# HYPERACTIVITY IN THE HAMSTER DORSAL COCHLEAR NUCLEUS: ITS RELATIONSHIP TO TINNITUS

*****Jinsheng Zhang1, Henry E. Heffner2, Gim Koay2 and James A. Kaltenbach1

1Department of Otolaryngology-Head and Neck Surgery, Wayne State University School of Medicine
2Department of Psychology, University of Toledo

Presented at the Twenty Seventh Meeting of the Association for Research in Otolaryngology, 2004.

# Note added November 4, 2006

This study was conducted to determine the relationship between tinnitus, spontaneous activity in the dorsal cochlear nucleus (DCN), and hearing loss. An analysis of the data indicated that the increase in spontaneous activity in the DCN following exposure to loud sound was correlated, not with DCN activity, but with hearing loss. However, a post hoc analysis performed by two of the authors (JZ and JK), in which animals were selectively removed from the analysis, came to the opposite conclusion.

It is the opinion of the other authors (HH and GK) that such a post hoc analysis is not appropriate; if one is not satisfied with the outcome of an experiment, one should reassess the procedures and perform a replication. In the meantime, we are making these results available so that others may reach their own conclusions.

This poster is presented as it was at ARO 2004, with the exception of notes that have been added to slides 4, 17-19, & 22.

## INTRODUCTION

Evidence continues to grow for the notion that noise-induced hyperactivity in the dorsal cochlear nucleus (DCN) represents an important neural component of sound-induced tinnitus (Brozoski et al., 2002; Kaltenbach et al., 2004). The aim of the current study was to examine the influence of hearing loss on the relationship between the level of spontaneous activity in the DCN and the strength of tinnitus using animals that had been tested behaviorally for tinnitus.

# METHODS

**Animal subjects:** Twenty-eight adult Syrian golden hamsters.
[N.B. Statistical analyses were based on 25 animals as complete physiological recordings were not available for three of the animals.]

**Behavioral training:** Animals were behaviorally trained to respond a sound using a two-choice sound localization paradigm. Each animal was trained to lick a waterspout, when a sound was presented either from left or right loudspeaker (Fig. 1). Correct responses were rewarded with water whereas incorrect responses were followed by a mild shock. Silent trials were inserted in which no sound was presented. After 42-47 training sessions, the animals were able to perform with 95% correct or better on the left-right discrimination for at least a week, which had 5 sessions of test trials during which no sound was presented.

**Tone exposure and control treatment:** The left ears of three groups of animals were exposed under anesthesia to a 10 kHz at 80, 110 or 125 dB SPL. The exposure duration was 4 hours. Animals in control group were only anesthetized, and not exposed to any sound.

# METHODS (cont.)

**Behavioral testing:** Behavioral testing was performed during silence one day after sound exposure/control treatment.

**Measurement of ABR thresholds:** Hearing loss was evaluated by measuring the ABR thresholds to a band-pass noise burst: 7.5-50 kHz; 1 ms in duration; pulsed 27.7 times per second.

**Electrophysiological recording:** Twelve to twenty days after sound exposure/control treatment and behavioral and ABR testing, spontaneous multiunit activity was recorded on the DCN surface (Fig. 2). In each animal, we recorded from 3 rows of 13-15 sites for a total of 39-45 sites. At each site, counts of spontaneous events were performed over an interval of 90 sec.

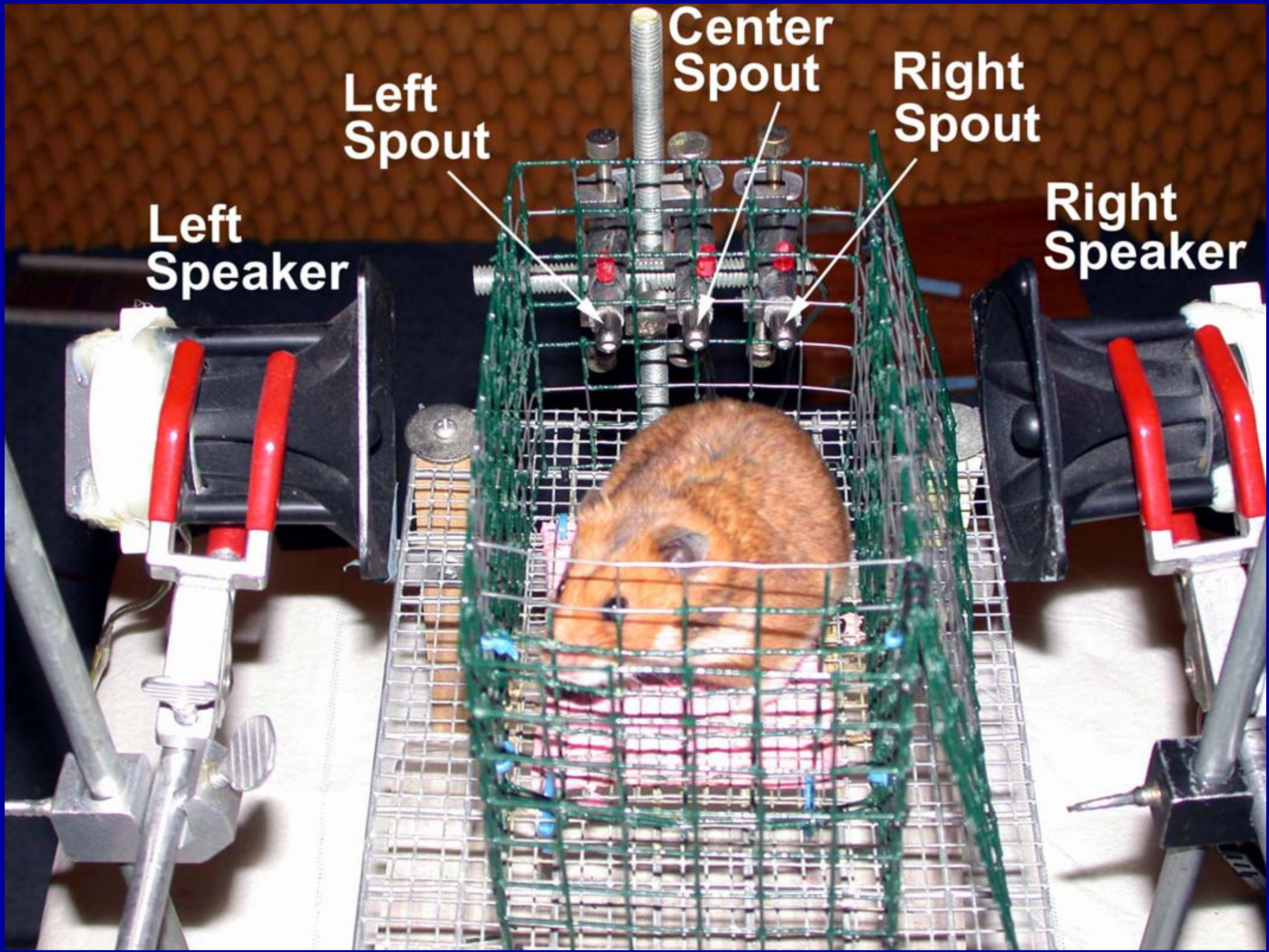
## Data analysis:

1) The behavioral performances were compared between the last pre-exposure/control treatment sessions and the first 4 post-exposure/control treatment sessions. High scores for animals going to the left indicated that they acquired tinnitus in the left, whereas low scores indicated these animals did not develop tinnitus.

