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Effects of aging and levodopa on the laryngeal adductor reflex in rats

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Abstract

Dopaminergic neurotransmission plays an essential role in sensorimotor function, and declines with age. Previously, we found the laryngeal adductor reflex (LAR) was increased in excitation by a dopamine receptor antagonist. If this airway-protective reflex is similarly affected by aging, it will interfere with volitional control in older adults. The current study tested whether the LAR was affected by aging, and whether such deficits were reversed by levodopa administration in aging rats. We recorded thyroarytenoid (TA) muscle activity at rest and during elicitation of LAR responses by stimulation of the internal branch of the superior laryngeal nerve (iSLN) in 6-, 18- and 30-month-old rats under alpha-chloralose anesthesia. Using paired stimuli at different inter-stimulus intervals (ISIs), LAR central conditioning, resting muscle activity, and reflex latency and amplitudes were quantified. Numbers of dopaminergic neurons in the substantia nigra pars compacta (SNpc) were measured using tyrosine hydroxylase staining. We found: (1) increased resting TA muscle activity and LAR amplitude occurred with fewer dopaminergic neurons in the SNpc in 18- and 30-month-old rats; (2) decreases in LAR latency and increases in amplitude correlated with reduced numbers of dopaminergic neurons in the SNpc; (3) test responses were greater at 1000 ms ISI in 18-month-old rats compared with 6-month-old rats; and (4) levodopa administration further increased response latency but did not alter muscle activity, response amplitude, or central conditioning. In conclusion, increases in laryngeal muscle activity levels and re-flex amplitudes accompanied age reductions in dopaminergic neurons but were not reversed with levodopa administration.

Keywords

Aging; Laryngeal adductor reflex; Dopaminergic neurons; Levodopa; Rat

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1. Introduction

Age-related swallowing disabilities in older adults can greatly limit their ability to eat, drink, or communicate comfortably; such disabilities are extremely difficult to treat (Ashley et al., 2006). The underlying pathophysiological mechanisms that contribute to these disabilities may be sensorimotor (Mortelliti et al., 1990). The laryngeal muscles may undergo sarcopenia (Kersing and Jennekens, 2004; McMullen and Andrade, 2006; Rodeno et al., 1993). In addition, age-related sensory desensitization (Aviv, 1997; Rosenberg et al., 1989) and denervation (McMullen and Andrade, 2009; Takeda et al., 2000) may lead to the deterioration of vocal fold closure reflexes (Shaker et al., 2003). These reflexes are necessary for adequate vocal fold closure to defend against repeated entry of substances into the lungs that could cause aspiration pneumonia. The laryngeal adductor reflex (Battaglia et al., 2006) is a bilateral brainstem-mediated sensorimotor reflex (Sun et al., 2011) and an important protective reflex for the upper airway (Sasaki et al., 1997). Its disturbance could result in increased risk of aspiration (Aviv et al., 2002; Sasaki et al., 2006). Poor control of this reflex has been reported in the elderly and in patients with aspiration (Aviv et al., 2002; Shaker et al., 2003; Zamir et al., 1996).

The afferent fibers in the internal branch of the superior laryngeal nerve (iSLN) involve cell bodies in the nodose ganglion terminating in the brainstem solitary nucleus tract (NTS). Stimulation of these afferents produces vocal fold adduction via the laryngeal motor neurons in the nucleus ambiguus (Ambalavanar et al., 2004; Gestreau et al., 1997; Jean, 2001; Mrini and Jean, 1995; Sessle, 1973; Sun et al., 2011). Sensory deficits and laryngopharyngeal muscle weakness associated with aging may increase the risk of aspiration in aging adults (Aviv, 1997; Aviv et al., 2002; Martin et al., 1994; Rosenberg et al., 1989). LAR modulation at the brainstem level is reflexive, and may interact with volitional control in higher levels of the central nervous system. It is unclear how LAR modulation is affected by the central nervous system changes in neurotransmission in aging.

Dopaminergic neurons are reduced with advancing age at a rate of 4.7% per decade (Fearnley and Lees, 1991), with more than one-third of the loss occurring between 20 and 90 years of age (McGeer et al., 1988). Loss of dopaminergic neurons in normal aging contributes to motor slowing, movement dysfunction, and memory loss (Emborg et al., 1998; Fozard et al., 1994; Winblad et al., 1985; Zhang et al., 2001). Although age-related loss of dopaminergic neurons and reduced dopaminergic metabolism (Qureshi et al., 1990) contribute to the decline in motor function in the elderly, it is unknown whether reduced laryngeal motor control is related to decreases in dopamine. The blink reflex is neurophysiologically similar to the LAR and has shown changes related to aging and dopamine loss in human and animal models (Basso et al., 1993; Battaglia et al., 2006; Peshori et al., 2001). Recent evidence suggests that dopamine may play an important role in the modulation of upper airway protection, as infarction in the basal ganglia can induce deficits in swallowing and the cough re-flex, increasing the risk of aspiration pneumonia in elderly patients (Kikawada et al., 2005).

