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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 CNS, 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 post-sectioning, 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.

Keywords

Chorda tympani nerve; taste bud; taste receptor cell; sensory; plasticity; neural-immune interactions

Introduction

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 to young rats (Verdu et al., 2000, Apel et al., 2009, Kovacic et al., 2009). Less is known about the

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Disclosure Statement

The authors have no actual or perceived conflicts of interest to disclose.

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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 post-injury 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., 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 Mistretta and McBride (1986) demonstrated similar CT responses in 24 month and 3 month old rats (1986). Likewise, the number of taste buds remains stable throughout the adult lifespan 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).

While 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 to 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 to 3 month old F344 rats. Remarkably, only a subset of aged rats exhibited CT responses even at day 80–85 post-injury. 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.

Methods

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). Female Fischer (F344) rats were purchased from the National Institute of Aging (Harlan, Bethesda, MD) at 24 or 3 months old. Additional 3 month old F344 rats were purchased from Charles River (Wilmington, MA). 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 one week of shipment. Rats were housed in a specified pathogen-free (SPF) environment from birth through death.

CT sectioning and 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; intraperitoneal (i.p.)] followed by a mixture of ketamine (40 mg/kg; i.p.) and xylazine (10 mg/kg; i.p.). 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 lipopolysaccharide (s.c.; E. coli 026:B6; Sigma, St. Louis, MO) 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) 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 and neutrophils identified with a well-characterized rabbit antibody to myeloperoxidase (MPO; 1:1:00; Abcam, Cambridge, MA)(Steen et al., 2010). Activated macrophages were stained with the ED1 antibody (1:400; Serotec, Raleigh, NC)(Dijkstra et al., 1985, McCluskey, 2004, Cavallin and McCluskey, 2005, Guagliardo et al., 2009). Sections were then incubated in biotinylated goat IgG (1:100; Jackson ImmunoResearch, West Grove, PA), avidin-biotin complex (Vector Labs, Burlingame, CA), and developed in diaminobenzidine (Sigma, St. Louis, MO). We assessed non-specific immunoreactivity for MPO and ED1 assays using equal concentrations of unimmunized rabbit serum or mouse IgG, respectively.

Lingual leukocyte responses were analyzed by investigators blinded to experimental condition using MetaMorph software (Molecular Devices/Olympus, Center Valley, PA) and a digital color camera (Cool Snap, Roper Scientific/Photometrics, Tuscon, AZ)(McCluskey, 2004, Cavallin and McCluskey, 2005, Guagliardo et al., 2009, Steen et al., 2010). We analyzed leukocytes on images captured at 50X from four tissue compartments per coronal section: (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 mm²) 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), with supplemental injections as necessary to maintain a surgical level of anesthesia. The CT nerve was dissected and multifiber recordings 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).

Taste stimuli included concentration series (0.05–0.50M) of NaCl, NaAc, KCl, and NH₄Cl. We also recorded responses to 0.025–0.20M citric acid, 0.10M monosodium glutamate (MSG), 0.01M quinine hydrochloride (QHCl), 0.01N HCl, and 1.0M sucrose. Taste stimuli (Sigma, St. Louis, MO) 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 0.50M NH₄Cl (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 sec after application of stimuli. Stable series bracketed by responses to NH₄Cl 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.

Taste bud identification and counting

We counted taste buds at day 50–65 post-injury according to previous methods (McCluskey and Hill, 2002, Guagliardo et al., 2009). Tongues were harvested and cryosectioned as described above. Mature taste buds were labeled with an antibody to cytokeratin (CK)-19 (1:200; Sigma, St. Louis, MO). CK-19 identifies mature taste receptor cells (Wong et al., 1994, Knapp et al., 1995, Zhang et al., 1995), and detailed methods using this antibody have been published elsewhere (McCluskey and Hill, 2002, Guagliardo et al., 2009). Slides were incubated in biotinylated secondary antibody (Jackson ImmunoResearch, West Grove, PA) followed by avidin-biotin complex (Vector Laboratories, Burlingame, CA), and developed using diaminobenzidine. Taste buds were defined as onion-shaped groups of immunopositive cells in the apical part of fungiform papillae. We counted taste buds that had immunopositive cells in at least four serial sections, which encompasses at least 50% of the total taste bud diameter (Mistretta, 1972). We required the presence of the entire papilla, which prevented counting incomplete taste buds at the beginning and end of collected sections. Counts were performed on the regenerated and intact sides of the tongue from young (n=6) and old (n=6) rats.

