Title

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Journal

Translational Psychiatry, 3(7)

ISSN

2158-3188

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Publication Date

2013-07-01

DOI

10.1038/tp.2013.47

Peer reviewed

Citation: Transl Psychiatry (2013) 3, e278; doi:10.1038/tp.2013.47

www.nature.com/tp

Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey

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Antibodies directed against fetal brain proteins of 37 and 73 kDa molecular weight are found in approximately 12% of mothers who have children with autism spectrum disorder (ASD), but not in mothers of typically developing children. This finding has raised the possibility that these immunoglobulin G (IgG) class antibodies cross the placenta during pregnancy and impact brain development, leading to one form of ASD. We evaluated the pathogenic potential of these antibodies by using a nonhuman primate model. IgG was isolated from mothers of children with ASD (IgG-ASD) and of typically developing children (IgG-CON). The purified IgG was administered to two groups of female rhesus monkeys (IgG-ASD; $n = 8$ and IgG-CON; $n = 8$) during the first and second trimesters of pregnancy. Another control group of pregnant monkeys ($n = 8$) was untreated. Brain and behavioral development of the offspring were assessed for 2 years. Behavioral differences were first detected when the macaque mothers responded to their IgG-ASD offspring with heightened protectiveness during early development. As they matured, IgG-ASD offspring consistently deviated from species-typical social norms by more frequently approaching familiar peers. The increased approach was not reciprocated and did not lead to sustained social interactions. Even more striking, IgG-ASD offspring displayed inappropriate approach behavior to unfamiliar peers, clearly deviating from normal macaque social behavior. Longitudinal magnetic resonance imaging analyses revealed that male IgG-ASD offspring had enlarged brain volume compared with controls. White matter volume increases appeared to be driving the brain differences in the IgG-ASD offspring and these differences were most pronounced in the frontal lobes.
Translational Psychiatry (2013) **3,** e278; doi:10.1038/tp.2013.47; published online 9 July 2013

Introduction

Autism spectrum disorder (ASD) affects over 1% of the children in the United States,[1] yet there remains relatively little understanding of its underlying causes. The prenatal environment, and particularly the fetal–maternal environment, has recently been highlighted as having a critical role in the etiology of some forms of ASD.[2] Although there are a number of potential environmental challenges to the maternal environment, our research group has been evaluating one prenatal risk factor implicated in ASD—maternal autoantibodies that target fetal brain tissue. During gestation, maternal immunoglobulin G (IgG) isotype antibodies normally cross the placenta and protect the immunologically naive fetus.[3] However, in addition to protective antibodies, autoantibodies that react to fetal 'self'-proteins can also cross the placenta resulting in a number of neonatal conditions.[4–7] We now know that in approximately 12% of women who have a child with autism, maternal antibodies exist that are reactive to fetal brain proteins at 73 and 37 kDa.[7,8] To date, this pattern of reactivity has not been observed in any mothers of typically developing children.

Attempts in other laboratories to evaluate maternal antibody effects in a mouse model found altered exploratory and motor behavior in the offspring born to dams injected with plasma from a single human mother of multiple children with autism.[9] A subsequent mouse study observed changes in anxiety, startle reflexes and sociability in the pups of mice that were gestationally treated with IgG derived from mothers of children with autism.[10] A more recent mouse study using IgG from mothers with antibodies directed specifically against the 37 and 73 kDa bands confirmed that the antibodies gain access to the fetal mouse brain and were associated with altered physical and social development of the offspring.[11] Although there are many benefits to studying mouse models of ASD, the nonhuman primate model offers unique translational advantages. Nonhuman primates, such as rhesus

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Keywords: ASD; animal models; autoantibody; nonhuman primate
A subset of the MRI data was presented at the 2012 International Meeting for Autism Research: Nordahl CW, Bauman MD, Braunschweig D, Iosif AM, Ashwood P, Van de Water J, *et al.* (2012): The Presence of Specific Maternal IgG Antibodies Is Associated with Abnormal Brain Enlargement in ASD and in Nonhuman Primate Model of ASD International Meeting for Autism Research, Toronto.
Received 18 December 2012; accepted 8 April 2013

Table 1 Experimental groups

Experimental group	*Behavioral data 0–6 months*	*Behavioral data 6–24 months*	*MRI analyses*	*Rearing conditions*	*Role on project*
IgG-ASD$^{37/73kDa}$	$n=8$ (4 males, 4 females)	$n=8$ (4 males, 4 females)	$n=4$ males	Maternally reared with daily access to a peer group consisting of treated ($n=2$) and control offspring ($n=2$).	Longitudinal behavior and MRI
IgG-CON[a]	$n=7$ (2 males, 5 females)	$n=7$ (2 males, 5 females)	$n=2$ males	Maternally reared with daily access to a peer group consisting of treated ($n=2$) and control offspring ($n=2$).	Longitudinal behavior and MRI
Untreated controls[a,b,c]	$n=9$ (5 males, 4 females)	$n=7$ (4 males, 3 females)	$n=4$ males	Maternally reared with daily access to a peer group consisting of treated ($n=2$) and control offspring ($n=2$).	Longitudinal behavior (MRI only at 2-year time point)
MRI library[d]	NA	NA	$n=5$ males	Maternally reared in separate field cage project.	Longitudinal MRI only

Abbreviations: ASD, autism spectrum disorder; IgG, immunoglobulin G; MRI, magnetic resonance imaging; NA, not applicable.
[a]Owing to limited availability of male fetuses, the original IgG-CON group included five female and three male fetuses. One of the male IgG-CON pregnancies spontaneously aborted and was subsequently replaced with an untreated mother–infant pair. Data collected from 0 to 6 months thus included seven IgG-CON animals and nine untreated controls. [b]Two untreated controls were removed from the project at 6 months of age because of poor weight gain/failure to thrive and were replaced with two age-matched colony animals. The replacement animals were accepted into their rearing groups; however, given that they had experienced a different rearing environment for the first 6 months of life, they were not included in the behavior or MRI studies from 6 to 24 months. [c]MRI only collected at final 2-year time point because of funding restrictions. [d]The archived MRI data were obtained from a previous study and thus not included in the rearing groups or behavioral data collection associated with this study.

macaques (*Macaca mulatta*), demonstrate many features of human physiology, anatomy and behavior, thus making it an ideal species to study human disorders.[12,13] The macaque monkey lives in a complex, hierarchical social system, and uses many forms of social communication such as facial expressions and postural gestures. Moreover, portions of the human brain, such as the prefrontal cortex, that mediate complex social behavior and are found to be heavily impacted by autism, are poorly developed or absent in the rodent brain, but highly developed in the rhesus monkey. It is likely that a human disorder such as autism that involves higher cognitive functions, might ultimately benefit from studies in animal species more closely related to humans.