On the other hand, in a rat model, dopamine receptor antagonists had a positive effect on the LAR, reducing its latency and increasing its amplitude (Feng et al., 2009). Levodopa, a dopamine agonist, is an established treatment for limb tremor in Parkinson's disease, and improves upper limb motor performance in aged non-human primates (Grondin et al., 2000). However, in patients with Parkinson's disease, levodopa does not improve voice and swallowing deficits to the same degree as limb control (Ho et al., 2008). Whether levodopa improves laryngeal motor activity in older patients has received little attention (Gallena et al., 2001).

The present study examined the effects of aging, age-related dopamine loss, and levodopa on the LAR in an established rat model. The LAR can be elicited either by electrical stimulation of afferents in the superior laryngeal nerve (SLN) or by deflection of mechanoreceptors in the laryngeal mucosa. Unilateral short-latency R1 responses and bilateral long-latency R2 responses in rats are somewhat similar to those in humans; only consistent R2 responses were elicited in the thyroarytenoid (TA) muscle with iSLN stimulation in rats in our previous study (Feng et al., 2009). Paired stimuli at different interstimulus intervals (ISIs) are used to estimate central conditioning effects on LAR by evoking activation of inhibitory inter-neurons on test responses. As blink oscillation increases with aging and dopamine loss, brain stem reflex conditioning may be modulated by dopaminergic neuronal pathways (Peshori et al., 2001). We hypothesized that (1) aging alters the LAR and its central conditioning; (2) these changes may be related to the loss of dopaminergic neurons in the brain, and (3) levodopa may modulate age-related changes in the LAR.

2. Methods

2.1. Animals

Twenty-four male Fischer 344×Brown Norway F1-hybrid (F344BN) rats were bought from the National Institute on Aging and were composed of three groups: 6, 18, and 30 months of age (8/each group). Rats were maintained on a 12-hour light/dark cycle and given ad libitum access to food and water. All procedures carried out in these experiments were approved by the Institutional Animal Care and Use Committee of Wake Forest University.

2.2. Surgical procedure

All surgical procedures were described in detail previously (Feng et al., 2009). Briefly, on the day of surgery, rats were anesthetized with isoflurane (3–4% mixed with 100% oxygen) (Sigma, St. Louis, MO) and maintained on a ventilator via tracheal intubation (Kent Scientific, Torrington, CT). The head of each rat was fixed on a stereotaxic frame (Stoelting, Indianapolis, IN) in the supine position. Due to the inhibitory effects of isoflurane on laryngeal response, its dosage was gradually reduced while alpha-chloralose (Sigma) solution was administered by i.v. drip into the tail vein (18 to 36 µl/min over 3 h). The right/left SLN was exposed and positioned over a hooked bipolar platinum stimulating electrode (FHC Inc., Bowdoin, ME) and connected to an A365 stimulus isolator (World Precision Instruments, Sarasota, FL). The stimulation site was immersed in warm mineral oil. For electromyography (EMG) recordings, two Teflon-coated stainless wires (0.011 mm coated

diameter) (California Fine Wire Co., Grover Beach, CA) with 1 mm bared at the tips and contained in a 27 gauge needle were inserted through the cricothyroid space into the right and left TA muscles, respectively. The response of the ipsilateral TA muscle (same side as the stimulation) was recorded throughout the study.

The external branch of the SLN was sectioned to eliminate cricothyroid responses, which would interfere with TA muscle recordings of the LAR. Electrical stimulation of the iSLN began at 15 μ A with a pulse width of 0.2 ms, and was increased until the threshold level for eliciting a reliable laryngeal adductor R2 response was determined. The SLN stimulus intensity level was then set at three times threshold to elicit the supramaximal R2 response for that rat throughout the experiment. Pairs of stimuli were administered at 250, 500, 1000, 2000, and 5000 ms ISIs, with the response to the first stimulus (conditioning stimulus), considered as the conditioning response, followed by the response to the second stimulus (test stimulus), considered as the test response. The percent change in a test response from a conditioning response at different ISIs was measured to estimate central conditioning effects on LAR. The stimulation rate was programmed using a Master-8 device (A.M.P. Instruments Ltd., Jerusalem, Israel). At least 40 s occurred between stimulus pairs to avoid habituation, and 5–6 trials were performed at each ISI. The EMG signals were amplified by Bio Amp (AD Instruments, Colorado Springs, CO) and recorded on a computer with Chart 7 for Windows (AD Instruments) for off-line analysis.

At the end of each experiment, the recording wires were cut from the outside, leaving the tips of the wires inside the laryngeal muscles for later verification. We analyzed only the data collected from rats in which the electrode position was confirmed to be in the TA muscle.

2.3. Levodopa administration

One set of LAR measurements was recorded in each rat, and then the rat was maintained under quiet conditions for 30 min. After a 0.25 ml bolus solution containing levodopa (6 mg/kg, Sigma) and dopa decarboxylase inhibitor benserazide (15 mg/kg, Sigma) was administered by i.v., another set of LAR measurements was recorded beginning 5 min after drug administration.