Statistical analyses

We compared leukocytes and neurophysiological response magnitudes between groups with one-way ANOVAs followed by Newman-Keuls post-tests using Prism 3.0 (GraphPad software, La Jolla, CA). To compare the number of neutrophils on the sectioned vs. intact

sides of the tongue within age groups, we used Student's *t* tests adjusted by the number of comparisons. Single comparisons between young and aged groups to determine (1) the effect of LPS injection on neutrophil numbers, or (2) age on taste bud regeneration (i.e. Figs. 1F and 6, respectively) were also performed with Student's *t* tests as specified in the text. The α level was set at $p = 0.05$.

Results

Leukocyte responses to nerve injury and inflammation in aged vs. young adult rats

We first determined whether aging alters early leukocyte responses to CT nerve injury. In both age groups receiving nerve injury, neutrophils are found at relatively equal levels in fungiform papilla, the lamina propria, and lingual mucosa. Qualitatively, the neutrophil response is dramatically increased on the injured side of the tongue in old rats (Fig. 1A–D). We confirmed this by counting neutrophils per standard area at Day 2 after sectioning, and statistically comparing age and surgical groups for each side of the tongue (ANOVAs followed by Newman-Keuls post-tests where appropriate). Indeed, the number of neutrophils is elevated on the injured side of the tongue in old rats compared to young and sham-sectioned groups ($p < 0.001$). Neutrophil numbers were higher in each fungiform field (i.e. from anterior to posterior tongue) in aged vs. young rats, though the response was most notable in the tip region. Neutrophils were also significantly elevated on the uninjured side of the tongue in aged animals, but only compared to young sham-sectioned rats ($p < 0.05$). Within age groups, there was a significant difference between the sides of the tongue in old, but not young rats at Day 2 post-sectioning ($p < 0.01$).

We analyzed the neutrophil response to an inflammatory stimulus, LPS, in a separate group of aged and young animals (Fig. 1F). We previously found that LPS injection to the ventral tongue attracts a significant, bilateral neutrophil response in young animals (Steen et al., 2010). However, the number of neutrophils was similar on each side of the tongue in old and young LPS-injected rats (Student's *t* tests; $p > 0.05$). Thus, the effects of aging on neutrophil entry are specific to denervation.

As the neutrophil response diminishes by day 2 post-injury, macrophages invade both sides of the tongue (McCluskey, 2004). At the same time, normal function returns in the neighboring, uninjured CT nerve (Wall and McCluskey, 2008). While aging alters the dynamics and magnitude of the neutrophil response to injury (above), macrophage responses are spared. Levels of activated macrophages in the anterior tongue were not significantly different in aged compared to young rats after CT sectioning ($p > 0.05$; not shown). Moreover, taste responses recorded from the uninjured CT were not significantly different in aged vs. young rats at this time ($p > 0.05$; not shown). Thus, aging resulted in fairly specific changes in the early post-injury period. The neutrophil response to injury was more robust on the injured side of the tongue in old vs. young rats, but only slightly elevated on the uninjured side of the tongue. Moreover, neither macrophage responses nor neural function in the contralateral nerve were altered in aged animals.

Long-term recovery of taste function in aged vs. young animals

The severed CT typically regenerates and functions normally within 40–45 days (Hill and Phillips, 1994). As shown in Figure 2A, robust responses were recorded from young F344 rats at day 45–50 post-sectioning. The regenerated CT was observed in aged rats, though the nerve is dissected for neurophysiology proximally to the injury site and presumably, Wallerian degeneration of distal fibers. Importantly, the regenerated nerve in aged rats failed to respond to tastants, cooling, warming, or tactile stimulation of the sensory field (Fig. 2A).