2) The lateral most recording point of each row was used as a reference point for topographical localization of all the recording sites. Spontaneous rates for each animal were obtained by averaging the data recorded from 3 rows of recording sites along their rostrocaudal axes of the DCN. The data were further averaged across animals to yield average spontaneous rates for each group.

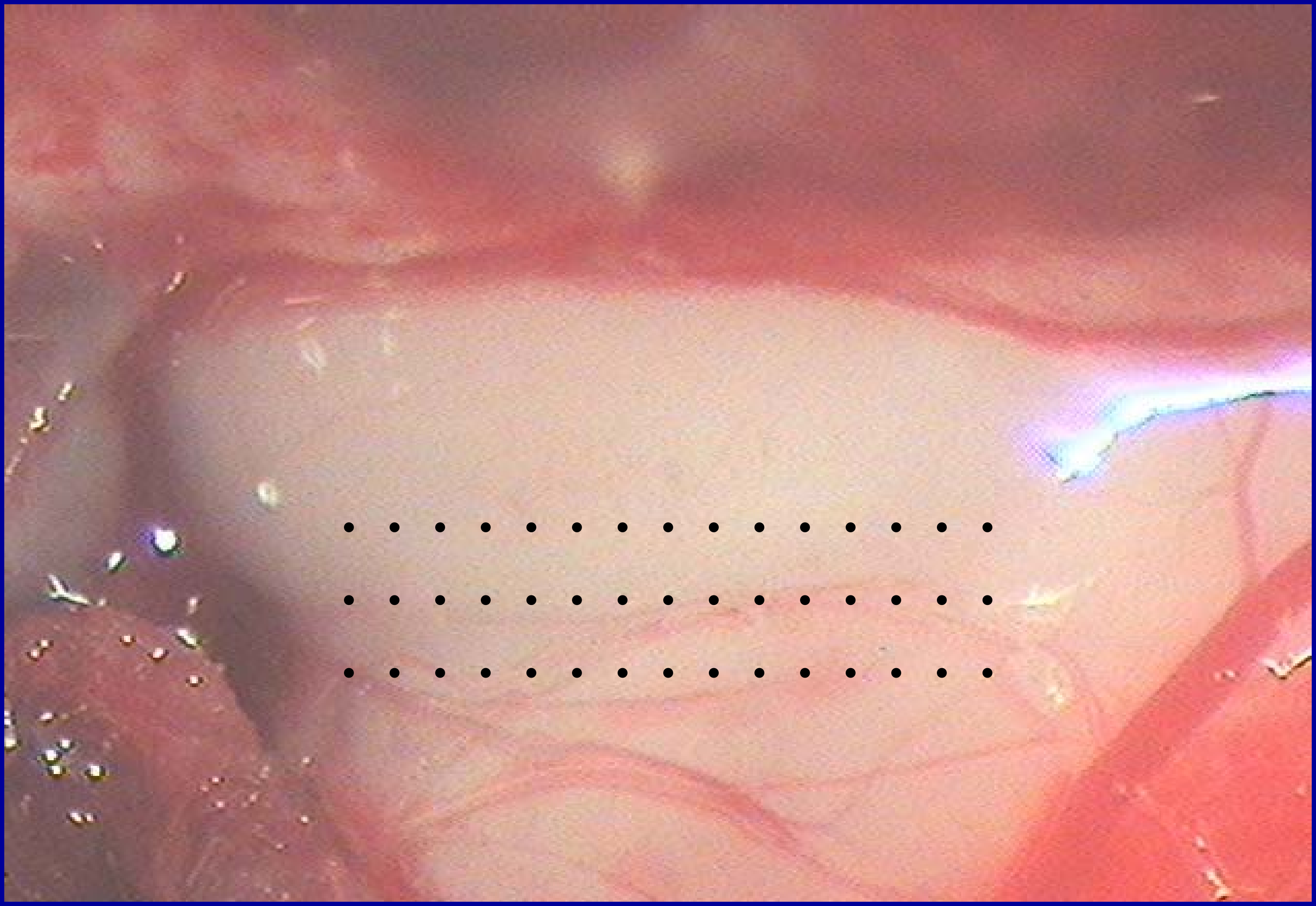
3) Linear regression and correlation (0 order and partial) analyses were performed to determine relationships among spontaneous rate, the strength of behavioral evidence for tinnitus and the degree of hearing loss as reflected by the level of ABR thresholds.

# Fig. 1 Behavioral setup




Setup for behavioral training and testing for tinnitus using the two-choice sound localization paradigm. Each animal was trained to lick a waterspout, when a sound was presented either from left or right loudspeaker

# Fig. 2 Recording setup



Dorsal view of the surgically exposed left DCN. Dots represent 3 rows of recording sites along the mediolateral axis of the DCN.

