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Research report

Microglia depletion in early life programs persistent changes in social, mood-related, and locomotor behavior in male and female rats

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HIGHLIGHTS

- We used liposomal clodronate to deplete microglia in neonatal rats and study the lifelong effects on motivated behavior.
- Central liposomal clodronate injection at P1 and P4 depleted forebrain microglia for approximately two weeks.
- Early-life microglia depletion led to increased locomotion and decreased anxiety and despair behaviors throughout life.
- Females that had microglia depleted neonatally had a blunted corticosterone response to acute stress in adulthood.

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ABSTRACT

Microglia, the innate immune cells of the central nervous system, regulate brain development by promoting cell genesis, pruning synapses, and removing dying, newly-born or progenitor cells. However, the role of microglia in the early life programming of behavior under normal conditions is not well characterized. We used central infusion of liposomal clodronate to selectively deplete microglia from the neonatal rat brain and subsequently assessed the impact of microglial depletion on programming of juvenile and adult motivated behaviors. Liposomal clodronate treatment on postnatal days one and four led to greater than 70% loss of forebrain microglia by postnatal day 6 that lasted for approximately ten days. Neonatal microglia depletion led to reduced juvenile and adult anxiety behavior on the elevated plus maze and open field test, and increased locomotor activity. On a test of juvenile social play, microglial depletion led to decreased chase behaviors relative to control animals. There was no change in active social behavior in adults on a reciprocal social interaction test, but there was decreased passive interaction time and an increased number of social avoidance behaviors in clodronate treated rats relative to controls. There was an overall decrease in behavioral despair on the forced swim test in adult rats treated neonatally with clodronate. Females, but not males, treated neonatally with clodronate showed a blunted corticosterone response after acute stress in adulthood. These results show that microglia are important for the early life programming of juvenile and adult motivated behavior.

1. Introduction

Microglia are the innate immune cells of the central nervous system. Microglia colonize the rodent brain beginning on embryonic day 9.5 and reach peak numbers by the third postnatal week [15,25,40]. They release a variety of diffusible factors, such as chemokines and cytokines, and express a variety of receptors that allow them to respond to and modulate events under normal and abnormal circumstances in the brain. In the developing brain, microglia have been shown to prune synapses and regulate neurogenesis, apoptosis, and axonal innervation [7,41,45,47,51]. While many of these studies have investigated microglia-regulated processes in a cellular developmental context, few studies have examined whether microglia influence behavioral development in the absence of inflammation, stress or other pathology.

Previous studies have investigated the behavioral effects of depleting microglia from the adult rodent brain, and these studies have found very limited and transient effects of microglial

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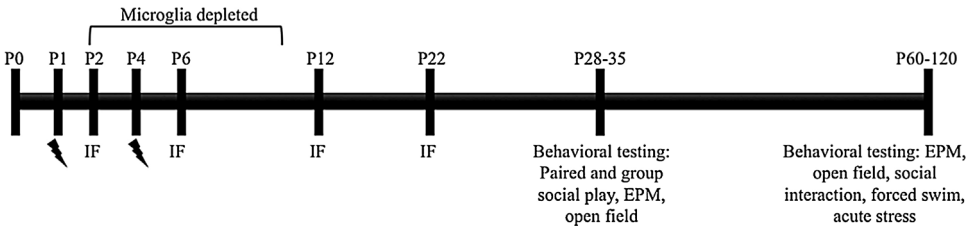

Fig. 1. Schematic of the treatment time, length of microglia depletion, time when microglia recolonized the brain, time when microglia density and morphology resembled control animals, and time when behavioral testing was performed.

depletion on social and anxiety behaviors [10,55]. In development, models that are thought to activate microglia or alter their function, such as perinatal stress or inflammatory challenge, show changes in social, anxiety and despair-like behavior, as well as stress reactivity [6,20,31,32,37,56,63,68]. These results suggest that microglia may be more important for developmental programming of behavior than the maintenance of behavior in adulthood. However, it is still unclear whether basal microglial function during development directly impacts the development of these behaviors and their expression later in life.

