Neuroscience 303 (2015) 299–311

EFFECTS OF ACOUSTIC TRAUMA ON THE AUDITORY SYSTEM OF THE RAT: THE ROLE OF MICROGLIA

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Abstract—Exposure to loud, prolonged sounds (acoustic trauma, AT) leads to the death of both inner and outer hair cells (IHCs and OHCs), death of neurons of the spiral ganglion and degeneration of the auditory nerve. The auditory nerve (8cn) projects to the three subdivisions of the cochlear nuclei (CN), the dorsal cochlear nucleus (DC) and the anterior (VCA) and posterior (VCP) subdivisions of the ventral cochlear nucleus (VCN). There is both anatomical and physiological evidence for plastic reorganization in the denervated CN after AT. Anatomical findings show axonal sprouting and synaptogenesis; physiologically there is an increase in spontaneous activity suggesting reorganization of circuitry. The mechanisms underlying this plasticity are not understood. Recent data suggest that activated microglia may have a role in facilitating plastic reorganization in addition to removing trauma-induced debris. In order to investigate the roles of activated microglia in the CN subsequent to AT we exposed animals to bilateral noise sufficient to cause massive hair cell death. We studied four groups of animals at different survival times: 30 days, 60 days, 6 months and 9 months. We used silver staining to examine the time course and pattern of auditory nerve degeneration, and immunohistochemistry to label activated microglia in the denervated CN. We found both degenerating auditory nerve fibers and activated microglia in the CN at 30 and 60 days and 6 months after AT. There was close geographic overlap between the degenerating fibers and activated microglia, consistent with a scavenger role for activated microglia. At the longest survival time, there were still silver-stained fibers but very little staining of activated microglia in overlapping regions. There were, however, activated microglia in the surrounding brainstem and cerebellar white matter.

Key words: deafness, noise exposure, inflammation, synaptic stripping, macrophages.

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Abbreviations: AT, acoustic trauma; CN, cochlear nuclei; DC, dorsal cochlear nucleus; IHC, inner hair cell; OHC, outer hair cell; PBS, phosphate-buffered saline; PFA, paraformaldehyde; SGN, spiral ganglion; VCN, ventral cochlear nucleus.

http://dx.doi.org/10.1016/j.neuroscience.2015.07.004

INTRODUCTION

The auditory system manifests a remarkable degree of plasticity in response to denervation. One form of denervation follows exposure to prolonged, loud sounds (acoustic trauma, AT); AT results in the death of hair cells and degeneration of the auditory nerve (reviews in Saunders et al., 1985; Kujawa and Liberman, 2009; Gold and Bajo, 2014). Axons of the acoustic nerve synapse in the three subdivisions of the cochlear nuclei (CN), the dorsal cochlear nucleus (DC), and the posterior (VCP) and anterior (VCA) subdivisions of the ventral cochlear nucleus (VCN) (Cohen et al., 1972; Kane, 1974; Jones and Casseday, 1979). Noise trauma therefore removes a major input to these structures. There are both anatomical and electrophysiological data showing plastic reorganization in the CN after AT. Changes include the formation of new synapses and axonal sprouting (Benson et al., 1997; Bilak et al., 1997; Michler and Illing, 2002; Kim et al., 2004a,b,c; Dong et al., 2010b). There is an increase of somatosensory input to the DC (Shore et al., 2008; Koehler et al., 2011; Dehmel et al., 2012; Zeng et al., 2012). Physiologically, there is an increase in spontaneous activity, suggesting significant reorganization of CN circuitry (Kaltenbach and McCaslin, 1996; Kaltenbach et al., 1998; Zhang and Kaltenbach, 1998; Kaltenbach, 2007; Mulders and Robertson, 2013; Groschel et al., 2014).

The critical mechanisms mediating this plasticity are not understood. Recent attention has focused on the possible role activated microglia may play in facilitating synaptic plasticity, both injury- and experience-induced, in several systems (Banati, 2002; Beggs and Salter, 2010; Tremblay and Majewska, 2011; Beynon and Walker, 2012). At present, little is known about the role of microglia in noise trauma-induced changes in the CN. Several studies have described activated microglia in the CN after cochlear lesions (Campos Torres et al., 1999; Fuentes-Santamaria et al., 2012; Janz and Illing, 2014) and proposed a role for activated microglia in CN plasticity. However, it is possible that the activated microglia were simply serving their traditional role as scavengers (Kreutzberg, 1996), removing the debris from auditory nerve degeneration. Auditory nerve degeneration was not examined in those reports. We therefore compared the distributions of degenerating auditory nerve fibers and activated microglia after noise exposure; overlap would support the ‘‘scavenger’’ hypothesis whereas more widespread microglia activation would be suggestive of a role in guiding neuroplasticity.

EXPERIMENTAL PROCEDURES

Animals

We followed the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the University at Buffalo. We used a total of 17 adult (ages 2–3 months at time of noise exposure) male, albino SASCO Sprague–Dawley rats from Charles River Laboratories (Wilmington, MA, USA). Animals were pair-housed and had *ad libitum* access to water and standard laboratory rodent chow. They were maintained on a 12-h light–dark cycle.