2.4. Histological staining

Dopaminergic neurons in the brains of rats in each age group were examined quantitatively in the substantia nigra par compacta (SNpc) by staining with tyrosine hydroxylase (TH). After the LAR study, animals were euthanized with alpha-chloralose overdose and perfused intracardially with phosphate buffer solution (PBS) followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Brains were removed, post-fixed in paraformaldehyde solution overnight, and cryoprotected in 30% sucrose PBS solution for 2 days. Coronal cryostat sections of 40 μ m were cut serially through the SNpc, blocked in goat serum for an hour, and incubated overnight in a rabbit polyclonal anti-TH antibody solution (1:2000, Sigma, St. Louis, MO). After they were washed with PBS, slices were incubated with a biotinylated goat anti-rabbit IgG as secondary antibody (1:200, Vector Laboratories) for an hour. Sections were washed in PBS before incubating for an hour with avidin–biotin–peroxidase complex (ABC kit,

Vector Laboratories, Burlingame, CA) in PBS. Slices were washed in PBS, and then incubated for 2–10 min in hydrogen peroxide and 3,3'-diaminobenzidine tetrahydrochloride (DAB kit, Vector Laboratories). Sections were washed in PBS before being mounted on slides, dehydrated, and prepared for light microscopy. TH+ soma were counted using TSVIEW 7.1 (Tucsen Imaging Technology, Elmsford, NY) by an investigator blinded to the age groups. The first ten sections were counted by two investigators and the results were analyzed. Because no significant differences in results were found between the two investigators, one investigator did all the subsequent counting. In all animals, each brain section was reconstructed with the help of a stereotaxic atlas of the rat brain (Paxinos and Watson, 2007). In each rat, 10 brain sections (a distance of 120 μ m between sections) spaced through the SNpc between –4.56 mm and –6.6 mm from the Bregma were chosen for evaluation. The optical fractionator method (Manfredsson et al., 2007) was used to quantify the total number of cell bodies containing TH+ neurons in the SNpc from both sides of 10 brain sections in each rat.

2.5. Data analysis

Analysis of the TA EMG signals was described previously (Feng et al., 2009). EMG signals were acquired, stored on a computer, and visually analyzed off-line by marking the onset and offset of each laryngeal response using Chart 7 software (AD Instruments). After rectification, the mean level of resting activity in the TA muscle was measured in microvolts. The resting levels of muscle activity over 20 ms before each conditioning stimulation were averaged and then normalized by the maximum peak of the R2 response at each trial accordingly (Normalized resting muscle activity = $\text{mean baseline} \times 100 / \text{maximum peak of R2 response}$). Normalized resting muscle activity, latency and normalized integrated amplitude (Normalized integrated amplitude = $\text{Integrated amplitude of response} - \text{Mean baseline} \times \text{Duration of the response}$) were measured and averaged over 25–30 trials (5–6 per ISI). Percent change in a test response from a conditioning response (Percentage change = $[\text{Integrated amplitude of test response} - \text{Integrated amplitude of conditioning response}] \times 100 / \text{Integrated amplitude of conditioning response}$) at each ISI was calculated over 5–6 trials.

SAS (version 9.2) was used for all data analysis. A mixed effect model was used to compare the LAR measures between different age groups. Using a repeated analysis, LAR changes due to levodopa administration within the same age group were examined. Post-hoc analysis was performed using a *t*-test with the Bonferroni adjustment method to compare the dopaminergic neurons in the SNpc among different age groups. Pearson correlation coefficients were used to detect correlations between LAR measures and the numbers of dopaminergic neurons in the SNpc. A *p* value of <0.05 was considered significant.

3. Results

3.1. Effects of aging on the LAR

No differences in the LAR threshold (A) and latency (C) were found between age groups ($p > 0.05$) (Fig. 1). TA muscle resting activity level (tone) was increased in 18-month-old rats ($p < 0.01$) and 30-month-old rats ($p < 0.05$) compared to 6-month-old rats (B). The integrated

amplitude of the LAR was increased with aging (D), and significant differences were found between 6- and 30-month-old rats ($p<0.05$).

3.2. Effects of aging on the central conditioning changes of LAR

In the 6-month-old rats, the test response percent change in amplitude from the conditioning response showed a central inhibitory effect on the LAR ($<0\%$) at 250 and 500 ms ISIs, and a central facilitation on the LAR at 2000 ms ISI with limited central effects at 1000 ms ISI or 5000 ms ISI (Fig. 2). In the 18- and 30-month-old rats, the test LAR was increased at 1000 ms ISI, suggesting a centrally facilitated effect on LAR conditioning with aging (Fig. 2). The increases were significant at 1000 ms ISI in the 18-month-old group compared with the 6-month-old group ($p=0.02$).

3.3. Effects of aging on dopaminergic neurons in SNpc

Fig. 3A and B shows TH staining in the SNpc. TH+ neurons in the SNpc decreased with aging (Fig. 3C). There were fewer TH+ neurons in the SNpc in the 30-month-old rats than in the 6-month-old rats ($p<0.01$) and a non-significant trend for a decrease in the 18-month-old rats compared to the 6-month-old rats ($p=0.07$). The mean percentage decrease of TH+ neurons was 26% in the 30-month-old rats and 16% in the 18-month-old rats compared to the 6-month-old rats.

