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AGING PROFOUNDLY DELAYS FUNCTIONAL RECOVERY FROM GUSTATORY NERVE INJURY

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Abstract—The peripheral taste system remains plastic during adulthood. Sectioning the chorda tympani (CT) nerve, which sends sensory information from the anterior tongue to the central nervous system, causes degeneration of distal fibers and target taste buds. However, taste function is restored after about 40 days in young adult rodents. We tested whether aging impacts the reappearance of neural responses after unilateral CT nerve injury. Taste bud regeneration was minimal at day 50–65 after denervation, and most aged animals died before functional recovery could be assessed. A subset (n=3/5) of old rats exhibited normal CT responses at day 85 postsectioning, suggesting the potential for efficient recovery. The aged taste system is fairly resilient to sensory receptor loss and major functional changes in normal aging. However, injury to the taste system reveals a surprising vulnerability in old rodents. The gustatory system provides an excellent model to study mechanisms underlying delayed recovery from peripheral nerve injury. Strategies to accelerate recovery and restore normal function will be of interest, as the elderly population continues to grow.

Key words: chorda tympani nerve, taste bud, taste receptor cell, sensory, plasticity, neural-immune interactions.

Injured peripheral nerves can regenerate, though age at the time of injury has a tremendous influence on recovery. Regeneration is typically delayed and incomplete in elderly patients and in aged animals sustaining neural injury (Kerezoudi and Thomas, 1999; Verdu et al., 2000). The impact of aging on regeneration varies depending on the model system, nerve, and even subpopulation of peripheral neurons injured (Kovacic et al., 2009). For example, injury leads to inefficient, partial recovery of motor responses in old compared with young rats (Verdu et al., 2000; Apel et al., 2009; Kovacic et al., 2009). Less is known about the regeneration of sensory afferent neurons, reinnervation of target cells, and recovery of sensory function.

The mechanisms underlying aging-induced deficits in regeneration are incompletely understood, but each stage in the process seems vulnerable (Kovacic et al., 2009). During the early postinjury period, aging delays the clearance of neuronal debris by leukocytes (Vaughan, 1992). Aging Schwann cells proliferate less, which decreases the supply of neurotrophic factors available for regenerating axons (Komiyama and Suzuki, 1992). Aging may also disturb neuronal functions necessary for regeneration, including axonal transport to distally growing fibers (McQuarrie et al., 1989). Finally, aged target cells may contribute to delayed or incomplete recovery of nerves and synapses, similar to the regenerating neuromuscular junction (Kawabuchi et al., 2011, Verdu et al., 2000).

The rodent gustatory system provides an excellent model to test the effects of aging on neural degeneration and regeneration. Distinct chorda tympani (CT) nerves innervate ipsilateral taste buds on the anterior tongue. Both the CT nerve and innervated taste buds degenerate following nerve section. Importantly, the CT nerve regenerates, induces the formation of new fungiform taste buds, and regains normal function (Cheal et al., 1977). In approximately 45 days (Hill and Phillips, 1994), gustatory information is successfully sent to the CNS via the regenerated nerve, resulting in control-like taste behavior in rats (St John et al., 1995; Kopka et al., 2000). Thus, functional recovery of a large population of neurons and sensory receptor cells can be assessed in aged animals.

Target organs and peripheral function are relatively resistant to the detrimental effects of aging compared with other sensory systems. Comprehensive studies by McBride and Mistretta (1986) demonstrated similar CT responses in 24- and 3-month-old rats. Likewise, the number of taste buds remains stable throughout the adult life span in rodents (Mistretta and Baum, 1984; Mistretta and Oakley, 1986) and rhesus monkeys (Bradley et al., 1985). Later, quantitative work also indicates that the number of taste buds is maintained in elderly people (Arvidson, 1979), though the number varies over 100-fold between subjects (Miller, 1988, 1989). Elderly subjects do exhibit deficits in gustatory perception (Boesveldt et al., 2011), due at least in part to the increasing frequency of medical conditions, interventional treatments, and medications (Schiffman, 2009). Interestingly, thresholds for some taste modalities may be more susceptible to the effects of aging than others (Murphy and Gilmore, 1989).

