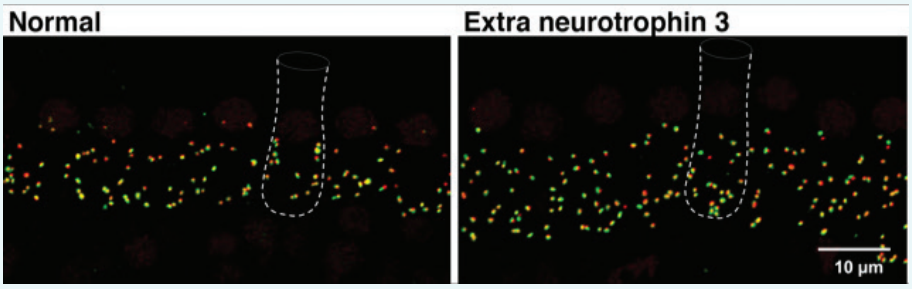
cochlear synapse loss, a.k.a. synaptopathy, could serve as a therapy for tinnitus.

However, studies linking hidden hearing loss and cochlear synaptopathy to tinnitus are based on noise exposure, thus making the connection between synapse loss and tinnitus indirect. Therefore, we believed it was important to test whether changes in cochlear synapses, per se, precipitate tinnitus in the absence of noise exposure. These studies became feasible when we generated mouse models in which we could increase or decrease the number of inner ear synapses independent of noise. We can now do this in genetically modified mice by increasing or decreasing the expression of a neurotrophic factor called neurotrophin 3 in the cochlea. Importantly, we have demonstrated that by increasing the amount of neurotrophin 3 in the cochlea, we can induce the regeneration of inner ear synapses and restoration of hearing after noise exposures that induce hidden hearing loss.

In the first year of our research supported by the American Tinnitus Association's grant, we used these novel mouse models and well-established behavioral and electrophysiological tests to analyze whether alterations in inner hair cell synapses contribute to tinnitus. We have established the necessary experimental procedures and have performed tests that strongly support our hypothesis. During the second year of this award, we will complete the testing of the effects of synapse changes on tinnitus. We will then test whether noise-induced tinnitus can be prevented by induction of synapse regeneration and hearing restoration by treatment with neurotrophin 3.



Increasing neurotrophin 3 levels in the inner ear increases the number of hair cell synapses. Representative images obtained with a confocal microscope of inner hair cells of mouse cochleas stained to visualize synaptic proteins illustrate the increased density of synapses (the red and green dots) in ears that have more neurotrophin 3 than controls. The dashed lines show the approximate outline of one inner hair cell. (Modified from Wan et al., eLife 2014;3:e03564 doi: 10.7554/eLife.03564)

Gabriel Corfas, PhD, is Director of the Kresge Hearing Research Institute and Associate Chair for Research for the Department of Otolaryngology and Head and Neck Surgery at the University of Michigan in Ann Arbor.

Using Electroencephalography (EEG) to Track Brain Responses to Gaps Embedded in Soft Sounds: A Stepping Stone Towards an Objective Measure of Tinnitus - YEAR 1

Principal Investigator: Sylvie Hébert, PhD
Co-investigator Brandon T. Paul, PhD

Presently, there is no method that enables us to detect the presence of tinnitus. For instance, if a person does not tell the clinician that she or he has tinnitus, then the clinician has no means to prove or refute this person's assumption. This lack of an objective tinnitus measure is a serious shortfall in both clinical and research settings: it prevents a firm diagnosis of tinnitus, prevents tracking the progress of an intervention over time, and precludes neuroscience from targeting an experimental outcome that would direct research toward a treatment or a cure.