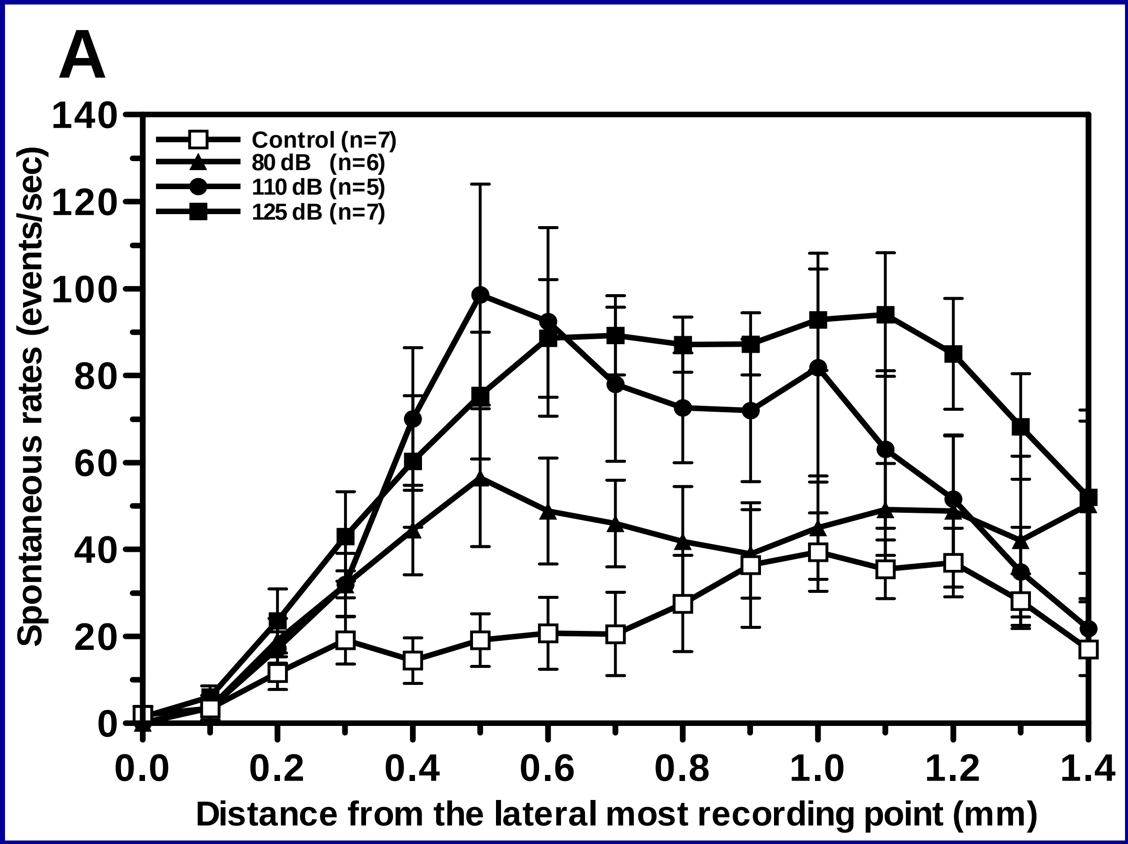
# RESULTS

Intense sound exposure caused corresponding changes in the level of spontaneous activity in the DCN, the probability that an animal tested positive for tinnitus, and the degree of ABR threshold shift. All three quantities increased in magnitude with increases in exposure level. (Fig. 3).

Correlation analyses revealed that the level of spontaneous activity in the DCN was significantly related to both behavioral evidence for tinnitus and the degree of hearing loss. The relationship between activity and hearing loss was slightly stronger than that between activity and tinnitus when the data were analyzed as a single sample (Fig. 4 and Table 1).

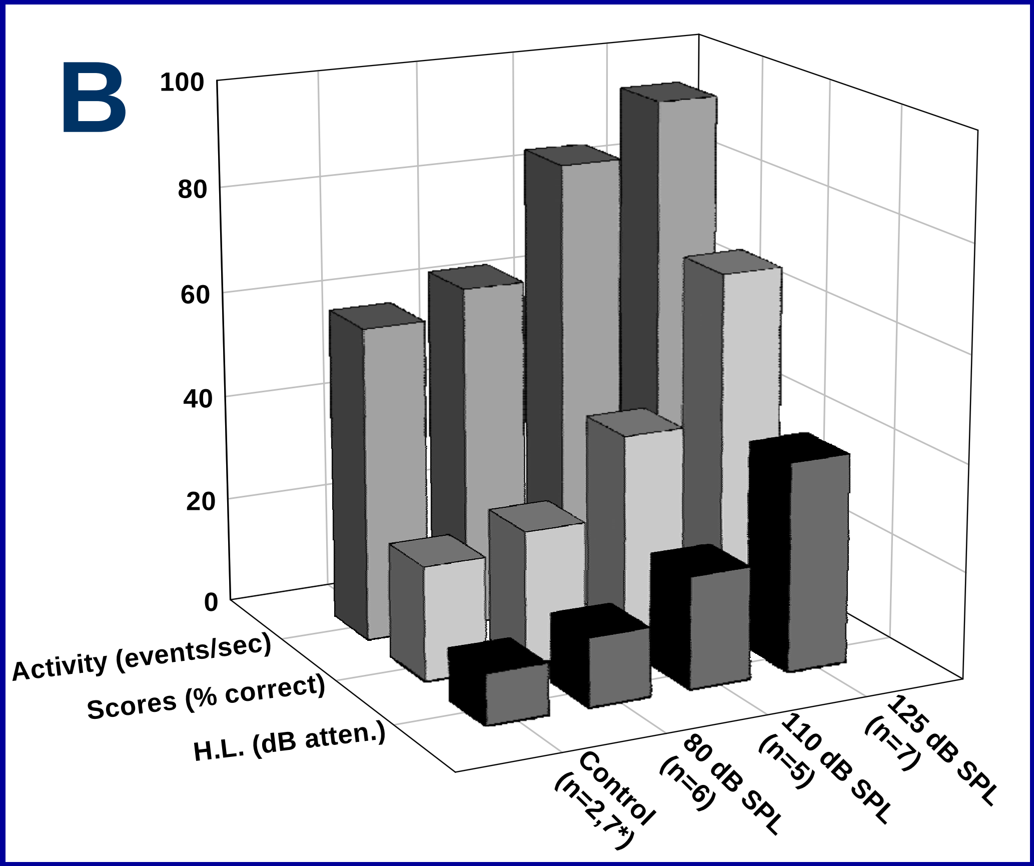
However, closer inspection of the data in Fig. 4A and B suggests that the point distribution falls into two segments as shown in Fig. 5A and B; this 2-segment pattern is similar to that found in a previous study examining the relationship between the level of activity in the DCN and the degree and type of hair cell loss (Fig. 5D). Correlational analysis of the major segment (segment A) suggests that the level of activity is more strongly correlated with the behavioral evidence for tinnitus than with the degree of hearing loss (JZ and JK, Fig. 5A and B, Table 1).

# Fig. 3A Activity, behavioral scores and ABR thresholds




A. Spontaneous rates (Mean ± S.E.M.) recorded from control animals and animals exposed to a 10 kHz tone at 80, 110 or 125 dB SPL. Note that spontaneous rates increased broadly as exposure intensity was increased.

