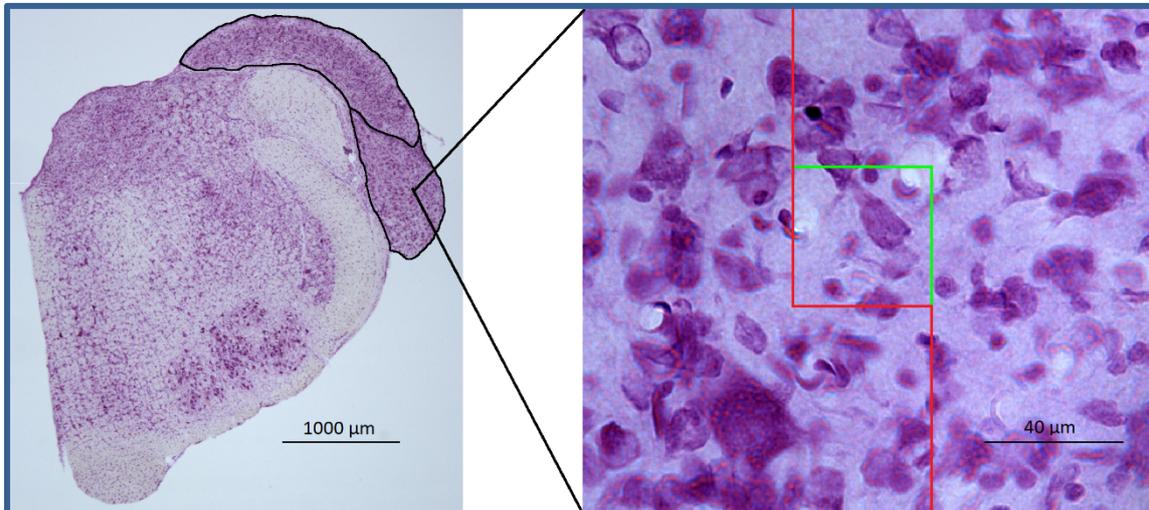


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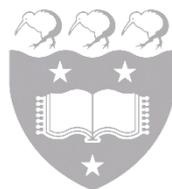
Structural changes in the auditory nuclei in the brainstem in a model of premature birth



The University of Auckland

SCHOLARSHIP IMPACT REPORT

15/02/2019



Thank You and Summary

I would like to express my gratitude to the University of Auckland for funding my research this summer into the effect of premature birth on the auditory brainstem. I would especially like to thank my supervisor, Dr Meagan Barclay, for your support. I really appreciated that you gave me the independence to work things out by myself but were also there when I needed guidance. I also need to acknowledge two previous Master of Audiology students, Manisha Nathu and Seungho Na, whose sectioned rat brain tissue I was fortunate to use.

This research project has been my first real venture into the world of academic research and I found it to be incredibly rewarding. I knew going into this project that neuroscience research is what I want to do; this project confirmed my passion and gave me valuable insights into a research career. I was also grateful for the opportunity to present my findings at the summer student forum and to the auditory neuroscience research group. Thank you.

Input

The Neurological Foundation of New Zealand gave \$115,394 to Dr Meagan Barclay and her collaborators to conduct research into auditory processing deficits in extreme prematurity in 2015. The University of Auckland gave \$6,000 for me to contribute to this research for 10 weeks.

Research Activity

Children who are born extremely prematurely sometimes have an auditory processing problem called auditory neuropathy spectrum disorder (ANSO). In this disorder, sound activates the sensory receptors in the ear normally, but the transmission of this information to the brain and its processing in the auditory brain are impaired (Berlin et al., 2003; Rance, 2005; Zeng et al., 1999). This results in deficits in language, literacy and learning due to difficulty processing speech and distinguishing similar or rapidly presented sounds (Berlin et al., 2003; Rance, 2005; Zeng et al., 1999). These deficits may be linked to damage to auditory brain nuclei due to their brains not receiving enough oxygenated blood flow early in their postnatal life (Laptook, 2016; Ravarino et al., 2014). This is called hypoxia-ischemia, and it occurs because the lungs of extremely premature infants are not properly developed at birth. The regions of the auditory brain that are affected by this lack of oxygenated blood flow are not yet well understood.