Objective measures for tinnitus also are a chief concern for animal research, which is needed to understand cellular and molecular mechanisms of tinnitus. Because animals cannot express verbally that they hear tinnitus, tinnitus must be inferred from their behavior. For example, one prominent method used to test for tinnitus in an animal measures the animal's response following the perception of a silent gap in a continuous sound.[1] If, after a manipulation used to induce tinnitus (e.g., noise exposure or toxic drugs), the animal does not behave in accordance

with normal perception of the gap, it is assumed the animal has tinnitus, because the perception of tinnitus has "filled-in" the silence. This gap-detection method has been adopted by several research groups and has been used in dozens of important scientific articles for more than a decade.[2]

If it is the case that gap detection-impairment can robustly identify tinnitus in an animal, can it be used as a diagnostic test in humans? Although this assumption is attractive, research has been unable to demonstrate that gap-detection deficits distinguish tinnitus in humans.[2] There are several reasons why efforts may have fallen short. For example, human studies simply may not have used an optimal design to test gap detection within sounds that match a person's tinnitus. Alternatively, the mechanism of gap detection in animals may not generalize well to humans, or, worse, the gap-detection method as employed in animals may not be measuring tinnitus, but rather some other attribute of the tinnitus-induction method. In either case, the lack of confirmatory evidence in humans that tinnitus can "fill-in" silent gaps in sounds threatens to undermine a large body of tinnitus research and impedes progress in clinical and scientific settings.

To address this concern, we proposed to use a neuroimaging technique called electroencephalography (EEG) to measure the brain's response to sounds, called *auditory evoked potentials*, which are fluctuations of electrical brain activity. We know from past research that silent gaps embedded in sound elicit measureable electric potentials with clear properties.[3] If we were to design a sound to be almost identical to the tinnitus that a person hears, would their tinnitus "fill-in" a silent gap in that sound, such that the brain would not detect it? Put another way, if auditory brain areas engaged in producing tinnitus-related neural activity are generating sounds where in the external environment there is silence, then detection of silence in an ongoing sound might be similarly masked. Consequently, the brain response indicating that a silent gap was perceived, which is clearly present in an individual without tinnitus, would be absent in an individual with tinnitus.

A potential challenge with this approach was the feasibility of measuring evoked potentials within sounds designed to match a person's tinnitus. In general, as sounds get weaker in both intensity level and as they increase in frequency (i.e., pitch), the brain's response size becomes smaller, eventually becoming difficult to separate from the inherent noise of the EEG recording. We also know that most cases of tinnitus whose acoustics are estimated with behavioural tests equate to a very low sensation level (sound level above threshold) and commonly are pitches of high frequency.[4] If we were to design a sound matching these properties, there is a danger that the brain's response to this sound would be undetectable, because the physical acoustics evoking the response were so weak. Thus, for many tinnitus sufferers, this test would not work. No previous data suggesting that gaps in sounds of low loudness and of high frequencies were observable in the EEG had been published.

During our first year of funding from the American Tinnitus Association, we conducted a pilot study on 12 normal-hearing (non-tinnitus) adults, providing the first proof of principle that such "weak" brain responses were indeed measurable. We took common properties of tinnitus, i.e. very soft, high-pitch sounds, and presented these sounds to participants. Silent gaps were randomly inserted into these sounds, and the EEG was recorded while participants listened. Using a novel signal-processing approach, we were able to detect that responses were present in all participants. Thus, our approach is feasible to conduct in humans, offers an individually diagnostic metric, and can be applied to tinnitus. This study is now published in the journal *Hearing Research*.[5]

A second challenge we addressed in our first year of funding from the ATA was how to ensure gap detection is tested when individuals hear their tinnitus, since tinnitus might only "fill-in" the gap at these sound frequencies. Measuring the acoustical features of tinnitus (for example frequencies, loudness, and "thickness," i.e., bandwidth of tinnitus) historically has been a challenge.[4] We developed a new method, based on randomly presenting sound features and then estimating the most likely features after sampling a large number of combinations of sound features. We tested the procedure on 14 adults with tinnitus and found that each individual rated our "estimate" of tinnitus as highly similar to their real tinnitus (an average of eight on a scale of 10). These findings were presented at the recent *Association for Research in Otolaryngology* meeting held in San Diego, California, in February 2018.[6]

Having laid the necessary groundwork, now we are equipped to apply our objective gap-detection test

on tinnitus sufferers to see if tinnitus can "fill-in the gap." Irrespective of the outcome, our findings will have important implications for the future of tinnitus research: Results that support the idea that gap detection is impaired in tinnitus not only would be an important step in validating animal research, but also would pave the way to a diagnostic tinnitus test that could be used in the clinic. However, if we fail to find evidence that tinnitus can fill in gaps, we will need to revisit the working paradigm used in animal models and find new avenues to objectively identify the presence of tinnitus.

