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Preliminary evidence of neuropathology in nonhuman primates prenatally exposed to maternal immune activation

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ABSTRACT

Maternal infection during pregnancy increases the risk for neurodevelopmental disorders in offspring. Rodent models have played a critical role in establishing maternal immune activation (MIA) as a causal factor for altered brain and behavioral development in offspring. We recently extended these findings to a species more closely related to humans by demonstrating that rhesus monkeys (*Macaca mulatta*) prenatally exposed to MIA also develop abnormal behaviors. Here, for the first time, we present initial evidence of underlying brain pathology in this novel nonhuman primate MIA model. Pregnant rhesus monkeys were injected with a modified form of the viral mimic polyI:C (poly ICLC) or saline at the end of the first trimester. Brain tissue was collected from the offspring at 3.5 years and blocks of dorsolateral prefrontal cortex (BA46) were used to analyze neuronal dendritic morphology and spine density using the Golgi-Cox impregnation method. For each case, 10 layer III pyramidal cells were traced in their entirety, including all apical, oblique and basal dendrites, and their spines. We further analyzed somal size and apical dendrite trunk morphology in 30 cells per case over a 30 μm section located 100 ± 10 μm from the soma. Compared to controls, apical dendrites of MIA-treated offspring were smaller in diameter and exhibited a greater number of oblique dendrites. These data provide the first evidence that prenatal exposure to MIA alters dendritic morphology in a nonhuman primate MIA model, which may have profound implications for revealing the underlying neuropathology of neurodevelopmental disorders related to maternal infection.

1. Introduction

Exposure to infection during pregnancy increases the risk of offspring developing neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia (Abdallah et al., 2012; Atladóttir et al., 2012, 2010; Brown et al., 2004; Lee et al., 2014; Mednick et al., 1988; Sørensen et al., 2009). The diversity of infections associated with altered neurodevelopment suggests that the mother's immune response, rather than a specific pathogen, underlies changes in fetal brain development. In support of this, maternal immune activation (MIA) in pregnant rodents yields offspring with behavioral abnormalities and brain pathology that parallel features of human neurodevelopmental disorders (Patterson, 2009). Aberrant development of rodent offspring can be induced by exposing the pregnant dam to influenza (Fatemi et al., 2008; Shi et al., 2003), the bacterial endotoxin lipopolysaccharide (LPS) (Baharnoori et al., 2009; Fortier et al., 2007) or the double stranded RNA viral mimic polyinosinic:polycytidylic acid (polyI:C) (Malkova et al., 2012; Piontkewitz et al., 2012; Shi et al., 2003; Zuckerman and Weiner, 2005, 2003).

Rhesus monkey (*Macaca mulatta*) models of human disorders provide an intermediate step between rodent models and clinical populations given the higher level of homology between humans and nonhuman primates in behavior, anatomy, and physiology (Watson and Platt, 2012). Previous nonhuman primate models

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have documented neurobehavioral abnormalities in macaque offspring following third trimester exposure to influenza or the bacterial endotoxin lipopolysaccharide (LPS) (Short et al., 2010; Willette et al., 2011). However, the effects of MIA at earlier gestational time points have not been explored in the nonhuman primate. We developed a novel, rhesus monkey MIA model using a modified form of poly I:C (poly ICLC), a double-stranded RNA that induces a transient innate inflammatory response in the primate immune system (Caskey et al., 2011; Levy et al., 1975). An initial cohort of animals was produced to establish dosing protocols, followed by a larger cohort that underwent comprehensive behavioral phenotyping from birth to four years of age. The MIA-treated offspring in the larger cohort demonstrated abnormal repetitive behaviors, altered vocal communication and atypical social interactions (Bauman et al., 2014). Non-invasive eye-tracking studies later revealed that the MIA-treated juvenile offspring fail to attend to salient social cues (Machado et al., 2014). The behavioral pathology in the monkey poly ICLC model extends the findings from rodent MIA models to more human-like behaviors resembling those in both ASD and SZ.

