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Title

Effects of deafness and cochlear implant use on temporal response characteristics in cat primary auditory cortex.

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Abstract

We have previously shown that neonatal deafness of 7-13 months duration leads to loss of cochleotopy in the primary auditory cortex (AI) that can be reversed by cochlear implant use. Here we describe the effects of a similar duration of deafness and cochlear implant use on temporal processing. Specifically, we compared the temporal resolution of neurons in AI of young adult normal-hearing cats that were acutely deafened and implanted immediately prior to recording with that in three groups of neonatally deafened cats. One group of neonatally deafened cats received no chronic stimulation. The other two groups received up to 8 months of either low- or high-rate (50 or 500 pulses per second per electrode, respectively) stimulation from a clinical cochlear implant initiated at 10 weeks of age. Deafness of 7-13 months duration had no effect on the duration of post-onset response suppression, latency, latency jitter, or the stimulus repetition rate at which units responded maximally (best repetition rate), but resulted in a statistically significant reduction in the ability of units to respond to every stimulus in a train (maximum following rate). None of the temporal response characteristics of the low-rate group differed from those in acutely deafened controls. In contrast, high-rate stimulation had diverse effects: it resulted in decreased suppression duration, longer latency and greater jitter relative to all other groups, and an increase in best repetition rate and cut-off rate relative to acutely deafened controls. The minimal effects of moderate-duration deafness on temporal processing in the present study are in contrast to its previously-reported pronounced effects on cochleotopy. Much longer periods of deafness have been reported to result in significant changes in temporal processing, in accord with the fact that duration of deafness is a major factor influencing outcome in human cochlear implantees.

Keywords

temporal processing; cochlear implant; cortical plasticity; neural prosthesis, sensorineural hearing loss

Abbreviations

ABR	Auditory brainstem response
BRR	Best repetition rate
AI	Primary auditory cortex
EABR	Electrically evoked auditory brainstem response
ICES	Intra-cochlear electrical stimulation
MFR	Maximum following rate
PSTH	Post stimulus time histogram+
SEM	Standard error of the mean

Highlights

- Cortical temporal processing in neonatally profoundly deafened unstimulated cats was near-normal after 7-13 months.
- High-rate intracochlear electrical stimulation had significant, but diverse, effects on temporal processing.
- Temporal processing in cats receiving low-rate electrical stimulation did not differ from that in controls.

1. Introduction

Cochlear implants are highly successful sensory prostheses, providing hearing and good levels of speech perception to more than 300,000 individuals with profound/severe sensorineural hearing loss. A congenital or neonatal hearing loss of this sort has effects on the morphology and function of neurons along the auditory pathway, which influence the effectiveness of a subsequently introduced cochlear implant (for reviews see Shepherd & Hardie, 2001; Fallon *et al.*, 2014a). At the cortical level, a period of 7-13 months of deafness in neonatally deafened cats results in complete loss of cochleotopy in the primary auditory cortex (AI) (Fallon *et al.*, 2009; Fallon *et al.*, 2014b); though cochleotopy can be maintained (Fallon *et al.*, 2009) or re-established (Fallon *et al.*, 2014b) by 6-8 months of cochlear implant use.

The perception of speech and of other complex acoustic signals involves the processing of both spectral and temporal information (e.g. Rosen, 1992; Shannon *et al.*, 1995; Moore, 2008), and temporal information plays a critically important role when spectral information is degraded, as is the case with a cochlear implant. These considerations have prompted a number of studies in animal models examining the way in which central auditory neurons process temporal information provided by intra-cochlear electrical stimulation (ICES), and of the effects of deafness and of experience with chronic ICES on temporal resolution in the auditory system. As elaborated below, the term “temporal resolution” is used here to refer to two aspects of processing of temporal information by the central auditory system. The first is the latency of the responses evoked by ICES and the variability of that latency (viz. latency “jitter”). The second is the precision with which trains of stimuli are represented in the neuronal discharge, which is quantified by measuring the stimulus repetition rate at which the maximum response is evoked and/or the highest rate at which the response can follow the stimulus (e.g. Eggermont, 1991; 1992).

Most studies of the effects of deafness and of chronic ICES on temporal resolution in the central auditory system have been carried out on neurons in the inferior colliculus (IC, e.g. Snyder *et al.*, 1991; Snyder *et al.*, 1995; Shepherd *et al.*, 1999; Vollmer *et al.*, 1999; Vollmer *et al.*, 2005). The general findings are of degraded temporal processing with deafness that is reversed with chronic intracochlear electrical stimulation. At the cortical level, Vollmer and Beitel (2011) reported that very long periods of deafness in two neonatally deafened cats (38 and 78 months, respectively) resulted in degradation in temporal following capacity and spike-timing precision. Vollmer and Beitel (2011) also reported that passive chronic ICES combined with training to detect ICES (but not passive ICES alone) over a period of 4-8 months after many years of deafness partially restored temporal resolution.

The primary aim of the present study was to investigate whether deafness of moderate duration (less than 14 months), known to effect cochleotopy, results in degradation of temporal resolution, and whether any such effects are offset by cochlear implant use that provides chronic ICES related to the acoustic environment. A secondary aim was to determine whether the effects of chronic ICES depend on stimulus rate. Preliminary findings have been presented in abstract form (Fallon *et al.*, 2007a; Fallon *et al.*, 2007b).

2. Materials and Methods

2.1 Experimental subjects

Seventeen healthy cats with otoscopically normal tympanic membranes were used in the present study. Data on the cochleotopic organization of the AI obtained from some of these cats were presented in Fallon et al. (2009), and the basic methods were as described in that paper. Those methods will therefore be described only briefly here. All procedures were in accordance with Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and with the Guidelines laid down by the National Institutes of Health in the US regarding the care and use of animals for experimental procedures and were approved by the Royal Victorian Eye and Ear Hospital Animal Research and Ethics Committee.

2.2 Deafening procedure

Fifteen cats were administered a daily subcutaneous (s.c.) injection of neomycin sulfate (60 mg/kg) from one day after birth for seventeen days (Leake *et al.*, 1991; Fallon *et al.*, 2009). Hearing status was then measured, and if the animal was not profoundly deaf neomycin injections were continued. The hearing status was repeatedly tested at three-day intervals until the animal was profoundly deaf. The criterion of profound deafness was the absence of a monaural click-evoked auditory brainstem response (ABR) at 93 dB peak equivalent sound pressure level in either ear. ABR-recording methods were as described previously (Coco *et al.*, 2007). The remaining two cats had normal hearing (ABR threshold of $\leq$ 32 dB peak equivalent sound pressure level), and were deafened acutely just prior to cortical recording as young adults. This cohort (acutely-deafened control (ADC) group) was unilaterally deafened by perfusion of neomycin sulfate (10% w/v solution) through the cochlea (Hardie & Shepherd, 1999).

