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Maternal immune activation during pregnancy in rats impairs working memory capacity of the offspring

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ABSTRACT

Maternal immune activation during pregnancy is an environmental risk factor for psychiatric illnesses such as schizophrenia in the offspring. Patients with schizophrenia display an array of cognitive symptoms, including impaired working memory capacity. Rodent models have been developed to understand the relationship between maternal immune activation and the cognitive symptoms of schizophrenia. The present experiment was designed to test whether maternal immune activation with the viral mimetic polyinosinic:polycytidylic acid (polyI:C) during pregnancy affects working memory capacity of the offspring. Pregnant Long Evans rats were treated with either saline or polyI:C (4 mg/kg; i.v.) on gestational day 15. Male offspring of the litters (2–3 months of age) were subsequently trained on a nonmatching-to-sample task with odors. After a criterion was met, the rats were tested on the odor span task, which requires rats to remember an increasing span of different odors to receive food reward. Rats were tested using delays of approximately 40 s during the acquisition of the task. Importantly, polyI:C- and saline-treated offspring did not differ in performance of the nonmatching-to-sample task suggesting that both groups could perform a relatively simple working memory task. In contrast, polyI:C-treated offspring had reduced span capacity in the middle and late phases of odor span task acquisition. After task acquisition, the rats were tested using the 40 s delay and a 10 min delay. Both groups showed a delay-dependent decrease in span, although the polyI:C-treated offspring had significantly lower spans regardless of delay. Our results support the validity of the maternal immune activation model for studying the cognitive symptoms of neurodevelopmental disorders such as schizophrenia.

1. Introduction

Maternal immune activation (MIA) during pregnancy may contribute to the development of schizophrenia, autism spectrum disorder, and bipolar disorder in the offspring (Brown, 2012; Brown, 2015; Brown & Derkits, 2010; Brown & Patterson, 2011; Fineberg & Ellman, 2013; Khandaker, Zimbron, Lewis, & Jones, 2013; Pearce, 2001). However, whether MIA contributes to the cognitive symptoms of these disorders remains poorly understood. In one study, Brown et al. (2009) showed that patients with schizophrenia whose mothers had confirmed viral infections during pregnancy performed worse on set-shifting as assessed by the Wisconsin Card Sorting Task than patients whose mothers did not have an infection. These findings raise the possibility that MIA may be particularly detrimental to higher order cognitive processes. One such process is working memory. Working memory impairments are consistently observed in schizophrenia patients in the domains of goal maintenance, interference control, and capacity (Barch, Moore, Nee, Manoach, & Luck, 2012; Barch & Smith, 2008). Interestingly, Gold et al. (2010) found that memory capacity (number of items stored) is impaired in schizophrenia patients while precision of stored representations and maintenance over different delays are intact. Thus, we sought to assess the effects of MIA on working memory capacity in a rodent model.

Rodent models of MIA during pregnancy recapitulate a variety of behavioral changes relevant to schizophrenia in the offspring (Meyer, Feldon, & Fatemi, 2009; Piontkewitz, Arad, & Weiner, 2012; Meyer, 2014). Following MIA in mice, working memory has been assessed using the alternating y maze (Krstic et al., 2012; Ribeiro et al., 2013) and matching-to-sample tasks using the cheeseboard maze (Bitanihirwe, Weber, Feldon, & Meyer, 2010; Richetto, Calabrese, Meyer, & Riva, 2013; Richetto, Calabrese, Riva, & Meyer, 2014) and Morris water maze (Meyer, Feldon, Schedlowski, & Yee, 2005; Meyer, Knuesel, Nyffeler, & Feldon, 2010; Meyer, Nyffeler, Yee, Knuesel, & Feldon, 2008). In general, working memory impairments in the offspring from MIA

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litters have been observed, particularly with longer delays and in older animals. In rats, no effects on a matching-to-sample working memory task in the water maze were observed in offspring following MIA during pregnancy (Vorhees et al., 2015). Importantly, the tasks used in these previous studies require rodents to remember a limited amount of information without manipulating capacity. Thus, we tested the effects of MIA on working memory capacity using the odor span task (OST) in rats (Dudchenko, Talpos, Young, & Baxter, 2013).

