] offspring, blunted reactivity in the MIA[1] offspring and abnormal social behavior observed only in MIA[1] offspring).

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