

## ABSTRACT

**Background:** Gentamicin is an aminoglycoside antibiotic widely used in clinical settings but associated with ototoxicity. Although its systemic and local effects on auditory structures have been studied extensively, the impact of intranasal administration on central auditory processing has yet to be fully examined. We have recently shown that intranasal delivery of therapeutic doses of gentamicin results in delayed auditory brainstem responses that were most severely impacted at the level of the auditory midbrain and inferior colliculus.

**Objective:** Based on these physiological observations, we hypothesized that intranasal gentamicin exposure results in changes in neuron morphology and number in the midbrain, specifically in the central nucleus of the inferior colliculus (CNIC), a critical midbrain relay that integrates auditory input from the brainstem and projects to the auditory thalamus.

**Methods:** Sprague-Dawley rats received bilateral intranasal irrigations with gentamicin or saline from postnatal day (P) 21–31. Animals were then anesthetized and perfused, and brains were harvested, coronally sectioned, and Nissl stained. CNIC neurons were hand-traced and manually counted under light microscopy.

**Results:** Gentamicin-exposed rats exhibited a significantly greater number of round neurons in the CNIC compared to controls.

**Conclusion:** These findings suggest that intranasal gentamicin affects central auditory pathways in addition to its known peripheral ototoxicity.

## INTRODUCTION

- Gentamicin is a commonly used aminoglycoside with known ototoxicity
- Systemic and local effects on auditory structures are well studied but little is known about intranasal delivery
- Our lab has shown that intranasal gentamicin delays auditory brainstem responses, most prominently at the level of the inferior colliculus

**Hypothesis:** Intranasal gentamicin alters neuron number and morphology in the central nucleus of the inferior colliculus (CNIC)

## METHODS

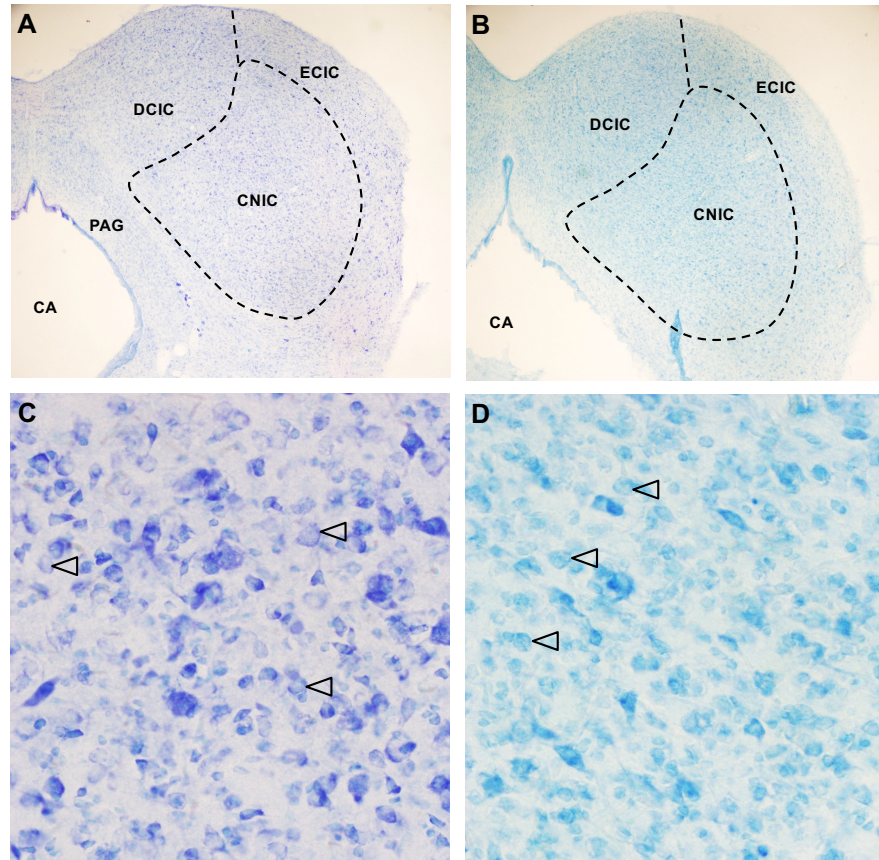
**Animals:** Sprague-Dawley rats received bilateral intranasal irrigations of gentamicin or saline from postnatal day (P) 21–31

**Perfusion & Histology:** On P32, animals were anesthetized with isoflurane and perfused through the ascending aorta with 4% paraformaldehyde in 0.1M sodium phosphate buffer. Brains were dissected from the skull and postfixed for at least 24 hrs. Before sectioning, brainstems were cryoprotected in 30% sucrose and then sectioned in the coronal plane at a thickness of 30  $\mu$ m. Sections were mounted on glass slides and stained for Nissl substance with Giemsa.

**Neuron Analysis:** CNIC neurons hand-traced and counted under light microscopy

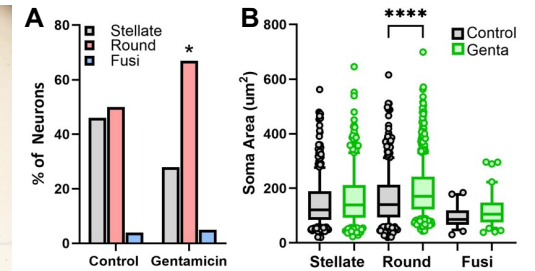
**Statistics:** Group comparisons analyzed with Chi-square and ANOVA ( $p < 0.05$ )

## NEURON MORPHOLOGY



Gentamicin exposure impacts neuron morphology in the CNIC. Shown in A is a low magnification view of the inferior colliculus from a control animal and shown in B is the inferior colliculus from a gentamicin-exposed animal. Shown in C is a higher magnification view of the CNIC from a control and in D from a gentamicin-exposed animal. Gentamicin exposure results in more round neurons and these were significantly larger in exposed animals.

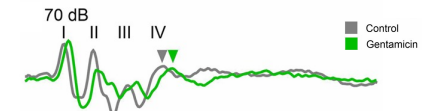
## QUANTIFICATION OF NEURON MORPHOLOGY



Gentamicin-exposed rats had significantly more round neurons and fewer stellate neurons (A; Chi square,  $p = .02$ ). Analysis of over 1,200 neurons revealed that cell bodies with round/oval morphology had significantly larger soma (B; Kruskal-Wallis,  $p < .0001$ ). There was no difference in the size of stellate or fusiform cell bodies.

## CONCLUSION

Intranasal gentamicin exposure is known to be ototoxic and specifically damaged cochlear hair cells. We have shown previously that intranasal gentamicin results in elevated hearing threshold and delays in the auditory brainstem response from waves I (auditory nerve) to waves IV and V (midbrain; see below).



Results from this study show that intranasal gentamicin exposure impacts neuron morphology throughout the entire auditory brainstem and highlight the consequences of intranasal antibiotic delivery.

## ACKNOWLEDGMENTS

Special thanks to Dr. Kulesza and the members of the Auditory Research Center Supported by the Lake Erie College of Osteopathic Medicine Based in part on methodology described in Breeden et al., *Exp Brain Res*, 2025.