Passive stimulation and behavioral training differentially transform temporal processing in the inferior colliculus and primary auditory cortex

Abbreviated title:
Temporal plasticity in inferior colliculus

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Abstract

In profoundly deaf cats, behavioral training with intracochlear electric stimulation (ICES) can improve temporal processing in the primary auditory cortex (AI). To investigate whether similar effects are manifest in the auditory midbrain, ICES was initiated in neonatally deafened cats either during development after short durations of deafness (8 wk of age) or in adulthood after long durations of deafness (≥3.5 yr). All of these animals received behaviorally-meaningless, 'passive' ICES. Some animals also received behavioral training with ICES. Two long-deaf cats received no ICES prior to acute electrophysiological recording.

After several months of passive ICES and behavioral training, animals were anesthetized, and neuronal responses to pulse trains of increasing rates were recorded in the central (ICC) and external (ICX) nuclei of the inferior colliculus. Neuronal temporal response patterns (repetition rate coding, minimum latencies, response precision) were compared with results from recordings made in the AI of the same animals (Beitel et al. 2011; Vollmer and Beitel 2011).

Passive ICES in long-deaf cats remediated severely degraded temporal processing in the ICC and had no effects in the ICX. In contrast to observations in the AI, behaviorally-relevant ICES had no effects on temporal processing in the ICC or ICX, with the single exception of shorter latencies in the ICC in short-deaf cats. The results suggest that independent of deafness duration passive stimulation and behavioral training differentially transform temporal processing in auditory midbrain and cortex, and primary auditory cortex emerges as a pivotal site for behaviorally driven neuronal temporal plasticity in the deaf cat.

New and Noteworthy

Behaviorally relevant versus passive electric stimulation of the auditory nerve differentially affect neuronal temporal processing in the central nucleus of the inferior colliculus (ICC) and the primary auditory cortex (AI) in profoundly short-deaf and long-deaf cats. Temporal plasticity in the ICC depends on a critical amount of electric stimulation, independent of its behavioral relevance. In
contrast, the AI emerges as a pivotal site for behaviorally-driven neuronal
temporal plasticity in the deaf auditory system.

**Introduction**

To recognize and comprehend natural sounds, including speech, humans
depend on the temporal features and the spectral content of sound (Ahissar et
al. 2001; Rosen 1992). However, in deaf humans who use a cochlear implant
(CI), spectral resolution is severely degraded (Drullman 1995; Shannon et al.
1995), and the ability to encode and resolve the temporal patterns of
intracochlear electric stimulation (ICES) provided by the prosthesis is critical for
speech recognition (Merzenich, 2015; Wilson and Dorman, 2008). Despite
degraded spectral information, many CI users, including profoundly deaf
children (Svirsky et al. 2000, 2004), are able to understand speech, indicating
the relevance of temporal fine structure and temporal envelope information in
prosthetic communication signals (Smith et al. 2002). Although long-duration
deafness in humans is associated with poor cortical temporal processing and
consequently with deficient speech discrimination performance (e.g., Busby et
al. 1993; Green et al. 2005; Sharma et al., 2009), even prelingually deaf adults
may show gradual improvement in speech discrimination with increasing
auditory experience after implantation (Busby et al. 1991; Klop et al. 2007;
Straatman et al. 2014). Auditory training and perceptual learning in deaf adult
CI users (Fu and Galvin 2007; Oba et al. 2011; Stacey et al. 2010) suggest the
potential for remediation of temporal information processing in the central
auditory system.

Neuronal plasticity in the central auditory system refers to dynamic
modifications of neurons that occur in response to changes in the significance
or the basic characteristics of their inputs (Beitel et al. 2011; Irvine 2007;
Vollmer and Beitel 2011, Vollmer et al. 1999). Studies in hearing animals have
shown that primary auditory cortex (AI) is involved in learning-induced temporal
plasticity. Behavioral training can markedly improve cortical temporal
processing and response-timing precision in the developing (Zhou and
Merzenich, 2009) and adult auditory systems (Bao et al., 2004; Beitel et al.,
2003; Engineer et al., 2008; Leon et al., 2008; Schnupp et al., 2006).

