



Tinnitus Research Initiative

**Applicant/
Principal investigator:**

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Grant ID :
Cooperation**

Treatments for Tinnitus Evaluated in Animal Models
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I Summary

I.1 Abstract

[Please formulate a publishable abstract that describes latest progress and final steps. Try to put the results in perspective to a treatment for tinnitus. – Abstract will be published on our website – 500 words maximum]

Subjective tinnitus affects approximately 6-11% or more of the population and approximately 0.7% experience severe or disabling tinnitus. Currently there are no known drugs that can reliably suppress tinnitus long term. Identifying potential drug therapies has been difficult because of the limited number of animal models to rapidly and reliably screen potential treatments. To address this problem, we had previously established two behavioral models to assess tinnitus in rats. One, Schedule-Induced Polydipsia Avoidance Conditioning (SIPAC), is a traditional animal operant technique. The other, Gap-Prepulse Inhibition of Acoustic Startle (GPIAS) utilizes a startle reflex response and is a very time efficient method. Using SIPAC, we have shown that NS1883, a potassium channel modulator, suppresses salicylate (aspirin) induced-tinnitus and does so in a dose dependent manner. The next logical step was to determine if NS1883 reliably suppressed other forms of tinnitus such as noise-induced tinnitus. Noise-induced tinnitus can be transient, occurring immediately after the exposure and then disappearing within a few days, or persistent, occurring immediately after noise exposure and lasting months or years.

The two major goals of the project were to determine if NS1883 can (1) suppress Transient Tinnitus induced by high level noise exposure and (2) Persistent Tinnitus induced by high level noise exposure (126 dB SPL, 2 kHz NBN, 2 h). To accomplish these 2 goals, we identified noise exposure conditions that generated either Transient Tinnitus (lasting 48 h) or Persistent Tinnitus (15 days) . Noise-induced tinnitus was induced in one ear using a unilateral, high-intensity noise exposure. The main result from Aim 1 was NS1883 (10 mg/kg) did not significantly reduce noise induced Transient Tinnitus. The main result of Aim 2 was that NS1883 (10 mg/kg) did not reduce noise-induced Persistent tinnitus. These negative results differ from earlier studies that showed that NS1883 reduced the magnitude of salicylate-induced tinnitus in a dose-dependent manner.

I.2 Describe deviations of the initial plan and why the plans were modified. Were new interesting results obtained or were new objectives created?

The same general plan remains largely in place except for (1) supplementary experiments to determine if NS1883 would suppress salicylate-induced tinnitus measured with the startle reflex method (GPIAS). (2) Determining the optimal acoustic conditions for inducing noise-induced tinnitus using the GPIAS method of assessing tinnitus.

I.3 Describe the efforts/goals for the final period.

The second phase of the project had two parts. During the first phase of our project we found that the noise exposure we employed only induced tinnitus in a small percentage of animals. Thus, part of our effort was devoted to optimizing the noise exposure conditions in order to increase the percentage of rats that developed Transient Tinnitus or Persistent Tinnitus. We found that noise exposures using 12 kHz NBN at 126 dB SPL for two hours induced transient tinnitus in nearly all animals and permanent tinnitus in more than 70% of animals. We hypothesized that NS1883 treatment would cause a suppression of noise-induced Transient Tinnitus measured at 48 h. For Aim II, we looked at rats that developed Persistent Tinnitus (>15 days) and treated them with a high dose of NS1883. We



hypothesized that NS1883 might cause suppression of noise-induced Persistent Tinnitus measured 15 days post noise.

I.4 What is your own assessment of the importance of your research regarding finding effective cures for tinnitus? [500 words maximum]

The SIPAC and GPIAS models that we developed to assess tinnitus in rats are reliable and efficient methods. The GPIAS model offers the potential to rapidly screen potential therapeutic drugs to treat tinnitus. Since tinnitus in humans is often induced by high level noise exposure, the noise-induced tinnitus studies are clinically relevant to large numbers of patients who develop noise-induced tinnitus. Since our previous studies showed that NS1883 suppressed salicylate induced tinnitus in a dose-dependent manner, it seemed highly important to determine if NS1883 would reliably suppress Transient or Persistent noise-induced tinnitus. .

I.5 Do you believe your research can lead to a patent application?

The compound that was tested, NS1883, is owned by a pharmaceutical company, Neurosearch that has patent rights to the use of this compound. If NS1883 proved successful in suppressing noise-induced tinnitus, it might encourage the company to continue drug development and to begin moving this compound or related drugs into clinical trials with human subjects with tinnitus.