3.4. Aging-associated correlation between the LAR and dopaminergic neurons in the brain

We found a trend toward an inverse correlation between the TH+ neurons in SNpc and resting levels of TA muscle activity (tone) ($r=-0.39$, $p=0.06$) (Fig. 4A). A significant correlation was found between the numbers of TH+ neurons in SNpc and LAR latency (Fig. 4B; $r=0.47$, $p=0.02$), indicating that fewer TH+ neurons were associated with a shorter LAR latency. In addition, a negative correlation between TH+ neurons and the LAR amplitude (Fig. 4C; $r=-0.50$, $p=0.014$) suggested that fewer TH+ neurons were associated with a larger LAR amplitude.

3.5. Effects of levodopa on LAR responses

Among all three age groups, we found no significant effects of levodopa on TA resting muscle activity (Fig. 5A), latency (Fig. 5B) or LAR response amplitude (Fig. 5C). Because no age-related differences were found on LAR latency, the age groups were combined for the analysis of levodopa effects on latency. When all groups were combined, LAR latency was increased after levodopa administration ($p<0.05$).

3.6. Effects of levodopa on the central control of LAR conditioning

No significant differences in the conditioning effects were found with the administration of levodopa at any ISIs in any of the age groups (Fig. 6A, B, and C).

4. Discussion

The purpose of the current study was to determine how the LAR, a laryngeal protective reflex, is altered by aging in the rat model. We examined the effects of dopamine depletion with aging as well as whether levodopa altered the LAR during normal aging. The major

findings were: (1) increased resting TA muscle activity with aging; (2) increased LAR (amplitude) and increased central facilitation of the LAR in aging rats; (3) fewer dopaminergic neurons in aging brains (SNpc) related to decreased response latency and increased response amplitude; and (4) levodopa increased LAR latency but had no effect on resting TA muscle activity, amplitude, or central conditioning. Overall, age-related LAR changes were correlated with deterioration of the dopaminergic neural pathway during aging.

These changes suggest an increased level of overall motor-neuron firing at rest in the laryngeal muscles and a general facilitation of the LAR with reduced latencies, and increases in response amplitude and central facilitation of laryngeal responses with aging and dopamine loss. These laryngeal responses to aging and dopamine depletion were similar to our earlier findings with selective D1 dopamine receptor blockade (Feng et al., 2009). Furthermore, a previous study of laryngeal muscle activity in early Parkinson's disease indicated increased laryngeal muscle activity in patients that decreased with acute levodopa administration (Gallena et al., 2001).

4.1. Aging and LAR

The aging effects found here were not predicted based on clinical reports, suggesting age-related deterioration in laryngeal function involves (1) desensitization of mucosa in the pharyngeal and supraglottic areas, as suggested by Aviv (1997); or (2) altered laryngeal motor unit characteristics, suggesting reinnervation on the left side with age (Takeda et al., 2000).

Although clinical evidence has indicated that the laryngeal reflex may be under the control of the central nervous system, a direct link between changes in brain system modulation by aging and LAR is still lacking. We addressed this question in the current study in aging rats. LAR was elicited by directly stimulating the iSLN (the afferent nerve), avoiding effects from possible desensitization of laryngeal mucosa seen with aging (Aviv, 1997).

We found increased resting muscle activity in the TA of older rats, which suggests a higher rate of spontaneous motoneuron firing with reduced dopaminergic neurons associated with aging. This is supported by the hypertonicity of laryngeal muscles reported in patients with Parkinson's disease (Gallena et al., 2001; Zarzur et al., 2007). Second, the integrated amplitude of LAR, when normalized by resting muscle tone, increased in older animals, suggesting that increased activation of inter-neurons in the LAR pathway is accompanied by increased TA muscle activity during normal aging. Third, the degree of change in the test response (from the conditioning response) increased at 1000 ms ISI in older compared to younger rats, suggesting a central facilitatory effect on the LAR with age. This increased central facilitation may also indicate reduced inhibition on the LAR pathway during normal aging.

The laryngeal response is suppressed after swallowing in normal people (Barkmeier et al., 2000), which may allow a period for residue clearing and a sufficient interval before creating another safe swallow. However, increased muscle tone and central excitatory effects on

laryngeal neurophysiology in the aged may disrupt this pattern, leading to some neural control abnormalities in swallowing in older individuals.

We did not study the effects of age-related changes on LAR modulation during swallowing in these experiments. Future studies of how upregulation of the LAR affects aging and the early stages of Parkinson's disease are needed to evaluate this possible interaction. The changes in the LAR with aging in this study are similar to previous reports on blink reflex, which showed that increased trigeminal blink excitability and blink oscillations may play an important role in benign essential blepharospasms associated with aging (Aramideh et al., 1994; Berardelli et al., 1985; Peshori et al., 2001).

4.2. Dopamine depletion and LAR changes during aging

Advanced aging is associated with declining motor function in Parkinson's disease (Bennett et al., 1996; Smith et al., 1999). During normal aging, death of dopaminergic neurons in the substantia nigra is increased due to apoptosis, mitochondrial DNA deletion, and oxidative damage—all changes similar to those in Parkinson's disease (Anglade et al., 1997; Kravtsov et al., 2006). In the present study, dopaminergic neurons in the SNpc of older rats decreased during aging, similar to previous findings in rats (Gao et al., 2011). We found a 16% decrease of dopaminergic neurons in 18-month-old rats and a 26% decrease in 30-month-old rats compared to 6-month-old rats. These rates of loss are comparable to those in the human dopaminergic system, reported at a rate of 4.7% per decade (Fearnley and Lees, 1991), with more than one-third of the loss occurring between 20 and 90 years of age (McGeer et al., 1988).