Although the normal taste system appears resilient to aging, the injured gustatory nerve system may be more vulnerable. Young patients (i.e. under 21 years old) recover taste sensitivity more quickly after undergoing middle ear surgery compared with older patients. However, cases in which the CT nerve was severed were excluded from analysis (Sone et al., 2001). In the current study, we tested whether recovery of normal taste function is delayed in 24-month-old compared with 3-month-old F344 rats.

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Abbreviations: ANOVA, analysis of variance; CT, chorda tympani; CK-19, cytokeratin-19; IgG, immunoglobulin; LPS, lipopolysaccharide; MPO, myeloperoxidase; NaAc, sodium acetate.

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Remarkably, only a subset of aged rats exhibited CT responses, even at day 80–85 postinjury. This doubles the normal recovery period into the end of life expectancy for this strain (Holmes, 2003). Functional delays were accompanied by belated taste bud regeneration. We suggest that the gustatory system will be useful to investigate mechanisms underlying deficits in regeneration. Strategies to promote functional recovery will be increasingly important, as the aging population maintains an active lifestyle and sustains peripheral nerve injuries.

EXPERIMENTAL PROCEDURES

Animals

The Animal Care and Use Committee at Georgia Health Sciences University approved all protocols, which followed guidelines set by the National Institutes of Health and the Society for Neuroscience (NIH Publications No. 80-23). Every effort was made to minimize the number of animals used and their suffering. Female Fischer (F344) rats were purchased from the National Institute of Aging (Harlan, Bethesda, MD, USA) at 24 or 3 months old. Additional 3-month-old F344 rats were purchased from Charles River (Wilmington, MA, USA). The F344 strain is widely used in aging studies, including those focused on the aging taste system (Mistretta and Baum, 1984; McBride and Mistretta, 1986; Mistretta and Oakley, 1986). CT sectioning or sham surgeries were performed within 1 week of shipment. Rats were housed in a specified pathogen-free (SPF) environment from birth through death.

CT sectioning and lipopolysaccharide (LPS) injection

Old and young rats received unilateral CT section or sham sectioning, as previously described (Hill and Phillips, 1994; McCluskey, 2004; Steen et al., 2010). Rats were injected with atropine sulfate (0.054 mg/kg; i.p.; Med-Pharmex, Inc., Pomona, CA, USA) followed by a mixture of ketamine (40 mg/kg; i.p.; Hospira, Inc., Lake Forest, IL, USA) and xylazine (10 mg/kg; i.p.; Akorn, Inc., Decatur, IL, USA). The CT nerve was exposed by a ventral dissection and transected after its branching from the lingual nerve, before entering the tongue. The CT nerve was visualized but remained intact in sham-sectioned rats. Separate groups of young (3-month-old; $n=4$) and old (24-month-old; $n=4$) rats received an injection of LPS (s.c.; *E. coli* 026:B6; Sigma, St. Louis, MO, USA) to the ventral tongue to stimulate neutrophil invasion. Injections consisted of 10 μg of LPS in 10 μl sterile PBS, as in previous work (Steen et al., 2010).