# Fig. 3B Activity, behavioral scores and ABR thresholds



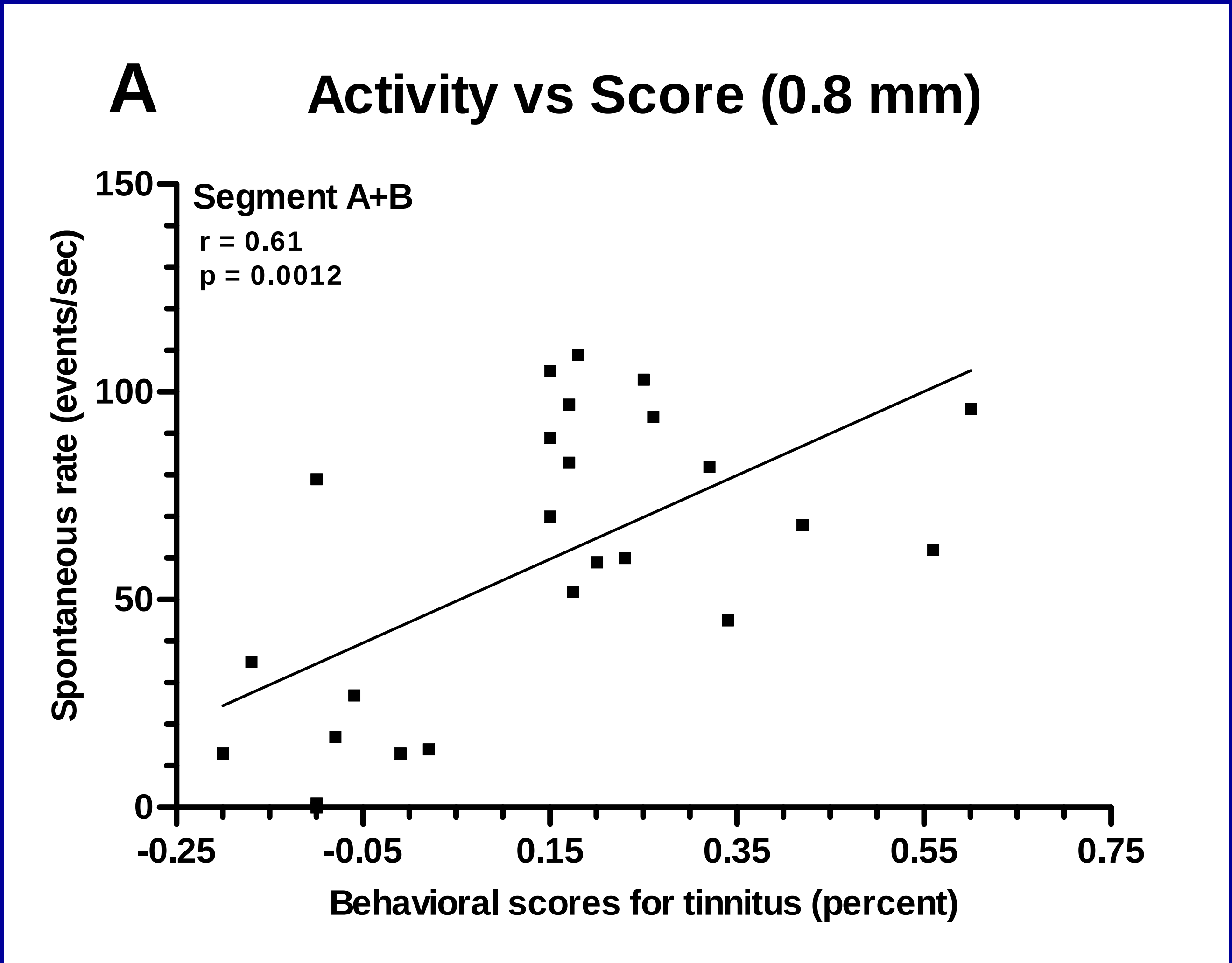

B. A three-dimensional plot shows trends towards higher spontaneous rates, behavioral scores for tinnitus and ABR thresholds with increasing exposure levels. Average spontaneous rates obtained at locus of 0.8 mm from the lateral most recording point of the DCN is used as a representative profile of changes in spontaneous rates.

# RESULTS

Intense sound exposure caused corresponding changes in the level of spontaneous activity in the DCN, the probability that an animal tested positive for tinnitus, and the degree of ABR threshold shift. All three quantities increased in magnitude with increases in exposure level. (Fig. 3). Correlation analyses revealed that the level of spontaneous activity in the DCN was significantly related to both behavioral evidence for tinnitus and the degree of hearing loss. The relationship between activity and hearing loss was slightly stronger than that between activity and tinnitus when the data were analyzed as a single sample (Fig. 4 and Table 1).