The aim of this project was to investigate the structural changes to the auditory nuclei in the brainstem in a model of premature birth. The following brainstem nuclei were investigated: the ventral and dorsal cochlear nuclei, the superior olivary complex, the lateral lemniscus, and the inferior colliculus. For reference, *Figure One* below shows the auditory system, and the regions of interest to this project have been circled.

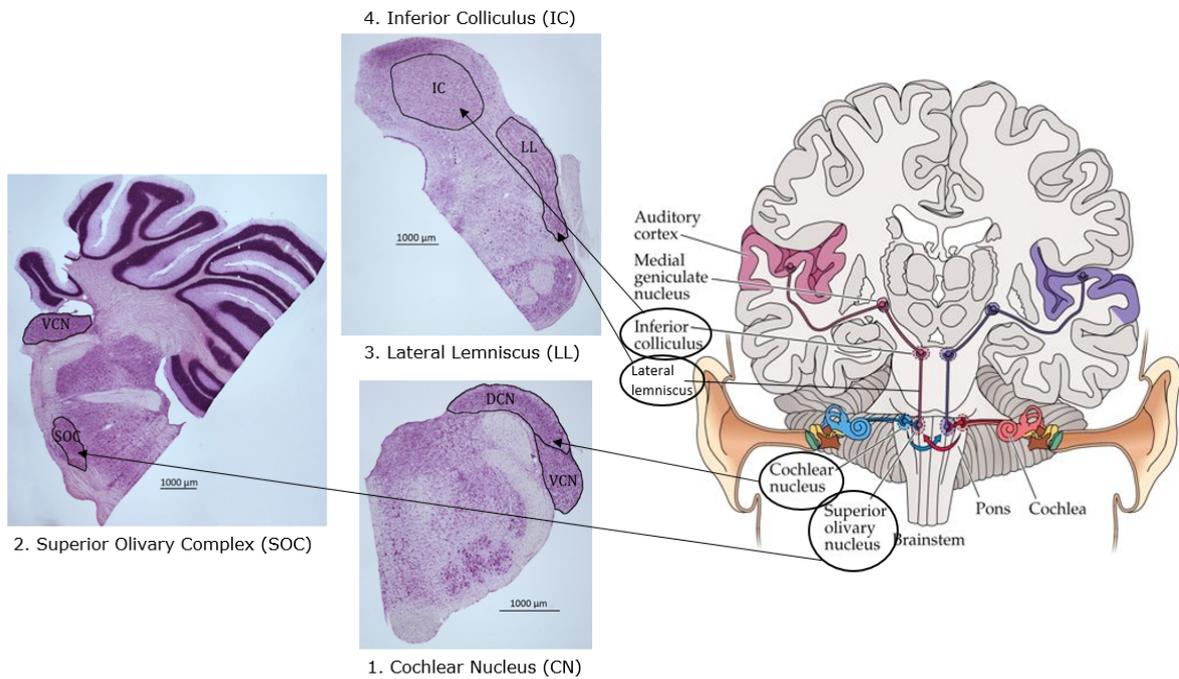


Figure One: The auditory system. Diagram adapted from Heeger (2006). Microscope images were stained with Nissl and imaged at 2x magnification.

This project used a novel rat model of premature birth developed by Dorothy Oorschot et al. (2013). *Figure Two* summarises this premature birth model. In brief, rats in their first three postnatal days were exposed to repeated episodes of hypoxia (low oxygen). This modelled the hypoxia-ischemia events that occur shortly after birth in human extreme premature birth, which occurs between the 22nd-28th weeks of gestation. The first three postnatal days of rodent brain development are considered to be developmentally equivalent to this period (Semple et al., 2014). On the 98th postnatal day, at approximately three months old, the rats were euthanized and their brains were fixed in paraformaldehyde.