In previous studies, we have induced temporal plasticity in the AI of
behaviorally trained short-deaf and long-deaf adult cats, indicating that perceptually relevant auditory experience (ICES) can effectively preserve and enhance neuronal temporal processing (repetition rate coding, minimum latencies, response precision) in the deaf central auditory system (Beitel et al. 2011; Vollmer and Beitel 2011). Exposure to ICES can also increase neuronal temporal processing in the inferior colliculus (IC) of both short-deaf and long-deaf adult cats (Vollmer et al., 1999, 2005). However, it is not known whether temporal processing in the IC is differentially affected by behaviorally-meaningless, passive ICES or by training with behaviorally-relevant ICES.

Ascending inputs from auditory brainstem (Joris et al. 2004; Krishna and Semple 2000; Schreiner and Langner 1988) and descending corticofugal projections (Aitkins 1986; Huffman and Henson 1990; Winer et al. 1998; Winer and Schreiner 2005; Bajo and King 2013) transform neuronal coding in the IC. In the cat, the IC includes three structures: the lemniscal central nucleus (ICC), the lateral or external nucleus (ICX), and the dorsal cortex (ICD) (Huffman and Henson 1990). Neurons in the ICD are broadly tuned with poorly defined best frequencies and a tendency to habituate rapidly (Aitkin et al. 1975; Merzenich and Reid 1974). Because the ICD has not been investigated in deaf animals, it is not included in the present study.

Analogous to the ‘core-belt’ distinction for the neocortex, the ICC is a designated ‘core’ structure, and the ICX corresponds to a ‘belt’ structure (see Edwards and Chang 2013 for review). The ICC and the ICX each have a dominant projection to the thalamic medial geniculate body (MGB). The ICC in the cat receives a modest descending input from the corticocollicular system and projects primarily to the tonotopic ventral MGB (Winer et al. 1998). The multisensory ICX receives intrinsic auditory input from the ICC (Aitkin et al., 1975, 1978, 1981) as well as somatosensory input from dorsal column and trigeminal nuclei (Aitkin et al. 1978; 1981) and descending input from the corticocollicular system (Bajo et al. 2007; Huffman and Henson, 1990; Winer et al. 1998). The ICX projects primarily to the non-tonotopic medial division of the MGB (Winer et al. 1998; Huffman and Henson 1990).

Our experimental goal in the present study is to evaluate in both short-deaf, early-implanted (~8 wk) cats and long-deaf, late-implanted (≥3.5 yr) cats the effects of passive, behaviorally-meaningless ICES versus behaviorally-
relevant ICES on neuronal temporal processing (repetition rate coding, minimum response latencies, response precision) in the ICC and the ICX. This allows us to compare the role of prosthetic auditory experience on functionally dissimilar collicular nuclei that have been studied extensively in both hearing cats (Aitkin 1986; Aitkin et al. 1978, 1981, 1994; Hind et al. 1963; Merzenich and Reid 1974) and deaf cats (Leake et al. 2000; Shepherd et al. 1999; Snyder et al., 1991, 1995; Vollmer et al., 1999, 2005, 2007).

Because sequential recordings were made in the IC and in cortical field AI of the same animals (Beitel et al. 2011; Vollmer and Beitel 2011), we have a unique opportunity to directly compare neuronal data recorded in the midbrain and cortex of the same subjects. In the Discussion (Fig. 8) we demonstrate that passive, behaviorally-meaningless versus behaviorally-relevant prosthetic auditory experience differentially transforms neuronal temporal processing in the ICC and the AI in profoundly deaf cats. Passively induced temporal plasticity in the ICC may be inherited in the AI as feed-forward sensory input, whereas, independent of an animal's age and duration of deafness, primary auditory cortex appears to be a pivotal site for learning-induced neuronal temporal plasticity in the deaf auditory system.