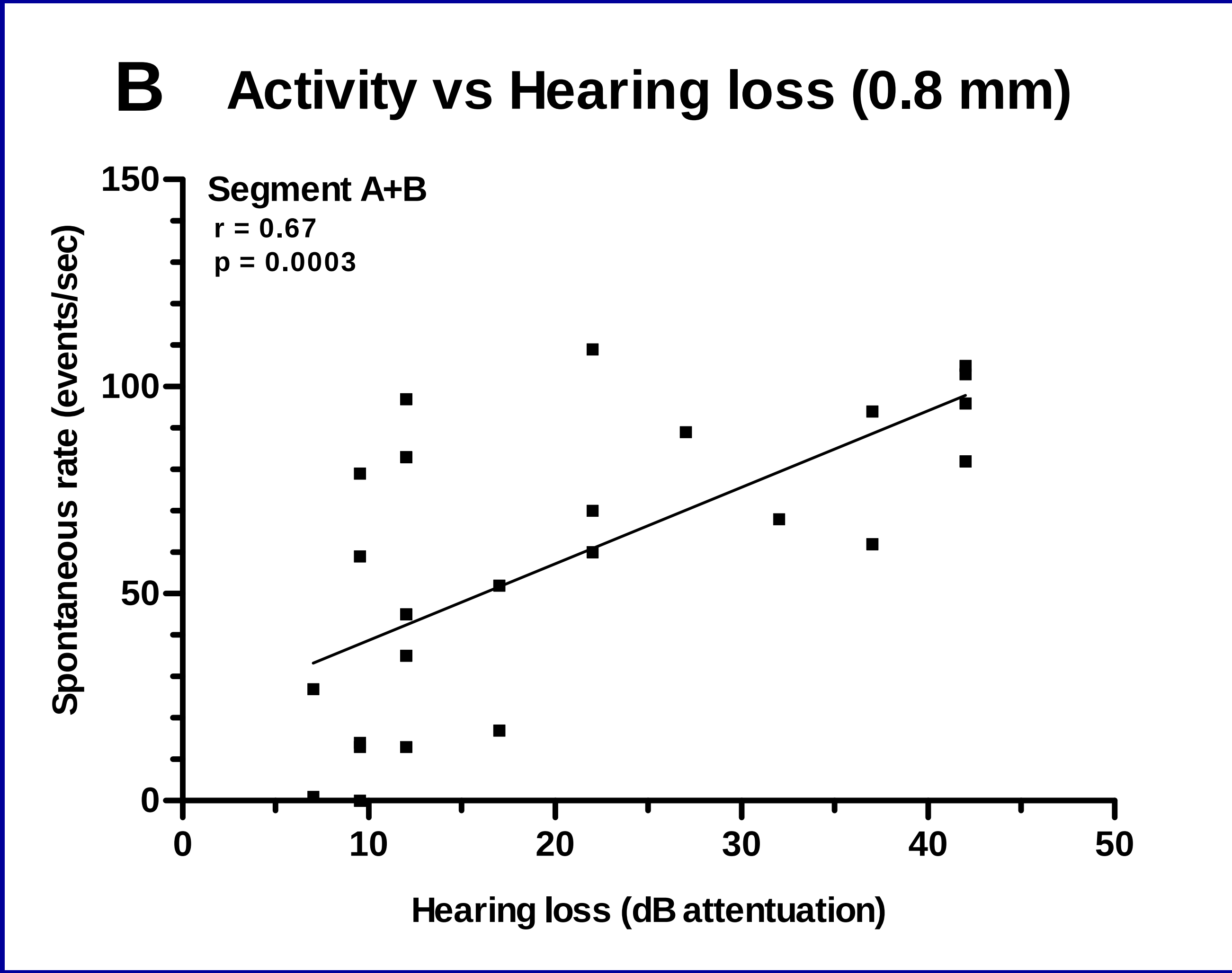
However, closer inspection of the data in Fig. 4A and B suggests that the point distribution falls into two segments as shown in Fig. 5A and B; this 2-segment pattern is similar to that found in a previous study examining the relationship between the level of activity in the DCN and the degree and type of hair cell loss (Fig. 5D). Correlational analysis of the major segment (segment A) suggests that the level of activity is more strongly correlated with the behavioral evidence for tinnitus than with the degree of hearing loss (JZ and JK, Fig. 5A and B, Table 1).

# Fig. 4A Correlation analysis



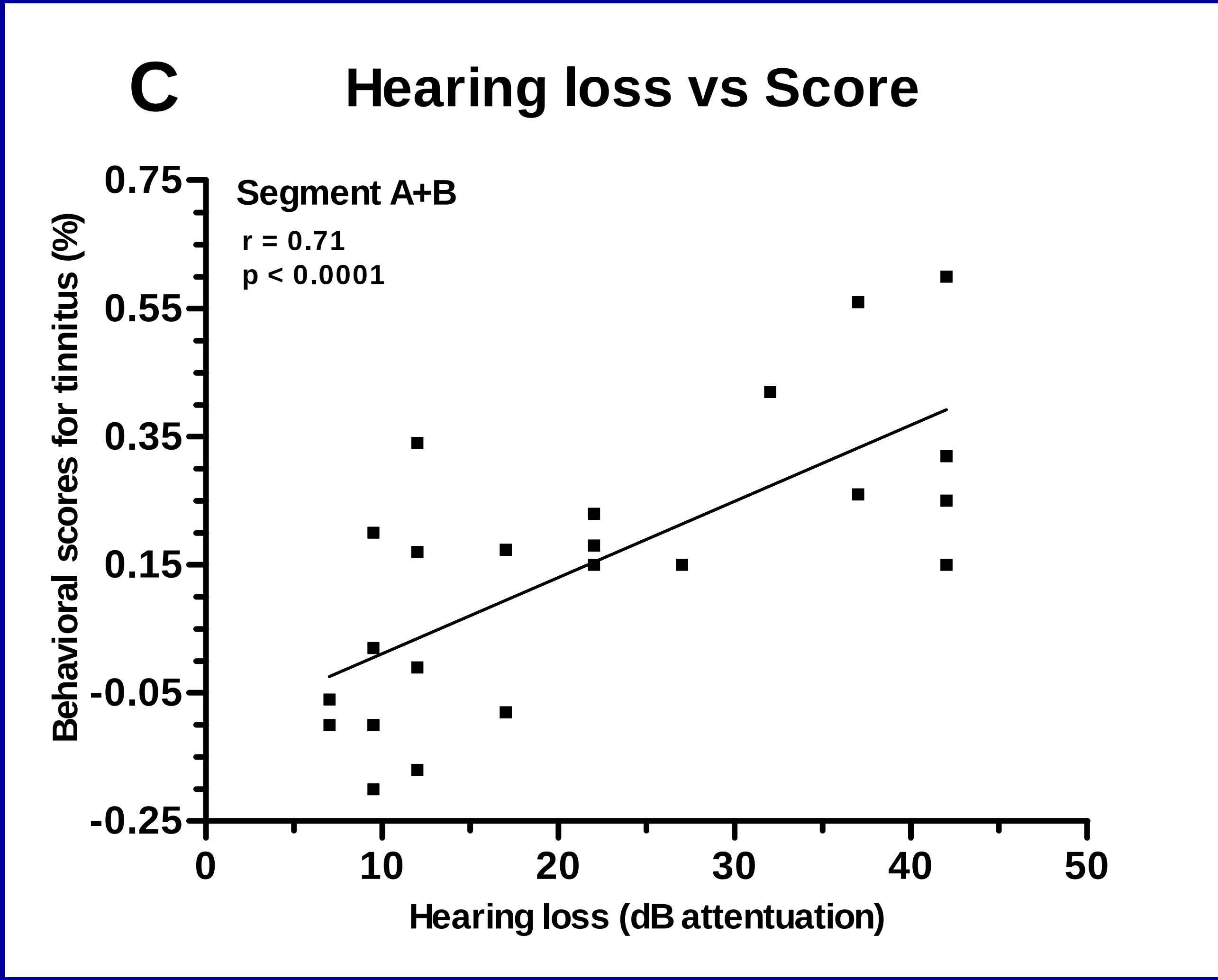

Relationship between DCN activity and behavioral evidence for tinnitus when the entire set of data is analyzed as a whole. Spontaneous rates for each animal were obtained from a recording site at the 0.8 mm locus.

