

# The hunt for nNOS in the auditory midbrain

Laura Kenny, Llwyd Orton & Adrian Rees Institute of Neuroscience, Newcastle University

(l.j.kenny@newcastle.ac.uk)



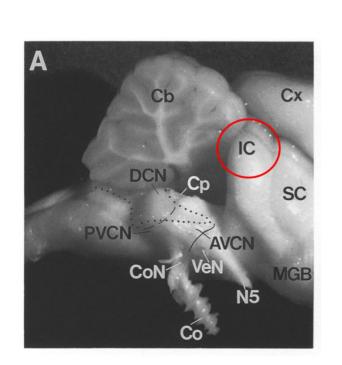
### INTRODUCTION

Nitric oxide is a gas that acts as a neurotransmitter, a chemical that enables neurones in the brain to communicate.

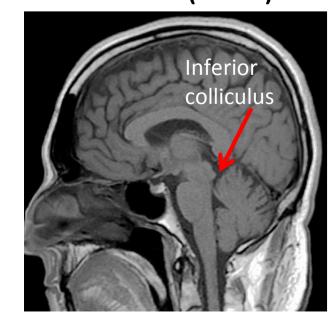
- It is involved in the auditory pathway, which enables us to hear and interpret sound.
- The inferior collliculus (IC) is the midbrain nucleus of the auditory system.
- Its receives inputs originating from the ear and sends information to the higher brain centres
- Nitric oxide is produced in the auditory system by its synthesising enzyme neuronal nitric oxide synthase (nNOS).
- The expression of nNOS has been hypothesised to change in tinnitus and deafness.

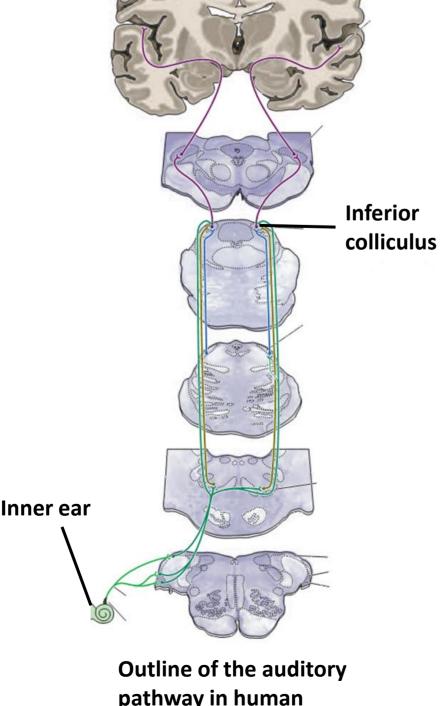
## **RESEARCH AIMS**

- The aim of this study was to determine the distribution of nNOS in the IC along with other neuronal markers.
- The IC is made up of 3 different parts; a central, dorsal and lateral division. Does nNOS expression differ between these regions?



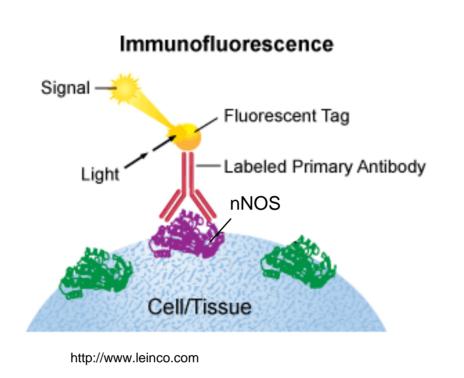
IC in guinea pig (above) and in human (below)