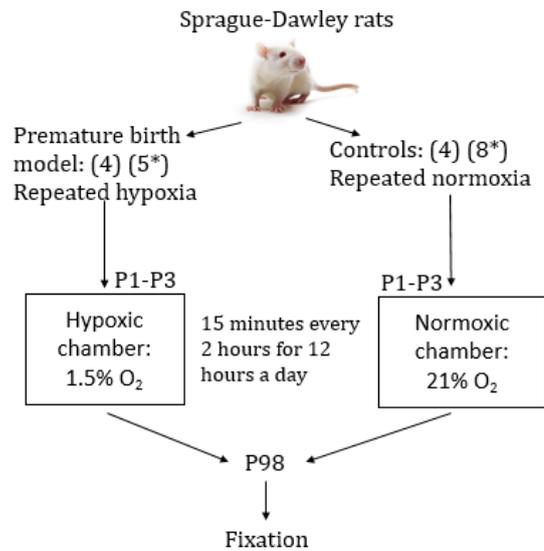


Figure Two: A novel repeated hypoxia rat model of human extreme premature birth. () indicates sample size; *is for the inferior colliculus only; P = days after birth.

Previous audiology students, Manisha Nathu and Seungho Na, cut these brains into 40 μm coronal sections. I stained these sections with Nissl, which stains neurons and glia (non-neuronal brain cells). I used StereoInvestigator software connected to a light microscope to determine the volume of and number of neurons in each region. To calculate the volumes, I used the Cavalieri estimator in the StereoInvestigator software. This places a grid to work out the cross-sectional area of each section, and calculates the volume using the formula shown in *Appendix Two*. The optical fractionator probe in the software was used to calculate the number of neurons in each nucleus. This generates a cube at regular intervals through the region of interest. Neurons that fell inside the cube were counted and the total number of neurons was calculated using the formula in *Appendix Two*. Neurons were identified by a darkly stained cytoplasm and lightly stained nucleus, compared to glia which have a darkly stained nucleus and very little or lightly stained cytoplasm. *Figure Three* below has been modified from screenshots of the software to show how these probes work.

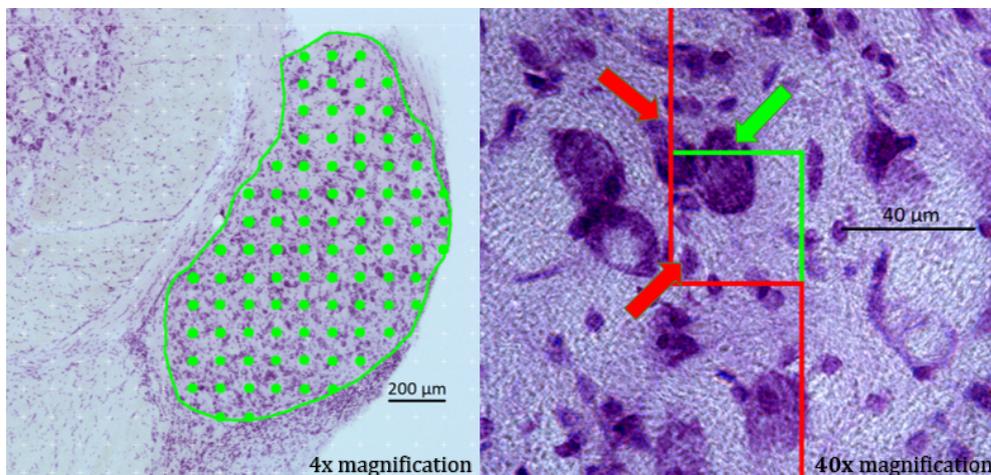


Figure Three: Using StereoInvestigator software to work out the volume and number of neurons. LEFT: the Cavalieri estimator. Green dots indicate points on the grid that were counted. RIGHT: the optical fractionator. Any neurons that fell entirely within this cube or crossed a green inclusion line were counted (green arrow). Any neurons that fell entirely outside the cube or crossed a red exclusion line were not counted (top red arrow). Glia were also excluded (lower red arrow).