2.3. Cochlear implantation and chronic stimulation

At eight weeks of age, ten randomly selected deafened animals were unilaterally implanted in the left cochlea with a custom built eight-ring scala tympani electrode array, an extra-cochlear ball electrode in the temporalis muscle, and lead-wire assembly, using previously published techniques (Coco *et al.*, 2007). Surgery was performed under aseptic conditions, with each animal premedicated using acepromazine maleate/atropine sulphate (0.05 ml/kg s.c.) and maintained at a surgical level of anesthesia using a closed circuit anesthetic machine delivering a mixture of halothane and oxygen. The array was inserted 8 mm into the scala tympani, placing the most apical electrode (E1) at the ~10-kHz place and the most basal electrode (E8) at the ~26-kHz place (Brown *et al.*, 1992).

Fourteen days after surgery, and every month thereafter during the chronic stimulation program, the cats were anesthetized with ketamine and xylazine (20 mg/kg i.m., 2 mg/kg s.c.), and an electrically evoked ABR (EABR) was recorded for each stimulating electrode.

EABR-recording methods were as described previously (Fallon *et al.*, 2009). After the first EABR recordings (i.e. at 10 weeks of age), the chronic stimulation program was initiated. Each animal received unilateral stimulation at multiple sites (typically 6 to 8 electrodes, depending on the number of functional electrode contacts) within the lower basal turn from a Nucleus® CI24 cochlear implant and Nucleus® ESPrit 3G speech processor (Fallon *et al.*, 2009; Fallon *et al.*, 2014b). The environmentally-derived stimulation was presented continuously (i.e., 24 hours per day) except for occasional periods when flat batteries had to be replaced. The speech processors were programmed using standard clinical frequency allocation tables and delivered monopolar stimulation at either 50 pulses per second (pps) (low rate electrical stimulation (LR-ES group)) or 500 pps (high-rate electrical stimulation (HR-ES group)) per electrode. Cats were randomly assigned to the HR-ES and LR-ES groups (n = 6 and 4, respectively). Each biphasic current pulse had a 25-μs phase duration and an 8-μs inter-phase gap, and stimulus level was varied from 3 dB below to 6 dB above the EABR threshold for that electrode. These stimulus levels were assessed for each animal and were confirmed to elicit altering responses, including head and pinna movements, with no evidence of discomfort. Both self-vocalizations and vocalizations by other animals housed in the facility produced modulations in the stimulus currents levels within the set 9 dB dynamic range. Similarly, other environmental sounds associated with the normal running of the facility resulted in modulation of the ongoing electrical stimulation similar to that which a cochlear implant patient would have received. Animals were chronically stimulated for periods up to 8 months, with stimulation continuing until the commencement of the acute electrophysiological experiments.

2.4. Cortical recording

Acute electrophysiological experiments to record the basic response properties of AI neurons were performed at 7-15 months of age in all but one animal in which damage to the chronic leadwire system required an earlier acute study (Table 1). Anesthesia was induced

with ketamine and xylazine (20 mg/kg i.m., 2 mg/kg s.c.), and a tracheal cannula was inserted. Sodium pentobarbitone (0.3 - 0.7 mg kg^{-1} h^{-1}, intravenous) via a slow-infusion pump was used to maintain a steady, light level of surgical anesthesia throughout the recording period. Heart rate, respiration rate, end-tidal CO_2, and core body temperature were maintained within normal levels. The ADC animals, and those in the neonatally deafened unstimulated (DU) group (n = 5), were acutely implanted (left ear) using the same surgical techniques as those used for the chronically implanted animals.

Animals were placed in a stereotaxic frame in a Faraday room, and a craniotomy was performed to expose the auditory cortex contralateral to the implanted cochlea. Multi-unit recordings were made from putative AI using a combination of single tungsten microelectrodes (WPI; Sarasota, Florida), and linear (A1x32-6mm-100-413-A32, NeuroNexus Technologies; Ann Arbor, Michigan) and planar (Cyberkinetics; Foxborough, Massachusetts) silicon arrays. In each case, the Cyberkinetics array (usually 7 by 7 electrodes with 400-µm inter-electrode spacing) was inserted in the middle of the medial ectosylvian gyrus, and single microelectrode penetrations were made into the gyrus at sites between the array and the anterior and posterior ectosylvian sulci. The vast majority of recording sites were therefore in the middle- to high-frequency region of putative AI, corresponding approximately to the location of the intracochlear stimulating electrodes. However, in the absence of information about tonotopic boundaries, the possibility that some of the most rostral recording sites might have been in the high-frequency region of the anterior auditory field cannot be excluded. The Cyberkinetics recording electrodes were 1.0 mm in length, and most recordings from the tungsten microelectrodes were made at depths from 600 – 1400 µm, so the vast majority of recordings were made from the middle (thalamorecipient) cortical layers. Although laminar differences in the temporal response properties of auditory cortical units have been described (e.g. Sakata & Harris, 2009; Christianson *et al.*, 2011), our experimental protocol did not allow us to examine depth effects in gyral penetrations.

Recordings were also made at a range of depths from tungsten microelectrodes and NeuroNexus arrays inserted into the low-frequency region of AI in the rostral bank of the posterior ectosylvian sulci. Multi-unit recordings from the tungsten microelectrodes were amplified by a factor of 10^4, filtered (high pass: 300 Hz, 24 dB/octave; low pass: 3 kHz, 6 dB/octave), and displayed on an oscilloscope. The oscilloscope trigger level was set to discriminate action potentials clearly above the noise level, and the trigger pulses were sampled at 20 kHz. Multi-unit recordings from the silicon arrays were captured at a sample rate of 30 kHz using the Cerebus system (Cyberkinetics; Foxborough, Massachusetts), and single- or multi-unit recordings were identified off-line using standard spike discrimination techniques in IgorPro (Wavemetrics; Lake Oswego, Oregon) after the removal of stimulus artifacts (Heffer & Fallon, 2008). Acute experiments were of typically of 2 days duration and at the end of the experiment the animals were terminated with an overdose of sodium pentobarbital (150 mg/kg, intravenous) and the location of the intracochlear electrode array within the cochlea confirmed.