I.6 Up-dated contact information

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II Report

II.1 List of initial goals and hypotheses of your project.

The goals and hypothesis of this project were to determine if NS1883, a BK potassium channel agonist and KCNQ antagonist could suppress noise-induced Transient or Persistent Tinnitus in a rat animal model. The behavioral readout of tinnitus is obtained from Gap-Prepulse Inhibition of Acoustic Startle (GPIAS)

- Hypothesis I: NS1883 will suppress noise-induced Transient Tinnitus, defined as tinnitus that is present 48 h after a noise exposure
- Hypothesis II: NS1883 will suppress noise-induced Persistent Tinnitus defined as tinnitus that is present 15 days after a noise exposure.

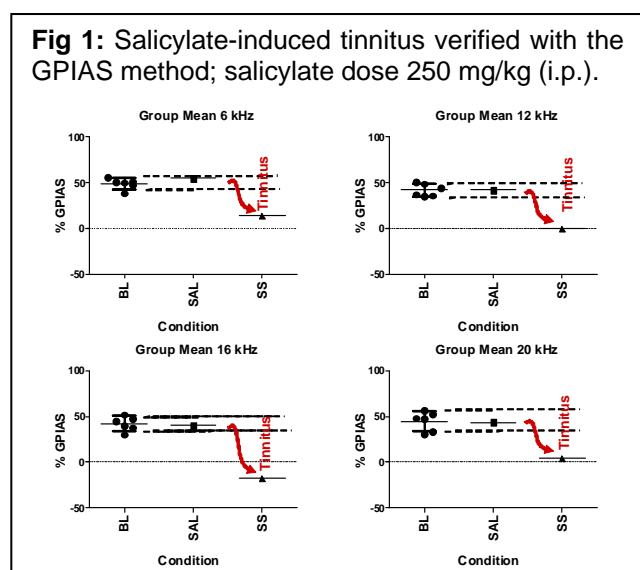
II.2 Problems encountered, how you attempted to solve the problems and your success in that?

We performed two pilot/supplementary studies in order to optimize the main studies being conducted with NS1883 and noise-induced tinnitus. (1) We performed supplementary experiments to determine if NS1883 would suppress salicylate-induced tinnitus measured with the startle reflex method (GPIAS). (2) We varied the noise exposure conditions in order to determine the optimal acoustic condition for inducing noise-induced tinnitus using the GPIAS method of assessing tinnitus.

II.3 Please describe the results of the research performed during the period covered by the report.

The studies described below present the main findings from the two main studies of noise-induced Transient tinnitus and Persistent tinnitus carried out with the GPIAS method of assessing tinnitus. In addition, we describe the supplementary studies carried out with the GPIAS method that were aimed at assessing salicylate-induced (aspirin) tinnitus and the ability of NS1883 to suppress salicylate-induced tinnitus. We will begin by describing the GPIAS data obtained with salicylate-induced tinnitus.

II.3.1 Salicylate-Induced Tinnitus: All of the rats studied in section II.3.2 below were given 250 mg/kg of sodium salicylate (i.p. 1 h pre-session) to determine if they would develop salicylate-induced tinnitus using the GPIAS method. Tinnitus was assessed at 6, 12, 16, 20 and 24 kHz. Noise-burst prepulse inhibition of acoustic startle (NBPIAS) was also assessed at the same test frequencies before and after salicylate treatment to confirm that the rats could indeed hear the noise stimulus used to assess tinnitus with the GPIAS methods. GPIAS measurements were obtained during baseline (BL), following a Saline (SAL) control treatment and after treatment with sodium salicylate (SS). During BL, the gap prepulse suppressed the mean (n=6) amplitude of the startle reflex by approximately 50% at all test frequencies (Figure 1) indicating an absence of





tinnitus in the baseline control condition. The horizontal lines in Figure 1 show the 95% confidence interval around the mean pre-exposure data. Treatment with saline, a control condition, had little or no effect on GPIAS indicating an absence of tinnitus; this rules out the possibility of an experimental artifact due to the injection. When the rats were treated with 250 mg/kg SS the GPIAS values dropped below the 95% confidence interval. The decrease was most pronounced at higher frequencies, but was also evident at 6 kHz. This group data indicated that the rats developed tinnitus and was consistent with our previous salicylate data obtained with SIPAC (Lobarinas et al., 2004). Inspection of individual data showed that 6 of 6 rats developed tinnitus like behavior at several frequencies consistent with our earlier findings. These results provided additional support for using GPIAS to assess tinnitus (Yang et al., 2007).