# Fig. 4B Correlation analysis




Relationship between DCN activity and hearing loss when the entire data is analyzed as a whole. Spontaneous rates for each animal were obtained from a recording site at the 0.8 mm locus.

# Fig. 4C Correlation analysis



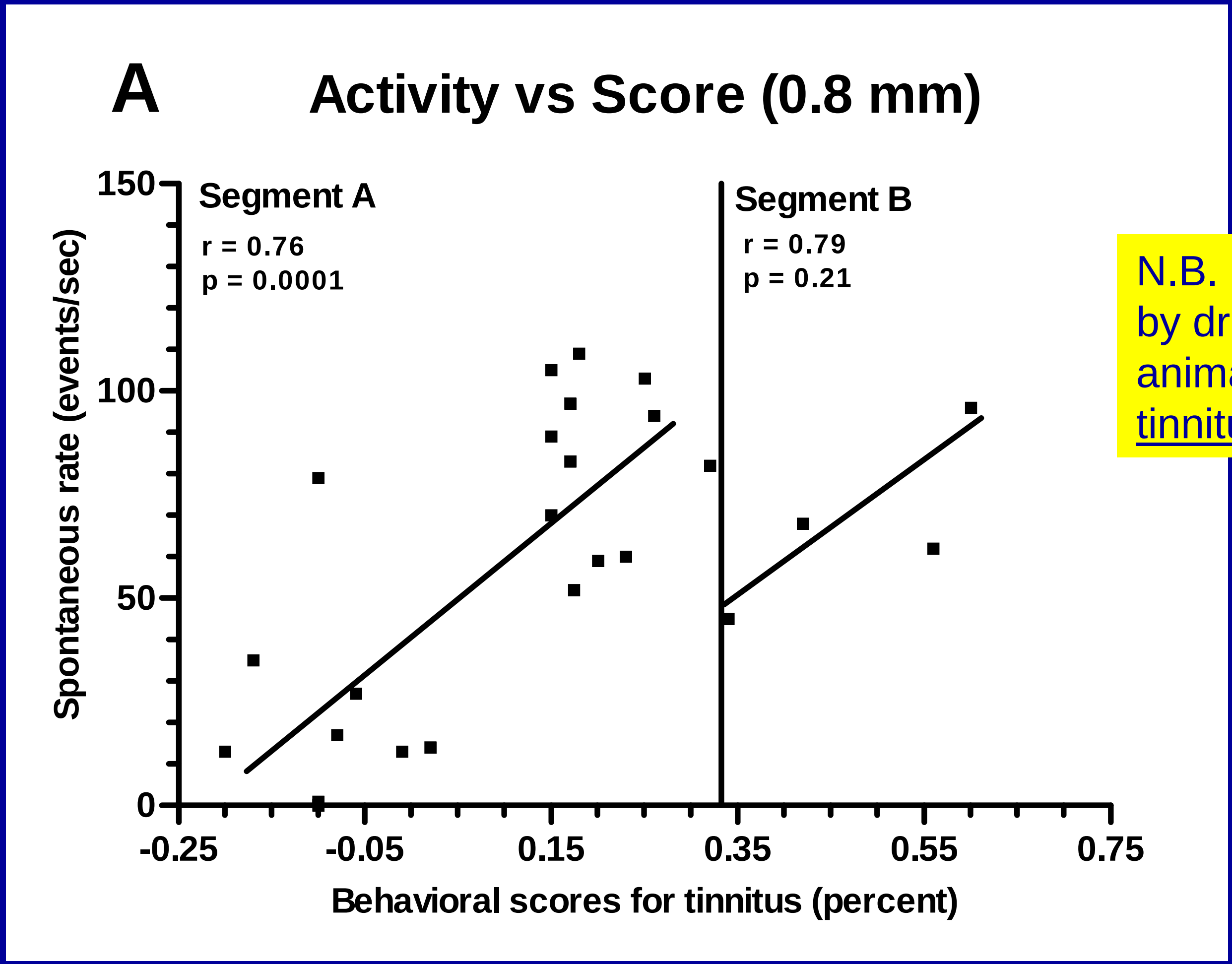

Relationship between behavioral evidence for tinnitus and hearing loss when the entire set of data is analyzed as a whole.

# Table for Fig. 4   Correlation analysis

| Correlations: | Entire data set | | Data in segment A | |
|---|---|---|---|---|
| **0 order** | r | p | r | p |
| 1. Activity x Score | 0.61 | <0.001 | 0.71 | <0.0001 |
| 2. Activity x Hearing Loss | 0.67 | <0.001 | 0.56 | <0.05 |
| 3. Score x Hearing Loss | 0.71 | <0.0001 | 0.59 | <0.001 |
| | | | | |
| **Partial** | $r_p$ | $p_p$ | $r_p$ | $p_p$ |
| 1. Activity x Score | 0.26 | N.S. | 0.57 | <0.05 |
| Remove Hearing Loss | | | | |
| | | | | |
| 2. Activity x Hearing Loss | 0.42 | <0.05 | 0.25 | N.S. |
| Remove Score | | | | |
| | | | | |
| 3. Score x Hearing Loss | 0.51 | <0.05 | 0.33 | N.S. |
| Remove Activity | | | | |

Correlation analyses (0 order and partial) among the level of spontaneous activity, behavioral evidence for tinnitus and the degree of hearing loss.