An essential next step is to determine if MIA-exposed macaque offspring also demonstrate brain neuropathology that parallels these human disorders. While behavioral studies were being carried out on the larger cohort, we initiated the neuropathological examination of brain tissue from the initial polyICLC dosing cohort. Offspring from this initial cohort were born to dams injected with polyICLC at six time points at the end of the first trimester to evaluate the maternal immune response ($n = 4$) or born to saline injected controls ($n = 4$). Brain tissue was collected at 3.5 years of age and stained with a modified Golgi-Cox technique (Glaser and Van der Loos, 1981). We have focused our initial studies of neuronal morphology on the dorsolateral prefrontal cortex (DLPC; Brodmann Area 46), an area known to show changes in layer III pyramidal neuron morphology in post-mortem populations of SZ patients (Glantz and Lewis, 2000; Glausier and Lewis, 2013; Kolluri et al., 2005; Pierri et al., 2001). The DLPFC is well-developed in primates and is a good candidate area to identify potential aberrant neuronal dendritic morphology in MIA-exposed offspring in a brain region implicated in human neuropsychiatric disease.

2. Materials and methods

All experimental procedures were developed in collaboration with the veterinary, animal husbandry, and environmental enrichment staff at the California National Primate Research Center (CNPRC) and approved by the University of California, Davis Institutional Animal Care and Use Committee. All attempts were made (in terms of social housing, enriched diet, use of positive reinforcement strategies, and minimizing the duration of daily training/testing sessions) to promote the psychological well-being of the animals that participated in this research.

2.1. Subjects

Eight pregnant rhesus monkeys were selected from the timed-mating program at the California National Primate Research Centre and randomly assigned to receive saline control injections ($n = 4$) or a modified form of the viral mimic polyI:C (polyinosinic: cytidylic acid, stabilized with poly-L-lysine (polyICLC)) (Oncovir Inc., Washington D.C.) ($n = 4$). PolyICLC is resistant to endogenous RNase activity present in primate blood that breaks down polyIC (de Clercq, 1979; Nordlund et al., 1970). Pregnancy was confirmed at approximately 20 days of gestation via ultrasound. Willingness to present an arm for intravenous injection while being temporarily restrained (less than 1 min) was assessed at gestational day 30. To minimize stress, only animals that readily complied were included in the study.

2.2. Maternal polyICLC administration

Pregnant dams received six intravenous injections of polyICLC or saline on gestational days 43, 44, 46, 47, 49 and 50 of pregnancy (at the end of the first trimester). The dose of polyIC utilized in rodent MIA models generally ranges from 4 to 20 mg/kg (Boksa, 2010). Lower doses were initially evaluated in the nonhuman primate model to establish parameters for stimulating a maternal immune response while minimizing spontaneous abortion. Three low doses were evaluated in this study: 0.25, 0.5 and 1 mg/kg ($n = 1, 2, 1$ respectively). The control group received saline injections to account for any differences that may arise from stress of receiving injections in pregnancy. Pregnancies of both MIA and control animals were monitored via ultrasound 24–48 h following the final polyICLC or saline injection, and again at approximately GD 100 and 150. Note that for the larger cohort of MIA-treated offspring utilized in the behavioral studies (Bauman et al., 2014; Machado et al., 2014) the lowest dose of polyICLC was used (0.25 mg/kg) and the number of polyICLC injections was reduced from six to three.

2.3. Interleukin-6 analysis

Blood was drawn 24–48 h prior to the initial polyICLC injection for baseline analysis, and then again 3 h after the first and last injections were administered (GD 43 and 50). A final sample was collected approximately 5-days after polyICLC injections as a second baseline measure (Table 1). Blood was separated and 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).

2.4. Offspring and behavioral scoring

The offspring (6 males, 2 females) were raised with their mothers and provided access to peers to facilitate species typical social development. While comprehensive behavioral phenotyping was not carried out on the initial dosing cohort, general health and development were monitored and the offspring were periodically screened for maladaptive behaviors, such as repetitive behaviors. Quantitative behavioral data were collected when the offspring were weaned from their mothers at 6 months of age. Trained observers, who were blind to the assigned experimental conditions, conducted 18 home cage observations (9 morning and 9 afternoon sessions) 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. 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". Behaviors included a subset of the standard rhesus monkey developmental ethogram (Bauman et al., 2014), focusing specifically on maladaptive motor stereotypies and self-directed behaviors.

2.5. Histological evaluation of tissue

Animals were perfused with saline at 3.5 years of age and the brains placed in 10% formalin prior to processing less than a week later. The left hemisphere was retained for the current study, while the right hemisphere was frozen and retained for future studies. Left hemisphere frontal lobe blocks were wrapped in gauze and placed in Golgi-Cox solution (working concentrations: 1%

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