II.3.2: NS1883 and Salicylate-Induced Tinnitus: Using the SIPAC technique, we previously demonstrated that NS1883 suppressed salicylate-induced tinnitus in a dose-dependent manner. Before starting the noise-induced tinnitus studies, we wanted to confirm that NS1883 would again suppress

salicylate-induced tinnitus using the more efficient GPIAS method. Figure 2 shows the mean data from a group of 6 rats treated with 150 mg/kg of sodium salicylate for one day. The 150 mg/kg dose of salicylate caused a significant reduction in GPIAS values at 6, 12, 16, 20 and 24 kHz. These results are consistent with earlier SIPAC data showing that 150 mg/kg induces tinnitus-like behavior in rats (Lobarinas et al., 2004). After a one week washout period, the rats were again treated with 150 mg/kg salicylate (1 day) plus 10 mg/kg of NS1883. Treatment with NS1883 resulted in an increase in GPIAS values at 4 or 5 frequencies (6, 12, 16 and 24 kHz), but had no effect at 20 kHz. The overall results in Figure 2 are generally consistent with our previous findings obtained with SIPAC; however, there are several minor differences. First, the ability of NS1883 to suppress salicylate-induced tinnitus was not as robust as that seen with the SIPAC technique. These differences could be due to the fact the measurements were only obtained for one day in this study versus 2 days in our earlier SIPAC study. Second, in our earlier SIPAC study, we measured the global effect across all stimulus frequencies whereas in the current GPIAS study we measured the effects at 5 different frequencies.

Individual Differences: Evaluation of the results from individual rats showed that NS1883 was very effective in reducing salicylate-induced tinnitus in 3 of 6 rats; in the remaining 3 rats, NS1883 had little effect on salicylate induced tinnitus.

II.3.3: Noise-Induced Transient Tinnitus: In preliminary experiments, we measured the time course of noise-induced

Fig 2: Tinnitus induced with 150 mg/kg sodium salicylate. Mean GPIAS values at Baseline, during 150 mg/kg salicylate treatment and with salicylate plus 10 g/kg of NS1883. Downward movement of red line indicates induction of tinnitus.

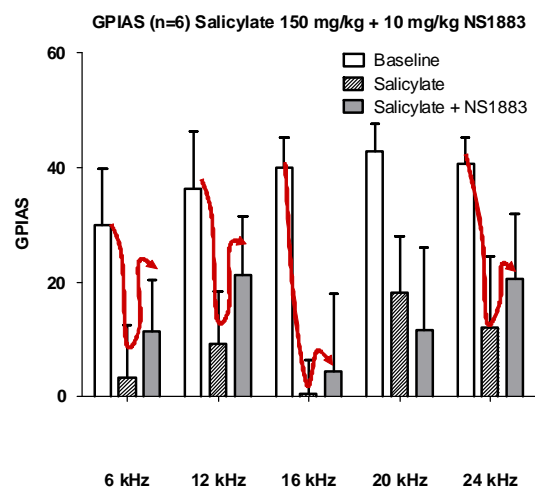
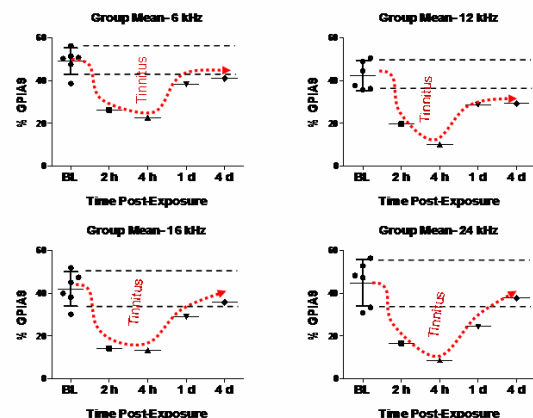


Fig 3: Time course of noise-induced tinnitus measured with GPIAS;





tinnitus induced by a 2 h exposure to narrow band noise centered at 12 kHz and presented at 123 dB SPL. Rats were anesthetized with 1-2% isoflurane during the noise exposure. The traumatizing noise was presented to just one ear in order to induce unilateral tinnitus. The opposite ear was plugged during the exposure in order to retain normal hearing in the unexposed ear. Tinnitus was assessed at 6, 12, 16, 20 and 24 kHz using the GPIAS technique. Noise-burst prepulse inhibition of acoustic startle (NBPIAS) was also assessed at 6, 12, 16, 20 and 24 kHz before and after the noise exposure to confirm that the rats could hear the continuous background noise used to assess GPIAS. (Note: Additional studies with distortion product otoacoustic emission showed that otoacoustic emissions were normal in the unexposed control ear.).