Research Outputs

The auditory brainstem nuclei showed no significant effect of hypoxia on their volume, except the inferior colliculus, which showed a 16% reduction in size (4.4 mm^3 in control tissue, compared to 3.7 mm^3 in the hypoxic rats). This is shown in *Figure Four*. This difference was statistically significant ($p = 0.013$). A summary of all of the data including sample sizes is shown in *Appendix One*.

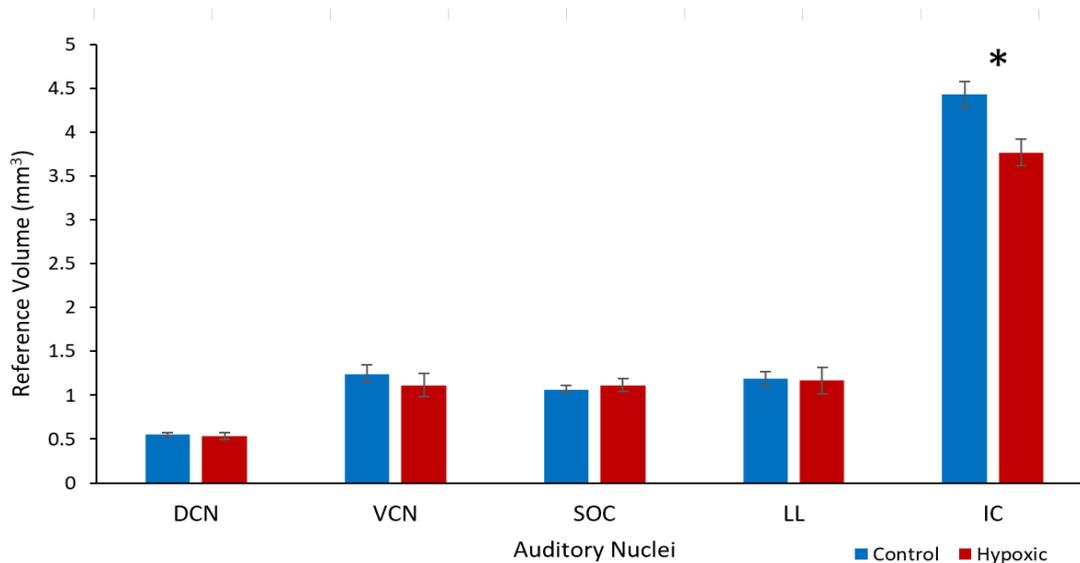


Figure Four: Reference volumes of the auditory brainstem nuclei. DCN = dorsal cochlear nucleus; VCN = ventral cochlear nucleus; SOC = superior olivary complex; LL = lateral lemniscus; IC = inferior colliculus. Results are expressed as means \pm SEM. * $p < 0.05$ (unpaired two-tailed t-test).

The number of neurons followed a similar pattern to the volume, as shown in *Figure Five*. There was no difference in the absolute number of neurons for all of the regions except the inferior colliculus, where 25% of the neurons were lost in the hypoxic brains. This difference was highly statistically significant ($p = 0.0017$).