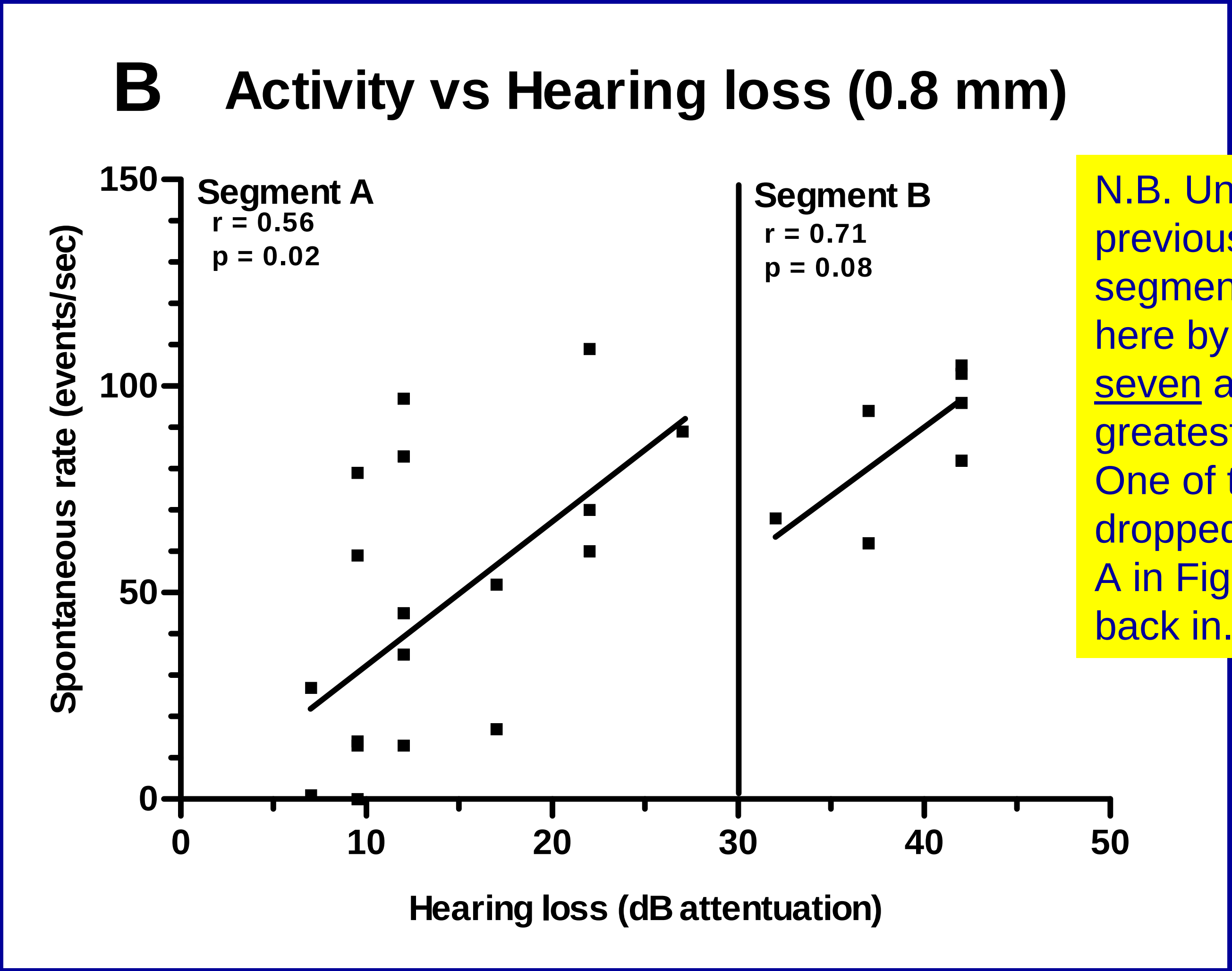
# Fig. 5A  A two-segment theory




N.B. Segment A is made by dropping the four animals with the highest tinnitus scores.

Relationship between DCN activity and behavioral evidence for tinnitus. Spontaneous rates for each animal were obtained from a recording site at the 0.8 mm locus. The point distribution is suggestive of a 2-segment function.

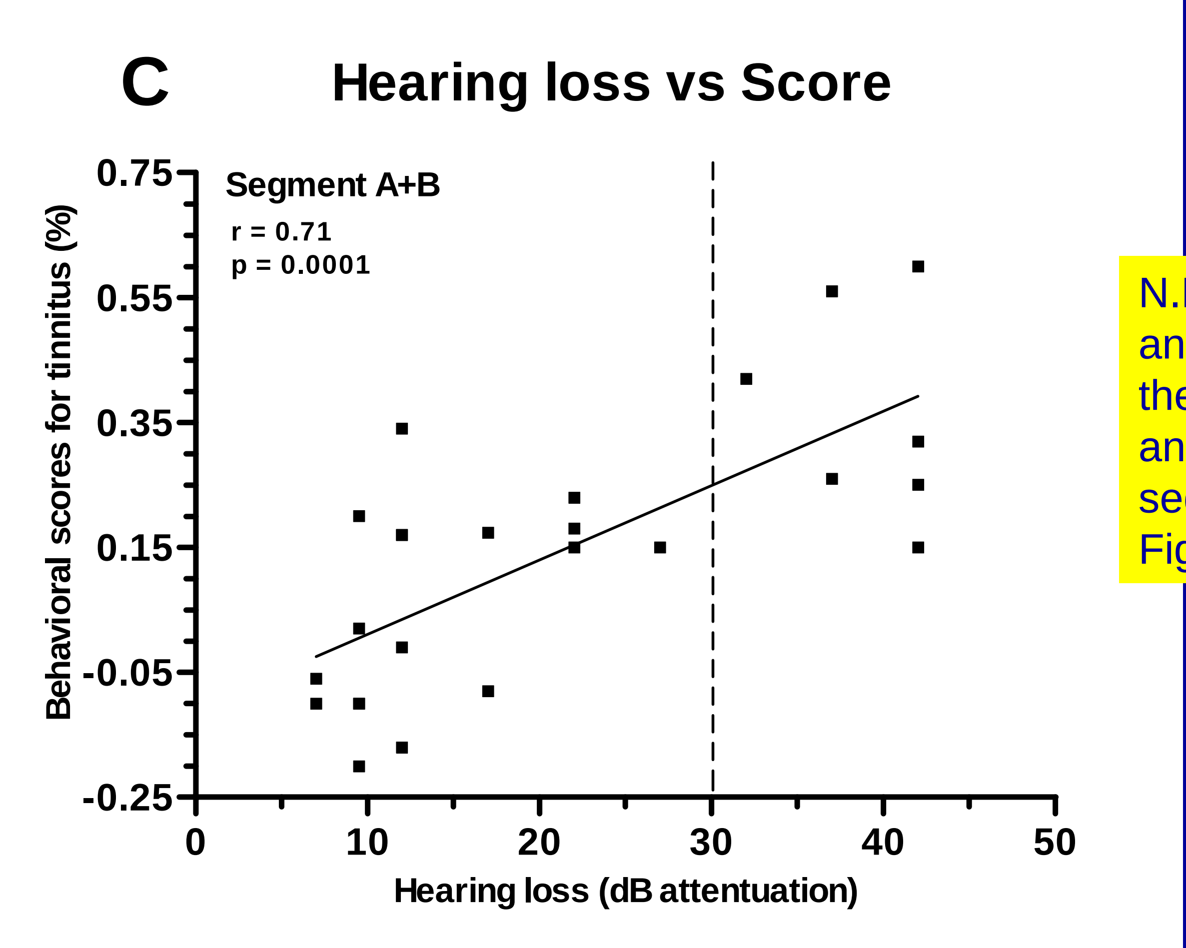
# Fig. 5B A two-segment theory




N.B. Unlike the previous graph, segment A is made here by dropping the seven animals with the greatest hearing loss. One of the animals dropped from segment A in Fig. 5A is now back in.