In 2006, we conducted an initial study of the effects of exposing gestating rhesus monkeys to IgG antibodies taken from human mothers of multiple children with ASD.[14] We found that animals prenatally exposed to antibodies from mothers of children with autism produced more whole body motor stereotypies and were hyperactive compared with both untreated control monkeys and monkeys prenatally exposed to IgG from mothers of typically developing children. In the past 5 years, substantial progress has been made in characterizing which maternal autoantibodies are specifically associated with ASD. Thus, the goal of this study was to evaluate the effects of fetal monkey exposure to the 37 and 73 kDa fetal brain autoantibodies that are now specifically associated with ASD.[7,8] A comprehensive assessment of the behavioral development of the treated offspring was carried out over a 2-year period, utilizing behavioral phenotyping tools that target the core features of human ASD. In addition, longitudinal magnetic resonance imaging (MRI) was also carried out to study brain development in the IgG-treated monkeys.

Materials and methods

All experimental procedures were developed in consultation with the veterinary staff at the California National Primate Research Center (CNPRC) and approved by the University of California, Davis Institutional Animal Care and Use Committee.

Antibody characterization. Serum was collected and IgG purified from mothers of children with autism who exhibited maternal autoantibody reactivity to fetal brain proteins at 37 and 73 kDa ($n=4$), or from mothers of typically developing children, who were devoid of these antibodies ($n=5$). Western blot for analysis of fetal brain reactivity and IgG purification processes have been described previously.[14]

IgG treatment and rearing conditions. Twenty-four pregnant multiparous rhesus macaques (*Macaca mulatta*) between 6 and 16 years of age (mean age = 11 years) were selected from the colony timed-mating program. Following fetal sex determination,[15] the pregnant dams were randomly assigned to one of three experimental groups: (1) IgG-ASD$^{37/73kDa}$ ($n=8$; four males, four females) received IgG purified from mothers of children with autism that exhibited fetal brain reactivity to 37 and 73 kDa proteins, (2) IgG-CON ($n=8$; three males, five females) received IgG purified from mothers of typically developing children and (3) untreated controls ($n=8$; four males, four females; Table 1). Purified IgG (15–20 mg) diluted in 3 ml of sterile saline was delivered intravenously following sedation with ketamine (10 mg kg^{-1}) on gestational day 30, 44, 58, 72, 86, 100 (normal gestation is 165 days). Experimental infants (IgG-ASD, IgG-CON and untreated controls) were raised with their mothers in standard home cages and weaned at 6 months of age. Offspring were provided 3-h daily access to a social rearing group as described in the Supplementary Material.

Neuroimaging. All MR imaging was performed at the University of California Davis Imaging Research Center using a 1.5 T GE Signa Horizon LX NV/I MRI system (GE Medical Systems, Waukesha, WI, USA). IgG exposed offspring underwent MRI scanning at six time points: 1 week, 1 month, 3 months, 6 months, 1 year and 2 years of age. MRIs from the male IgG-ASD$^{37/73kDa}$ offspring ($n=4$) were

compared with a combined control group consisting of male IgG-CON ($n=2$) and archived MRI data from an existing library of outdoor colony animals that were matched for age, sex and weight parameters ($n=5$). The indoor, untreated controls were not scanned at the first five time points. To ensure that the differences in total brain volume were not attributable to unanticipated environmental factors, we also collected MRI data from available indoor, untreated male controls animals ($n=4$) at the final MRI time point (2 years of age). Significant findings were unaltered with the addition of the four indoor, untreated animals, therefore data in Figure 6 and Table 6 are presented using the same control group ($n=7$) that was used for the longitudinal model. All MRI image sets underwent partial correction for intensity in homogeneity. Total brain volume was acquired by manually tracing the brain, including cerebellum and brainstem, using Analyze 11.0 Software (Biomedical Imaging Resource at the Mayo Clinic). To acquire lobar volumes, a single subject's MRI scan served as a template on which the lobes of the cerebrum were delineated. The lobes were manually traced on the template brain (see Supplementary Methods), then propagated onto each subject via an affine registration followed by a cubic B-spline high-dimensional warping procedure (Imaging of Demential & Aging (IDeA) Lab: High Dimensional Warping using Cubic B-Splines - http://idealab.ucdavis.edu/software/spline_warp.php).

Behavioral observations. Behavioral data were collected on all animals throughout the first 2 years of life using assays from our standardized infant rhesus developmental battery[16–19] summarized in Table 2. All data were collected using the Observer software (Noldus, Sterling, VA, USA) by trained observers demonstrating an inter-observer reliability $>85\%$ (agreements/(agreements + disagreements) $\times 100$) who were blind to the experimental condition of the offspring. Unless noted in the detailed Supplementary Material description, behavioral data were collected using focal animal samples[20] in a pre-determined pseudo-random order using a catalog of behaviors commonly used for this species (Supplementary Table S1).

Statistical analysis. Preliminary analyses revealed that behaviors of the control IgG monkeys and the untreated control monkeys were very similar. They were thus pooled to form a single control group. Statistical analyses used a linear model approach. Transformations were performed if the normality assumption of the data appeared to be violated. In cases where transformations were not successful, behaviors were dichotomized into present or absent. For behaviors collected repeatedly for an animal, random-effects regression models[21] were used to test group differences and assess whether covariates were related to these variables. This approach allowed the use of all available data for each animal, while accounting for the correlated nature of the data because of the repeated measurements. Similar random-effects models were used to analyze the MRI data. Age was log transformed to improve the linearity of the relationship between brain volume and age. For the paradigms with only one observation per animal, linear or logistic (in the case of dichotomized variables) regression models were used. Residual analyses and graphical diagnostics were used to check the validity of all model assumptions. All statistical analyses were implemented in SAS Version 9.3 (SAS Institute, Cary, NC, USA). See Supplementary Material for in-depth description of the models used.

Results

For the sake of brevity, only significant behavioral results obtained from the comprehensive behavioral assessment (Table 2) are presented in detail. See Supplementary Material for behavioral definitions (Supplementary Table S1) and in-depth description of the statistical models used.

Infant development measures. There were no differences in physical growth, neonatal motor or reflex development, adrenal activity, repetitive behaviors, development of threat detection (Supplementary Table S2), attachment to the mother, response to novel objects, activity level, dominance rank or fear and aggression related behaviors (data not shown).

Pre-weaning group observations (0–6 months). Compared with controls, the IgG-ASD$^{37/73kDa}$ offspring were more frequently approached by their mothers ($P<0.01$), they were more commonly in close proximity ($P=0.03$) to them, and they were more often contacted ($P<0.01$) by their mothers (Figure 1; Table 3; Supplementary Table S3). There was no group difference in the amount of time spent interacting with other animals in their rearing group (Supplementary Table S4; Figure 1; Table 3).