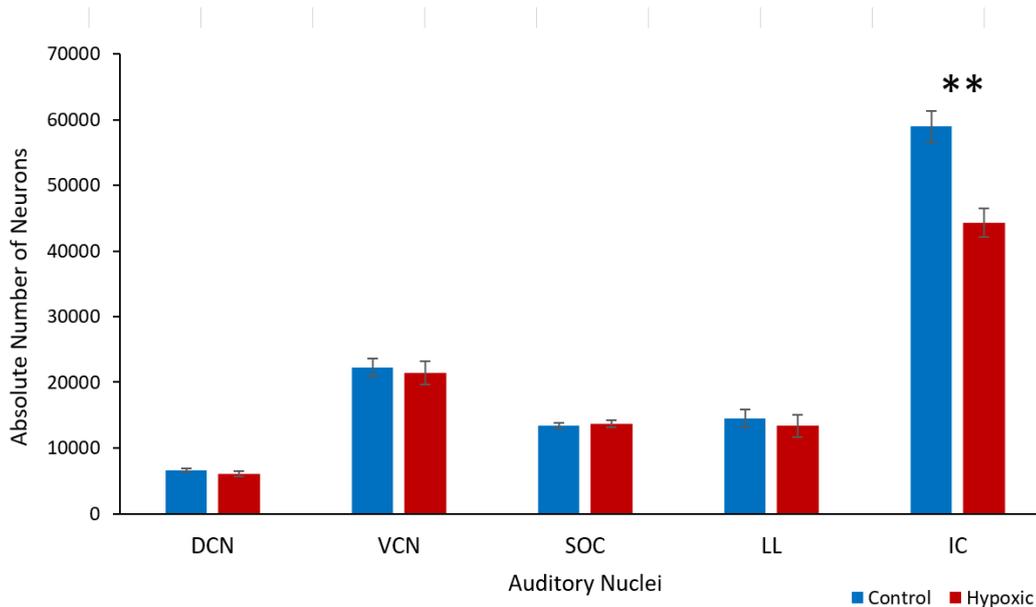


Figure Five: Absolute number of neurons in the auditory brainstem nuclei. DCN = dorsal cochlear nucleus; VCN = ventral cochlear nucleus; SOC = superior olivary complex; LL = lateral lemniscus; IC = inferior colliculus. Results are expressed as means \pm SEM. ** $p < 0.01$ (unpaired two-tailed t-test).

Research Outcomes

My research suggests that the inferior colliculus is affected by the episodes of hypoxia-ischaemia that occur following premature birth. This is because both the volume of, and the number of neurons in this region was significantly reduced by hypoxia. I did not find any statistically significant differences in the other auditory brainstem nuclei (the cochlear nuclei, inferior colliculus and lateral lemniscus). This suggests that these regions are resistant to the effects of hypoxia that occurs following premature birth.

The inferior colliculus receives sound information from both ears and plays an important role in the localization and temporal characteristics of sound, enhancing speech from background noise, frequency discrimination, and more (Akimov et al., 2017; Felix et al., 2018; Litovsky et al., 2002; Malmierca & Young, 2014). By damaging the inferior colliculus, premature birth may impair the ability of the brain to make the small distinctions necessary for speech, for example, being unable to identify the very small difference in timing and frequency between "bad" and "Dad", resulting in poor language skills.

Many studies have investigated changes to the auditory brainstem response (a measure of the electrical activity of neurons in the auditory brainstem) following premature birth or neonatal hypoxia-ischemia. Their main finding was a reduced wave V amplitude (Jiang & Chen, 2017; Jiang et al., 2006, 2009), which represents a reduced number of neurons firing in the inferior colliculus (Abadi et al., 2016; Litovsky et al., 2002; Starr & Hamilton, 1976). The reduction in neurons in the inferior colliculus that I found is consistent with these studies and enables us to combine information on where the lesion is with information on what the effect of the lesion is to gain a better understanding of how premature birth affects auditory processing.

Future Impact

This research has shown significant structural changes in the inferior colliculus due to premature birth. This leads to the future possibility of hearing interventions for premature birth targeted at the inferior colliculus. I also showed that the dorsal and ventral cochlear nuclei, superior olivary complex and lateral lemniscus were not structurally changed by premature birth. This suggests that any interventions targeting only these regions may not improve hearing outcomes in children with ANSD.

