Cochlear involvement in tinnitus
Tinnitus

Tinnitus affects 5-15% of population.
Can severely affect quality of life
No cure yet
Strong correlation with hearing loss - Prevalence increasing

What is the neural substrate of tinnitus?
Perception-abnormal neuronal activity

(Axelsson and Ringdahl, 1989)
Changes in the brain after hearing loss: Possible mechanisms for tinnitus

1. Changes in tonotopic maps

2. Synchronous activity between central structures

3. Increased spontaneous activity (hyperactivity) in central auditory pathway

Note: several theories suggest these changes in the auditory system are accompanied/modulated by changes in non-auditory parts of the brain.

Eggermont and Komiya, 2000; Robertson and Irvine 1989; Norena and Eggermont, 2003
Our guinea pig model to study central hyperactivity and tinnitus

- Recovery 0-12 weeks
- Record single neuron activity in inferior colliculus
- Behavioural tinnitus test

Pure tone acoustic trauma

Record cochlear neural thresholds (CAP) to establish hearing loss
Our animal model to study tinnitus:

Our results:

Increased neural activity in IC without sound present:

HYPERACTIVITY

Control data

High activity

Low activity

Mulders and Robertson Neurosci. 2010; Mulders et al. J. Neurosci. 2010
The University of Western Australia

Hyperactivity shows correlation with region of hearing loss

Human studies:
**Audiogram vs tinnitus pitch:** frequencies of hearing loss closely match pitch of perceived tinnitus

Robertson et al 2013 hearing Res
Measuring tinnitus in animals

gap prepulse inhibition of the acoustic startle (GPIAS)

Turner et al. 2006
Our guinea pig model: central hyperactivity and tinnitus

Correlation hearing loss and hyperactivity

Tinnitus: GPIAS

Can we modulate the central hyperactivity?

Stop activity auditory nerve

Measure single neuron activity **Hyperactivity**

Acoustic trauma
1-6 weeks recovery: Acute destruction of auditory nerve after recovery period eliminates hyperactivity

Post recovery-pre-ablation

High activity

Low activity

Pitch increase

Mulders and Robertson. 2009 Neuroscience
8-12 weeks recovery: Acute destruction of auditory nerve does **NOT** completely eliminate hyperactivity

**Post-recovery-pre-ablation**

**After acute cochlear ablation**

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Mulders and Robertson 2011 Neurosci
Central Hyperactivity—a two stage process?

Cochlear trauma

central neurons become hyperexcitable

hyperactivity requires spontaneous afferent input from cochlea

A therapeutic window?

central neurons become intrinsically overactive

hyperactivity becomes "centralized" -independent of spontaneous afferent input from cochlea
The Big Question

In stage 1:
Reduction spontaneous activity in auditory nerve: hyperactivity

In stage 1:
Reduction spontaneous activity in auditory nerve: tinnitus
How can we suppress spontaneous activity of the auditory nerve fibres?

Possibility: **Furosemide**

- Loop diuretic (affecting membrane transport).
- Known to affect kidney and inner ear
- Decreases spontaneous firing rate auditory nerve fibres (Sewell 1984)
- Can suppress tinnitus in human subjects (Risey et al 1995; Caesarani et al. 2002)
Can we modulate hyperactivity and tinnitus in our animal model using furosemide?

- Recovery 0-12 weeks
- Measure brain activity: effect of furosemide
- Behavioural tinnitus test: effect of furosemide

Partial deafness

Measure hearing loss

Adapted from Lee Kishnan and Winer
Furosemide acutely decreases spontaneous firing auditory afferent nerve fibres (SNN) and central hyperactivity

Furosemide (80 mg/kg i.p.)

Spontaneous firing (spikes/sec)

N=4

Time re furosemide injection (min)

% initial SNN

Spontaneous firing (spikes/sec)

before furosemide

after furosemide

Mulders, Barry and Robertson 2014 Plos One
Saline i.p. has no effect on behavioural signs of tinnitus

Mulders, Barry and Robertson 2014 Plos One
Furosemide i.p. eliminates behavioural signs of tinnitus
In conclusion

- Our data suggest that furosemide can suppress the behavioural signs of tinnitus in our animal model.

- Our data strengthens the argument that hyperactivity is involved in the generation of tinnitus.

- Our data supports the notion that there may be a therapeutic window for some time after acoustic trauma.
What’s next?

- Can we show proof of principle in human tinnitus sufferers? (collaboration Prof Friedland; Ear Science Institute Australia)

- Investigations into more chronic effects of furosemide on tinnitus. (Mulders et al. 2014 Frontiers in Neuroscience)

- Other options beside furosemide?

- What about treatments for centralized tinnitus?
A different way to modulate activity in the cochlea: Extra-cochlear electrical stimulation (ECES)

- ECES with positive current can suppress activity of auditory nerve
- Suppression of tinnitus reported using ECES with positive direct current
- Mechanism unknown-due to reduction of central hyperactivity?