Post-weaning group observations (6–12 months). IgG-ASD$^{37/73kDa}$ offspring were similar to controls in their frequencies of all behaviors initiated and received (see ethogram Supplementary Table S1), with one notable exception. IgG-ASD$^{37/73kDa}$ offspring consistently deviated from species-typical behavior by more frequently approaching infants in their rearing group (Figure 2a; Table 4). For each 5-minute observation period, the IgG-ASD$^{37/73kDa}$ initiated two more approaches ($P=0.02$) than similar aged controls. The IgG-ASD$^{37/73kDa}$ actually increased their approaches to other animals as they got older while control animals remained relatively stable. Despite the increased frequency of approaches, there were no differences in the frequency of other affiliative behaviors (such as playing or grooming) that generally follows an approach (Supplementary Table S5). It was also striking that the increased approaches from the IgG-ASD$^{37/73kDa}$ offspring were not reciprocated because there was no difference in the frequency of affiliative behaviors received by the IgG-ASD$^{37/73kDa}$ animals or in the duration of social interactions (Supplementary Table S6). There was actually a trend for the IgG-ASD$^{37/73kDa}$ offspring to receive less grooming from other animals than the control animals received ($P=0.08$).

Juvenile group observations (12–18 months). As the IgG-ASD$^{37/73kDa}$ offspring matured, they continued to demonstrate deviant social behavior by approaching familiar

Table 2 Behavioral phenotyping assays

Behavioral assay	*Brief description*	*Relevance to ASD*
0–6 Months of age		
Neonatal motor and reflex development[a]	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.[22–24]	Measures of physical health, neurological reflexes, locomotion and muscle strength serve as control parameters to rule our global effects on development.
Biobehavioral assessment[a]	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.[25,26]	Measures of response to separation, adrenal regulation and temperament serve as control parameters.
Human intruder paradigm[a]	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.[27–29] This paradigm can be used to identify animals with an anxious temperament.[30]
Pre-weaning home cage observations[b]	Each infant was observed approximately twice daily while with their mothers in their home cages using a 1–0 scoring checklist (approximately 135 observations per subject).	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.[31]
Pre-wean social group observations[c]	Five-minute focal observations were collected twice weekly while the infants interacted with members of the 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.[17]
6–12 Months of age		
Mother preference[a]	Following weaning, each infant was tested for 4 days to evaluate one aspect of mother–infant attachment, the infant's preference for its own mother vs another familiar adult female (12 2-min trials per subject).	Measures of attachment serve as control parameters for species-typical development and response to separation.[17]
Solo observations[b]	At approximately 8 months of age, the animals were observed alone in a large, unfamiliar cage for two 5-min focal samples on 2 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.[18,32,33]
Home cage observations[b]	Each infant was observed approximately twice daily while alone in their home cages using a 1–0 scoring checklist (approximately 190 observations per subject).	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.[31]
Post-weaning social group observations[c]	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.[16]
12–18 Months of age		
Novel dyads[c]	At 12 months of age, each animal was individually paired once with eight unfamiliar stimulus animals for a 20-min session (160 min per subject).	Interactions with novel conspecifics provide an opportunity to evaluate the emergence of reciprocal social interactions.[16]
Juvenile social group observations[c]	Five-minute focal observations were collected on 10 occasions while the infants interacted with members of the social rearing group, consisting of four infant pairs and one adult male.	Formal observations of the animals provide a developmental account of the emergence of social behavior within a familiar rearing group.[16]
Restricted interests[b]	At 14 months of age, animals were allowed to freely explore a large cage containing eight novel objects. Frequency of approach and contact of the objects, as well as the number of transitions from one object to another were quantified as an index of restricted interests.	Exploration of novel toys has been used to quantify restricted patterns of interests in children with ASD[34] and have been translated into assays for mouse models of ASD.[35]
Dominance[c]	At approximately 14 months of age, the rank of each animal within their social group was evaluated.[36]	Systematic changes in social rank can be indicative of altered social behavior in rhesus monkeys.[19,37]
Three-chambered social approach[c]	At approximately 16 months of age, social interactions with a novel nonspecific were evaluated using a modified version of the mouse three-chambered social approach assay (20 min per subject).	The high-throughput social approach assay used in mouse models[38] paired with the fine-grained focal observations utilized in our nonhuman primate studies[16,17] provide a screen for sociability as indexed by the amount of time spent in a chamber with a constrained novel conspecific.
Activity monitoring[a]	At approximately 20 months of age the animals were fitted with collars in which activity monitors were attached.	Measures of global activity serves as a control parameter to rule our changes in behavior associated with hyperactivity.[14,18]

Abbreviation: ASD, autism spectrum disorder.
[a]Assays used to control for changes in physical development, reflexes, fear system development, attachment and activity levels that are not directly related to the core features of ASD. [b]Behavioral assays targeting repetitive behaviors and restricted interests domains of ASD. [c]Behavioral assays targeting social and communication domains of ASD.

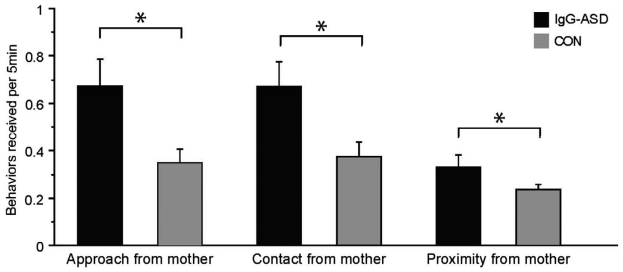

Figure 1 Immunoglobulin G (IgG)-autism spectrum disorder (ASD) offspring receive approach, contact and proximity more frequently from their mothers during daily rearing group socialization. Bars represent the mean frequency ± s.e.m. per 5-min observation period across all observations from 0 to 6 months of age. $^{*}P<0.05$.

Table 3 Descriptive summary of mother–infant interactions

	IgG-ASD (n = 8)		*Control (n = 16)*	
	Mean (s.d.)	*Median (range)*	*Mean (s.d.)*	*Median (range)*
Behaviors[a]				
Approach from mom	0.7 (0.4)	0.7 (0.2–1.3)	0.4 (0.2)	0.4 (0.0–0.8)
Contact from mom	0.7 (0.3)	0.7 (0.2–1.1)	0.4 (0.2)	0.3 (0.0–0.9)
Proximity from mom	0.4 (0.2)	0.4 (0.1–0.5)	0.2 (0.1)	0.2 (0.1–0.5)

Abbreviations: ASD, autism spectrum disorder; IgG, immunoglobulin G.
Summary of the behaviors' frequency for the IgG-ASD and control monkeys.
[a]Behaviors were first averaged within an animal over the same period of time (from 9 to 27 weeks).

peers more frequently (Figure 2b; Table 4). For each 5-minute observation period, the IgG-ASD$^{37/73kDa}$ initiated four more approaches ($P=0.01$) than similar age controls. Within each of the 5-min observation periods, the IgG-ASD$^{37/73kDa}$ initiated approximately two more physical contacts ($P=0.01$) than similar age controls. However, the IgG-ASD$^{37/73kDa}$ did not generate an increased frequency of affiliative behaviors that required sustained interaction (all $P>0.11$; Supplementary Table S7). Moreover, as when the animals were younger, they did not receive any greater social interactions from their peers. In fact, there was a trend for the IgG-ASD$^{37/73kDa}$ offspring to receive fewer approaches and proximity from their peers ($P=0.08$ and 0.09, respectively). Although the IgG-ASD$^{37/73kDa}$ were approaching other animals more, there was no overall difference in the amount of time spent interacting with other animals (Supplementary Table S8).