Methods

Female and male cats were obtained from a UCSF feline breeding colony. Experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Francisco, and were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (8th Edition, 2011). Procedures for deafening, implantation, chronic stimulation, surgical preparation, and recording techniques in the IC have been described in previous reports (e.g., Snyder et al., 1995; Vollmer et al., 1999, 2005) and will be repeated only as required. Detailed descriptions of psychophysical procedures and AI recording techniques are described in two companion papers (Beitel et al., 2011; Vollmer and Beitel, 2011) and will not be repeated in the present report.

Experimental design
An important feature of our experimental design is recognition that specific experimental treatments (e.g., behavioral training) as well as an animal's level of development (juvenile or adult) and duration of deafness can affect neuronal temporal plasticity in the central auditory system during an animal's entire lifetime (Bonham et al. 2004; Kral 2007). Table 1 shows individual experimental histories for 13 neonatally deafened cats included in this study. The table includes group and cat identifications, and provides details about deafness histories, passive behaviorally-meaningless ICES, and behaviorally-relevant ICES used in the feline signal detection program.

The upper part of the table includes six neonatally deafened short-deaf cats (SDpass and SDtrain groups). Cats in both groups received passive ICES starting at a mean age of 8 wk±1 (SD). Passive ICES was discontinued in the SDtrain group of cats when psychophysical training was initiated using behaviorally-relevant ICES. At a mean age of 9 months, acute electrophysiological recording was conducted in the anesthetized short-deaf cats.

The lower part of the table includes six neonatally deafened long-deaf cats (LDpass, LDtrain and LDU groups). As mature adults (≥3.5 yr) the LDpass group received only passive ICES, whereas the LDtrain group also received behaviorally-relevant ICES during psychophysical training. The LDU group received no ICES until the final, acute electrophysiological recording session. At a mean age of about 68 mo, acute electrophysiological recording was conducted in the anesthetized long-deaf cats.

In addition to the five groups of animals included in the experimental design for this study, data for a unique neonatally deafened long-deaf cat, K55, are shown at the bottom of Table 1. This cat received behaviorally-relevant ICES during psychophysical training (Vollmer and Beitel 2011). Importantly, cat K55 was not stimulated with passive ICES. The electrophysiological data recorded in this cat address an important question: How did behavioral training alone, in the complete absence of passive ICES, in a long-deaf animal affect temporal neuronal plasticity in the ICC and the AI?

The sequence of procedures used during study of the five groups of animals was as follows. Cats in the SDpass and LDpass groups were neonatally deafened, unilaterally implanted with a feline scala tympani cochlear
prosthesis, and received passive ICES; cats in the SDtrain and LDtrain groups and cat K55 (no passive ICES) were neonatally deafened, implanted, received passive ICES, and also were trained psychophysically with behaviorally-relevant ICES; cats in the LDU group were neonatally deafened, and a cochlear implant was implanted acutely in each cat one week prior to the terminal electrophysiological recording experiment. With the exception of cat K190, neurons were recorded first in the IC and then in the AI of each animal in Table 1. In K190, neurons were recorded first in the AI followed by recordings in the IC.