One strategy to determine the role of microglia on the development of later life behavior is to selectively deplete microglia through the use of liposomal clodronate. Clodronate is a cytotoxic drug that, when encapsulated in lipid droplets, is selectively taken up by phagocytic cells, where it subsequently induces apoptosis [60]. Liposomal clodronate specifically depletes macrophages while sparing other cell types, such as neurons, oligodendrocytes and astrocytes, when centrally injected into the central nervous system [12,28,59]. In the current studies we used central infusion of liposomal clodronate to deplete microglia from the developing brain and test whether microglia regulate the development of motivated behaviors in male and female rats. We found that microglia depletion in the early postnatal period led to decreased anxiety behavior and depressive-like behavior and increased locomotor activity in male and female rats, and reductions in stress-induced corticosterone release in females. The current studies underscore that microglia are necessary for the normal development of several motivated behaviors.

2. Materials and methods

2.1. Animals

All procedures were conducted in accordance with The Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and approved by The Ohio State University Institutional Animal Care and Use Committee. One cohort of age matched juvenile and virgin adult male and female Sprague Dawley rats from four separate litters were used for all behavioral testing. Adult Sprague Dawley rats (Harlan) were mated in our facilities, or timed pregnant animals were ordered to deliver within a week of arrival at the animal facility (Harlan). Two experimental litters yielded from in house breeding and two litters yielded from ordered timed-pregnant females. Animals were housed in sex-matched pairs in a temperature and humidity controlled room with *ad libitum* access to food and water, and the room was maintained on a 12 h/12 h light/dark cycle (lights on at 20 h). Pregnant females were allowed to deliver naturally and the day of birth was designated as postnatal day (P) 0.

2.2. In vivo *manipulations*

Bilateral intracerebroventricular (icv) injections were performed on neonates on P1 and P4 under brief cryoanesthesia. A 23 gauge Hamilton syringe attached to a stereotaxic manipulator was placed 1 mm caudal to Bregma and 1 mm lateral to the midline, lowered 3 mm into the brain, and then backed out 1 mm. A total of 1 μl of liposomal clodronate (Encapsula NanoSciences, Cat. 8092) or control liposomes (vehicle) was infused over 60s, and the procedure was repeated on the other hemisphere. For all procedures, the separation of pups from the dam was kept to a minimum (<1 h). Other than icv treatment of pups on P1 and P4, experimental animals were otherwise left undisturbed with the maternal dam until sacrifice or weaning. The experimental timeline is shown in Fig. 1.

2.3. Immunohistochemistry

A time-course study of microglial numbers following injection of liposomal clodronate was performed to (1) verify that liposomal clodronate effectively depleted microglia and (2) to determine the time course of microglial depletion and repopulation. After liposomal clodronate treatment, rats were sacrificed on either P2 (after receiving only one injection on P1), P6, P12, or P22 (after receiving injections on P1 and P4). Rats were deeply anesthetized with FatalPlus (Vortech Pharmaceuticals), transcardially perfused with 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Brains were then removed, post-fixed overnight in the same fixative, and cryoprotected in 30% sucrose in 0.1 M PBS until they sank. Brains were coronally sectioned on a cryostat at a thickness of 45 μm and mounted on charged slides into two alternate series. Brain sections underwent immunofluorescence staining for the microglia/macrophage specific marker, ionized calcium-binding adaptor molecule 1 (Iba1; Wako Chemicals). Slide mounted sections were extensively rinsed with 0.1 M PBS then incubated for 20 min in 50% methanol. Sections were rinsed in 0.1 M PBS then treated in 10 mM sodium citrate solution (pH 9.5) heated to 70° for 20 min, rinsed in 0.1 M PBS then blocked for 1 h in 0.1 M PBS + 0.4% Triton-X + 5% normal donkey serum (NDS). Sections were then incubated at 4 °C overnight in antiserum to Iba1 (1:1000) in 0.1 M PBS + 0.4% Triton-X + 2.5% NDS. On day 2, sections were rinsed in 0.1 M PBS and incubated in the dark for 2 h at room temperature with anti-rabbit AlexaFluor 488 (ThermoFisher Scientific, 1:333) in 0.1 M PBS + 0.4% Triton-X + 2.5% NDS, rinsed in the dark in 0.1 M PBS, counterstained with DAPI, and coverslipped with VectaShield HardSet mounting medium (Vector Laboratories). Sections were then imaged using a Zeiss AxioImager.M2 microscope, Zeiss AxioCam MRm camera, and StereoInvestigator software (MBF Biosciences).

2.4. Densitometry and microglia cell counts

Six animals from each treatment group (three male, three female) were imaged for each time point, except one clodronate treated male was removed from the P22 time point due to low body weight. To quantify gross changes in microglia within the brain following liposomal clodronate treatment, digital image analysis (DIA) of Iba-1 staining was performed [9]. The amygdala and