2.4. Electrical stimulation and data analysis

To characterize the basic temporal response properties of AI neurons to ICES, monopolar stimulation at a rate of 1 to 1.5 pps using a range of stimulus currents (0 – 2 mA in 25 μA steps) and intracochlear electrodes, was utilized. Stimuli were generated by a laboratory-based stimulator that delivered charge-balanced, optically-isolated biphasic stimuli. The mean first-spike latency and jitter (the standard deviation of the first-spike latency across trials) of the responses was determined at every stimulation electrode - current level combination. For each intracochlear electrode an input-output function was also determined and was fitted with a saturating Gaussian function (Sachs & Abbas, 1974), from which the threshold (defined as the current required to achieve a half maximal response) and dynamic range (defined as the current range required to achieve an increase in response from 10% to 90% of maximal) could be determined. The input-output function was also used to

determine the current levels at which statistical comparisons of temporal response characteristics were made.

To investigate the response properties of AI neurons to repetitive ICES, 0.5-s pulse trains at rates of 1-20 pps over a range of stimulus currents and intracochlear electrodes were presented. The same randomised matrix of stimulating electrode - current level combinations was used as for the basic response properties and responses were recorded at every stimulation electrode - current level combination. The total number of spikes evoked over the entire 0.5-s stimulus duration was used to determine the best repetition rate (BRR; i.e. the stimulation rate that evoked the most spikes) and the cut-off rate (CutOff; i.e. the stimulation rate at which the response dropped to just less than 50% of the response at BRR). In some cases it was not possible to determine a CutOff value (i.e. the response had not dropped below 50% of the response at BRR by 20 pps). Data from these units were excluded from the CutOff analysis, and the reported values will therefore tend to underestimate the true CutOff value for each group. The response to individual stimuli within the train was used to determine the maximum following rate (MFR; i.e. the highest stimulation rate at which the percentage of pulses in the train that evoked at least one spike within a 5 – 40 ms window following each pulse was $\geq$ 80%).

The effects of the different experimental treatments on the temporal resolution of AI units were assessed by analyzing the responses to stimulation on the intra-cochlear electrode with the lowest threshold (the best electrode) with one-way ANOVAs; post-hoc analyses of significant effects used Bonferroni-corrected t-tests.

3. Results

3.1. Duration of deafness

As there is ongoing degeneration of spiral ganglion neurons following a profound hearing loss, it is important to ensure that all groups had a similar duration of deafness. The duration of deafness for the DU, HR-ES and LR-ES groups of animals is equivalent to their

age at acute study (Table 1; mean ages 9.6, 8.0, and 10.2 months, respectively), and there was no significant difference in age between the groups (one-way ANOVA, $F_{2,14} = 2.2$, $P = 0.16$). However, given that duration of stimulation can also affect cortical responses to cochlear electrical stimulation (e.g. Kral *et al.*, 2006), it should be noted that the duration of stimulation in the HR-ES group (6.0 ± 1.5 months) was significantly shorter than in the LR-ES group (8.3 ± 1.0 months; t-test; $t_9 = 2.6$, $P = 0.033$).

3.2. Basic Response Characteristics

The number of recording sites at which basic latency and jitter data were recorded are shown for each animal in Table 1. Off-line spike discrimination techniques were able to isolate a total of 23 single-units that were responsive to electrical stimulation. The average spontaneous firing rate, determined from a 40-ms window preceding each stimulus for the 1 to 1.5 pps stimuli, for the single-units was less than 1 spike/s and, there was no difference between the experimental groups (one-way ANOVA, $F_{3,19} = 0.60$, $P = 0.62$). The data for driven single-units exhibited the same trends evident in the multi-unit data presented below, but because of the low numbers separate statistical analysis of these data was not appropriate.

The response of the majority (over 95%) of multi-unit clusters to individual pulses at 1 pps comprised an onset response (with a latency between 5 and 40 ms) followed by a period of suppression of spontaneous activity and a rebound response (Figures 1A, B and 2A). This response pattern is identical to that evoked by click stimuli in AI of hearing cats (Eggermont, 1991; 1992). Consistent with previous reports (Fallon *et al.*, 2009; Fallon *et al.*, 2014b), there was typically a monotonic increase in the number of spikes in the onset response with increasing current level, which was well approximated by a sigmoidal function (Figure 1C).

3.3. Suppression duration

In many cases, the duration of post-onset suppression could not be reliably measured in the post-stimulus time histogram (PSTH) based on only the 10 stimulus presentations at a single current level. The measure for a given multi-unit was therefore derived from a PSTH (bin width 1 ms) constructed from the pooled responses to stimuli presented at currents that produced a response of at least 90% of the maximum response (Figure 2A). Mean suppression durations are shown in Figure 2B. There was a significant difference in suppression duration between the treatment groups (one-way ANOVA, $F_{3,456} = 16$, $P < 0.001$); post-hoc analysis revealed that suppression duration in the HR-ES group (115 ± 3 ms) was significantly shorter than those in the other three groups (ADC: 142 ± 4 ms; DU: 132 ± 4 ms; LR-ES: 143 ± 4 ms: corrected P values < 0.001), and that no other differences were significant (corrected P values > 0.70).

3.4. Latency and Jitter

All clusters exhibited a near-monotonic decrease in first spike latency and jitter with increasing stimulus current and a plateau region at the highest current levels (Figure 1D). Mean latency (Figure 3A) and jitter decreased with stimulus intensity up to approximately 150% of the dynamic range (DR150) for all treatment groups, and statistical analysis of the latency and jitter data are therefore based on the values at this level. There was a significant difference in mean latency between the treatment groups (Figure 3B; one-way ANOVA, $F_{3,441} = 7.3$, $P < 0.001$); post-hoc analysis revealed that the latency in the HR-ES group (12.9 ± 0.3 ms) was significantly longer than in the other three groups (ADC: 11.1 ± 0.5 ms; DU: 11.2 ± 0.4 ms; LR-ES: 11.5 ± 0.3 ms: corrected P values < 0.02), and that no other differences were significant (corrected P values > 0.5). There was also a significant difference in mean jitter between the treatment groups (Figure 3C; one-way ANOVA, $F_{3,440} = 12$, $P < 0.001$); post-hoc analysis revealed that the jitter in the HR-ES group (3.3 ± 0.3 ms) was significantly larger than in the other three groups (ADC: 2.2 ± 0.3 ms; DU: 2.0 ± 0.2 ms;

LR-ES: 1.5 ± 0.1 ms: corrected P values < 0.04), and that no other differences were significant (corrected P values > 0.12).