The central nigrostriatal dopaminergic system plays an important role in the control of motor activity. Although dopamine loss during aging does not necessarily induce Parkinson's disease, deficiencies of limb motor behavior related to dopaminergic system decline in normal aging have been reported (Gash et al., 1999; Yue et al., 2012; Zhang et al., 2000). Whether this age-related decline in the dopaminergic system affects the laryngeal system and limb muscles similarly is not clear.

The current study found a positive correlation between the number of dopaminergic neurons in SNpc and LAR latency, and an inverse correlation between the number of dopaminergic neurons in SNpc and LAR amplitude, suggesting that the laryngeal reflex pathway is less controlled with decreased dopaminergic neurons in the brain during normal aging, similar to findings with blink reflex (Peshori et al., 2001). These findings are consistent with and further explain our previous finding that blockage of dopamine receptors could affect laryngeal muscle activity and laryngeal sensorimotor reflexes (Feng et al., 2009). Importantly, the existence of reciprocal connections between the basal ganglia and the laryngeal motor cortex in squirrel and rhesus monkeys further indicates a possible direct linkage between the dopaminergic pathway and laryngeal reflexes (Jurgens, 2009; Simonyan and Jurgens, 2002, 2003).

4.3. Levodopa and LAR

The current study examined, for the first time, the effects of levodopa on laryngeal neurophysiology in a rat model of aging. The dose of levodopa used in this study was based

on previous reports on levodopa pharmacokinetics and similar studies in rats (Avila et al., 2010; Bredberg et al., 1994). Six $\mu\text{g/kg}$ levodopa plus 15 $\mu\text{g/kg}$ benserazide creates an efficient plasma concentration of levodopa and increases the activity of SNpc neurons. The half-life of levodopa at this dose is over 30 min, assuring its effectiveness throughout the 20-min LAR recording process.

Levodopa partially suppressed the laryngeal sensorimotor reflex by increasing response latency, suggesting an inhibitory effect of levodopa on the laryngeal response. However, we found no effects of levodopa on resting TA muscle activity, LAR amplitude, or central conditioning effects of LAR in any age groups. Thus, levodopa did not completely reverse all effects of aging-related dopaminergic neuron loss on laryngeal response. A possible explanation for this result is that levodopa activates both dopamine 1 and dopamine 2 receptors in the brain. Considering that blockage of the dopamine receptor 1 (but not 2) increased the resting tone of laryngeal muscles and LAR (Feng et al., 2009), a partial effect of levodopa could be related to its higher affinity to the dopamine 2 receptor (Jenner, 2002). Therefore, the current finding, in combination with our previous study (Feng et al., 2009), suggests that the dopamine receptor 1 neural pathway may play a more important role in the control of laryngeal motor activity. In comparison, both dopamine receptors 1 and 2 are involved in blink modulation, but may have opposing effects on that function (Jutkiewicz and Bergman, 2004). These findings suggest that different dopamine receptors may be involved in the modulation of these two brainstem reflexes, and may even have different effects in the same reflex.

To further support our hypothesis on the differential role of D1 and D2 dopamine receptors in LAR regulation, an increased central excitatory conditioning effect on LAR at 1000 ms was found in aging rats, an effect that occurred at 2000 ms ISI in our previous report. This difference suggests that degeneration of the dopaminergic system during aging does not affect the laryngeal reflex conditioning in the same way as the blockage of specific dopamine receptor 1 or as in Parkinson's disease. These discrepancies could be explained by the fact that the two dopamine receptors are involved in the direct and indirect dopaminergic neural pathways in the brain, respectively (Deng et al., 2006). Whether these two receptors oppose, synergize, or have independent effects on laryngeal motor control was not addressed in the current study. Specific dopamine receptor agonist or brain mapping of dopamine receptors on the laryngeal sensorimotor neuromotor pathway will be valuable for further determination of the dopaminergic pathways involved in the modulation of laryngeal neurophysiology.

4.4. Summary

LAR changes with aging were correlated with the loss of dopaminergic neurons in SNpc in rats. The level of activity in the TA muscle, the laryngeal response, and its excitatory central conditioning effects all increased with aging, when stimulating the afferent nerve directly. Those changes may be involved in the impaired control of laryngeal function during aging and may interfere with other functions, such as the normal suppression of the LAR during swallowing (Barkmeier et al., 2000). The decline in volitional control of the larynx – as shown by changes in phonation, swallowing, and respiration during normal aging – may

involve both peripheral denervation (muscle weakness and atrophy) and central nervous system changes in control. Dopaminergic neural pathways are partially involved, with a possible preferential pathway through dopamine receptor 1 (Feng et al., 2009).

5. Conclusions

Age-related changes in laryngeal muscle control and LAR suppression were related to deteriorations in dopaminergic neural pathways and were not modulated by levodopa, possibly because of inadequate numbers of dopaminergic neurons remaining to be modulated by levodopa. These findings question the potential benefits of levodopa for improving laryngeal functions in aging, but open the door to future studies on how specific dopamine receptor agonists affect laryngeal sensorimotor modulation.