To examine the relationship between initiating and receiving social gestures, we combined scores for all affiliative facial expressions, vocalizations, and body movements (Supplementary Table S1) initiated and received into composite scores. We found that the control subjects demonstrated a significant positive correlation ($r=0.69$, $P<0.01$) between the number of affiliative behaviors that they produced and the number that they received. This was not true, however, for the IgG-ASD$^{37/73kDa}$ offspring ($r=-0.20$, $P=0.63$; Figure 3). Although the IgG-ASD$^{37/73kDa}$ generated more social

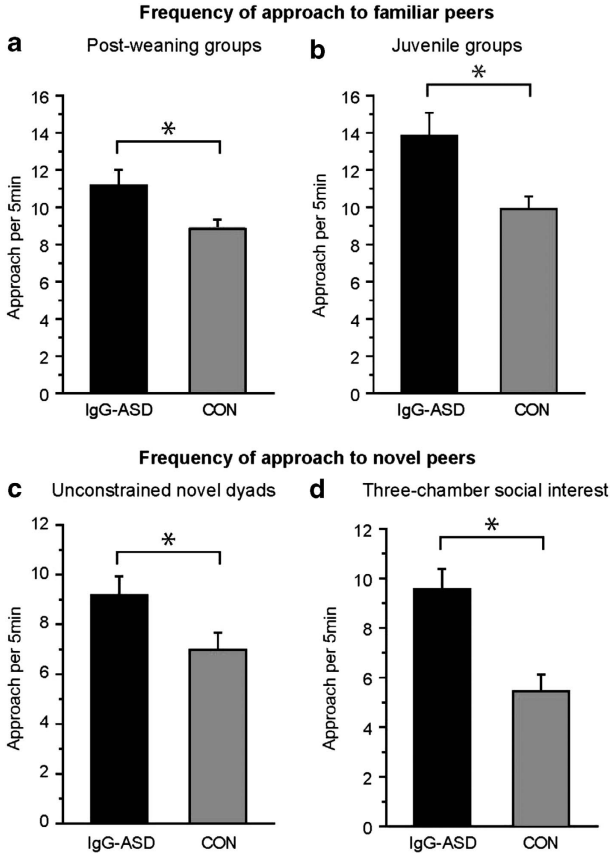

Figure 2 Immunoglobulin G (IgG)-autism spectrum disorder (ASD) offspring consistently deviate from species-typical social interactions by more frequently approaching familiar and unfamiliar peers. Bars represent the mean frequency ± s.e.m. per 5-min observation period across all observations. (**a**) Approach to familiar peers during daily post-weaning group observations 6–12 months of age. (**b**) Approach to juvenile familiar peers during daily juvenile group observations 12–18 months of age. (**c**) Approach to unfamiliar peers during unconstrained novel dyads at 12 months of age. (**d**) Approach to unfamiliar peers housed in a stimulus cage during the three-chambered social approach assay at 16 months of age. $^{*}P<0.05$.

gestures, they were not reciprocated by the untreated animals (Figures 2 and 3; Table 4).

Novel dyadic interactions. To this point, we have described the social interactions of the experimental animals with conspecifics that they knew well. We also evaluated their social interactions with eight novel, age-matched conspecifics. Consistent with the earlier observations, the IgG-ASD$^{37/73kDa}$ offspring approached the unfamiliar partners more frequently (Figure 2c; Table 5) than control animals. But again, there were no differences in the frequency of reciprocal social interactions (Supplementary Table S9). Moreover, the IgG-ASD$^{37/73kDa}$ offspring did not differ from controls in the total duration of time spent interacting with unfamiliar conspecifics (Supplementary Table S10).

Three-chambered social approach. We have developed a task that is modeled after the sensitive rodent assay of sociability developed by Crawley *et al.*[38–41] This task allows

Table 4 Descriptive summary of familiar peer interaction frequency for post-weaning group (6–12 months) and juvenile group (12–18 months) observation periods

	IgG-ASD (n = *8*)		*Control* (n = *14*)	
	Mean (s.d.)	*Median (range)*	*Mean (s.d.)*	*Median (range)*
Post-weaning group[a]*: behaviors directed to peers*				
Approach	11.1 (2.3)	10.9 (6.9–14.7)	8.8 (1.8)	8.9 (5.4–12.2)
Contact	5.9 (1.8)	5.3 (4.0–9.6)	4.9 (1.2)	4.7 (3.3–7.5)
Proximity	2.1 (0.7)	2.2 (1.0–3.0)	1.8 (0.5)	1.8 (1.1–2.5)
Groom	0.3 (0.1)	0.3 (0.1–0.5)	0.3 (0.2)	0.3 (0.0–0.6)
Play	9.4 (2.3)	10.0 (5.6–12.4)	7.5 (2.7)	8.0 (1.9–11.9)
Post-weaning group[a]*: behaviors received from peers*				
Approach	7.5 (1.4)	7.6 (5.2–9.9)	7.9 (2.3)	8.6 (4.0–12.9)
Contact	4.3 (0.9)	3.9 (3.3–6.0)	4.7 (1.5)	5.0 (2.2–6.7)
Proximity	1.1 (0.3)	1.1 (0.7–1.7)	1.4 (0.4)	1.3 (0.7–2.2)
Groom	0.2 (0.2)	0.1 (0.0–0.5)	0.3 (0.2)	0.3 (0.1–0.6)
Play	7.5 (1.8)	8.0 (5.0–10.5)	7.0 (2.9)	6.2 (2.3–13.4)
Juvenile group: behaviors directed to peers				
Approach	13.9 (3.4)	13.8 (7.6–18.3)	9.8 (2.4)	9.5 (4.9–15.2)
Contact	7.8 (1.9)	7.7 (4.3–10.3)	5.5 (1.8)	4.9 (3.4–8.6)
Proximity	2.1 (0.5)	2.3 (1.3–2.7)	1.7 (0.4)	1.8 (1.1–2.4)
Groom	0.4 (0.4)	0.3 (0.0–1.3)	0.2 (0.2)	0.2 (0.0–0.6)
Play	10.5 (2.9)	9.9 (6.6–14.5)	8.1 (3.9)	8.2 (2.2–14.3)
Juvenile group: behaviors received from peers				
Approach	8.9 (1.7)	9.0 (6.3–11.4)	10.6 (2.7)	10.7 (4.7–14.2)
Contact	4.7 (1.5)	4.3 (3.1–7.4)	5.9 (2.0)	5.6 (2.5–10.5)
Proximity	1.4 (0.4)	1.3 (1.0–2.1)	1.5 (0.5)	1.5 (0.5–2.4)
Groom	0.3 (0.2)	0.3 (0.1–0.5)	0.4 (0.5)	0.3 (0.0–1.8)
Play	8.8 (2.0)	8.5 (5.5–12.2)	8.7 (3.7)	8.5 (3.1–15.8)