3.5. Responses to pulse trains

As stimulation level can affect the response of AI neurons to repetitive ICES, responses for each recording site to the 0.5-s pulse trains presented at DR150 on the best electrode were analysed. Figure 4A illustrates the response of a multi-unit recording from an HR-ES cat to 0.5-s pulse trains at a range of different rates. It is clear that the number of spikes evoked by each pulse train increased as the stimulation rate was increased from 1 to 10 pps. At stimulation rates above 10 pps, the number of spikes evoked by the pulse train decreased, until at 20 pps the response was less than half that at 10 pps. The band-pass nature of the response, defined as a drop of at least 50% from the maximum response at both the highest and lowest stimulation rates used, is illustrated by the plot of the number of spikes evoked by the pulse train as a function of stimulation rate (Figure 4B). Band-pass type responses were observed in 98 % of all multi-units, and the proportion did not vary between treatment groups ($\chi^2 = 4.0$, $P = 0.27$). The following analysis will therefore be restricted to this type of response. Mean functions for the four experimental groups are shown in Figure 5A. Due to time constraints, responses to pulse trains were only recorded using the silicon arrays, and the data are therefore based on a smaller number of recordings, as indicated in Figures 5 & 6.

The rate response of each multi-unit was characterised by determining two parameters of the band-pass response (illustrated by data from a single recording site in Figure 4B): the best repetition rate (BRR) and the cut-off rate (CutOff). Mean BRR and CutOff values are shown in Figure 5. There was a significant difference in BRR between the treatment groups (one-way ANOVA, $F_{3,161} = 3.7$, $P = 0.014$); post-hoc analysis revealed that the mean BRR in the HR-ES group (8.2 ± 0.3 pps) was significantly higher than that in the ADC group (6.3 ± 0.2 pps; $t_{66} = -5.4$, corrected $P < 0.001$) and that no other differences were significant (DU:

7.6 ± 0.3 pps; LR-ES: 7.4 ± 0.5 pps; corrected P values > 0.07). The same pattern of effects was seen in CutOff; there was a significant difference between the treatment groups (one-way ANOVA, $F_{3,153} = 3.8$, $P = 0.011$), and post-hoc analysis revealed that the mean CutOff in the HR-ES group (14.1 ± 0.5 pps) was significantly higher than that in the ADC group (10.6 ± 0.6 pps; $t_{60} = -4.6$, corrected $P < 0.001$) and that no other differences were significant (DU: 12.4 ± 0.5; LR-ES: 12.5 ± 0.8 pps; corrected P values > 0.10).

The ability of cortical multi-units to follow repeated stimulation is illustrated by the recording from an HR-ES cat in Figure 4A & C. In Figure 4A, the response to each stimulus in the 0.5-s pulse train is similar for stimulation rates up to 6-8 pps, after which it declines, until at 20 pps there is essentially only a response to the first stimulus in the train. The rate-following function in Figure 4C reveals that percent following is above 80% up to 10 pps, and then declines. Low-pass functions were observed in 93% of all multi-units, and the proportion did not vary between treatment groups ($\chi^2 = 3.3$, $P = 0.35$). The mean rate-following functions for the four treatment groups are shown in Figure 6A and were all low-pass. There was a significant effect of treatment group on MFR (one-way ANOVA, $F_{3,87} = 8.3$, $P < 0.001$); post-hoc analysis revealed that the mean MFRs in the ADC and LR-ES groups (7.2 ± 0.5 and 6.3 ± 0.4 pps, respectively) were significantly higher than that in the DU group (4.2 ± 0.4 pps; $t_{44} = 4.8$, corrected $P < 0.001$ and $t_{54} = -3.8$, corrected $P = 0.002$), and that no other differences were significant (corrected P values > 0.2). It should be noted that the mean MFR in the HR-ES group (5.7 ± 0.6 pps) was intermediate between those of the DU and LR-ES groups and not significantly different from any group.

4. Discussion

We have examined the effects of neonatal deafness and cochlear implant use on the temporal resolution in the AI of young adult cats. The only effect of profound deafness of moderate (7-13 months) duration, known to result in a complete loss of cochleotopic organisation (Fallon *et al.*, 2009; Fallon *et al.*, 2014b), was a decrease in the ability to

respond to every stimulus in a pulse train (MFR). Chronic low-rate (50 pps) stimulation from a cochlear implant resulted in an increase in MFR compared to the unstimulated deaf control animals, such that temporal resolution in this group was the same as in the acutely deafened controls. In contrast, chronic high-rate (500 pps) stimulation (similar to rates used in contemporary clinical implants) resulted in: a decrease in suppression duration; increases in response latency and jitter; and increases in two measures of responses to repetitive stimulation (viz., BRR and CutOff). Overall, temporal processing appears to be far more robust than spatial/spectral processing with respect to both a moderate period of deafness and cochlear implant use.

4.1. Comparison with previous data

There are only limited cortical data with which our results can be compared. Although there have been a number of studies of the responses of neurons in the AI to the rate of ICES pulses and pulse trains (e.g. Schreiner & Raggio, 1996), and to amplitude modulation of such pulse trains (Middlebrooks, 2008b; a), there appear to have been only two studies of the effects of deafness and chronic ICES on temporal resolution at the cortical level. Beitel et al. (2011) and Vollmer and Beitel (2011) investigated the effects of chronic ICES in neonatally deafened cats, but there are major differences between their studies and ours that constrain comparison of the results. While Beitel et al. (2011) used cats of approximately the same age as those in our study, their experimental design did not include groups equivalent to our DU and ADC groups. Rather, the adult-deafened cats in their study received chronic ICES unrelated to their acoustic environment for approximately 7 months prior to recording. Consequently the effects of deafness *per se* cannot be determined from their data. Vollmer and Beitel's (2011) study did include both a long-term deaf unstimulated group and an acutely deafened control group; however, the duration of deafness of the two cats in their long-term deaf unstimulated group (viz., 38 and 78 months) and in their stimulated groups (60-86 months) was much longer than that in our study (range: 7-13 months). Finally, it is

worth noting that there are differences in data analyses and definitions for latency, BRR and CutOff between the current work and the Beitel et al. (2011) and Vollmer and Beitel (2011) studies.

