Uncovering the Neural Basis of Tinnitus: Using Laboratory Animal Models in Tinnitus Research

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Subjective tinnitus is characterized by the perception of a sound which has no acoustic source in the environment. Often, this phantom sensation is described as a “ringing” or “buzzing” in one or both ears. Nearly all adults will experience tinnitus at some point in their life, albeit perhaps only for a short time, and most likely as a consequence of exposure to loud noise. For ~10% of the general population, however, tinnitus is a chronic condition that can lead to disturbance of sleep, difficulty concentrating and, in some cases, severe forms of depression, all of which can negatively affect one’s quality of life.

Unfortunately for tinnitus sufferers, a lack of understanding of its neural basis has hindered efforts to devise completely effective treatments. Early theories of tinnitus speculated that its signals originated in the cochlea and propagated to the brain via the auditory nerve; however, this theory has been challenged because tinnitus often persists after the auditory nerve has been surgically transected. Moreover, support for a central generator of tinnitus has emerged from several neuroimaging1–3 and magnetoencephalography4–6 studies on tinnitus patients which have found that their brain activity is altered compared to that of non-tinnitus subjects, during both quiet conditions as well as in response to acoustic stimuli. At present, however, it remains difficult to conclude from such non-invasive studies to what extent each of the reported changes is responsible for the onset and persistence of tinnitus.

To help uncover the neural basis of tinnitus, a number of laboratory animal models have been developed over the past ~25 years, with the vast majority using rodents (e.g., rats, mice and hamsters). It is important to note that prior to assessing any changes in brain activity that may underlie tinnitus, it was necessary that researchers first overcome the challenge of developing behavioural tests that were capable of determining whether or not animals were actually experiencing tinnitus. Since Jastreboff and colleagues7 first established a rat model of tinnitus, a variety of behavioural paradigms have been developed to screen rats and other laboratory animals for noise- and drug-induced tinnitus. In general, the majority of these initial behavioural paradigms involved training an animal to perform a distinct behaviour when sound was presented in its environment, and a different behaviour during quiet conditions. Then, following noise or drug exposure, if the animal mistakenly behaved during quiet conditions as though it was “hearing” an acoustic stimulus, the researchers concluded that the animal was experiencing tinnitus. Based on these behavioural paradigms, it is now well-established that, similar to humans, excessive exposure to loud noise and ototoxic drugs can induce tinnitus in laboratory animals.

Once researchers were able to reliably assess tinnitus-like behaviour in laboratory animals, numerous studies followed in which the animals that had screened positive for tinnitus were then anesthetized and microelectrodes were inserted into various regions of their brains to record how their neural activity...
was altered compared to non-tinnitus animals. In recent years, there has been a significant increase in the number of studies that have used laboratory animal models to investigate the putative neural mechanisms underlying tinnitus. Despite this increased attention, there is still debate as to whether subcortical or cortical mechanisms are responsible for generating tinnitus. That said, there is mounting support from human studies as well as laboratory animal models that abnormal cortical activity is likely associated with tinnitus.8

As it is beyond the scope of this article to discuss the findings from the numerous studies that have used laboratory animal models for tinnitus research, interested readers are encouraged to refer to comprehensive review articles on the topic.9–11 In the following sections, I will highlight the results from some recent experiments that my colleagues and I conducted while at the Center for Hearing and Deafness at the University at Buffalo, in which we used rat models to study noise- and drug-induced tinnitus.

In a series of experiments led by Daniel Stolzberg,12–14 we investigated the relationship between drug-induced tinnitus and changes in neural activity in the auditory cortex. To induce tinnitus, we treated rats with a high dose of salicylate, which is a component of Aspirin that is known to induce temporary tinnitus in both rats and humans. Once anesthetized, we inserted microelectrodes into the rat’s auditory cortex, and recorded the activity of neurons before and after salicylate treatment. In our first study,12 we found that salicylate caused the majority of neurons in the auditory cortex to become particularly sensitive to sound frequencies that matched the previously established tinnitus pitch in rats; findings which suggest that abnormal cortical activity underlies salicylate-induced tinnitus. In a follow-up study,13 we recorded the activity of neurons located at different depths of the auditory cortex, and found that the abnormal cortical activity observed during salicylate-induced tinnitus was not simply inherited from subcortical brain regions, but was also generated within the cortex itself via altered processing in its upper layers. This finding further supported our suggestion that abnormal auditory cortex activity contributes to salicylate-induced tinnitus.