Leukocyte immunohistochemistry and analyses

Since aging dysregulates innate immune function (Shaw et al., 2010), we tested whether leukocyte responses to nerve injury differed in young and old animals receiving nerve injury or sham sectioning ($n=3–8$/group). Rats were euthanized by overdose with pentobarbital (80 mg/kg i.p.; Hospira, Inc., Lake Forest, IL, USA) at day 2 following nerve sectioning or LPS injection. Both neutrophil and macrophage responses are significantly elevated at this time (McCluskey, 2004; Steen et al., 2010). Tongues were dissected, and 8-μm coronal cyrosections were collected from the anterior, mid, and posterior fungiform fields, as previously described (McCluskey, 2004). Sections were fixed in 0.2% glutaraldehyde (Fisher Bioreagents, Fair Lawn, NJ, USA), and neutrophils identified with a well-characterized rabbit antibody to myeloperoxidase (MPO; 1:1:00; Abcam, Cambridge, MA, USA) (Steen et al., 2010). Activated macrophages were stained with the ED1 antibody (1:400; Serotec, Raleigh, NC, USA) (Dijkstra et al., 1985; McCluskey, 2004; Cavallin and McCluskey, 2005; Guagliardo et al., 2009). Sections were then incubated in biotinylated goat immunoglobulin (IgG) (1:100; Jackson ImmunoResearch, West Grove, PA, USA), avidin-biotin complex (Vector laboratories, Burlingame, CA, USA), and developed in diaminobenzidine (Sigma, St. Louis, MO, USA). We assessed nonspecific immunoreactivity for MPO and ED1 assays using equal concentrations of unimmunized rabbit serum or mouse IgG (AbDserotec, Raleigh, NC, USA), respectively.

Lingual leukocyte responses were analyzed by investigators blinded to experimental condition using MetaMorph software (Molecular Devices/Olympus, Center Valley, PA, USA) and a digital color camera (Cool Snap, Roper Scientific/Photometrics, Tuscon, AZ, USA) (McCluskey, 2004; Cavallin and McCluskey, 2005; Guagliardo et al., 2009; Steen et al., 2010). We analyzed leukocytes on images captured at 50× from four tissue compartments per coronal section as follows: (1) the denervated/sham denervated epithelium and lamina propria; (2) the denervated/sham denervated submucosa and muscle; (3) the intact epithelium and lamina propria; (4) the intact submucosa and muscle. A standardized region of interest (12.9 mm^2) was placed in the region visually determined to contain the highest density of leukocytes. Immunopositive neutrophils were counted in each region. Since macrophages have fine processes that are difficult to count, we analyzed the percentage of immunoreactive pixels/standard area, as in previous studies (McCluskey, 2004; Cavallin and McCluskey, 2005; Guagliardo et al., 2009). Images were minimally processed to enhance color and contrast.

Neurophysiology

We assessed short-term changes in the function of the intact CT ($n=3–5$/group), and longer-term recovery from injury by recording neural responses from the regenerated CT ($n=3–8$/group). We recorded responses from the intact CT of the same rats when possible, though additional rats were used to supplement the data sets as necessary (aged rats frequently died before the lengthy recordings could be completed). Rats were anesthetized with chloral hydrate (525 mg/kg; i.p.; Stacy's Compounding Pharmacy, Atlanta, GA, USA), with supplemental injections as necessary to maintain a surgical level of anesthesia. The CT nerve was dissected, and multifiber recordings were performed, as previously described (Hill and Phillips, 1994; Wall and McCluskey, 2008; Steen et al., 2010). Neural activity was summated and analyzed with PowerLab software (AD Instruments, Inc., Colorado Springs, CO, USA).

Taste stimuli included concentration series (0.05–0.50 M) of NaCl, sodium acetate (NaAc), KCl, and NH_4Cl. We also recorded responses to 0.025–0.20 M citric acid, 0.10 M monosodium glutamate (MSG), 0.01 M quinine hydrochloride (QHCl), 0.01 N HCl, and 1.0 M sucrose. Taste stimuli (Sigma, St. Louis, MO, USA) were dissolved in distilled water and applied to the tongue at room temperature. In longer-term recovery studies, we also tested responses to thermal stimulation (4 °C and 40 °C distilled water) and tactile stimulation with a probe swept across the ipsilateral fungiform taste bud field. Test responses were bracketed by responses to 0.50 M NH_4Cl (i.e. our standard response) and expressed as a percentage of the mean of the two standard responses. Tonic, steady-state responses were measured at a single point 20 s after application of stimuli. Stable series bracketed by responses to NH_4Cl within 10% of each other were included in statistical analyses. Rats were euthanized following the completion of recordings (80 mg/kg pentobarbital, i.p.), and tongues harvested for immunohistochemical staining and analyses.