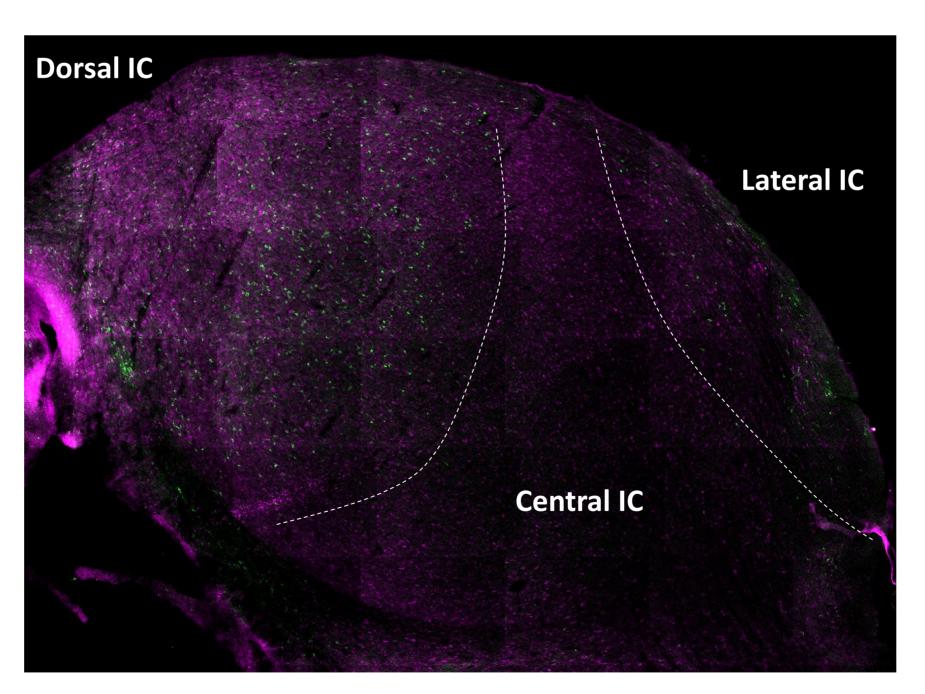


#### **METHOD**

- Thin sections of frozen guinea pig brain were taken and then processed with a primary antibody which binds selectively to the nNOS enzyme.
- A secondary antibody was added which binds to the primary antibody and fluoresces when exposed to a particular wavelength of light (see figure below).
- Neurones containing nNOS could be visualised under a confocal microscope.



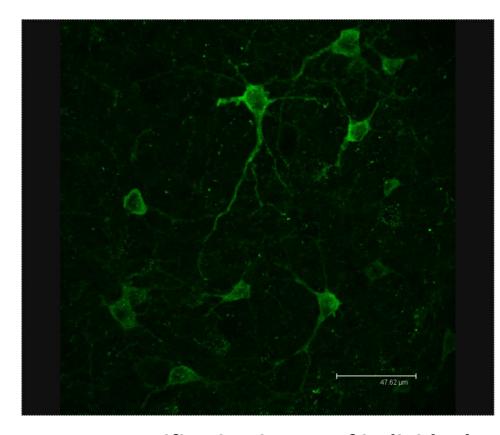
## **RESULTS**

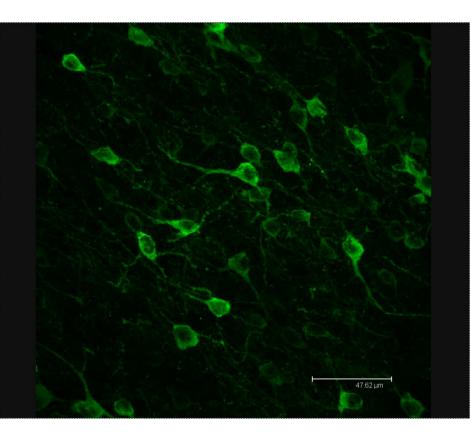


A x10 magnification image of the whole of the right IC of guinea pig, seen in cross-section. Neurones containing nNOS are labelled in green. (The purple shows staining for the neurochemical serotonin).

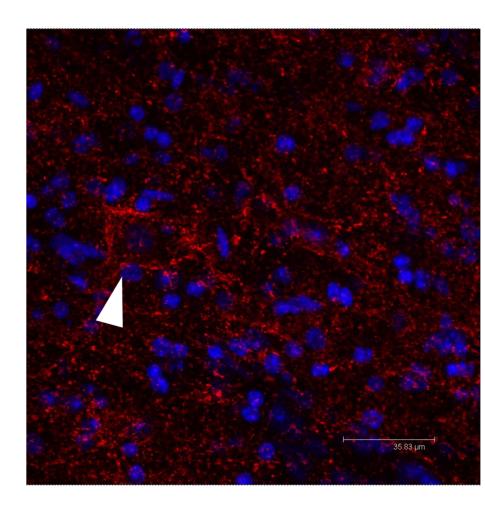
## **RESULTS** continued

High magnification images showed nNOS staining of individual neurones.





A x63 magnification image of individual nNOS containing neurones in the dorsal part of the IC. Both the cell bodies and the dendrites (wire-like parts of the neurone that receive inputs from other neurones) stained for nNOS. nNOS is found in a variety of different neurone types, as characterised by their shapes.



X63 magnification image showing in red the distribution of vesicular transport protein VGAT. This marker labels inhibitory inputs that can be seen here surrounding the cell body of a neurone in the IC (arrowhead).

# **DISCUSSION & CONCLUSION**

- The experiments demonstrate the presence of nNOS in the IC.
- The regions expressing the highest nNOS levels are those which receive feedback connections from higher auditory centres.
- Further experiments are required to correlate nNOS expression with other markers that show the excitatory and inhibitory inputs that neurones receive.
- This work will provide a basis for further research into the role of nitric oxide in deafness and tinnitus.