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References

- Ambalavanar R, Tanaka Y, Selbie WS, Ludlow CL. Neuronal activation in the medulla oblongata during selective elicitation of the laryngeal adductor response. *J Neurophysiol.* 2004; 92:2920–2932. [PubMed: 15212423]
- Anglade P, Vyas S, Hirsch EC, Agid Y. Apoptosis in dopaminergic neurons of the human substantia nigra during normal aging. *Histol Histopathol.* 1997; 12:603–610. [PubMed: 9225140]
- Aramideh M, Devisser BWO, Deriese PP, Bour LJ, Speelman JD. Electro-myographic features of levator palpebrae superioris and orbicularis oculi muscles in blepharospasm. *Brain.* 1994; 117:27–38. [PubMed: 8149212]
- Ashley J, Duggan M, Sutcliffe N. Speech, language, and swallowing disorders in the older adult. *Clin Geriatr Med.* 2006; 22:291–310. viii. [PubMed: 16627080]
- Avila I, Parr-Brownlie LC, Brazhnik E, Castaneda E, Bergstrom DA, Walters JR. Beta frequency synchronization in basal ganglia output during rest and walk in a hemiparkinsonian rat. *Exp Neurol.* 2010; 221:307–319. [PubMed: 19948166]
- Aviv JE. Effects of aging on sensitivity of the pharyngeal and supraglottic areas. *Am J Med.* 1997; 103:74s–76s. [PubMed: 9422628]
- Aviv JE, Spitzer J, Cohen M, Ma GG, Belafsky P, Close LG. Laryngeal adductor reflex and pharyngeal squeeze as predictors of laryngeal penetration and aspiration. *Laryngoscope.* 2002; 112:338–341. [PubMed: 11889394]
- Barkmeier JM, Bielamowicz S, Takeda N, Ludlow CL. Modulation of laryngeal responses to superior laryngeal nerve stimulation by volitional swallowing in awake humans. *J Neurophysiol.* 2000; 83:1264–1272. [PubMed: 10712454]
- Basso MA, Strecker RE, Evinger C. Midbrain 6-hydroxydopamine lesions modulate blink reflex excitability. *Exp Brain Res.* 1993; 94:88–96. [PubMed: 8101493]
- Battaglia F, Ghilardi MF, Quartarone A, Bagnato S, Girlanda P, Hallett M. Impaired long-term potentiation-like plasticity of the trigeminal blink reflex circuit in Parkinson's disease. *Mov Disord.* 2006; 21:2230–2233. [PubMed: 17078045]
- Bennett DA, Beckett LA, Murray AM, Shannon KM, Goetz CG, Pilgrim DM, et al. Prevalence of parkinsonian signs and associated mortality in a community population of older people. *N Engl J Med.* 1996; 334:71–76. [PubMed: 8531961]

- Berardelli A, Rothwell JC, Day BL, Marsden CD. Pathophysiology of blepharospasm and oromandibular dystonia. *Brain*. 1985; 108(Pt 3):593–608. [PubMed: 4041776]
- Bredberg E, Lennernas H, Paalzow L. Pharmacokinetics of levodopa and carbidopa in rats following different routes of administration. *Pharm Res*. 1994; 11:549–555. [PubMed: 8058614]
- Deng YP, Lei WL, Reiner A. Differential perikaryal localization in rats of D1 and D2 dopamine receptors on striatal projection neuron types identified by retrograde labeling. *J Chem Neuroanat*. 2006; 32:101–116. [PubMed: 16914290]
- Emborg ME, Ma SY, Mufson EJ, Levey AI, Taylor MD, Brown WD, et al. Age-related declines in nigral neuronal function correlate with motor impairments in rhesus monkeys. *J Comp Neurol*. 1998; 401:253–265. [PubMed: 9822152]
- Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain*. 1991; 114(Pt 5):2283–2301. [PubMed: 1933245]
- Feng X, Henriquez VM, Walters JR, Ludlow CL. Effects of dopamine d-1 and d-2 receptor antagonists on laryngeal neurophysiology in the rat. *J Neurophysiol*. 2009; 102:1193–1205. [PubMed: 19535485]
- Fozard JL, Vercryssen M, Reynolds SL, Hancock PA, Quilter RE. Age differences and changes in reaction time: the Baltimore Longitudinal Study of Aging. *J Gerontol*. 1994; 49:P179–P189. [PubMed: 8014399]
- Gallena S, Smith PJ, Zeffiro T, Ludlow CL. Effects of levodopa on laryngeal muscle activity for voice onset and offset in Parkinson disease. *J Speech Lang Hear Res*. 2001; 44:1284–1299. [PubMed: 11776365]
- Gao J, Miao H, Xiao CH, Sun Y, Du X, Yuan HH, et al. Influence of aging on the dopaminergic neurons in the substantia nigra pars compacta of rats. *Curr Aging Sci*. 2011; 4:19–24. [PubMed: 21204780]
- Gash DM, Zhang Z, Umberger G, Mahood K, Smith M, Smith C, et al. An automated movement assessment panel for upper limb motor functions in rhesus monkeys and humans. *J Neurosci Methods*. 1999; 89:111–117. [PubMed: 10491941]
- Gestreau C, Bianchi AL, Grelot L. Differential brainstem Fos-like immunoreactivity after laryngeal-induced coughing and its reduction by codeine. *J Neurosci*. 1997; 17:9340–9352. [PubMed: 9364079]
- Grondin R, Zhang Z, Gerhardt GA, Gash DM. Dopaminergic therapy improves upper limb motor performance in aged rhesus monkeys. *Ann Neurol*. 2000; 48:250–253. [PubMed: 10939577]
- Ho AK, Bradshaw JL, Iansek R. For better or worse: the effect of levodopa on speech in Parkinson's disease. *Mov Disord*. 2008; 23:574–580. [PubMed: 18163453]
- Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. *Physiol Rev*. 2001; 81:929–969. [PubMed: 11274347]
- Jenner P. Pharmacology of dopamine agonists in the treatment of Parkinson's disease. *Neurology*. 2002; 58:S1–S8. [PubMed: 11909980]
- Jurgens U. The neural control of vocalization in mammals: a review. *J Voice*. 2009; 23:1–10. [PubMed: 18207362]
- Jutkiewicz EM, Bergman J. Effects of dopamine D1 ligands on eye blinking in monkeys: efficacy, antagonism, and D1/D2 interactions. *J Pharmacol Exp Ther*. 2004; 311:1008–1015. [PubMed: 15292458]
- Kersing W, Jennekens FGI. Age-related changes in human thyroarytenoid muscles: a histological and histochemical study. *Eur Arch Oto-Rhino-L*. 2004; 261:386–392.
- Kikawada M, Iwamoto T, Takasaki M. Aspiration and infection in the elderly: epidemiology, diagnosis and management. *Drugs Aging*. 2005; 22:115–130. [PubMed: 15733019]
- Kraytsberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW, Khrapko K. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat Genet*. 2006; 38:518–520. [PubMed: 16604072]
- Manfredsson FP, Burger C, Sullivan LF, Muzyczka N, Lewin AS, Mandel RJ. rAAV-mediated nigral human parkin over-expression partially ameliorates motor deficits via enhanced dopamine neurotransmission in a rat model of Parkinson's disease. *Exp Neurol*. 2007; 207:289–301. [PubMed: 17678648]