4.2. Effects of deafness on temporal resolution

In contrast to our results with moderate-duration deafness (less than 14 months), Vollmer and Beitel (2011) reported that deafness of a much longer duration (greater than 36 months) resulted in a significant increase in latency and jitter, and a decrease in BRR and CutOff. It is likely that the different findings are related to the difference in the duration of deafness in the two studies, and reflect differences in the severity of deafness-induced degenerative changes in the auditory pathway. After the 7–13 months of neonatal deafness the number of surviving spiral ganglion neurons in our animals would be expected to be approximately 20-30% compared to as little as 5% after more than 36 months (Fallon *et al.*, 2014a). Other peripheral changes such as axonal shrinkage and demyelination that result in increased latency and jitter (Miller *et al.*, 2010; Kim *et al.*, 2013) and decrease responsiveness to trains of stimulation (Shepherd & Javel, 1997; Miller *et al.*, 2010) also increase with greater durations of deafness (e.g. Leake & Hradek, 1988; Hardie & Shepherd, 1999). It is also likely that deafness-induced changes in central synapses (e.g. Ryugo *et al.*, 2010) increase with duration of deafness.

Further evidence of the importance of duration of deafness is provided by cortical evoked-potential studies in deaf white cats and single unit studies in the inferior colliculus of neonatally ototoxically-deafened cats. Kral et al. (2002) reported that the mean latency of the first positive wave (Pa) of the AI field potential in four congenitally deaf white cats (three of which were in the age-range 4-8 months) was not significantly different from that in normal hearing controls. In a later study, Kral et al. (2009) reported that Pa latency was longer in deaf white cats implanted acutely at the time of testing as adults (> 12 months) than in acutely-deafened control cats. With respect to the IC, Snyder et al. (1991) reported that mean

latency in unstimulated neonatally-deafened cats (mean age at testing: ~12 months) was not significantly different from that in normal hearing controls. In contrast, Vollmer et al. (2005) reported that inferior colliculus latencies in long-term deaf cats (mean age at testing ~ 46 months) were significantly longer than in normal hearing controls. These cortical and inferior colliculus data confirm the importance of duration of deafness.

Ours is the only cortical study of the effects of deafness in which data on the response to individual stimuli within a train were obtained. All rate-following functions were low-pass, as previously described for AI single-unit responses in hearing cats to click trains (Eggermont, 1991) or trains of very brief characteristic-frequency tone pulses (Phillips *et al.*, 1989). The finding that long-term deafness reduced MFR, but did not affect BRR or CutOff might reflect a difference in the sensitivity of the two measures to response failures. BRR and CutOff are based on the total number of spikes during the pulse train and are thus relatively insensitive to occasional failures to respond to stimuli. Conversely, the rate-following measure is insensitive to the overall magnitude of the response but is highly sensitive to response failures. It is likely that deafness-induced changes in central synapses would play a role in any increase in response failure (e.g. Ryugo *et al.*, 2010).

4.3. Effects of chronic ICES on temporal resolution

Nine to eleven months of low-rate stimulation from a cochlear implant in neonatally deafened animals resulted in no significant changes in basic temporal response characteristics or rate-based measures of periodicity coding; but it prevented (or reversed) the deafness-induced reduction in MFR. Given the relatively short-duration of deafness prior to initiation of stimulation it seems more likely that low-rate stimulation prevented degradation of MFR rather than MFR having degenerated and being subsequently restored by stimulation. The similar effects of chronic ICES on deafness-induced changes in central synapses in brainstem structures (e.g. Ryugo *et al.*, 2010) suggest that these structure may be involved.

In contrast to low-rate stimulation, chronic high-rate stimulation in neonatally deafened animals resulted in increases in latency and latency jitter (relative to control and neonatally deafened unstimulated cats). Beitel et al. (2011) also reported that latency was significantly longer in neonatally-deafened cats that received high-rate ICES (with or without training), than in adult-deafened cats, but reported no significant differences in median jitter. It is possible that the very high activation rates produced by high-rate ICES result in some form of homeostatic plasticity, such as synaptic scaling (Turrigiano, 2008), in the cochlear nucleus or more centrally. Synaptic scaling would be expected to result in the downscaling of excitatory synaptic connections in response to the chronic stimulation regime, which could result in reduced excitability, and hence longer latency and greater jitter, when lower stimulation rates were used in the acute experiments.

Chronic high-rate ICES also resulted in a significantly shorter suppression duration and an increase in BRR and CutOff. The mean BRRs in our and Schreiner and Raggio's (1996) acutely deafened control cats are similar to peak in the modulation transfer functions in normal hearing cats (Eggermont, 1991; 1992). Eggermont (1992) suggested that the suppression period could be the limiting factor in the modulation transfer functions. Our findings are in agreement with this suggestion; as if there were a simple inverse relationship between these variables, the mean suppression durations of 142ms and 115 ms in our ADC and HR-ES groups, respectively, would predict BRRs of 7.0 and 8.7 pps, which are similar to our observed values of 6.3 and 8.2 pps.

Due to the difference in the duration of stimulation between the groups, the effects of high- and low-rate stimulation were confounded with the total duration of stimulation. However, as we have frequently reported, chronic ICES does not alter the rate of spiral ganglion neuron loss (e.g. Ryugo *et al.*, 2010; Fallon *et al.*, 2014a), and the HR-ES group had the shorter duration of deafness and stimulation. Yet, it was the LR-ES that exhibited no significant effects on temporal response characteristics compared to the acutely deafened

control group. Therefore, it is unlikely that changes observed in the HR-ES group were a result of the shorter period of chronic stimulation.

4.4. Conclusions and clinical implications

The consistent finding in our study is that moderate duration deafness, with or without chronic low-rate ICES, had relatively little effect on temporal response characteristics in contrast to its previously reported effects on cochleotopic organisation (Fallon et al., 2009, 2014). The only effect was a decrease in the maximum following rate (i.e., the ability to respond to every stimulus in a pulse train) with moderate duration deafness that was prevented by chronic low-rate stimulation. In contrast to our results, Vollmer and Beitel (2011) have shown that much longer durations of deafness result in significant degradation of a number of features of temporal processing. Duration of deafness has also been identified as a major factor influencing the speech perception performance of both child and post-linguistically deaf adult implantees (e.g. Dowell *et al.*, 1995; Blamey *et al.*, 1996; Sarant *et al.*, 2001). Although our and Vollmer and Beitel's (2011) studies can be related only to neonatally or early-deaf implantees, they suggest that the contribution of deafness-induced degradation of temporal processing on speech perception performance is likely to increase with longer periods of deafness.