Deafening

All cats included in the study were deafened by systemic injections of neomycin sulfate (60-70 mg/kg IM SID) for 16-25 days starting the day after birth (Leake et al. 1999). Profound bilateral deafness (threshold >105 dB SPL) was documented by the absence of evoked auditory brainstem responses (ABRs) to acoustic clicks (Moore et al. 2000). Because cats are born deaf due to the immaturity of the auditory system, it is assumed that these animals had no normal auditory experience and provide a model of congenital deafness in humans. The cats included in this study also did not demonstrate any residual acoustic hearing as judged by the absence of pinna reflexes or head movements to environmental sounds. In Table 1 the durations of deafness (mo) are identical to the animals’ age at the time of the acute, terminal electrophysiological experiments. The absence of auditory hair cells in the cochlea of the deafened animals was confirmed by post-mortem histological analyses (Leake et al. 1999; Vollmer et al. 2005). Spiral ganglion cell (SGC) density was evaluated as described previously (e.g., Leake and Hradek 1988; Leake et al. 1991) and was about 44% in the short-deaf cats (mean: 43.9±7.1 SD % of normal) and only about 6% in the long-deaf animals (mean: 6.0±6.2 SD) (Table 1). The cochleae of two deafened animals (K190 and K55) were not available for histological analysis.

Feline cochlear prostheses design and implantation

Feline cochlear electrodes were fabricated in our laboratory from Teflon coated platinum-iridium wires embedded in a silicone rubber carrier (Rebscher
et al. 2007). The carrier was molded to the size and shape of the scala tympani of the basal turn of the left cochlea. The electrode array consisted of four hemispherical contacts (~250 µm diameter) arranged as apical and basal offset-radial bipolar pairs (contact separation 1-mm; center-to-center separation of pairs 5-mm).

Prior to surgical implantation using aseptic procedures, each animal was sedated (IM, ketamine: 22-33 mg/kg; acepromazine maleate: 0.1 mg/kg). Anesthesia was induced by pentobarbital sodium (IV, 7-20 mg/kg) and maintained at an areflexic level during surgery. Vital parameters (temperature, oxygen saturation, heart rate and respiration rate) were monitored and maintained within the physiological range. A prophylactic antibiotic and buprenorphine HCl (0.005 mg/kg) for analgesia were administered. Meloxicam was given postsurgically, and the animal was monitored during recovery.

A feline prosthesis was implanted in the left scala tympani of the six neonatally deafened, weaned juvenile cats (SDpass group and SDtrain group), and the animals were studied in acute electrophysiological experiments as young adults (range: 7-11 mo). Prostheses were also implanted in the seven long-term neonatally deafened adult cats (LDU group, LDpass group, LDtrain group and K55). LDU cats were implanted one week before the acute electrophysiological experiment. The long-deafened animals were studied after prolonged periods of deafness (≥3.5 years; range: 38-86 mo).

**Intracochlear electric stimulation**

Intracochlear electric stimuli (ICES) were charge-balanced, biphasic rectangular current pulses (0.2 ms/phase) generated by a computer and delivered through an audio attenuator to an optically-isolated constant current stimulator. For passive stimulation, electric pulses were delivered at 300 pulses/sec (pps), sinusoidally amplitude-modulated at 30 Hz (SAM 300/30; modulation depth=100%; Snyder et al., 2000).

The choice of the stimulus (SAM 300/30) used in the present study was based on two considerations: A temporal modulation rate of 30 Hz is within the upper range of the temporal following ability of AI neurons, and a carrier rate of 300 pps is within the upper range of temporal following ability of IC neurons (e.g., Beitel et al. 2011; Vollmer et al. 1999, 2005; Vollmer and Beitel 2011).
The peak intensity of the stimuli was set at 2 dB above each animal’s electrically evoked ABR (eABR) threshold. This intensity was also above behavioral signal detection thresholds as well as IC and AI minimum neuronal response thresholds (Beitel et al. 2000a, b). In one long-deaf cat (CH618), an analogue speech processor (SP) was used to transduce environmental sounds into ICES (band-pass filter 250 Hz to 3 kHz; peak-to-peak output set at 6 dB above eABR threshold). EABR thresholds and electrode impedances were measured at regular intervals to assess the stability and reliability of the CIs and to adjust stimulation intensity if thresholds shifted. Passive ICES provided activation of the auditory nerve and the central auditory system prior to the acute electrophysiological experiments. Because passive ICES was not behaviorally-relevant, it appeared to be unattended by the deaf animals. A regimen of continuous ICES (~4 h/d, 5 d/wk) was applied in each animal, usually beginning within one week after implantation surgery. The durations (wk) of passive ICES are shown in Table 1. Animals were stimulated in bipolar mode using the apical electrode pair 1,2. Two cats (CD393 and CH539) were stimulated with electrode pairs 2,3 and 1,4, respectively, during the final few weeks of passive stimulation due to lead failure. The ICES was applied to the cochlear electrodes via a percutaneous cable that exited the skin of a cat’s neck at the midline. As noted above, the cats in the LDU group and cat K55 were the only animals in this study that did not receive passive ICES (see Table 1).