- Martin JH, Diamond B, Aviv JE, Jones ME, Keen MS, Wee TA, et al. Age-related-changes in pharyngeal and supraglottic sensation. *Ann Otol Rhinol Laryngol*. 1994; 103:749–752. [PubMed: 7944164]
- Mcgeer PL, Itagaki S, Akiyama H, Mcgeer EG. Rate of cell-death in parkinsonism indicates active neuropathological process. *Ann Neurol*. 1988; 24:574–576. [PubMed: 3239957]
- McMullen CA, Andrade FH. Contractile dysfunction and altered metabolic profile of the aging rat thyroarytenoid muscle. *J Appl Physiol*. 2006; 100:602–608. [PubMed: 16239605]
- McMullen CA, Andrade FH. Functional and morphological evidence of age-related denervation in rat laryngeal muscles. *J Gerontol A Biol Sci Med Sci*. 2009; 64:435–442. [PubMed: 19223602]
- Mortelliti AJ, Malmgren LT, Gacek RR. Ultrastructural changes with age in the human superior laryngeal nerve. *Arch Otolaryngol Head Neck Surg*. 1990; 116:1062–1069. [PubMed: 2200442]
- Mrini A, Jean A. Synaptic organization of the interstitial subdivision of the nucleus tractus solitarii and of its laryngeal afferents in the rat. *J Comp Neurol*. 1995; 355:221–236. [PubMed: 7541810]
- Paxinos, G.; Watson, C., editors. *The Rat Brain in Stereotaxic Coordinates*. 6th.. Elsevier Inc; 2007.
- Peshori KR, Schicatano EJ, Gopalaswamy R, Sahay E, Evinger C. Aging of the trigeminal blink system. *Exp Brain Res*. 2001; 136:351–363. [PubMed: 11243477]
- Qureshi S, Ageel AM, Al-Yahya MA, Tariq M, Mossa JS, Shah AH. Preliminary toxicity studies on ethanol extracts of the aerial parts of *Artemisia abyssinica* and *A. inculta* in mice. *J Ethnopharmacol*. 1990; 28:157–162. [PubMed: 2329806]
- Rodeno MT, Sanchezfernandez JM, Riverapomar JM. Histochemical and morphometrical aging changes in human vocal cord muscles. *Acta Oto-Laryngol*. 1993; 113:445–449.
- Rosenberg SI, Malmgren LT, Woo P. Age-related changes in the internal branch of the rat superior laryngeal nerve. *Arch Otolaryngol Head Neck Surg*. 1989; 115:78–86. [PubMed: 2909234]
- Sasaki CT, Yu Z, Xu J, Hundal J, Rosenblatt W. Effects of altered consciousness on the protective glottic closure reflex. *Ann Otol Rhinol Laryngol*. 2006; 115:759–763. [PubMed: 17076098]
- Sasaki H, Sekizawa K, Yanai M, Arai H, Yamaya M, Ohnui T. New strategies for aspiration pneumonia. *Intern Med*. 1997; 36:851–855. [PubMed: 9475237]
- Sessle BJ. Excitatory and inhibitory inputs to single neurones in the solitary tract nucleus and adjacent reticular formation. *Brain Res*. 1973; 53:319–331. [PubMed: 4350322]
- Shaker R, Ren J, Bardan E, Easterling C, Dua K, Xie P, et al. Pharyngoglottal closure reflex: characterization in healthy young, elderly and dysphagic patients with predeglutitive aspiration. *Gerontology*. 2003; 49:12–20. [PubMed: 12457045]
- Simonyan K, Jurgens U. Cortico-cortical projections of the motor cortical larynx area in the rhesus monkey. *Brain Res*. 2002; 949:23–31. [PubMed: 12213296]
- Simonyan K, Jurgens U. Efferent subcortical projections of the laryngeal motor cortex in the rhesus monkey. *Brain Res*. 2003; 974:43–59. [PubMed: 12742623]
- Smith CD, Umberger GH, Manning EL, Slevin JT, Wekstein DR, Schmitt FA, et al. Critical decline in fine motor hand movements in human aging. *Neurology*. 1999; 53:1458–1461. [PubMed: 10534251]
- Sun QJ, Chum JM, Bautista TG, Pilowsky PM, Berkowitz RG. Neuronal mechanisms underlying the laryngeal adductor reflex. *Ann Otol Rhinol Laryngol*. 2011; 120:755–760. [PubMed: 22224318]
- Takeda N, Thomas GR, Ludlow CL. Aging effects on motor units in the human thyroarytenoid muscle. *Laryngoscope*. 2000; 110:1018–1025. [PubMed: 10852524]
- Winblad B, Hardy J, Backman L, Nilsson LG. Memory function and brain biochemistry in normal aging and in senile dementia. *Ann N Y Acad Sci*. 1985; 444:255–268. [PubMed: 2861774]
- Yue F, Zeng S, Wu D, Yi D, Alex Zhang Y, Chan P. Age-related decline in motor behavior and striatal dopamine transporter in cynomolgus monkeys. *J Neural Transm*. 2012; 119:943–952. [PubMed: 22327353]
- Zamir Z, Ren J, Hogan WJ, Shaker R. Coordination of deglutitive vocal cord closure and oral–pharyngeal swallowing events in the elderly. *Eur J Gastroenterol Hepatol*. 1996; 8:425–429. [PubMed: 8804869]