To determine the time course of noise induced tinnitus, GPIAS measurements were obtained in a group of rats before and at 2 h, 4 h, 1 d and 4 d after the noise exposure. Prior to the noise exposure, the gap prepulse suppressed the amplitude of the startle reflex by approximately 50%. As shown in Figure 3, the mean (n=6) pre-treatment GPIAS amplitudes (baseline control; BL) measured at 6, 12, 16 and 24 kHz were approximately 50%.

The horizontal lines in Figure 3 show the 95% confidence interval around the mean pre-exposure data. The 123 dB exposure produced a significant decline in GPIAS at all frequencies at 2 and 4 h post-exposure; the largest decreases in GPIAS occurred at 12, 16 and 24 kHz; the decrease in GPIAS was smaller at 6 kHz. These results suggested that the rats had developed noise-induced tinnitus shortly after the exposure and that the pitch profile of the tinnitus had a broad maximum between 12-24 kHz; i.e., the pitch of the tinnitus was located at and above the exposure frequency. After 1 day of recovery, mean GPIAS values had recovered significantly and by 4 days of recovery GPIAS values were within the 95% confidence limits at 16 and 24 kHz, but still below the 95% confidence interval at 12 kHz and just barely below the 95% confidence limits at 6 kHz. These group data results suggest that tinnitus was still present near 12 kHz 4 days post-exposure; however, inspection of individual data provided a different perspective.

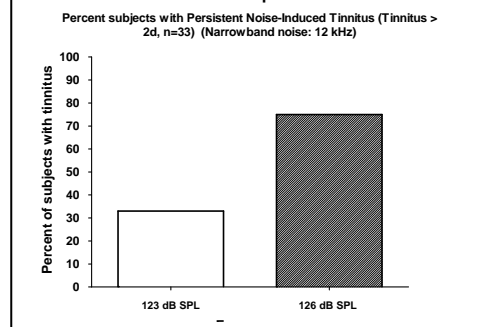
Individual Subjects: Analysis of the data from individual animals indicated that the 2 h, 123-dB exposure caused Transient Tinnitus in all 6 animals at one or more frequencies during the first few days following the exposure. However, by 4 days post-exposure, tinnitus has disappeared spontaneously at all frequencies in 3 of 6 rats. In the 3 remaining rats, tinnitus was still present at one or more frequencies. Tinnitus was present in 3 of 3 rats at 12 kHz, the exposure frequency. In addition, 2 of 3 rats showed evidence of tinnitus at 6 kHz, below the exposure frequency, and at 1 of 3 rats showed evidence of tinnitus at 16, 20 and 24 kHz. These results suggest that the 123 dB SPL exposure should produce Transient Tinnitus in nearly all rats during the first hours and days following the exposure. However, less than half of the rats developed tinnitus that persisted for 4 days. Since testing was not continued beyond 4 days, it is unclear what percentage of these animals would have developed persistent tinnitus, defined as tinnitus that persists out to 15 days post-exposure.

Noise-Exposure Level: Additional studies were carried out with a larger group of subjects and two exposure levels, 123 dB and 126 dB, to determine the percent of rats that developed Persistent Noise Induced Tinnitus. Persistent tinnitus was defined as tinnitus lasting more than 15 days following the exposure. These results indicate that increasing the exposure level from 123 to 126 dB SPL significantly increased the probability of inducing persistent tinnitus.