Mean response ratios from the functional CT nerves are shown in Figure 3. Responses to some concentrations of sodium were statistically different in the regenerated CT of young rats compared to other nerves/groups. Specifically, mean relative responses to 0.10M NaCl were slightly lower in the young regenerated CT ($p < 0.05$ vs. responses from the intact CT nerve of the aged group), while responses to 0.25 NaCl and 0.50M NaAc were significantly elevated ($p > 0.05$ vs. the intact nerve from young or both groups, respectively). ANOVA revealed a significant main effect in responses to 0.50 NaCl, but no significant differences between groups emerged in posttests. Altered responses in the regenerated CT were unexpected given previous work (Hill and Phillips, 1994, McCluskey and Hill, 2002), but may relate to the enhanced salt sensitivity of the F344 strain (Grill and Bernstein, 1988, Bernstein et al., 1991).

At day 65–70 post-sectioning, the regenerated CT was still unresponsive to taste, thermal, and tactile stimuli in the aged group (Fig. 2B). The regenerated CT from young rats responded robustly, as expected. Mean responses from young rats and the intact CT of old rats were generally similar, with a few exceptions (Fig. 4). First, the trend toward an altered NaCl dose-response function in the regenerated CT of young rats continued during this post-injury period, though only the response to 0.25 M NaCl differed significantly from other group's responses ($p < 0.05$ vs. young regenerated CT). Second, responses to 0.05 M NaCl and 0.10 M NH_4Cl were increased in the intact CT from old rats ($p < 0.05$ vs. both nerves/groups). Third, responses to bitter and sour stimuli were slightly but significantly different from other responses as shown (Fig. 4; $p < 0.05$).

Few aged rats survived until 80–85 days post-injury, and fewer survived the surgical anesthesia necessary for neurophysiology. However, the regenerated CT responded to taste, tactile, and thermal stimuli in 3/5 old rats in which functional recovery could be assessed. Recovery was “all or nothing” in this group. Control-like neural responses were obtained in a subset of aged animals (Fig. 2C), while the regenerated CT of others was unresponsive. Mean relative responses are shown in Figure 5 for comparison, though the variance was high given the small size of the aged group ($n=3$). As such, there were no significant differences between groups at this period ($p > 0.05$). In sum, normal gustatory function returns in a subset of aged rats at day 80–85 post-injury, which is double the recovery period of young rats and extends into the median life expectancy for this strain (Holmes, 2003).

Regeneration of taste buds

Rodent taste buds degenerate soon after CT sectioning, and reappear following regeneration of the nerve (Cheal and Oakley, 1977). However, this process has not been examined in aged animals. To determine whether taste bud regeneration is impaired along with deficits in neural function, we counted taste buds on the both sides of the tongue at day 50–65 post-sectioning. As shown in Figure 6, the taste cell marker (CK-19) identifies sensory cells that form well-organized taste buds in young rats, and on the uninjured side of the tongue in aged rats. In old rats, few or no immunopositive taste cells were observed on the previously denervated side of the tongue. Indeed, the number of regenerated taste buds was drastically reduced in aged vs. young rats (Student's t test; $p < 0.001$). The few taste buds present, however, appeared morphologically similar to those on the intact side, and to those in young rats. Taste bud numbers on the intact sides of the tongue were comparable between age groups (Student's t test; $p > 0.05$). Thus, few regenerated taste buds are available for transmitting sensory information to the CT nerve and CNS in aged animals. At the same time post-injury, taste buds have regenerated and peripheral gustatory input to the brain is restored in young adults.