Abbreviations: ASD, autism spectrum disorder; IgG, immunoglobulin G.
Summary of the average[a] frequency of behaviors for the IgG-ASD and control monkeys.
[a]Behaviors were first averaged within each monkey, from 32 to 50 weeks.

an animal to choose to spend time in a chamber with a conspecific (the 'stimulus' monkey) or in a chamber with an empty cage. All subjects, irrespective of experimental condition, spent significantly more time in the chamber with the novel conspecific compared with the chamber containing the empty cage (Supplementary Table S11). However, IgG-ASD$^{37/73kDa}$ offspring approached and contacted the cage with the animal enclosed nearly twice as frequently as the control animals (Table 5; both $P=0.02$; Figure 2d). The abnormal approach behavior was specific to the social cage because there were no differences in the frequency of approaching the empty cage ($P=0.49$). Despite the increased frequency of approach, the IgG-ASD$^{37/73kDa}$ offspring did not remain near the stimulus monkey for the 3 s required to score 'proximity' (Supplementary Table S12; Table 5).

Neuroimaging. Initial analyses of volumetric imaging data that included male and female animals did not produce significant developmental differences. However, recent data that we have acquired on alterations of developmental brain trajectories in children with autism indicate that there are substantial differences in the neuropathology of boys and girls with ASD.[42] This prompted us to carry out separate analyses with male and female subjects. Although we have not identified differences in female subjects, very interesting findings emerged from our analyses of male subjects and we will restrict our remaining comments to these analyses. MRIs from the male IgG-ASD$^{37/73kDa}$ offspring ($n=4$) were compared with a combined control group consisting of male IgG-CON ($n=2$) and archived MRI data from an existing library of outdoor colony animals ($n=5$) that were matched for age, sex and weight parameters. Trajectories of brain volume development in the male subjects from 1 week to 2 years were estimated using mixed-effects regression

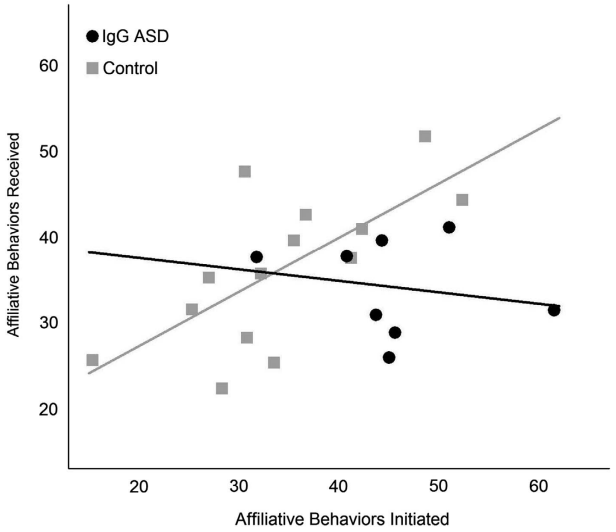

Figure 3 Unlike control juveniles, immunoglobulin G (IgG)-autism spectrum disorder (ASD) juveniles fail to show a species-typical correlation between the total number of affiliative behaviors initiated and the total number of affiliative behaviors received from peers. Total affiliative behaviors represent a composite score of all positive vocalizations (coo, grunt, girney), facial expressions (lipsmack, play, threat) and movements (approach, follow, play, groom, mount, approach, contact, proximity) not associated with negative behaviors (aggressive contact, and so on).

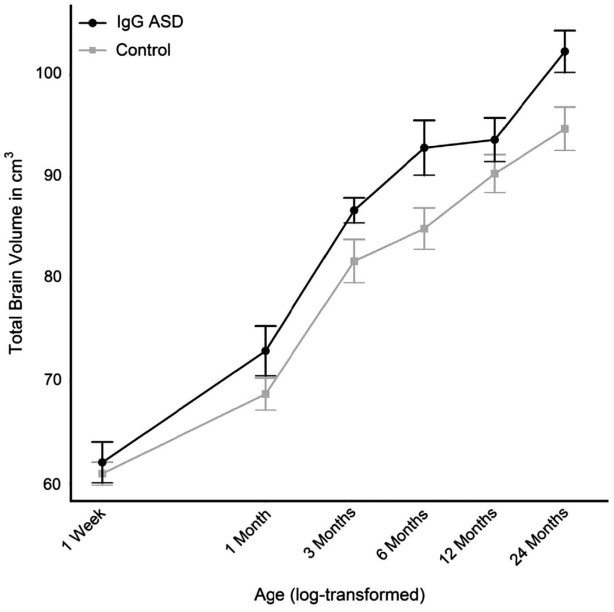

Figure 4 Immunoglobulin G (IgG)-autism spectrum disorder (ASD) males demonstrate a higher rate of brain growth resulting in significant differences in total brain volume emerging between 3 and 6 months of age.

models (Supplementary Table S13). As illustrated in Figure 4, the two groups of male monkeys had similar brain volumes at 1 week of age, but the rate of growth was significantly faster in the IgG-ASD$^{37/73kDa}$ offspring ($P=0.01$). The precocious growth of the IgG-ASD$^{37/73kDa}$ animals resulted in significant group differences in total brain volume that emerged between 3 and 6 months of life. At 2 years, the mean total brain volume of the IgG-ASD$^{37/73kDa}$ was 7072 mm^3 greater than the control animals ($P=0.01$). For the 2-year data, we investigated whether the major cerebral lobes of the brain were contributing equally to the increased size (Table 6). Further analyses explored whether the group differences observed in brain volume were generalized across the four lobes (Figure 5). Wilcoxon exact tests revealed that the male IgG-ASD$^{37/73kDa}$ offspring had increased frontal ($P=0.04$), occipital ($P=0.04$), but not parietal ($P=0.11$) and temporal lobes ($P=0.11$). We also evaluated whether the increased brain volume was preferentially associated with increases in gray or white matter. We found a significant difference in total white matter volume ($P=0.01$) and a trend difference in gray matter volume ($P=0.08$; Figure 6). IgG-ASD$^{37/73kDa}$ offspring had increased frontal ($P=0.01$), occipital ($P=0.04$) and parietal