II.3.4: NS 1883 and Noise-Induced Transient Tinnitus:

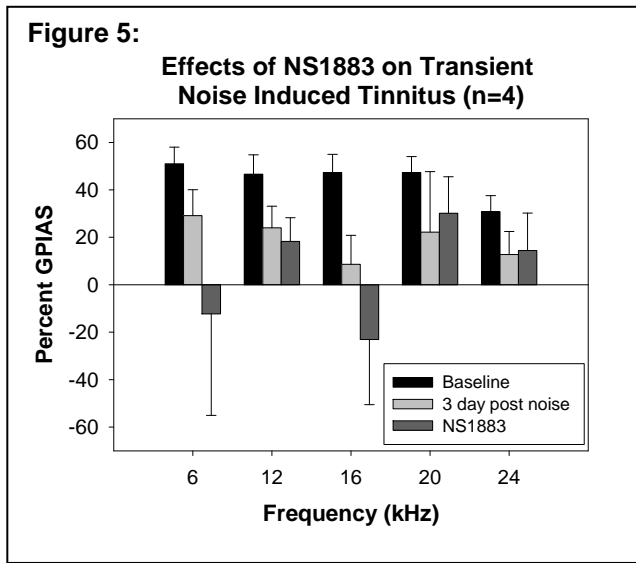
Additional studies were carried out to determine if NS1883 could suppress noise-induced Transient Tinnitus, defined as tinnitus that was present 48 h post-exposure. Tinnitus was assessed with the GPIAS technique. Rats were exposed to a 12 kHz narrow band noise

Figure 4: Percent of subjects that developed Persistent Tinnitus as a function of noise exposure level.



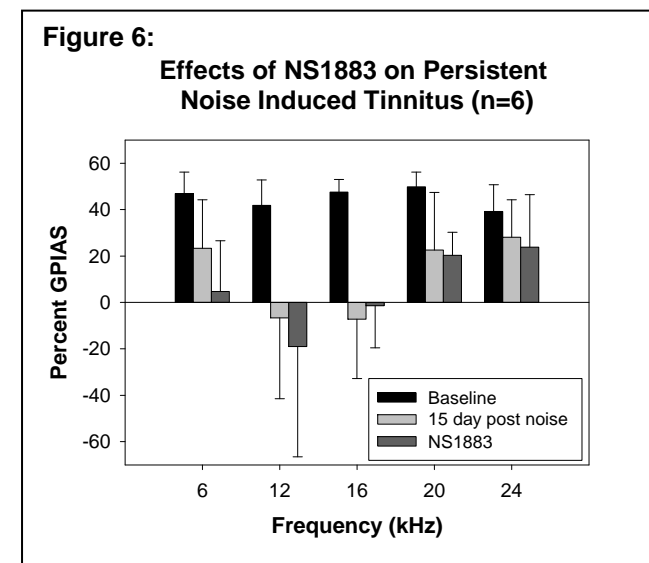


for 2 h at 126 dB and those animals (n=4) showing tinnitus at 48 h post-exposure (Note: 2 of 6 animals failed to develop Transient Tinnitus) were treated on day 3 with 10 mg/kg of NS1883 to determine if it would noise-induced Transient Tinnitus. A two-way repeated measure ANOVA was carried out to evaluate the main effects of treatment (baseline, noise exposure and NS1883). The results showed that the noise exposure cause a significant reduction in GPIAS at 48 h post-exposure indicating that the rats had developed Transient Tinnitus (Figure 5) ($p < 0.05$). Transient tinnitus, indicated by % reduction in GPIAS was most pronounced at 12 and 16 kHz suggesting that the pitch of the tinnitus profile had a peak from 12-16 kHz ($p < 0.05$, Student Newman Keuls post-hoc analysis). Rats with Transient Tinnitus on day 3 were treated with 10 mg/kg of NS1883 to determine if the drug would reduce tinnitus. The Transient Tinnitus behavior observed at 48 h post-exposure was not significantly different from the tinnitus behavior observed after treatment with NS1883 at 3 days post-exposure ($p > 0.05$, n.s.). Thus, NS1883 did not help to suppress noise-induced Transient Tinnitus. Transient Tinnitus was still present 3 days post-exposure compared to normal baseline measures ($p < 0.05$). These results indicate that NS1883 cannot suppress noise-induced Transient Tinnitus induced by the 126 dB, 2 h noise-exposure. These negative results were unexpected since this dose of NS1883 suppressed salicylate-induced tinnitus. However, care must be taken not to over interpret these negative results since NS1883 might suppress tinnitus induced by less severe noise exposures.