**Psychophysical procedures: Signal detection training**

Animals in the SDtrain group, the LDtrain group, and cat K55 were trained to detect temporally modulated electric signals. Detailed descriptions of conditioned avoidance signal detection training in the deaf animals included in this report have been published previously (Beitel et al. 2000a, 2000b, 2011; Vollmer and Beitel, 2011). The training ICES, the ages of the cats at the onset of training, their mean detection thresholds at the end of training on the initial signal detection task and the behavioral paradigms are presented in Table 1. Trained animals typically received less than 1-min total duration of behaviorally-relevant ICES during a daily training session of 30-40 minutes.
Perceptual learning was documented by a reduction in detection thresholds across training sessions (Beitel et al. 2011, Vollmer and Beitel 2011). Mean detection thresholds were determined from three or four threshold sessions for each cat at the end of training on the signal detection task (Table 1). The mean thresholds are within the range of thresholds reported previously in juvenile deaf cats trained on detection of temporally modulated ICES (Beitel et al., 2000a,b). Session thresholds obtained in the two long-deaf LDtrain cats (6 sessions, median=47.2 dB) were higher than the session thresholds obtained in the three short-deaf SDtrain cats (11 sessions, median=40.8 dB) (Mann-Whitney Rank Sum Test, P<0.001). Differences in age, duration of deafness, percent surviving spiral ganglion cells or stimulating electrode position in the cochlea may have contributed to signal detection thresholds in the trained short-deaf and long-deaf cats.

**Acute electrophysiological procedures**

The animals included in this study were sexually mature adults at the time of the acute electrophysiological recording experiments. An areflexic level of anesthesia was established as described above for cochlear implantation surgery and was maintained throughout the recording sessions. Both in hearing animals and in animals implanted with a cochlear prosthesis, anesthesia can alter neuronal temporal processing in the IC and the AI (Chung et al. 2014; Ter-Mikaelian et al. 2007). However, the expression of neuroplasticity in the auditory system is not prevented by anesthesia. Different anesthetics and various animal species are used in neurophysiological research, complicating interpretation of anesthetic effects on neuronal responses. For the present study we used an anesthesia protocol that was identical in all cats to ensure that the expression of stimulus-driven changes in the IC and the AI was not confounded by variation in the anesthesia protocol.

The animal’s head was immobilized using a customized head holder. Following a craniotomy contralateral to the cochlear prosthesis, the right IC and cortex were exposed (e.g., IC: Snyder et al. 1995; Vollmer et al. 1999, 2005; AI: Beitel et al. 2011; Schreiner and Raggio 1996; Vollmer and Beitel 2011). The exposed neural structures were protected with silicone oil.
Electric charge-balanced biphasic rectangular current pulses (0.2 ms/phase) generated by a computer (TMS32010) at a sampling rate of 60 kHz were delivered to the intracochlear electrodes via an audio attenuator and optically-isolated constant current stimulator. Prior to each recording session, the system was calibrated to a common reference level (0 dB=1 µA_{peak-peak}).