Relationship between DCN activity and the degree of hearing loss. Spontaneous rates for each animal were obtained at the 0.8 mm locus. The point distribution is suggestive of a 2-segment function.

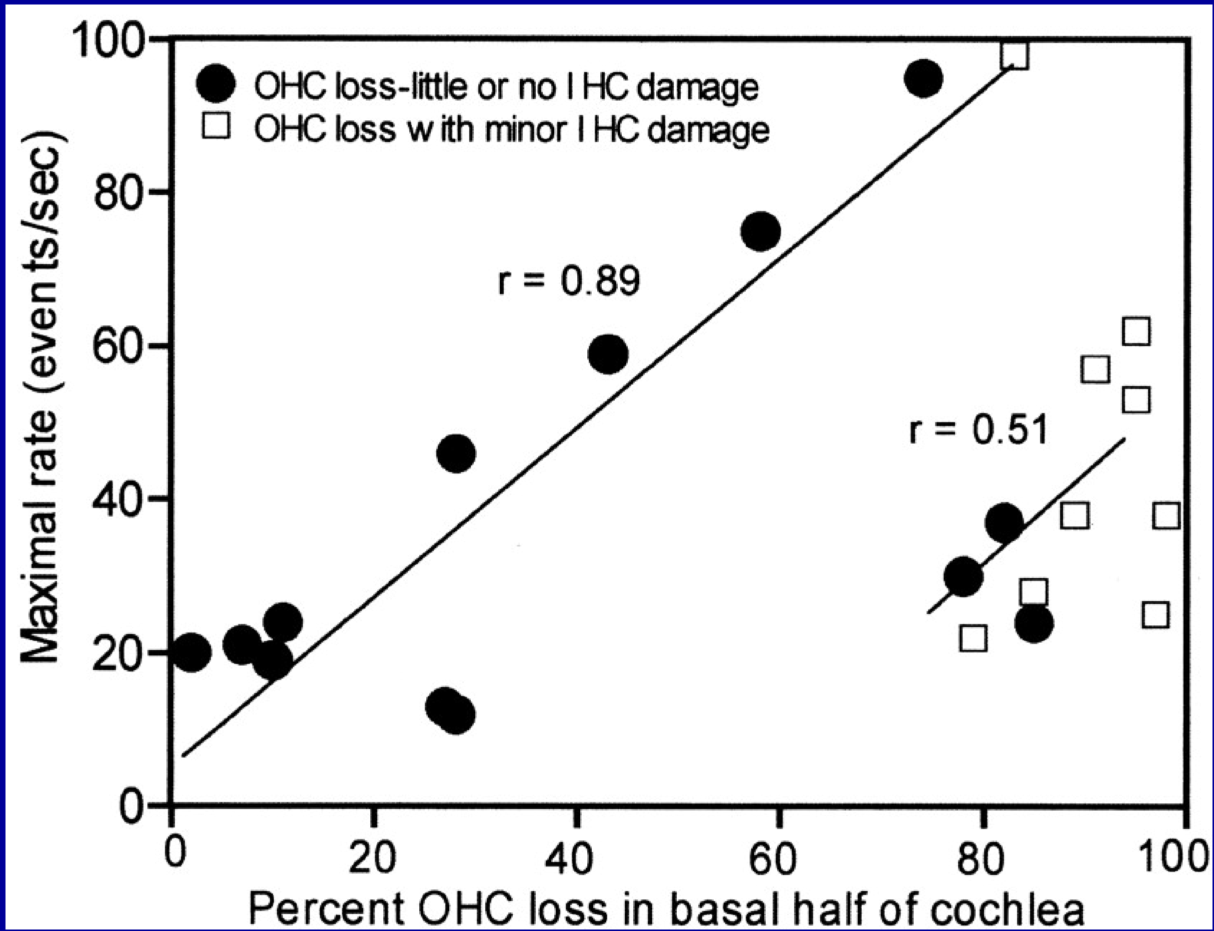
# Fig. 5C A two-segment theory




N.B. This analysis drops the same seven animals from segment A as Fig. 5B.

Relationship between the strength of tinnitus and the degree of hearing loss. Each point indicates the behavioral score for a single animal whose hearing loss, measured as the magnitude of the ABR threshold shift, is indicated on the abscissa.

# Fig. 5D   A two-segment theory




Relationship between spontaneous rate and hair cells loss as a result of cisplatin treatment. Spontaneous rate changes manifested with an offset as loss of IHCs was involved (adapted from Kaltenbach et al., 2002 )

# SUMMARY

The results are in line with our previous findings (Kaltenbach et al., 2004) by showing that the strength of evidence for tinnitus correlated with the degree of hyperactivity in the DCN.

The results of the correlational analysis are as yet unclear regarding the cause and effect relationship between hyperactivity and tinnitus. The partial correlation results for the data taken as a whole suggest that the relationship between DCN activity and tinnitus may be dependent on the presence of hearing loss. Removal of the effects of hearing loss reduces the significance of the relationship between DCN activity and tinnitus. This could be interpreted to mean either that hyperactivity and tinnitus are independent effects of hearing loss, or that hearing loss may lead to tinnitus indirectly by causing hyperactivity as well as directly by removing the masking effects of external sound on the perception of tinnitus.

# SUMMARY (cont.)

An alternative view, based on the two-segment model of Fig. 5, is that hyperactivity and tinnitus have a significant cause and effect relationship independent of hearing loss, over most of the hearing loss range, but that with severe hearing loss, the level of activity in the DCN becomes less important than the degree of hearing loss in determining the occurrence of tinnitus (JZ and JK).

This two-segment hypothesis also offers to explain why some cases of tinnitus are not associated with hearing loss and why hearing loss is not always accompanied by tinnitus (JZ and JK).

N.B. An issue not addressed here is the discrepancy in the time course of the increased spontaneous activity in the DCN, which does not occur until days after exposure to loud sound, whereas the onset of the resulting tinnitus is instantaneous. For a discussion of this and other issues, see Heffner, H. E. and Koay, G. (2005). Tinnitus and hearing loss in hamsters exposed to loud sound. *Behavioral Neuroscience*, 119, 734-742.

## Acknowledgement

This work was supported by the Tinnitus Research Consortium and NIDCD grant R01 DC03258.