Table 5 Descriptive summary of novel peer interaction frequency for unconstrained novel dyads (12 months) and three-chambered social approach (16 months)

	IgG-ASD (n = *8*)		*Control* (n = *14*)	
	Mean (s.d.)	*Median (range)*	*Mean (s.d.)*	*Median (range)*
Novel dyads[a]*: behaviors directed to novel peers*				
Approach	9.2 (2.1)	9.4 (6.0–11.4)	6.9 (2.5)	7.4 (2.8–10.8)
Contact	3.4 (1.7)	2.8 (1.9–6.7)	2.6 (1.4)	2.9 (0.7–5.3)
Proximity	2.4 (1.2)	2.0 (1.2–4.5)	2.0 (1.0)	2.1 (0.7–3.8)
Groom	0.0 (0.0)	0.0 (0.0–0.1)	0.1 (0.1)	0.0 (0.0–0.4)
Play	1.6 (2.2)	0.5 (0.0–6.2)	0.8 (1.2)	0.1 (0.0–4.6)
Novel dyads[a]*: behaviors received from novel peers*				
Approach	1.9 (0.9)	1.9 (0.6–3.0)	2.0 (0.9)	2.0 (0.5–3.5)
Contact	1.0 (0.7)	0.9 (0.2–2.2)	1.0 (0.6)	1.0 (0.2–2.2)
Proximity	0.5 (0.4)	0.4 (0.1–1.1)	0.4 (0.3)	0.4 (0.0–0.9)
Groom	0.0 (0.0)	0.0 (0.0–0.1)	0.1 (0.2)	0.1 (0.0–0.6)
Play	0.8 (0.8)	0.3 (0.0–2.0)	0.4 (0.6)	0.2 (0.0–2.0)
Three-chambered social approach[b,c]*: behaviors directed to novel peer in cage*				
Approach	19.2 (4.3)	18.0 (14.5–28.0)	10.8 (5.4)	9.5 (1.5–22.0)
Contact	16.6 (6.4)	18.0 (4.9–24.0)	8.4 (4.5)	7.5 (0.5–19.0)
Proximity	4.1 (2.2)	4.0 (0.0–70–7.5)	3.8 (3.0)	3.5 (0.5–12.5)

Abbreviations: ASD, autism spectrum disorder; IgG, immunoglobulin G.
Summary of the average[a] frequency of behaviors for the IgG-ASD and control monkeys.
[a]Behaviors were first averaged within each monkey, from the behaviors exhibited by an animal across the eight different stimulus monkeys. [b]Averages calculated after behaviors were first averaged within each monkey, from behaviors exhibited during two different days (presented as average behavior per 10-min trial). [c]As the stimulus animal was constrained in a small cage, the behavioral definitions were modified for the three-chambered social approach paradigm as follows: Approach, move within arm's reach of the cage containing the unfamiliar peer; Contact, contact the cage containing the unfamiliar peer; Proximity, remain within arm's reach of the cage containing the unfamiliar peer.

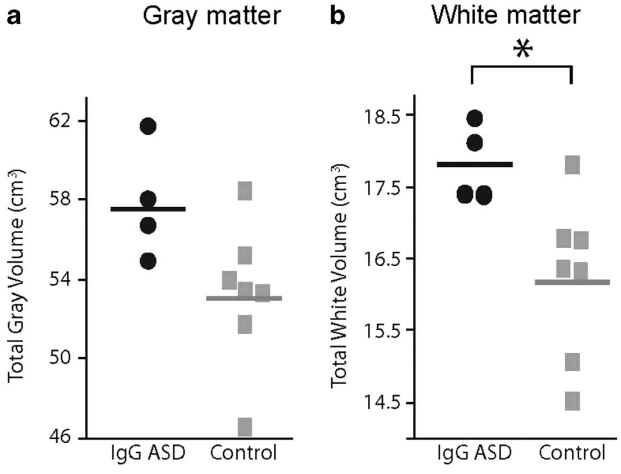

Figure 6 At two years of age, IgG-ASD male offspring demonstrate (**a**) trend towards increased total gray matter and (**b**) significantly more white matter than controls. *$P<0.05$.

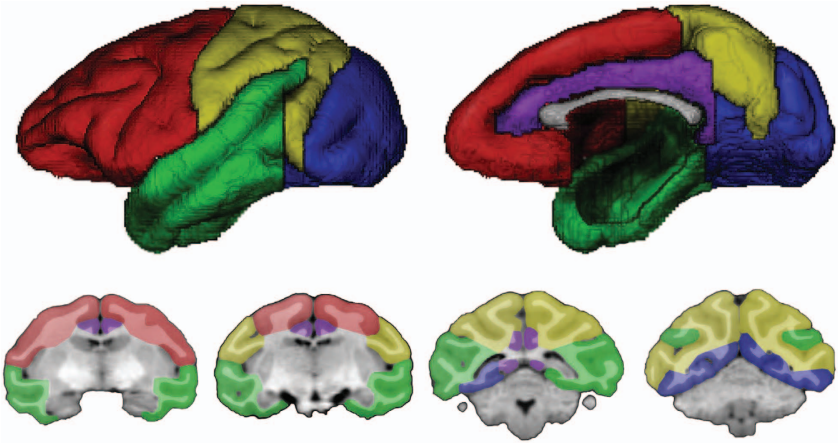

Figure 5 Parcellation of five cerebral lobes: frontal (red), cingulate (purple), temporal (green), parietal (yellow), occipital (blue) on the lateral and medial surface on an magnetic resonance imaging (MRI) three-dimensional reconstruction of the macaque brain. Coronal images from rostral (left) to caudal (right) indicating segmentation of lobes into gray (dark exterior color band) and white (light interior color) matter within each lobe parcellation.