Discussion

We demonstrate that the aged peripheral gustatory system is more vulnerable to injury than expected, as summarized in Fig. 7. Aging increases early neutrophil response to injury in the peripheral taste system. More profoundly, taste bud regeneration and recovery of sensory responses is delayed by at least 35 days. Most aged rats died before demonstrating functional recovery, which provides the most clinically relevant indicator of regeneration. These aging-induced deficits in regeneration contrast with the enormous capacity for regeneration in the adult taste system (Guth, 1971, Cheal et al., 1977). Normal aging (i.e. in the absence of axotomy) has little effect on peripheral taste function in animal models (Mistretta, 1989), though elderly humans demonstrate more significant changes in taste perception (Schiffman, 2009). Thus, these findings were unexpected in light of the stability of the aging taste system and the well-known ability to recover from nerve injury.

In general, the recovery of CT and taste bud function is more severely affected than in other injured peripheral systems. After sectioning, CT fibers regenerate, induce taste bud regeneration, and reform synapses with new taste receptor cells before function is restored. This process was prolonged or failed to occur in some animals. Recovery from motor nerve injuries tends to be more efficient in aged animals, though studies vary in animal age and type of injury. For example, reappearance of whisker movements was delayed by just 4–5 days in aged (15 month old) compared to young (3 month old) rats after facial nerve crush (Vaughan, 1992). Likewise, the digital extension reflex recovers 1–2 weeks later in aged (24 month old) vs. young (1 month old) rats after sciatic nerve compression (Drahota and Gutmann, 1961). The latter authors noted that the period to achieve functional recovery varied more in old rats, similar to our observations at day 80–85 post-sectioning (Drahota and Gutmann, 1961). However, the aged taste system's exaggerated vulnerability to injury is not a feature of all chemosensory systems. The injured vomeronasal epithelium retains a remarkable ability to regenerate new neurons, even in 24 month old mice (Brann and Firestein, 2011).

Aging may impair regeneration and recovery at several sites, including the denervated taste buds. We demonstrate that taste buds regenerate by day 50–65 post-sectioning in young adult but not aged animals. The progenitor population that generates new taste receptor cells may be reduced by the end of the lifespan. However, CT responses and taste bud size remain fairly stable in aged rats, suggesting that any changes in normal turnover are subtle in aged animals (Mistretta, 1984). Yet, denervation of taste receptor cells places additional demands on progenitors as the ipsilateral target population must be replaced. Perhaps taste precursors, unlike vomeronasal progenitors, are unable to proliferate efficiently to replenish taste buds (Brann and Firestein, 2011). Withdrawal of trophic support upon CT sectioning may lead to taste progenitor cell de-differentiation or even death (Hosley et al., 1987).

Injury reveals deficits in the ability to upregulate needed trophic factors in aged peripheral nerves. Aging may also reduce trophic support available for taste progenitor cells, new taste receptor cells, and/or the regenerating CT nerve. Vascular endothelial growth factor (VEGF) expression is inhibited in the regenerating sciatic nerve of aged mice, which diminishes angiogenesis necessary for regeneration and functional recovery (Pola et al., 2004). Fortunately, aging nerves may retain the potential for efficient regeneration. For example, exogenous insulin-like growth factor (IGF)-1 improves morphological measures of regeneration in the aged sciatic nerve (Apel et al.). Treatment with nerve growth factor (NGF) permits control-like sympathetic innervation of aged blood vessel transplants (Gavazzi and Cowen, 1993). Trophic interactions between taste targets and innervating nerve fibers are under active investigation in the developing peripheral taste system (Krimm, 2007), and will be of interest for regenerative studies.

The aged CNS is vulnerable to injury, and may hold clues to mechanisms responsible for delayed recovery in the PNS. Studies by Cotman and colleagues reveal surprising parallels between hippocampal denervation in aged rats and our results in the gustatory system. When neural inputs to the dentate gyrus are lesioned, nearby fibers sprout to reinnervate the damaged region and form new synapses with target granule cells. Notably, reinnervation is slower and incomplete in aged (26 month old) compared to young adult (3 month old) rats (Scheff et al., 1978, Cotman and Scheff, 1979). Elevated levels of circulating glucocorticoids in aged rats appear to play an important role in the delayed reinnervation response. Young animals treated with corticosterone or hydroxycortisone, to mimic levels in aged rats, exhibit impaired hippocampal reinnervation (Scheff et al., 1980, Scheff and Cotman, 1982). Clearance of degenerated debris is also compromised in aged rats after denervation (Hoff et al., 1982). These effects may be system dependent, as others report that glucocorticoids promote the myelination and regeneration of the sciatic (Morisaki et al.) and olfactory nerves (Kobayashi and Costanzo, 2009).

