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BRAIN RESEARCH

Research Report

Prenatal inflammatory effects on nigrostriatal development in organotypic cultures

Abigail Snyder-Keller, Peri F. Stark*

Wadsworth Center, David Axelrod Institute, New York State Department of Health, 120 New Scotland Avenue, Albany, NY 12208, USA

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ABSTRACT

Maternal intrauterine infection, and the accompanying inflammation in the fetal brain, represent a significant risk to the developing fetus. Dopamine (DA) neurons have been shown to be particularly vulnerable to inflammation induced by injection of the bacterial endotoxin lipopolysaccharide (LPS). In order to further examine the nature of this vulnerability, we used a combination of *in vivo* prenatal LPS exposure, and *in vitro* analysis of nigrostriatal development in organotypic cultures prepared from LPS-exposed rat fetuses. Control co-cultures prepared from unexposed E14 substantia nigra (SN/VTA) and E21 striatum exhibited numerous DA neurons in the nigral piece and robust ingrowth into the striatal piece. When E14 SN/VTA was obtained from fetuses exposed to LPS (0.1 mg/kg) on E10, initial DA cell numbers and striatal innervation in co-cultures were normal, but at longer durations *in vitro*, a reduction in DA neurons was observed. When striatal tissue from fetuses exposed to LPS on E14 or E18 was used in combination with non-exposed SN/VTA, DA neurons initially exhibited a normal pattern of ingrowth into LPS-exposed striatum. However, with longer durations *in vitro*, DA neurons were lost more rapidly when co-cultured with LPS-exposed striatum. Despite the loss of DA neurons, striatal DA innervation was only reduced in cultures prepared with striatum exposed to LPS at E18, at the longest time period examined. Experiments in which unexposed SN/VTA was given the choice to grow toward control striatum or toward LPS-exposed striatum supported the idea that the tropic qualities of the striatum were not altered by LPS-induced inflammation. Thus, the inflammation induced by LPS not only affects the SN/VTA DA neurons, but also alters the neurotrophic – although not the neurotropic – characteristics of the striatum. Such alterations in nigrostriatal development may demonstrate how adverse perinatal events predispose the developing brain toward the later development of Parkinson's disease.

1. Introduction

The idea that prenatal events can adversely contribute to an individual's susceptibility to adult-onset neurological disorders has received considerable attention in recent years. Parkinson's disease, in particular, is known to involve inflammatory processes within the substantia nigra (SN), the locus of dopamine (DA) neuron degeneration (Liu and Hong, 2002; Dauer and Przedborski, 2003). Microglial activation is a common finding in the SN of Parkinson patients (McGeer et al., 1988); cells here express high levels of the cytokines TNFα and IL-1β (Boka et al., 1994; Hunot et al., 1999). The possibility that inflammation begins early in life is just beginning to be addressed.

* *Corresponding author*. Fax: +1 518 402 4773.
E-mail address: snykell@wadsworth.org (A. Snyder-Keller).

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Experimental models using the bacterial endotoxin lipopolysaccharide (LPS), a constituent of gram-negative bacteria that induces an acute inflammatory response, have demonstrated that those DA neurons that give rise to the nigrostriatal pathway are particularly vulnerable to inflammatory reactions (Kim et al., 2000; Herrera et al., 2000; Zhou et al., 2005). As a means to address developmental susceptibility, maternal LPS administration has been used to model the effects of maternal infectious exposure. Ling and coworkers demonstrated that LPS exposure at embryonic day (E)10.5 produces a lasting increase in microglial activation and neurotoxic effects on DA neurons in the offspring (Ling et al., 2002; Carvey et al., 2003). In addition, synergistic neurotoxic effects on DA neurons were observed with the combination of prenatal exposure to LPS and adult exposure to rotenone (Ling et al., 2004).

In order to more effectively delineate regional differences in response to the initial inflammatory insult, as well as to separate primary from secondary effects, we have turned to *in vitro* techniques for studying neural development after *in vivo* exposure. Specifically, we remove fetuses a few days after maternal LPS administration, and then prepare organotypic cultures of various combinations (exposed vs. non-exposed) of ventral mesencephalon (hereafter referred to as 'SN/VTA') and striatum. Cortex is also included, as we have found this to enhance the growth and physiological attributes of these co-cultures (Tseng et al., 2007; Snyder-Keller et al., 2008). This approach allows us to examine whether inflammation directly affects the developing DA neurons, or whether it disrupts target-derived factors that influence nigrostriatal connectivity. In previous studies we demonstrated that the DA neurons in the SN/VTA piece readily innervate the co-cultured striatal piece, and exhibit morphological, biochemical, and physiological features characteristic of DA neurons *in vivo* (Snyder-Keller et al., 2001; 2008; Snyder-Keller, 2004; Lyng et al., 2007; Tseng et al., 2007).