- Zarzur AP, Duprat AC, Shinzato G, Eckley CA. Laryngeal electromyography in adults with Parkinson's disease and voice complaints. *Laryngoscope*. 2007; 117:831–834. [PubMed: 17473678]
- Zhang Z, Andersen A, Grondin R, Barber T, Avison R, Gerhardt G, et al. Pharmacological MRI mapping of age-associated changes in basal ganglia circuitry of awake rhesus monkeys. *Neuroimage*. 2001; 14:1159–1167. [PubMed: 11697947]
- Zhang Z, Andersen A, Smith C, Grondin R, Gerhardt G, Gash D. Motor slowing and parkinsonian signs in aging rhesus monkeys mirror human aging. *J Gerontol A Biol Sci Med Sci*. 2000; 55:B473–B480. [PubMed: 11034220]

Fig. 1.

Age-related changes in the laryngeal adductor reflex (LAR) in rats at 6, 18, and 30 months of age. No significant differences were found in the response threshold (A) and latency (C). Resting muscle tone increased at 18 and 30 months compared with 6 months (B) (* $p < 0.05$, ** $p < 0.01$). (D) Amplitude increased in 30-month-old rats compared with 6-month-old rats (* $p < 0.05$). TA, thyroarytenoid muscle.

Fig. 2.

Age-related changes in LAR conditioning. Conditioning facilitation increased in 18-month-old rats compared to 6-month-old rats ($*p < 0.05$) at 1000 ms ISI. No differences in conditioning changes were found in 30-month-old rats at all ISIs. ISI = interstimulus interval.

Fig. 3.

Age-related decline of dopaminergic neurons in substantia nigra par compacta (SNpc) of rats in the three age groups. (A, B) Representative TH staining in the SNpc of a rat brain section (scale bar: 300 μ m in A and 30 μ m in B). (B) is the enlarged white square in (A). (C) Neurons that stained positive for tyrosine hydroxylase (TH+) in the SNpc decreased with aging (** $p < 0.01$, $n = 8$ /each age group).

Fig. 4.

Correlations between dopaminergic neurons in SNpc and LAR. (A) No significant correlation was found between resting muscle tone of the thyroarytenoid muscle (TA) and dopaminergic neurons. (B) Positive correlation between LAR latency and (C) negative correlation between the integrated amplitude of LAR and dopaminergic neurons in SNpc.

Fig. 5.

Effects of levodopa on the LAR. No significant differences between resting muscle tone of TA (A), latency (B), and amplitude (C) of LAR were associated with levodopa administration at separate age groups ($p > 0.05$). Latency of LAR increased after levodopa administration ($p < 0.05$) when data from all age groups were combined.

Fig. 6.

Effects of levodopa on LAR conditioning. No significant effects of levodopa on LAR conditioning at 6- (A), 18- (B), or 30- (C) month-old rats were seen at each interstimulus interval (ISI) ($p > 0.05$).