Tested in our animal model using round window electrical stimulation

Schreiner et al. 1986; Cazals et al., 1978
Only small effect on thresholds and tone-induced activity of IC neurons. ECES may be a viable approach for suppressing some forms of (peripheral-dependent) tinnitus.
Acknowledgements:

Action on Hearing Loss (UK)

Neurotrauma Research Program

NHMRC

MHRIF

Auditory lab members

A/Prof. Jenny Rodger (University of Western Australia)

Dr Arnaud Norena (Universite de Marseille, France)

Prof Tony Paolini (RMIT, Australia)

Prof Richard Salvi (University of Buffalo, USA)
1-6 weeks recovery: Acute but temporary silencing of auditory nerve after recovery period eliminates hyperactivity.

Single neuron recordings in midbrain 2 weeks after acoustic trauma:

Cochlear perfusion with kainic acid or CoCl$_2$ suppresses spontaneous activity.
What’s next?

- Can we show proof of principle in human tinnitus sufferers? (collaboration ESIA)

- Investigations into more chronic effects of furosemide on tinnitus.

- Other options beside furosemide?

  - What about treatments for centralized tinnitus?
Repetitive Transcranial Magnetic Stimulation (rTMS)

- Therapeutic effects on many neurological and psychiatric disorders
- Non-invasive
- Some success reported in tinnitus patients

Vooys 2014; Huerta and Volpe, 2009; (Khedr et al., 2008, Langguth et al., 2008, Khedr et al., 2010)
rTMS frequency protocol

High frequency rTMS – excites neuronal activity

Low Frequency rTMS – inhibits neuronal activity

Low-frequency rTMS:
Ideal treatment for disorders involving excessive cortical excitability

Hallet (2000); Huerta and Volpe (2009); Hoffman (2002); Eichhammer et al. (2003)
Can rTMS suppress hyperactivity after hearing loss?

10 Guinea Pigs

Week0
Acoustic Trauma

Week1
Recovery

Week2–3
Treatment
rTMS n=5
sham n=5

Week4
Electrophysiology and samples for ELISA
Coil size and position

10 minute sessions, 1 Hz, Monday – Friday for 2 weeks
rTMS does not affect hearing loss but reduces hyperactivity

- **rTMS group (n=5)**
- **Sham group (n=5)**

**rTMS** vs **Sham**

- **SFR (Spikes/sec)**
  - **Sham** and **rTMS**
    - Mean with SEM
    - Median

*Vooys 2014*
Can rTMS reduce the behavioural signs of tinnitus in our animal model?
Preliminary data: possible effect rTMS on tinnitus?

- Is rTMS affecting descending pathways from the cortex?
- Direct effect on IC?

N=3/group
Further ongoing studies

- Modulation of hyperactivity by paraflocculus (Darryl Vogler)
- Modulation of hyperactivity and tinnitus by limbic system (Kristin Barry and Prof Tony Paolini RMIT University Melbourne)
- Projection patterns of descending auditory systems (Ahmaed Bashaar)
- Effects of cochlear electrical stimulation on hyperactivity and tinnitus
- Testing validity of GPIAS in human subjects (Prof. Geoff Hammond, ESIA)
- Effects of rTMS on hyperactivity and tinnitus (A/Prof. Jenny Rodger)
- Proof of principle experiment effect of furosemide in tinnitus subjects (ESIA and Prof Peter Friedland)
Neural substrates of tinnitus

Human studies:

Neuroimaging data: Excessive spontaneous activity in auditory structures

Audiogram vs tinnitus pitch: frequencies of hearing loss closely match pitch of perceived tinnitus

Animal studies: (models of hearing loss)

Electrophysiology: Increased spontaneous activity in auditory structures

Audiogram vs tinnitus pitch: Increased spontaneous activity associated with frequency range of hearing loss and behavioral signs of tinnitus correlate with increased spontaneous activity/ frequency range of hearing loss
RESULTS (STUDY2): SPONTANEOUS FIRING RATE

- Mean with SEM
- Median

SFR (spikes/second)

- Sham
- rTMS
RESULTS (STUDY1): BDNF ELISA

Brain Area

rTMS Treatment (n=5)
Sham Treatment (n=5)
Measuring brain activity

- Depth
- CF (characteristic frequency) and threshold
- Spontaneous firing rate
- 90-120 neurons per animal

Picture courtesy C. Bester
Experiment 1:

Guinea pig model
→ Establish hyperactivity in the brain
→ Measure excitatory and inhibitory systems in the brain:
  Gene products and brain chemicals
→ Main result: Inhibitory systems

Dong et al. Neurosci. 2009
Dong et al. Brain Res. 2010