The OST (Fig. 3A), developed by Dudchenko, Wood, and Eichenbaum (2000), is an incremental nonmatching-to-sample task in which rats or mice receive a food reward by choosing to dig in a bowl of sand with a novel scent (Davies, Greba, & Howland, 2013; Davies, Molder, Greba, & Howland, 2013; Dudchenko et al., 2000; Rushforth, Allison, Wonnacott, & Shoaib, 2010; Rushforth, Steckler, & Shoaib, 2011; Young et al., 2007) or by flipping scented lids (April, Bruce, & Galizio, 2013; Galizio, Deal, Hawkey, & April, 2013; MacQueen, Bullard, & Galizio, 2011). If the subject chooses the novel bowl, additional bowls are added one at a time with the previous bowl(s) repositioned on the platform until the subject chooses a previously rewarded bowl (recorded as an error). The number of bowls correctly selected minus 1 is the span of the rat. Average spans of approximately 7–9 odors are reported when rats are stopped after their first error (Dudchenko et al., 2000 but see April et al., 2013; Davies, Greba et al., 2013; Davies, Molder et al., 2013). Odor span capacity is decreased following reversible inactivation of the medial prefrontal cortex (mPFC) (Davies, Molder et al., 2013) and dorsomedial striatum (Howland, Davies, Greba, Selk, & Syed, 2014), but not permanent lesions of dorsal hippocampus (Dudchenko et al., 2000) in rats. Studies in rats have shown that span capacity is impaired following various treatments, such as acute stress (Davies, Molder et al., 2013), 192 IgG-saporin-induced cholinergic lesions of the basal forebrain (Turchi & Sarter, 2000), N-methyl-D-aspartate (NMDA) receptor antagonists (Davies, Greba et al., 2013; Galizio et al., 2013; MacQueen et al., 2011; Rushforth et al., 2011), and the γ-aminobutyric acid (GABA) A receptor modulator chlordiazepoxide (April et al., 2013). Odor span capacity is also increased in rats by systemically administered nicotinic (Rushforth et al., 2010). Given the neural substrates mediating the OST, we expected that the offspring of rats treated with the viral mimetic polyinosinic:polycytidylic acid (polyI:C) during pregnancy would be impaired on the task.

2. Materials and methods

2.1. Subjects

Timed pregnant Long–Evans rats [gestational day (GD) 7; Charles River Laboratories, Quebec, Canada] were individually housed in clear plastic cages in a temperature-controlled (21 °C) colony room on a 12/12-h light/dark cycle (lights on at 0700 h). Food (Purina Rat Chow) and water were available ad libitum. Male offspring of 3 separate squads of dams were used in the current experiments. The experiments were conducted during the light phase and offspring were handled 3 times before experiments commenced. Offspring had water available ad libitum and were food restricted to maintain 85% of their free feeding weight during behavioral experiments. Experimenters were blind to the treatment of the dams and pups during the course of all experiments. All experiments were performed in accordance with the Canadian Council on Animal Care and were approved by the University of Saskatchewan Animal Research Ethics Board.

2.2. Gestational and neonatal treatment

Treatment methods closely followed those reported previously (Ballendine et al., 2015; Howland, Cazakoff, & Zhang, 2012; Sangha, Greba, Robinson, Ballendine, & Howland, 2014; Zhang, Cazakoff, Thai, & Howland, 2012). On GD 15, dams were individually transported to a room where weight and rectal temperature (Homeothermic Blanket System, Harvard Instruments, MA, USA) were measured. Dams were then anesthetized with isoflurane (5% induction and 2.5% maintenance) and injected intravenously with a single dose of either saline (n = 8) or polyI:C (4.0 mg/kg, high molecular weight; InVivoGen, San Diego, CA, USA; n = 8) via the tail vein. This procedure took an average of 10 min/animal, and care was taken to ensure the saline treated dams were anesthetized for the same duration as the polyI:C-treated dams. Weight and temperature were measured again at 8, 24, and 48 h after the injection. Dams were otherwise left undisturbed until the day after parturition. The day of parturition was designated postnatal day (PND) 0. On PND 1, litters were weighed and culled to a maximum of 10 pups per litter (six males and four females where possible). Other than routine husbandry (including recording litter weights on PND 8, 14, and 21), litters were left undisturbed until weaning on PND 21. Weaned male pups from the same litter were housed in same-sex cages of 2–4 animals. Care was taken to ensure that one offspring per litter was included in each group to reduce the influence of litter effects. Training was initiated when the offspring were young adults (2–3 months of age).

2.3. Apparatus

Training and testing followed previously established protocols (Davies, Greba et al., 2013; Davies, Molder et al., 2013). A 91.5 cm^2 platform covered with black corrugated plastic with a 2.5 cm tall border around the outer edge was used. The platform was secured to a metal frame with casters and stood 95 cm above the floor. The platform was surrounded by a beige curtain to block visual cues in the room. Velcro was used to secure white porcelain bowls (4.5 cm high, 9 cm in diameter) to the platform and prevent the rats from spilling the sand. Pieces of Velcro were equally spaced along the edge of the platform (one piece in each corner and five additional pieces on each side). The bowls for a given trial were randomly positioned on the pieces of Velcro.

2.4. Odors

Premium Play Sand (Quikrete Cement and Concrete Products, Atlanta, GA) was sifted to remove rocks and then odors were mixed into the sand. Sand (100 g) was scented by mixing it with 0.5 g of a single dried spice. The odor and sand mixtures were stored in separate Ziploc bags when unused and new batches of sand and odors were freshly mixed every 7 days. Twenty-four different spices were used in the experiments: allspice, anise seed, basil, caraway, celery seed, cinnamon, cloves (0.1 g), cocoa, coffee, cumin, dill, fennel seed, garlic, ginger, lemon and herb, marjoram, mustard powder, nutmeg, onion powder, orange, oregano, paprika, sage, and thyme. Spices were purchased from a local grocery store. The sequence of the odors used each day were selected randomly and rats were regularly exposed to all odors. Sand filled bowls were placed on the platform as needed for each trial.

2.5. Training on the odor span task

Dig Training/Shaping. First, rats were trained to dig for a food reward (Kellogg's Froot Loops) in a bowl filled with 100 g of unscented sand. Rats were placed opposite to a bowl on the platform for three separate phases. In the first phase, the food reward