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Short Communication

Sensory deprivation increases phagocytosis of adult-born neurons by activated microglia in the olfactory bulb

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ABSTRACT

The olfactory bulb (OB) is a highly plastic structure that can change organizational networks depending on environmental inputs in adult mammals. Particularly, in rodents, adult neurogenesis underlies plastic changes in the OB circuitry by continuously adding new interneurons to the network. We addressed the question of whether microglia, the immune cells of the brain, were involved in pruning OB neurons. Using lentiviral labeling of neurons in neonatal or adult mice and confocal analysis, we showed that microglia engulfed parts of neonatal-born and adult-born neurons in the healthy OB. We demonstrated that OB deafferentation by Dichlobenil administration induced sensory deprivation. It also increased phagocytosis of adult-born, but not neonatal-born neurons, by activated microglia. Conversely, intranasal lipopolysaccharide administration induced activation of microglia but changed neither adult neurogenesis nor olfaction. Our data reveal that steady-state microglia eliminate adult-born neurons and their synapses in both healthy and sensory deprived OBs, thereby adapting neuronal connections to the sensory experience.

1. Introduction

The olfactory bulb (OB) is a highly plastic structure that modifies organizational networks depending on the degree of environmental inputs in adult mammals. Particularly, the olfactory system is characterized by a unique turnover of granule cell (GC) interneurons, a feature called adult neurogenesis, which significantly contributes to the functional plasticity of the system (Lledo et al., 2006; Imayoshi et al., 2008). Every day, thousands of new GCs are added in the adult mammalian OB circuit; however, only 50% of them survive for longer than a month after their birth, as many undergo apoptosis (Petreanu et al., 2002; Mouret et al., 2008). Neuronal activity instructs the survival or death of young GCs (Yamaguchi and Mori, 2005; Lazarini and Lledo, 2011) but the mechanisms involved in the permanent sculpting of neuronal connections in the OB are only beginning to be deciphered.

Microglia are the immune resident cells of the brain and play a major role in innate immunity in the Central Nervous System (CNS) (Nimmerjahn et al., 2005; Hughes, 2012). Increasing evidence supports that, aside their phagocytic activity, microglia promote developmental neuronal apoptosis and synaptic pruning in several brain regions, in both healthy and pathological CNS (Marín-Teva et al., 2004; Schafer et al., 2012; Ueno et al., 2013). Recent evidence suggests that microglia regulate several steps of adult neurogenesis in the olfactory system and hippocampus (Chesnokova et al., 2016). Microglia play a central role in eliminating adult-born neurons in healthy and inflamed brain (Sierra et al., 2010; Paolicelli et al., 2011).

We have previously demonstrated that the Dichlobenil (Dichlo) toxin induces chemical ablation of the olfactory epithelium, resulting in the loss of sensory olfactory afferents to the OB and olfactory deprivation (Lazarini et al., 2012). It also disrupts adult OB neurogenesis and activates OB microglia. Suppression of microglial activation in this sensory deprivation model restored adult neurogenesis in the deafferented OB. Here we focus on the interactions between microglia and GCs and show that microglia engulf adult-born but not neonatal-born GCs in the OB after sensory deafferentation.

2. Methods

2.1. Animals and treatments

Experiments were performed using male C57BL/6JRj (B6) mice (Janvier, France) and in-house CX3CR1-GFP mice propagated on a B6 background (Jung et al., 2000), in compliance with the EU

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Directive 2010/63/EU for animal experiments. Experimental designs and procedures were approved by the local ethics committee (2013-0081, CETEA 89). Groups consisted of 3–5 animals for histology, 4–12 animals for behavior.

Chemical ablation of olfactory epithelium using Dichlo was achieved as previously described (Lazarini et al., 2012). Vehicle mice were injected with DMSO only.

Anti-inflammatory treatment with Minocycline (Mino) was performed as previously described (Lazarini et al., 2012).

Intranasal injection of lipopolysaccharide (LPS) was administered to evoke neuroinflammation (Kapetanovic et al., 2010). LPS from *Salmonella enterica* serotype Typhimurium (Sigma-Aldrich) was dissolved in Limulus Amebocyte Lisate reagent water (Lonza) at the stock concentration of 5 $g.L^{-1}$. LPS was freshly diluted in endotoxin-free saline to the concentration of 1 $g.L^{-1}$ before use and 0.8 $\mu L.g^{-1}$ of this solution were intra-nasally instillated by presenting drops in front of nostrils under anesthesia (60 $mg.kg^{-1}$ ketamine (Merial) and 1.2 $mg.kg^{-1}$ xylazine (Merial)). Vehicle mice received saline only.

2.2. Behavior

To assess olfaction we used the Burried Food Finding test after 20 h of food deprivation (Lazarini et al., 2012). About 10 pieces of "Coco Pops" cereals were hidden in a corner of the test cage under 1.5 cm bedding. The tested mouse was placed in the test cage in the opposite corner and the latency to find the food (defined as the time to locate cereals) was recorded.

Open Field behavior was assessed by individually video-tracking animals in 43 × 43 cm^2 containers during 30 min (Noldus Ethovision 3.0, Siopi et al., 2016).