Table 6 Two-year old MRI

	IgG-ASD (n = 4)	*Control* (n = 7)	P-*value*
Frontal lobe	25 289 (1160)	23 178 (1437)	0.04
Frontal gray	18 987 (944)	17 534 (1105)	0.16
Frontal white	6302 (250)	5644 (395)	0.01
Parietal lobe	16 555 (683)	15 217 (1358)	0.11
Parietal gray	12 119 (548)	11 208 (1054)	0.16
Parietal white	4437 (147)	4009 (313)	0.02
Temporal lobe	15 300 (868)	14 400 (1130)	0.11
Temporal gray	11 939 (785)	11 189 (856)	0.11
Temporal white	3360 (97)	3211 (287)	0.32
Occipital lobe	15 989 (725)	14 405 (855)	0.04
Occipital gray	12 245 (499)	11 048 (701)	0.02
Occipital white	3744 (236)	3356 (191)	0.04
Cingulate cortex	2543 (309)	2217 (278)	0.16
Total cortical gray	57 442 (2873)	52 857 (3620)	0.07
Total cortical white	18 233 (545)	16 560 (1145)	0.02

Abbreviations: ASD, autism spectrum disorder; IgG, immunoglobulin G; MRI, magnetic resonance imaging.
Male IgG-ASD monkeys have significantly larger frontal and occipital lobes. Total white matter volume was also significantly larger in the IgG-ASD and trend level difference in total gray matter.

($P = 0.02$) white matter volumes (Figures 4, 5 and 6; Table 6).

Discussion

Anti-fetal brain antibodies directed at proteins of 37 and 73 kDa molecular weight are found in approximately 12% of mothers who have children with ASD, but not in mothers of typically developing children.[7,8] We have hypothesized that these anti-brain antibodies cross from the mother through the placenta and the immature fetal blood–brain barrier,[43,44] bind antigens expressed in the fetal brain, disrupt neurodevelopment and ultimately contribute to one form of ASD. If the antibodies are pathologically significant in ASD, we would predict that monkeys prenatally exposed to the antibodies would exhibit perturbations in behavior related to the diagnostic features of ASD.[38,45] We would also predict that some of the abnormal patterns of brain development identified in children with ASD[46] might also be observed in the monkey model. We report here evidence both of aberrant social development and abnormal brain growth in monkeys exposed to the human anti-brain antibodies. These outcomes are supportive of our hypothesis that the antibodies are pathogenic for one form of autism.

Before discussing the significance of these findings to the etiology of ASD, it is important to indicate the limitations of the present experiments. Many of these limitations are a result of the paradox of conducting research in nonhuman primates. Although the macaque monkey is an ideal model for analysis of human neuropsychiatric disorders because of similarities in brain organization and cognitive and social function with human beings, there are ethical and pragmatic considerations that limit what is feasible in the development of a nonhuman primate model. The primary limitation of this study is the small sample size. This is particularly true for the imaging results which, we discovered during these studies, are apparent only in the male subjects. An essential next step is to replicate these findings with a sample of male subjects sizable enough to carry out both more sophisticated imaging studies as well as histopathological studies aimed at investigating the neural substrates of the abnormal brain growth. Another limitation of this study is that it is largely descriptive and we are not able to provide a mechanistic explanation of how the administered antibodies disrupt brain development leading to altered postnatal behavior. In fact, neither we, nor anyone else, have data indicating the efficiency with which maternal antibodies enter the fetal brain. In addition, while exogenously administered antibodies are a first step in developing this maternal immune model, the ideal replication of the human condition would be to have female rhesus monkeys produce antibodies themselves to the identified antigens in order to have titer of antibody circulating throughout the entire pregnancy. Nonhuman primate studies are inherently lengthy and costly and the development of this model will necessitate an iterative process that will increasingly approximate the human condition.

In spite of these limitations, specific changes in behavior and brain development were found in the macaque offspring prenatally exposed to the 37/73 kDa antibodies. Our first indication of differences between the groups was that the macaque mothers responded to their IgG-ASD$^{37/73kDa}$ offspring with heightened protectiveness during early development. Compared with control offspring, the IgG-ASD$^{37/73kDa}$ offspring were more frequently approached and contacted by their mothers and they were more commonly in close proximity to their mothers in peer groups. The behaviors of the IgG-ASD$^{37/73kDa}$ offspring mothers are consistent with a protective maternal style that has been well documented in macaques and other nonhuman primates.[47] Although it is possible that the eight mothers randomly assigned to the IgG-ASD$^{37/73kDa}$ treatment group were innately more protective, this seems unlikely as there were no systematic differences in maternal characteristics such as age, previous maternal experience or dominance status that might be associated with over protection.[48] Another possibility is that the IgG-ASD$^{37/73kDa}$ antibody injections somehow directly altered the mothers' behavior, though this too seems unlikely given that the mature blood–brain barrier would likely inhibit access of the antibodies into their brains. The protective maternal style was observed only when other animals were present, suggesting that the mothers perceived a greater risk to their infants in the context of the group interaction.[47] It is plausible that the mothers detected subtle behavioral abnormalities in the IgG-ASD$^{37/73kDa}$ offspring that eluded our observations, but nonetheless induced them to adopt a more protective maternal style.

The nature of behavioral perturbations in an animal model of ASD may be complex and species-specific, especially in the social and communication domains. In mice, for example, the default response to an unfamiliar conspecific is to approach and investigate. Thus, decreased time spent investigating a novel animal is taken as evidence of diminished sociability.[38] The social protocol for nonhuman

primates is much more complex and nuanced. For rhesus monkeys, the decision to approach and interact with another animal depends on a number of internal and external factors such as temperament, rank of the social partner or the presence of kin.[49–53] Deviations from species-typical social behaviors, such as immediately approaching an unfamiliar conspecific or behaving impulsively with familiar animals, are consistently associated with negative outcomes, such as serious aggression, in a number of nonhuman primate species.[54–61] This is why it is so unusual that across multiple paradigms, we observed the IgG-ASD$^{37/73kDa}$ offspring to more frequently approach both familiar and unfamiliar peers.

Following weaning from the mothers at 6 months, IgG-ASD$^{37/73kDa}$ offspring were observed to approach familiar peers more frequently than controls during daily group socialization time. The abnormal social approach was only directed to age-matched conspecifics (and not adults) and was not associated with global changes in activity or exploration. Although an increase in approach to peers may initially be perceived as heightened sociability, closer examination of the behavior revealed that the approach to peers by the IgG-ASD$^{37/73kDa}$ offspring was not effective in eliciting social interaction. An approach is scored when the animal under observation moves within arm's length of another animal. Animals that approach more frequently would thus have more opportunity to initiate sustained social interactions, such as play or grooming. In spite of the increased opportunity to interact with peers, however, the approaches failed to manifest in sustained social interactions. As the animals matured, control offspring demonstrated a positive correlation between the frequencies of affiliative behaviors initiated and received. This is what would be expected if these gestures are intended to engage the peer in reciprocal social interaction. In contrast, the IgG-ASD$^{37/73kDa}$ animals generated unreciprocated social approaches. We could not determine why the approaches were not reciprocated. Likely there were subtleties in the demeanor of the IgG-ASD$^{37/73kDa}$ subjects that dissuaded their peers from engaging in the proffered social interactions. The instances of increased peer approach described above occurred during interactions with familiar conspecifics from their natal rearing group, which posed little threat to the IgG-ASD$^{37/73kDa}$ offspring. Even more surprisingly, the same pattern of increased peer approach was observed during interactions with unfamiliar conspecifics during dyadic interactions and again during the three-chamber social approach assay. Inappropriately approaching a novel animal is highly unusual and potentially dangerous for rhesus monkeys[61,62] and reflects a clear deviation from species-typical social development.