Increased glucocorticoid levels and/or delayed removal of cellular debris could contribute to delayed recovery of taste function in aged rats, though reinnervation of target taste cells is accomplished by regenerating CT fibers, rather than collateral sprouting from the intact CT (Kinnman and Aldskogius, 1988). While macrophage responses to CT nerve injury were similar in old and young rats at day 2 post-injury, glucocorticoids could affect other phagocytes, Schwann cells, regenerating neurons, or sensory cells. Aging could also impair macrophage *function* in aged animals, including their ability to scavenge debris resulting from degeneration. Though little is known of glucocorticoid signaling in the peripheral taste system, aldosterone modulates the amiloride sensitivity of CT nerve responses to sodium in young adult rats (Herness, 1992). Aldosterone also suppresses CT responses to sodium and macrophage responses to injury in young adults sustaining contralateral CT sectioning (Guagliardo et al., 2009). These results hint that corticosteroids could impair regeneration in the peripheral taste system by acting through mineralocorticoid receptors.

The immune system plays an important role in neural degeneration and regeneration, and also undergoes significant changes with aging (Shaw et al., 2010). In young adults, CT sectioning upregulates the expression of IL-1 by multiple cell types, including taste receptor cells (Shi et al., in press). Leukocytes also infiltrate the anterior tongue in parallel with increased expression of vascular adhesion molecules and elevated levels of macrophage chemotactic protein (MCP)-1 (McCluskey, 2004, Cavallin and McCluskey, 2007, Cavallin and McCluskey, 2007, Steen et al., 2010). We show here that neutrophil invasion of the denervated side of the tongue is elevated at Day 2 post-injury in old compared to young rats. In previous work using young adult rats, the neutrophil response peaked earlier (12 hr) on the denervated side of the tongue, and returned to baseline by Day 1 (Steen et al., 2010). Moreover, neutrophil numbers were ~8-fold higher in aged rats than previously observed. Taken together, these findings suggest that the neutrophil response to nerve injury is amplified, and extended or delayed in aged animals.

Future studies will determine whether neutrophils are implicated in the delayed taste bud regeneration and functional recovery in aged animals. Neutrophils have a short response period (i.e. days) and would be amenable to suppressive treatment. Our results suggest that nerve injury and inflammation differentially upregulate neutrophil recruitment signals in aged rats, which may also be exploited to control their response. Neutrophil responses to sterile, thermal injury in other tissues may provide clues. Sterile injury, such as nerve degeneration, elicits overlapping but distinct neutrophil recruitment pathways compared to infection. For example, thermal injury in the liver induces the release of formylated peptides ringed by a gradient of the CXC-chemokine ligand 2 (CXCL2) (McDonald et al.). This allows focused neutrophil recruiting to sterile injury sites, which protects intact tissue from

the detrimental effects of neutrophils. Divergent homing signals may also contribute to our results showing a localized response to nerve injury vs. the bilateral response to bacterial LPS in aged rats, though the signals may be tissue-specific (Harding and Kubes, 2011).

In sum, we demonstrate that the aged taste system is surprisingly vulnerable to the effects of nerve injury given the plasticity exhibited in young adults (Hill and Phillips, 1994, Hendricks et al., 2002). This fragility at the end of the lifespan is reminiscent of that in the early neonatal taste system, where taste buds fail to regenerate after nerve injury (Hosley et al., 1987, Sollars and Bernstein, 2000). The outlook for functional recovery after peripheral nerve injury may be less bleak. While only a few rats survived to the longest post-sectioning period, 60% of those who did survive demonstrated normal neural responses to taste, thermal, and tactile stimuli. Application of exogenous growth factors has also been successful in promoting morphological regeneration, as discussed above. Together, these findings suggest that aging peripheral nerves and target cells retain the capacity for regeneration and recovery after injury. The peripheral gustatory system will be advantageous for future work to determine whether intracellular and intercellular signaling components are retained and can be manipulated to promote and accelerate recovery throughout the lifespan.