ANSD results in about 10% of permanent hearing loss in children (Berlin et al., 2003; Rance, 2005). Not only do these children have poor auditory processing, but their literacy, learning, educational achievement, memory and IQ are also negatively affected (Davis & Hind, 1999; McClure et al., 2005; Theunissen et al., 2015). Children with hearing disorders have also been shown to have an increased risk of developing behavioural, attentional, emotional and mental health disorders (Barker et al., 2009; Davis & Hind, 1999; Hogan et al., 2011; Stevenson et al., 2015; Theunissen et al., 2015; Wake et al., 2004). Although this relationship may not be causal, it is possible that developing interventions to improve hearing after premature birth could also improve other aspects of these children's wellbeing and development.

Assistive hearing devices like hearing aids and cochlear implants target the auditory system peripheral to the inferior colliculus and have limited and highly variable efficacy in children with ANSD (Madden et al., 2003; Rance & Barker, 2008). Improvement in language development has been shown by combining these devices with a frequency modulation system (Rocha & Scharlach, 2017; Schafer et al., 2014; Schafer & Kleineck, 2009). This system amplifies speech relative to background noise, which is one of the roles of the inferior colliculus. Future research is needed to assess if these systems are of benefit to children with auditory processing disorders following premature birth, and to design new devices that target the inferior colliculus or its role in auditory processing.

Future research is also needed to determine whether there are structural changes to any nuclei higher up the auditory pathway, such as the medial geniculate nucleus of the thalamus or the auditory cortex. It could also be possible that there are other changes to the auditory brainstem nuclei that do not affect the volume of or number of neurons in these regions. For example, there could be changes in the levels of neurotransmitters (brain messenger molecules) or their receptors that affect these regions, without significantly changing their structure. Further research is needed before the cochlear nuclei, superior olivary complex and lateral lemniscus can be said to be unaffected by premature birth.

This research demonstrated structural changes to the inferior colliculus of the auditory brainstem in a repeated hypoxia rat model of premature birth. These findings increase our understanding of ANSD in extreme prematurity by showing the structural changes underlying the physiological auditory processing deficits. This could lead to the future development of devices that target the role of the inferior colliculus in auditory processing to improve hearing outcomes for children born extremely prematurely.

Appendix

Appendix One: Summary Data

| Region of interest | Number of animals available | Mean volume; controls | Mean volume; hypoxia | t-test on volumes | Mean number of neurons; controls | Mean number of neurons; hypoxia | t-test on number of neurons |
|--------------------|-----------------------------|-----------------------|----------------------|-------------------|----------------------------------|---------------------------------|-----------------------------|
| DCN | 8 (4C, 4H) | 0.595 | 0.534 | 0.760 | 6602 | 6075 | 0.272 |
| VCN | 8 (4C, 4H) | 1.243 | 1.115 | 0.475 | 22237 | 21438 | 0.731 |
| SOC | 8 (4C, 4H) | 1.065 | 1.116 | 0.925 | 13419 | 13708 | 0.690 |
| LL | 8 (4C, 4H) | 1.186 | 1.169 | 0.925 | 14535 | 13358 | 0.604 |
| IC | 13 (8C, 5H) | 4.432 | 3.772 | 0.0133 | 58962 | 44278 | 0.00167 |

Appendix One: Summary data: mean volumes and number of neurons for the auditory brainstem nuclei. C = control animals; H = hypoxic animals; DCN = dorsal cochlear nucleus; VCN = ventral cochlear nucleus; SOC = superior olivary complex; LL = lateral lemniscus; IC = inferior colliculus. t-tests were unpaired and two-tailed.

Appendix Two: Stereological Formulae

Reference volume = $P \cdot A_p \cdot sei \cdot t$

Where:

- P = sum of grid points counted
- A_p = area associated with a point
- sei = section evaluation interval
- t = section cut thickness

Number of neurons = $Q \cdot (1/asf) \cdot (1/sei)$

Where:

- Q = sum of neurons counted
- asf = area sampling fraction (includes the dissector height divided by the section thickness, and the total area of the counting frames divided by the total cross-sectional area.)
- sei = section evaluation interval

Appendix Three: References

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