Through variation of the age of LPS administration prior to removal of the tissues, it is possible to identify critical periods with respect to prenatal infectious exposure. LPS was administered at particular ages chosen to preferentially influence different developmental processes that affect nigrostriatal development: E10, an age when DA neurons are beginning to be born; E14, an age when nigral DA neurons are fully generated but just beginning to project their axons into the striatum; and E18, an age when DA innervation is present in the developing striatum, but when the segregation of DA afferents into patches has not yet occurred (Snyder-Keller, 1991).

2. Results

The first set of experiments examined the effect of *in vivo* fetal inflammatory exposure on the developing SN/VTA, after

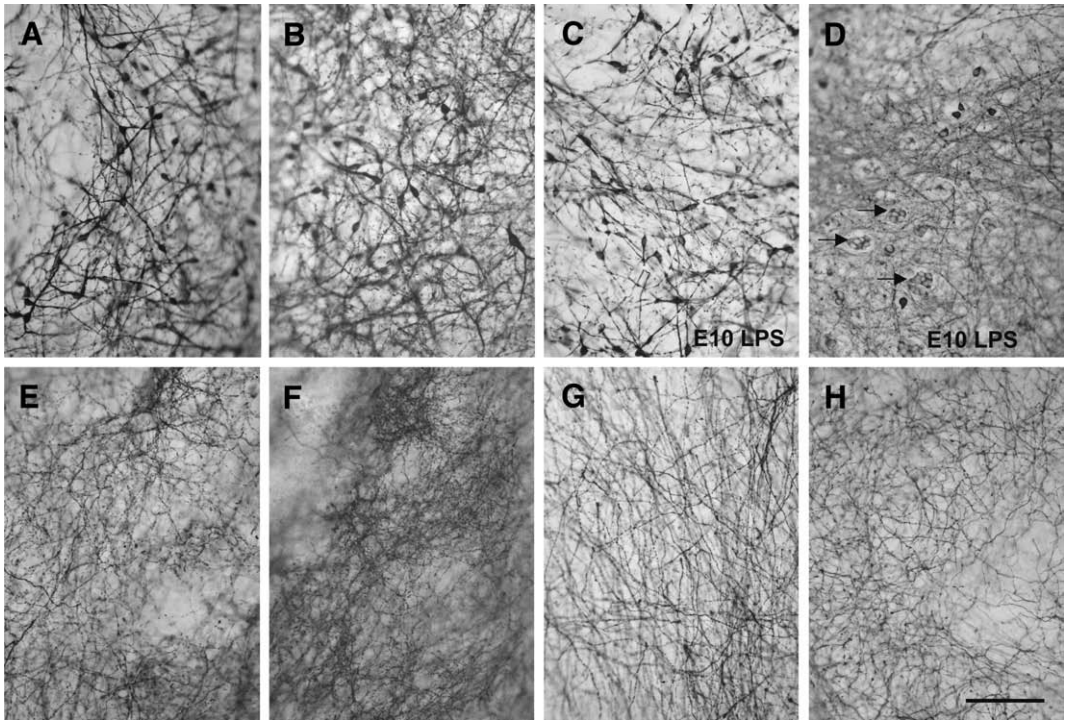

Fig. 1 – Dopamine neurons and their striatal projections, in organotypic cultures prepared from LPS-exposed SN/VTA. TH immunostaining of SN/VTA (A–D) and striatum (E–H) in co-cultures prepared from untreated tissues (A, B, E, F) and in co-cultures prepared with SN/VTA from fetuses exposed to LPS on E10 (C, D, G, H). A, B) DA neurons in an unexposed SN/VTA at 1 week (A) and 2 weeks (B) *in vitro*. C, D) DA neurons in SN/VTA exposed to LPS at E10 prior to dissection at E14, after 1 week (C) and 2 weeks (D) *in vitro*. Note numerous degenerating neurons with fragmented nuclei in D (arrows). E, F) DA innervation in the striatal piece in co-cultures prepared with unexposed SN/VTA, at 1 week (E) or 2 weeks (F) *in vitro*. G, H) DA innervation in the striatal piece in co-cultures prepared with SN/VTA exposed to LPS at E10, at 1 week (G) or 2 weeks (H) *in vitro*. Scale bar in H = 100 μm for all.