Main reference: Paul, B.T., M. Schoenwiesner, and S. Hébert (2018). Towards an objective test of chronic tinnitus: Properties of auditory cortical potentials evoked by silent gaps in tinnitus-like sounds. *Hearing Research*.

1 Turner JG, et al. 2006. *Behav. Neurosci.* 120 (1), 188.
2 Galazyuk A, Hébert S. 2015. *Front. Neurol.* 6.
3 Pratt H, et al. 2005. *Clin. Neurophysiol.* 116 (11), 2648-2663.
4 Vernon JA, Meikle MB. 2003. *Otolaryngol. Clin.* 36 (2), 293-305.
5 Paul BT, Schoenwiesner M, Hébert S. 2018. *Hearing Research*.
6 Paul BT, Schoenwiesner M, Hébert S. 2018. Poster #528, 41st Midwinter Meeting of Association for Research in Otolaryngology.

Sylvie Hébert, PhD, is professor at the School of Speech Pathology and Audiology at Université de Montréal, Montreal, Canada. She is member of the International laboratory of research on Brain, Music, and Sound (BRAMS) and Center of Research on Brain, Language, and Music (CRBLM). Dr. Hébert has published pioneering studies on the comorbidities of tinnitus, including stress and sleep disorders.

Unlocking the Basis of Tinnitus Sound Therapy: Informational Masking and Tinnitus Adaptation - YEAR 2

Principal Investigator: Grant D. Searchfield, PhD

The tinnitus research team at the University of Auckland in New Zealand is exploring the basis of tinnitus sound therapy. In this research, we are collaborating with the Knowledge Engineering and Discovery Research Institute, Auckland University of Technology, to analyze brain activity working toward the goal of predicting the types of tinnitus that respond best to different masking sounds. This research is investigating where in the brain the fundamental processes explaining tinnitus are located, and in particular the effect of sound therapy on these. Informational masking is being studied as a clinical approach to suppressing tinnitus. We hope to be able to identify types of tinnitus that do best with different types of sound therapy.

Sound Therapy

Sound therapy along with counseling is currently one of the most widely used methods for tinnitus management. Sound has been used to treat or suppress tinnitus for centuries. The brain mechanisms explaining how sound interferes with and helps reduce tinnitus are, perhaps surprisingly, not known. Some sounds work to reduce tinnitus for some people and for some of the time. What is the basis of these effects? Can we predict them? Who will benefit? Perhaps the most basic form of sound therapy is masking. Masking is presumed to cover tinnitus, making it more difficult to hear. There may be two processes involved: a process called "energetic" masking and another called "informational" masking. Energetic masking occurs at the ear when the energy in sound covers the area thought to be the trigger for tinnitus activity. Informational masking acts at the level of the brain, interrupting how the brain is analyzing tinnitus. Informational masking uses information, such as the meaning and location of sound, to be effective.

We set out to investigate and observe the changes in the brain responsible for the effectiveness of masking sounds. Using the information obtained from these studies, we hope to create more effective sound therapies and tailor sounds to individuals to maximize treatment effectiveness.

New Sounds and Analysis

Three different sorts of sound were used, and their effect on tinnitus measured over a three-month period using questionnaires (the *Tinnitus Functional Index* [TFI] and rating scales). The sounds consisted of rain or noise, changed to provide:

- Energetic masking — sound presented in stereo (heard equally in both ears)
- Informational masking — sound presented with a critical band notch applied around the tinnitus pitch (to diminish the contribution of energetic masking) and presented