2.3. Stereotaxic lentiviral injections

Subventricular neurogenesis continuously produces new interneurons for the OB (Lledo et al., 2006). These new neurons migrate via the rostral migratory stream (RMS) toward the OB. To label them, we injected 200 nl of fluorescent lentivectors into each RMS at 3.30 mm anteroposterior, ±0.82 mm mediolateral from Bregma and −2.90 mm dorsoventral from skull as previously described (Siopi et al., 2016). A replication-deficient lentiviral vector was used to express GFP driven by the Cytomegalovirus promoter (LV-CMV-GFP, 13.8 $ng.L^{-1}$ of viral antigen) or TdTomato driven by the ubiquitin C promoter (LV-UbC-TdTomato, 8.10^{11} $VG.L^{-1}$, constructed using Addgene plasmid # 22771). Infected neuroblasts express GFP or TdTomato and migrate along the RMS into the OB.

For injections in neonatal mice, male pups at Postnatal day 6 (P6d) were isoflurane-anesthetized and positioned in a homemade cast providing isoflurane for anesthesia maintenance. Bilateral stereotaxic injections of lentiviral vectors (350 nl/site) were performed as previously described (Alonso et al., 2012).

2.4. Immunohistochemistry, confocal imaging and quantification

Sixty micrometer coronal brain sections were obtained using a vibrating microtome (VT1000S, Leica) or a microtome (SM2010 R, Leica). Immunostaining was performed on free-floating sections as previously described (Lazarini et al., 2012) using anti-IBA1 (1:400; Wako), anti-CD68 (1:2000; Serotec), anti-OMP (1:2000; Wako), anti-GFP (1:1000; Molecular probes), and anti-RFP (1:4000 Rockland) antibodies.

For spine density, eight sections 180 μm apart were selected for each animal, using the accessory OB as landmark. About 10 GCs

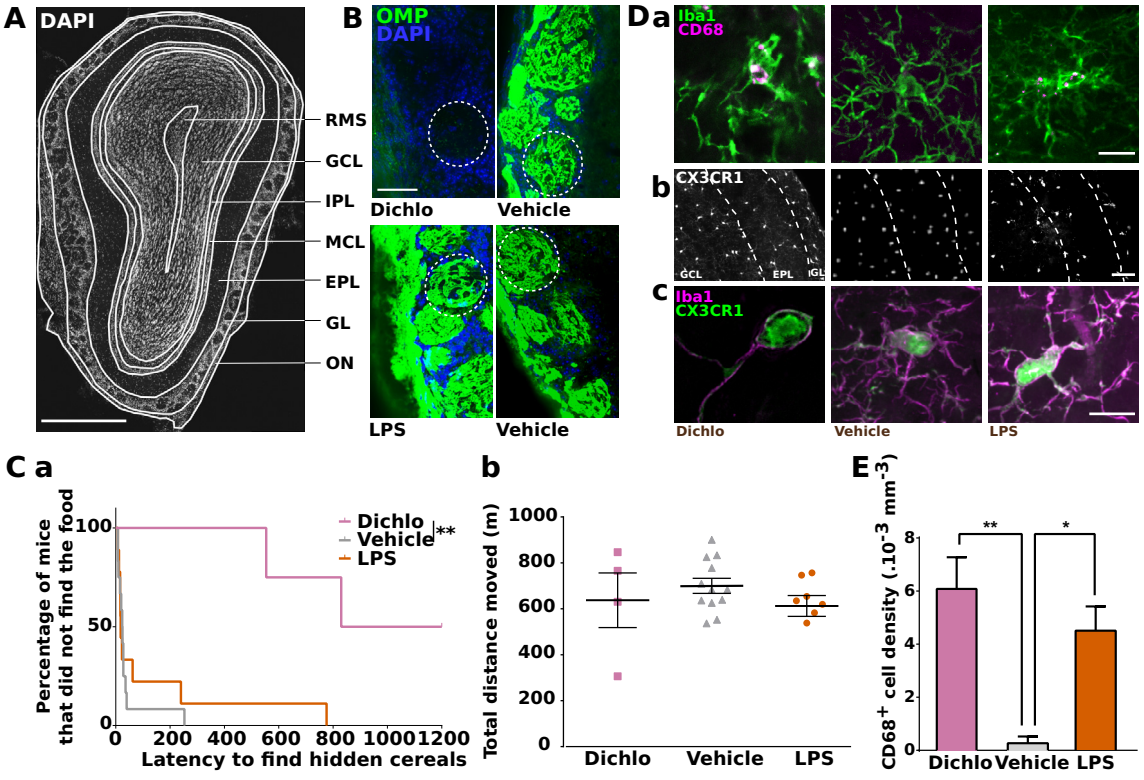

Fig. 1. Dichlo but not LPS drives olfactory deficits. (A) OB coronal slice stained with DAPI, showing the different layers of the OB; scale bar: 300 μm. (B) Representative staining of coronal OB slices with OMP antibody, showing the projections of olfactory sensory neurons on the glomeruli of the GL; upper panel: Vehicle = DMSO; lower panel: Vehicle = intranasal saline; dotted circle: one glomerulus; scale bar: 50 μm. (C) Behavioral assessments (n = 4–12); (a) latency to find hidden cereals in the buried food finding test (at 3 d.p.i. of Dichlo or LPS); (b) total distance moved in open field (at 6 d.p.i.). (D) Representative images of (a) CD68 and Iba1 co-staining, (b) CX3CR1 expression and (c) IBA1 staining in the GL of B6 (a) or CX3CR1-GFP transgenic (b, c) mice 3 days after treatment with Dichlo, LPS or respective vehicles; scale bars: 20 μm (a, c), 100 μm (b) (E) CD68+ cell density in the GL 3 days after treatment with Dichlo or LPS. Vehicle = intranasal saline. n = 3. $^{*}p < 0.05$; $^{**}p < 0.01$.