We found that chronic high-rate ICES had diverse effects on a number of the temporal response characteristics of AI neurons (viz., it produced increases in latency, and latency jitter, but also increases in BRR and CutOff). It is worth noting that the majority of contemporary CI systems stimulate at rates at or above our high-rate of 500 pps, and therefore this group represents the majority of the clinical population. However, there is renewed interest in lower rates of stimulation, particularly for providing temporal fine structure (Chen *et al.*, 2013). The differential effects of low- vs. high-rate chronic stimulation on latency and jitter might be attributable to differences in the rate and/or the synchrony of the activity generated in the auditory nerve and at higher centres by the two stimulus regimes.

Finally, it should be noted that our and Vollmer and Beitel's (2011) data on neural responses to ICES were obtained in acute experiments on barbiturate anesthetized animals. Barbiturate anaesthesia has profound effects on inhibitory synaptic conductances in AI (Tan *et al.*, 2004; Wehr & Zador, 2005), and synaptic inhibition is an important determinant of suppression duration and BRR. The effects of anaesthesia are also highlighted by the recent report that temporal neural coding of ICES in the inferior colliculus of the acutely deafened, awake rabbits is better than that in anesthetized cats (Chung *et al.*, 2013).

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Table 1. Details of electrical stimulation regimes, age, and number of recording sites for individual animals

Group	Animal ID	Deafening	Chronic Stimulation (Duration in weeks)	Age (months)	Recording Sites
Acutely Deafened Control (ADC)	970	Adult (Acute)	-	9	33
	974	Adult (Acute)	-	15	65
Neonatally deafened unstimulated (DU)	952	Neonatal	-	13	38
	953	Neonatal	-	11	53
	957	Neonatal	-	9	43
	959	Neonatal	-	7	29
	961	Neonatal	-	8	56
High-Rate ICES (HR-ES)	958	Neonatal	High-rate (23)	8	34
	962	Neonatal	High-rate (12)	5	54
	965	Neonatal	High-rate (30)	9	34
	984	Neonatal	High-rate (25)	8	51
	992	Neonatal	High-rate (27)	9	55
	993	Neonatal	High-rate (26)	9	81
Low-Rate ICES (LR-ES)	968	Neonatal	Low-rate (38)	11	46
	971	Neonatal	Low-rate (35)	11	38
	972	Neonatal	Low-rate (34)	10	39
	977	Neonatal	Low-rate (27)	9	60

Individual animals in the experimental groups are specified by identification (ID) number; Deafening is the time of deafening; Chronic Stimulation is the ICES regime received and was provided from 10 weeks of age; Age is the age at the time of the acute experiment and corresponds to the duration of deafness for the neonatally deafened groups; Recording Sites is the number of recording sites at which basic latency and jitter data were obtained.

Figures Legends

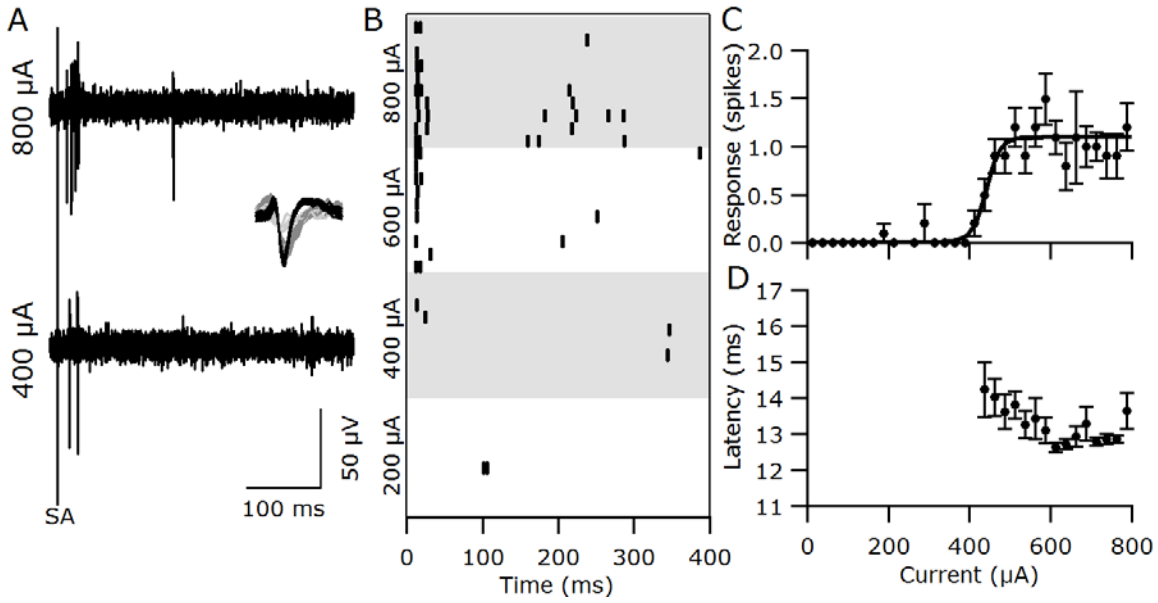

Figure 1. Example response: A) Raw recording from a DU animal at near-threshold (400 µA) and saturated (800 µA) current levels. Insert shows two single units (black and dark grey) that could be isolated from this multi-unit recording. B) Spike rasters (over 400 ms) of one of the single units (black) illustrated in (A) to individual pulses presented at 1.5 pps. Each shaded/unshaded region shows responses to 10 stimuli at a different current level (200, 400, 600, and 800 µA respectively from bottom to top). At higher current levels, post-onset suppression of activity and rebound responses are apparent. C and D) Input-output and latency functions for the same unit. C: Mean number of spikes and standard error of the mean (SEM) for onset response component (5 – 40 ms) and fitted sigmoid. D: First spike latency (mean ± SEM) as a function of current level.