Are the observed deviations from macaque social development relevant to human ASD? The social and communication deficits described in the proposed Diagnostic and Statistical Manual of Mental Disorders (5th edn; DSM-5) diagnosis for ASD include: (1) deficits in social–emotional reciprocity, (2) deficits in nonverbal communicative behaviors used for social interaction and (3) deficits in developing and maintaining relationships, appropriate to developmental level. Although deficits in social and emotional reciprocity are a diagnostic feature of ASD, manifestation of these social impairments varies greatly among individuals with ASD. In recognition of the complexity of social impairments, Wing and Gould[63] proposed a system to categorize three common subtypes of social interaction styles within the ASD population: aloof, passive and active-but-odd. Although both the aloof and passive subtypes were described as rarely initiating social approaches to others, members of the active-but-odd subgroup were described as making spontaneous social approaches to others, but in a naive and one-sided manner. Subsequent studies relying on parental questionnaires,[64–67] diagnostic tools[68] and direct observations[69] have confirmed these subtypes within the ASD patient population. We suggest that the inappropriate social approach behaviors observed in the animal model are highly reminiscent of the active-but-odd subtype of social interaction style. Moreover, the lack of reciprocal affiliative interactions observed in the IgG-ASD monkeys parallels the reciprocity deficits described in the DSM-5 as 'ranging from abnormal social approach and failure of normal back and forth conversation through reduced sharing of interests, emotions, and affect and response to total lack of initiation of social interaction'. Although we recognize that the rhesus macaque behaviors are, at best, rough approximations of human social interactions, recent studies have highlighted the importance of reciprocity in forming and maintaining 'friendships' among juvenile macaque monkeys.[70] Reciprocal interactions between juvenile monkeys are more likely to persist over time compared with unidirectional interactions that are primarily initiated by one individual. Reciprocity is also a key component of establishing and maintaining friendships in typically developing human children, and has been consistently noted as a deficit in children with ASD.[71–73]

The notion that prenatal exposure to autism-specific antibodies alters normal development is corroborated by our MRI data demonstrating increased brain volumes in male IgG-ASD$^{37/73kDa}$ offspring. These data are consistent with numerous reports from human neuroimaging demonstrating that increased brain size is found in boys with ASD.[42,74–78] Analysis of our longitudinal MRI data revealed significant group differences in brain volume that emerge between 3 and 6 months of age. This age in monkeys is roughly equivalent to a 2-year-old child—a period where numerous MRI studies have documented accelerated brain growth in human children with ASD. It is important to note, however, that increased brain size is not found in all children with ASD. Nordahl *et al.*[42] have found that approximately 10% of boys with ASD have megalencephaly whereas few, if any, girls demonstrate that abnormal growth pattern. The most striking convergence between the monkey model and clinical population comes from an analysis of MRI data from children with ASD born to mothers who have the 37/73 kDa antibodies. Volumetric evaluation of the MRI data indicate that male children with autism who were exposed prenatally to the same 37/73 kDa antibodies have significantly larger brains than male children with autism born to mothers without the 37/73 kDa antibodies and typically developing control groups.[79] Similar to the situation with children with ASD, we found that differences in brain volume were accounted for predominantly by increases in the frontal lobe, most notably of frontal lobe white matter. Consistent with our findings of increased cortical white matter in male IgG-ASD$^{37/73kDa}$ offspring, excess superficial/radiate

white matter has been reported in older children and adolescents with ASD.[80–84]

Although the convergence of abnormal brain growth in both clinical populations and the nonhuman primate model lends credence to this animal model, there are many unanswered questions remaining. For example, it is not clear why prenatal exposure to these antibodies would alter brain volume in males, but not females, when both males and females have altered behavioral profiles. We also do not understand the mechanism by which prenatal exposure to maternal antibodies leads to larger brain size. Maternal IgG can be detected in human fetal circulation as early as 13 weeks of gestation[85,86]—a time that coincides with both neurogenesis and neuronal migration, followed by a dramatic increase in placental transport of maternal IgG throughout mid to late pregnancy[87–89]—overlapping with neuronal differentiation, synaptogenesis, dendritic and axonal arborization, myelination and apoptosis. Slight alterations in any of these processes could dramatically disrupt the trajectory of brain development. Although we do not know how, or when, these autoantibodies develop, retrospective analysis of banked blood samples indicate that a subset of mothers of children with ASD have anti-brain antibodies in their circulation during pregnancy[90] and these antibodies can remain in circulation for as long as 18 years after the pregnancy.[91]

Although we are still at a relatively early stage with the development of this model, we find the promise to be twofold: (1) this program is the first to utilize nonhuman primates to test a specific etiology of ASD that is derived directly from patient populations. Potential outcomes of this model are the development of diagnostic procedures for autism risk factors and a viable model for implementing therapeutic interventions and preventative measures that could be quickly adapted for human patients. (2) This nonhuman primate model will allow, for the first time, a histological evaluation of the neural changes associated with precocious brain growth that is a consistent, albeit not universal, feature of human ASD. Identification of the cellular and molecular mechanisms underlying the abnormal brain growth in the animal model will allow us to potentially identify the neural circuits disrupted in at least one form of ASD. This vital information could provide targets for pharmacological interventions that may limit, reverse or prevent the course of behavioral pathology associated with this and perhaps other forms of the disorder.

Conflict of interest

Drs Van de Water and Amaral are members of the scientific advisory board for Pediatric Bioscience, a company that has licensed the maternal antibody technology from UC Davis. Pediatric Bioscience did not contribute in any way to the current studies. The remaining authors declare no conflict of interest.

Acknowledgements. This research was supported by funding from the National Institute of Mental Health (R01MH80218). Additional support was provided by the base grant (RR00169) of the California National Primate Research Center. Human subject serum collection was supported by the UC Davis MIND Institute Pilot Grant Program and the National Center for Research Resources, National Institutes of Health, through grant #UL1 RR024146. Special acknowledgements are due for the Research Services and Primate Medicine staff at the California National Primate Research Center for care of the animals, J Buser, G Moadab and A Lopez for behavioral data collection and J Bennett, S Liston for MRI data collection and K Wells for MRI analyses. Biobehavioral assessments of the macaque offspring were carried out by Dr John Capitanio and funded in part by the National Center for Research Resources (R24RR019970 to JPC and P51RR000169 to CNPRC) and is currently supported by the Office of Research Infrastructure Programs/OD (R24OD010962 and P51OD011157, respectively).

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)