Bilateral noise exposure (AT)

We exposed animals to bilateral noise. Under isoflurane anesthesia (Webster Veterinary Supply Inc., Sterling, MA, USA, 4% induction 1.5–2% maintenance) rats were exposed to narrowband noise (bandwidth 100 Hz) centered at 12 kHz, 126 SPL for 2 h. Sound stimuli were generated by Tucker-Davis Technology (TDT, Alachua, FL, USA) hardware and presented by a super compression driver (D-59l GMI Sound Corp., Brooklyn, NY, USA). The SPL was calibrated with a sound level meter coupled to a half-inch condenser microphone (Model 824 Audiometer, Larson Davis, Depew, NY, USA). The control animals were anesthetized but not exposed to the noise stimulus.

We used four different survival times, 30 days ($n = 3$ experimental, $n = 3$ controls), 60 days ($n = 3$), 6 months (200 days; $n = 2$ AT; $n = 2$ controls) and 9 months (284 days; $n = 2$ AT; $n = 2$ controls). At the end of the survival time rats were deeply anesthetized with 86 mg/kg i.p. of Fatal-plus (Vortech, Pharmaceutical Ltd.) and perfused through the heart with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. The brains were removed and divided into two blocks in the coronal plane at approximately Bregma −5.30 (Paxinos and Watson, 1997). The blocks were post-fixed in 4% PFA overnight and then cryoprotected in 15% sucrose and then 30% sucrose in PBS. We made a small slit on the ventral surface of the brain on one side to be able to distinguish left and right. Frozen sections 40 -μm-thick were cut in the coronal plane on an AO sliding microtome equipped with a custom built freezing platform. All sections were collected and stored in tissue culture wells in a cryoprotection solution of 30% ethylene glycol and 30% glycerol in phosphate buffer at −20 °C.

Immunohistochemistry

All processing was performed on free-floating sections. Sections were removed from the cryoprotectant and rinsed in PBS (all rinses were 3 × 10 min). Non-specific binding of primary antibodies was blocked by incubating the sections in a solution of 1% bovine serum albumin (BSA, Sigma), 1.5% normal horse serum (NHS, Vector Laboratories, Burlingame, CA, USA) and 0.1% TritonX-100 (TX) in PBS for 30 min. The primary antibody was added and the sections incubated overnight at 4 °C. Table 1 shows the primary antibodies and dilutions used. Sections were then rinsed and incubated in the appropriate biotinylated secondary antibody (Vector Laboratories following the manufacturer's instructions); further processing was with the Vector ABC method using the Vectastain Elite kit. Immunoreactivity was visualized using the glucose oxidase modification of the diaminobenzidine (DAB) method (Shu et al., 1988; Van Der Gucht et al., 2006).

Antibody characterization and specificity

CD11b (Serotech #MCA275R). The immunogen was resident in rat peritoneal macrophages. The antibody recognized the rat integrin alpha-M, the receptor for the iC3b component of complement, expressed on most macrophages, and recognizes brain microglia (Manufacturer's data sheet and Robinson et al., 1986). The antibody immunoprecipitates polypeptide chains of 160 and 95 kDa (Robinson et al., 1986). Antibodies to CD11b have been used to immunostain microglia in the brain (Jeong et al., 2010; Maroso et al., 2011; Fuentes-Santamaria et al., 2013).

CD68 (Serotech #MCA341GA). The protein ED1/cd68 was first recognized in the cytoplasm of peripheral macrophages and is considered a macrophage marker, with the level of expression correlated with phagocytosis (Damoiseaux et al., 1994). It is only weakly expressed on the cell surface. The properties of this antibody were summarized by Damm et al. (2011), based in part on information from the manufacturer. The antibody recognizes a glycosylated protein of 90–110 kD; the level of CD68 expression in a cell is correlated with phagocytic activity. The antibody has been used specifically to immunostain microglia in rat or mouse brain sections and in primary glial cultures (Bauer et al., 1994; Hughes et al., 2002; Wuchert et al., 2009).

OX6 (Serotech #MCA46R). As summarized by Berglof et al. (2009), the antibody was raised against rat thymocyte membrane, purified by affinity chromatography on Protein G from tissue culture supernatant and recognizes a monomorphic determinant of the rat 1-A antigen. It has been used to label activated microglia (Ogura et al., 1994; Cho et al., 2006).

SV2 (DSHB, supernatant form). The monoclonal antibody SV2, developed by Dr. K. M. Buckley, was

Table 1. Antibodies and dilutions

Antigen	Company, Catalogue #	Host	Dilution
CD11b	AbD Serotech Cat # MCA275R	Ms	1:300
CD68	AbD Serotech Cat #: MCA341GA	Ms	1:500
OX6	AbD Serotech Cat #: MCA46R	Ms	1:300
SV2	Developmental Studies Hybridoma Bank, supernatant form	Ms	1:50