II.3.5: NS 1883 and Noise-Induced Persistent Tinnitus:

Additional studies were carried out to determine if NS1883 could suppress noise-induced Permanent Tinnitus defined as tinnitus that was present 15 days post-exposure. Tinnitus was assessed with the GPIAS technique. Rats were exposed to a 12 kHz narrow band noise for 2 h at 126 dB and those animals (n=6) showing tinnitus at 15 day post-exposure (day 2) were treated on day 16 with 10 mg/kg of NS1883 to determine if it would suppress noise-induced Permanent Tinnitus. A two-way repeated measure ANOVA was carried out to evaluate the main effects of treatment (baseline, noise exposure and NS1883). The results showed that the noise exposure caused a significant reduction in GPIAS at 15 days post-exposure indicating that the rats had developed Permanent Tinnitus at 12 and 16 kHz, as indicated by a large depression in %GPIAS ($p < 0.05$, Student Newman Keuls post-hoc analysis) (Figure 6). Rats with Permanent Tinnitus on day 15 were treated with 10 mg/kg of NS1883 on day 16 to determine if the drug would reduce tinnitus. The Permanent Tinnitus behavior observed on day 15 post-exposure was not significantly different from the tinnitus behavior observed after treatment with NS1883 on day 16 ($p > 0.05$,





n.s.). Thus, NS1883 did not suppress noise-induced Permanent Tinnitus. The GPIAS measures observed during NS1883 treatment were significantly different from normal baseline measures indicating that the tinnitus was still present on day 16. These results indicate that NS1883 cannot suppress noise-induced Permanent Tinnitus induced by the 126 dB 2 h noise-exposure. These results were unexpected given that this dose of NS1883 suppressed salicylate-induced tinnitus. However, care must be taken not to over interpret these results since NS1883 might suppress tinnitus induced by less severe noise exposures.

II.4 Please indicate the percentage of the used resources vs total planned.

100% of the resources were used to complete the study.

II.5 How do you judge the importance of your results regarding developing effective treatments for tinnitus and hyperacusis?

Since noise-induced tinnitus is one of the most common types of tinnitus, identifying drugs that can suppress noise-induced transient tinnitus or noise-induced persistent tinnitus are extremely important. The compound we investigated was effective in suppressing salicylate-induced tinnitus and therefore it seemed plausible that it might suppress noise-induced tinnitus.

II.6 What is your justification for continuing your research project?

The proposed project was completed and NS1883 was not found to be effective at reducing transient or persistent noise induced tinnitus. However, we have determined that the noise levels used caused more damage than previously expected. It is possible that the extensive damage reduced the ability of NS1883 to reduce milder forms of noise induced tinnitus. Further studies would need to be carried out to determine this. In addition, the basic strategy for evaluating drugs to suppress tinnitus in an animal model is a reasonable approach for drug screening.

II.7 List of publications, poster presentations and other dissemination activities.

Stolzberg, D., Lu, J., Schlee, W., Weisz, N., Sun, W., Salvi, R. 2008 Salicylate-Induced Tinnitus: Spectral Changes in Spontaneous Ensemble Activity in Auditory Cortex of Awake Rats Association for Research In Otolaryngology, Phoenix.

Lobarinas, E., Langguth, B., Sun, W., Lu, J., Salvi, J. 2008 Repetitive Transcranial Magnetic Stimulation (rTMS) on Persistent Noise Induced Tinnitus in Rats: a pilot study, Association for Research In Otolaryngology, Phoenix.

II.8 What are your expectations from TRI (in addition to economic support)?

Our expectations from TRI are to continue exchanging information and ideas related to the neural and biological mechanisms that give rise to tinnitus and to identify potential treatments that can be used to suppress tinnitus. Our research mainly involves animal models at this time, but we are interested in having some ongoing human studies that provide points of intellectual interaction with other members of TRI and make use of the results derived from our animal data. During the past year, we have had several TRI members (Berthold Langguth, Nathan Weisz, Winfried Schlee, and Tanit Schlee) visit our labs and give talks to our research group at the University at Buffalo. In addition, we presented two posters at the Association for Research in Otolaryngology meeting in Phoenix in February of 2008 in collaboration with other TRI members, Berthold Langguth, Nathan Weisz and Winfried Schlee.



References

- Lobarinas, E., Sun, W., Cushing, R., Salvi, R. 2004. A novel behavioral paradigm for assessing tinnitus using schedule-induced polydipsia avoidance conditioning (SIP-AC). *Hearing Res.* 190, 109-14.
- Yang, G., Lobarinas, E., Zhang, L., Turner, J., Stolzberg, D., Salvi, R., Sun, W. 2007. Salicylate induced tinnitus: Behavioral measures and neural activity in auditory cortex of awake rats. *Hearing research* 226, 244-53.