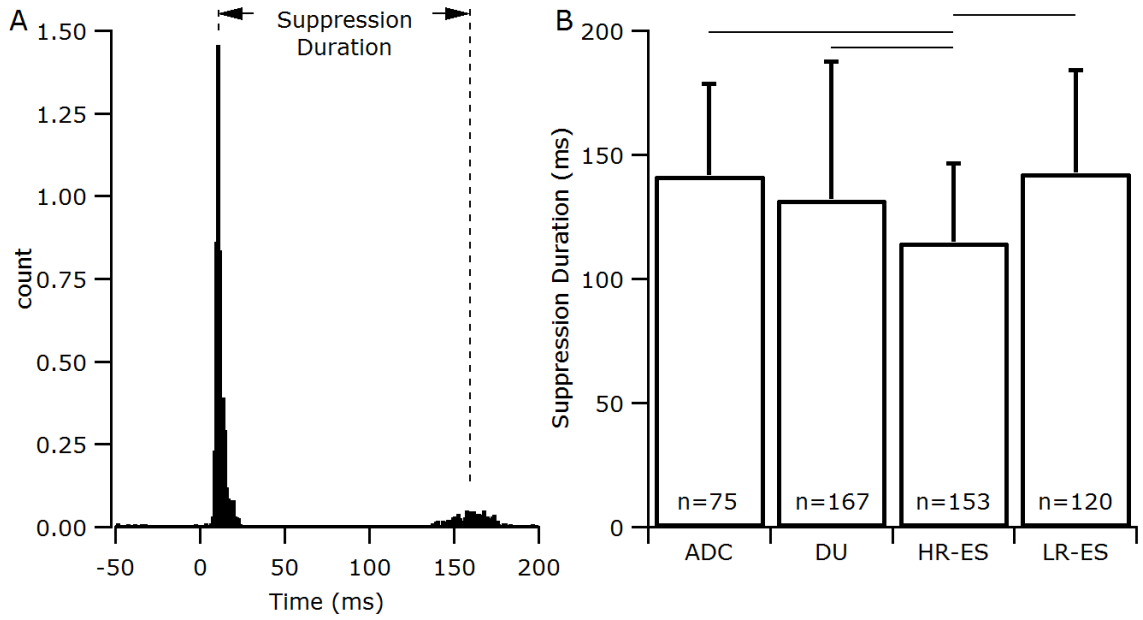

Figure 2. Effect of treatments on suppression duration. A) Example peri-stimulus time histogram of response to suprathreshold stimulation from a multi-unit recording in a control cat (bin width 1 ms). As detailed in the text, the histogram is based on summed responses over a range of stimulus levels. Suppression duration was measured from the peak in the onset response to the peak in the rebound response (dashed lines) and in this case was 145 ms. B) Mean + SD for suppression period. Lines indicate statistically significant differences (post-hoc analysis, $p < 0.001$). n = number of recordings.

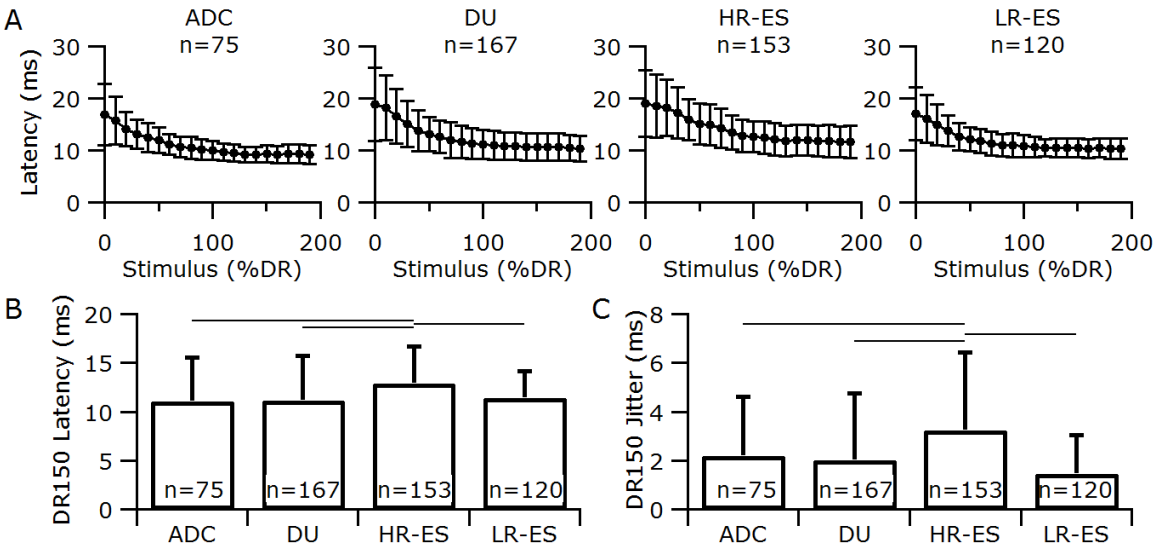

Figure 3. Effect of treatments on latency and jitter. A) Mean latency for different treatment groups (error bars: SD). B and C) Mean + SD for DR150 latency and jitter, respectively.

Lines indicate statistically significant differences (post-hoc analysis; $p < 0.04$). n = number of recordings.