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Figure 1.

Neutrophil responses to nerve injury and inflammation in old and young rats collapsed across fungiform regions. (A–D) Myeloperoxidase (MPO)-positive neutrophils (brown) are shown in the lingual mucosa of the denervated (A, B) and intact (C, D) sides of the tongue at day 2 after CT nerve sectioning. More neutrophils infiltrate the injured side of the tongue in aged rats (B). (E) The number of neutrophils/standard area is significantly increased on the injured side of the tongue compared to the injured side of other groups (** $p < 0.001$). A significant increase is also observed on the uninjured sides of the tongue in aged rats compared to young rats receiving sham sectioning (* $p < 0.05$). (F) The number of neutrophils responding to lingual injection of lipopolysaccharide (LPS) is unaffected by age. Scale bar in D = 50 μm .

Figure 2.

Representative response traces from the regenerated CT nerve of young and old rats at increasing post-injury periods. Responses to 0.50 M NH_4Cl bracket responses to test stimuli, and demonstrate the stability of the preparation. (A) At 45–50 days after injury, the CT regenerates and exhibits robust responses to multiple tastants in 3 month old rats. The regenerated CT fails to respond to taste stimuli in 24 month old rats. Traces from this nonfunctional nerve show neural artifacts not associated with stimulus onset. Increased gain (in an attempt to amplify potential neural activity) results in a more variable baseline in the old vs. young rats. (B) At day 65–70 post-sectioning, the regenerated CT responds well to multiple tastants in young animals. The regenerated nerve still fails to respond to stimuli in old rats at this time. (C) By day 80–85 post-injury, the regenerated CT responds to taste stimuli in young rats and a subset of old rats. Bar under initial NH_4Cl responses = 20 sec.

Day 45-50 Post-Sectioning

Figure 3.

Mean relative responses $\pm$ S.E.M. from the regenerated and intact chorda tympani (CT) nerves at 45–50 days post-injury. The regenerated CT of old animals was unresponsive at this period. Most response ratios from the functional nerves ($n=5-6$ /group) were not significantly different, with the exception of responses to 0.10 M and 0.25 M NaCl, 0.50 M sodium acetate (NaAc) and 0.10 M monosodium glutamate (MSG). (* $p < 0.05$).

Day 65-70 Post-Sectioning

Figure 4.

Mean relative responses $\pm$ S.E.M. from the regenerated and intact chorda tympani (CT) nerves at 65–70 days post-injury. The regenerated CT of aged rats did not exhibit responses. Responses from young CT nerves and the intact CT of old rats were generally similar, though a few statistical differences were observed as noted (* $p < 0.05$).

Figure 5.

Mean relative responses $\pm$ S.E.M. from the regenerated and intact chorda tympani (CT) nerves at 80–85 days post-injury. The regenerated CT responded to taste, tactile, and thermal stimuli in a subset ($n=3/5$) of aged rats at this period. Response magnitudes were not significantly different between groups at this period ($p<0.05$).

Figure 6.

Taste bud regeneration in young and aged animals at 50–65 days post-injury. Cytokeratin (CK)-19 positive taste buds (brown) are present on both sides of the tongue in young rats, and on the uninjured side in old rats, during this period. Few immunopositive taste buds regenerated in old rats. The number of taste buds is significantly reduced on the previously sectioned side of the tongue in old vs. young animals (** $p < 0.001$).

Figure 7.

Timeline of aging-induced changes in the injured peripheral taste system. Neutrophil responses to nerve sectioning are amplified and extended during the early post-injury period. Aging induces more drastic changes during regeneration as the time to recovery is doubled in aged compared to young adult rats.