Unlike salicylate, which reliably induces tinnitus in rats in a dose-dependent manner, in order to study the neural basis of noise-induced tinnitus it is very important to screen each animal behaviourally because not all noise-exposed animals develop tinnitus. To date, the most commonly used behavioural tool to screen animals for noise-induced tinnitus has been the gap-startle paradigm, which was developed by Turner and colleagues.15 In contrast to the previously-described behavioural tests which involved training animals prior to inducing tinnitus, the gap-startle paradigm does not require overt training, as it is based on an animal’s ability to detect a silent gap in a background sound as well its reflexive “flinching” response to a loud sound (i.e., its acoustic startle reflex). A key feature of the gap-startle paradigm is the well-established finding that if an animal is able to detect a brief silent gap in a background noise prior to the loud startle stimulus, its acoustic startle reflex will be suppressed (i.e., it “flinches” less in response to the loud sound). Supporters of the gap-startle paradigm suggest that if the animal’s tinnitus pitch is qualitatively similar to the background sound, then it should be unable to detect the silent gap, and consequently, its acoustic startle reflex will not be suppressed. It should be noted, however, that this notion of tinnitus “filling in” the silent gap has been challenged recently by a study that used the gap-startle paradigm on humans with tinnitus.16 Moreover, in a study on rats that was co-led by Edward Lobarinas and Sarah Hayes,17 we identified an additional caveat of the gap-startle paradigm: it is not resilient to the hearing loss that often accompanies noise exposure, and as a consequence, animals with only hearing loss can be falsely-screened as having tinnitus. Clearly, a failure to accurately screen animals for the presence/absence of tinnitus represents a significant concern for researchers who intend to subsequently investigate its neural basis.

While it is certainly more challenging to have a laboratory animal behaviourally report whether or not it is experiencing tinnitus than it is to simply ask a person, there are some distinct benefits of using laboratory animal models for tinnitus research. For example, laboratory animal models allow researchers to (1) directly record neural activity from various brain regions using microelectrodes (as described above), (2) precisely control how tinnitus is induced, and (3) evaluate the efficacy of novel treatments for tinnitus. Furthermore, my colleagues and I contend that an effective way to investigate the neural basis of noise-induced tinnitus involves a longitudinal study design in which a given subject’s brain activity is recorded both before and after traumatic noise exposure; an experimental approach that is unacceptable for human studies. To that end, Daniel Stolzberg recently led the development of the first laboratory animal model that would not only allow for us to monitor a rat’s cortical activity before and after tinnitus induction, but would also permit us to record this cortical activity at the very moment when
tinnitus was reported behaviourally.

As described in our recent publication,\textsuperscript{14} we validated the efficacy of our novel rat model by exposing them to a high dose of salicylate. Because subjects with tinnitus no longer perceive “quiet,” we designed the behavioural paradigm so that a rat would screen positive for tinnitus if it mistakenly reported that it was “hearing” a steady noise during an actual quiet period. Briefly, rats were trained to self-initiate a trial by poking their nose into a center port located on the front wall of a behavioural chamber, and wait ~6 seconds for a light to provide a “go” command. During this “holding time,” the rat attended to the sound being presented from an overhead speaker, and then made the corresponding behavioral choice once the light illuminated. Rats were trained to go to a left-side feeder trough for various steady noises (which sounded like continuous hissing), and the right-side feeder for both quiet (speaker off) and amplitude-modulated noise (which sounded like a pulsing effect). As predicted, after salicylate treatment, the rats correctly identified the steady noises (left-side feeder) and amplitude-modulated noises (right-side feeder), which confirmed that they could still accurately process auditory stimuli; however, they mistakenly went to the left-side feeder during the quiet trials, which indicated that they perceived a steady phantom sound (i.e., tinnitus). Using microelectrodes that were chronically implanted into the auditory cortex, we recorded the neural activity during the “holding time” of the quiet trials. Importantly, during salicylate-induced tinnitus, we observed complex changes in the pattern of spontaneous cortical activity in the quiet trials that largely paralleled that which has been reported in non-invasive studies on humans with tinnitus.\textsuperscript{5,6,18} Consequently, we now have a strong rationale for using our rat model to investigate the molecular mechanisms that underlie this abnormal neural activity associated with tinnitus.

Motivated by our recent findings, my colleagues and I are preparing to use our novel behavioural paradigm to track the changes in cortical activity associated with the onset and persistence of noise-induced tinnitus. Moreover, we will extend our rat model to investigate whether there are particular risk factors that can increase one’s susceptibility to developing chronic tinnitus after exposure to loud noise. Ultimately, given the expanding international community of scientists and clinicians devoted to uncovering the neural basis of tinnitus, I anticipate that laboratory animal models will continue to serve an important role in future tinnitus research.

REFERENCES