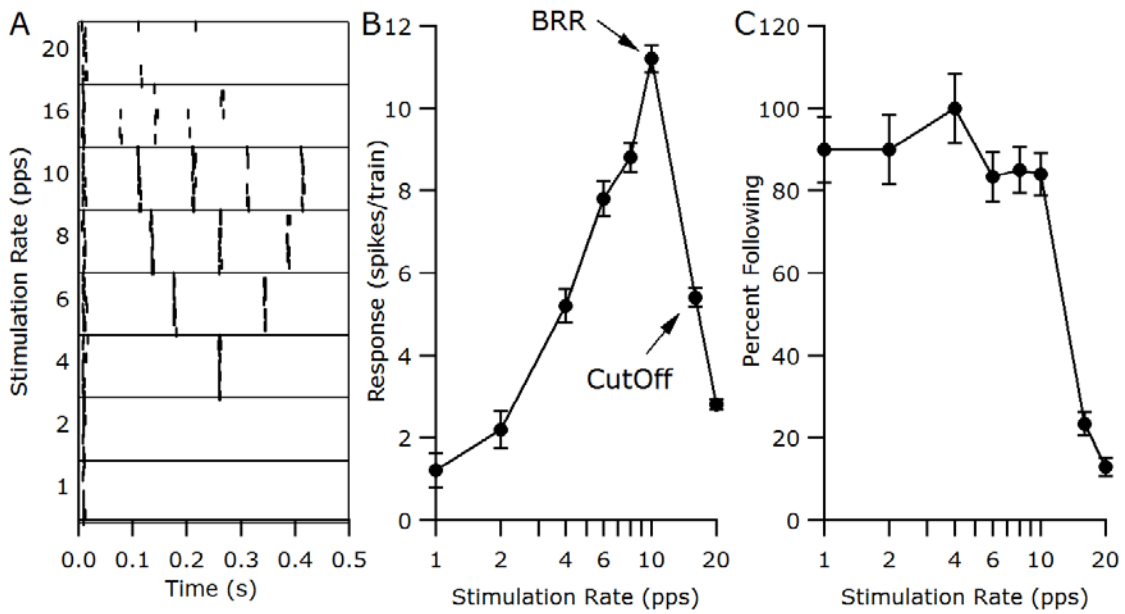

Figure 4. Example of responses to 0.5-s stimulus trains at different rates from a multi-unit recording in a HR-ES cat. A) Spike rasters (over 0.5 s) to ten pulse trains at different stimulation rates. B and C) Two measures of this unit's response to repetitive stimulation. B: The mean (± SD) response to the 0.5-s stimulus train as a function of stimulation rate. The best repetition rate (BRR) and cut-off rate (CutOff), as defined in the text, are indicated. C: Percent following (i.e., the percentage of pulses in the spike train that evoked at least one spike) as a function of stimulation rate.

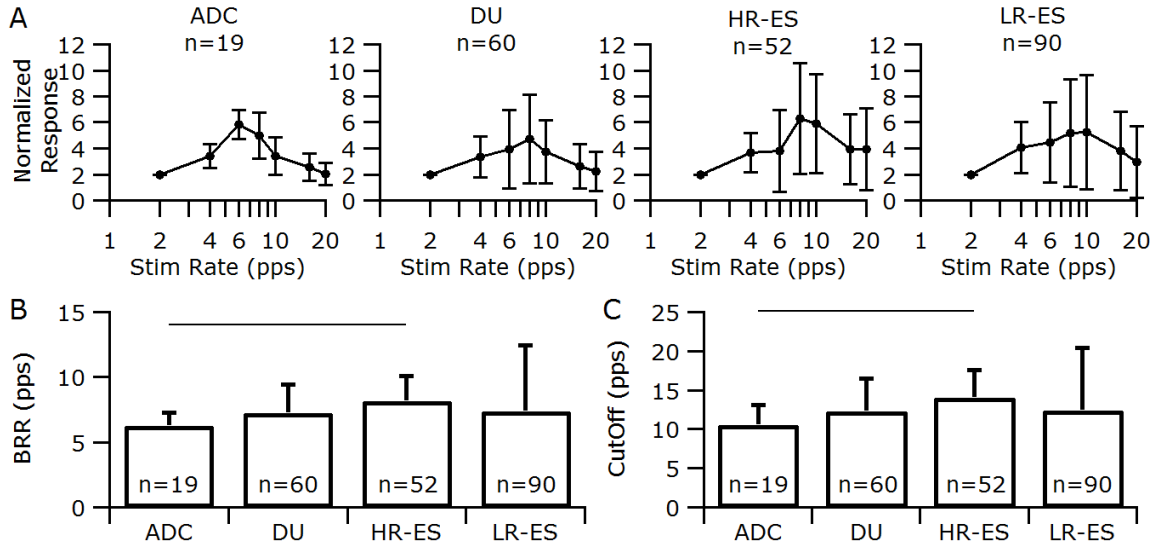

Figure 5. Effect of treatments on responses to pulse trains. A) Mean ± SD response to a 0.5-s pulse train at different stimulation rates, normalized to the 1-pps response. The mean functions show a band-pass type response for all treatments groups. B and C) Mean + SD values for BRR and CutOff, respectively.. Lines indicate statistically significant differences (post-hoc analysis, $p < 0.05$). n = number of recordings

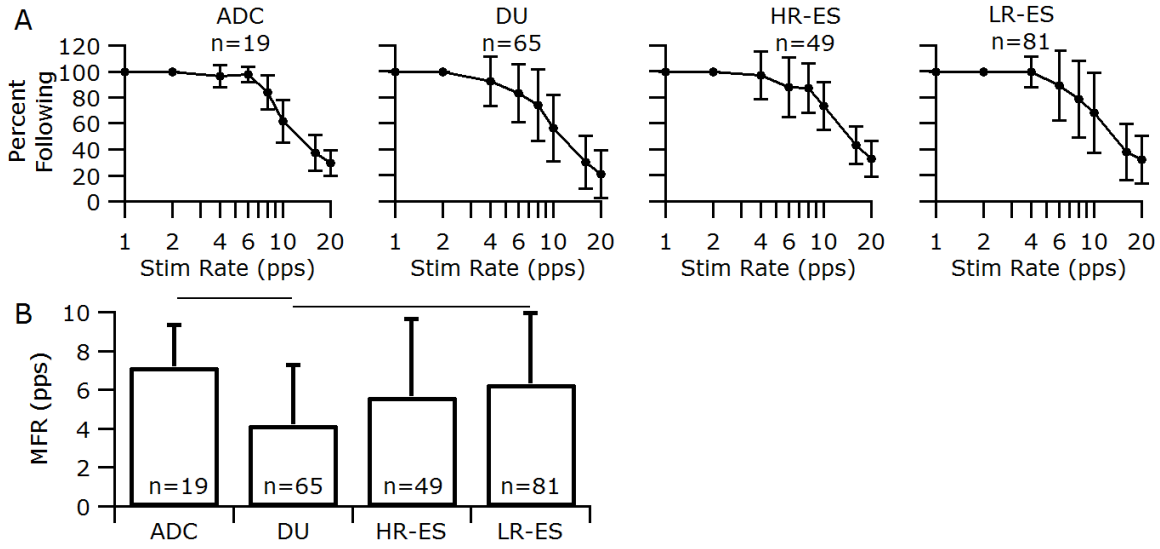

Figure 6. Effect of treatments on rate-following. A) Mean ± SD percent following. (B) Mean + SD maximum following rates (MFR). Lines indicate statistically significant differences (post-hoc analysis, $p < 0.01$). n = number of recordings