Neuronal activity was recorded differentially from single neurons or small multunit clusters using two impedance matched tungsten microelectrodes (0.8-1.2 M Ohm). For recording in the IC the active electrode was advanced ventromedially at ~45 degrees (re sagittal plane; Fig. 1A), and a high threshold region between two locations of minimum thresholds was defined as the border between the external nucleus (ICX) and the central nucleus (ICC) of the IC (Fig. 1B; Vollmer et al. 1999, 2005, 2007). Spiking activity was isolated from background noise using a window discriminator (BAK-DIS-1) set to the positive and negative amplitudes of action potentials. The response threshold to a single electric pulse delivered at the minimal intensity required to elicit a spiking response with a probability of ≥50% were determined using audiovisual criteria (Beitel et al. 2000a, b; Snyder et al. 1995, 2000; Vollmer et al. 2001). To study temporal processing, trains of unmodulated biphasic current pulses (ICES) were applied at increasing pulse rates (10 to ~200 pps, in 5, 10 or 20 pps increments, 320 or 500 ms duration, 20 or 40 repetitions, 1-s interval between repetitions). Stimulus intensities were set at 2-6 dB above audiovisual thresholds at a level that elicited reliable spiking responses to electric pulses delivered at the lowest pulse rate used in the IC (10 pps). The arrival time of each spike was recorded and stored in a computer.

Spontaneous activity was not recorded in this study. However, two studies have reported that in barbiturate-anestheized long-deaf cats, spontaneous activity in the inferior colliculus is significantly higher than in acutely deafened control cats (Hancock et al. 2013; Shepherd et al. 1999).

**Neuronal data analysis**

To prevent electric pulse artifacts or evoked potentials from contaminating the neuronal responses, an initial window (IC: 4.5 ms) after each stimulus pulse was excluded (zeroed) from analysis. The response to the first
pulse in each repetition was excluded from the vector strength analyses to prevent stimulus onset effects from dominating the neural responses (Fig. 2A, D; gray zones).

Responses recorded in the IC were analyzed using customized software (Matlab, The Math Works) to determine the number of phase-locked spikes (Phase-Locked Spikes=Total Spikes*Vector Strength; Eggermont 1991) evoked by different pulse rates for each unit studied. We plotted the number of phase-locked spikes versus the stimulus pulse repetition rate to obtain repetition rate transfer functions (RRTFs) in the ICC and the ICX (Fig. 2B, E).

From the RRTF, two measures of phase locking were used to estimate temporal following responses in a neuronal unit: (1) The best repetition rate (BRR) was defined as the pulse rate that evoked the largest number of phase-locked spikes; (2) the cutoff rate (CR) is the highest pulse rate at which the number of phase-locked spikes was equal to or just less than 50% of the number of spikes at BRR (Fig. 2B, E). For all units, only significant vector strength values were used to calculate the two phase-locking parameters (P≤0.01; Rayleigh test; Mardia and Jupp 2000).

Neuronal response latency and temporal precision were analyzed using the lowest stimulus pulse rate (10 pps) to determine the mean minimum latency (mean first spike latency) and the standard deviation of minimum latencies (temporal precision or jitter of the response onset) across stimulus repetitions (Fig. 2C, F). Finally, for the entire population of units, the mean phase-locked spike rates in the ICC and the ICX were determined as a function of pulse rate for each experimental group. Mean phase-locked spike rates (Fig. 3) were calculated as proportional values (mean phase-locked spike rate * proportion of contributing neurons).

**Statistical analyses of temporal processing and spike-rate data**

For comparison of neuronal temporal following responses (BRRs and CRs), mean minimum latencies and temporal precision ('jitter') we used either the nonparametric Mann-Whitney Rank Sum Test (two groups) or the Kruskal-Wallis one-way ANOVA on Ranks (three groups) followed by all pairwise multiple comparison procedures (Dunn’s test; SigmaStat 3.0). Rank order statistics are shown in box plots that illustrate the median and four percentiles.
(10th, 25th, 75th, and 90th) of the ranked data. Differences in cumulative
distributions were tested using the two-sample Kolmogorov-Smirnov test. Chi-
square tests were used to compare the proportions of units with band-pass
filter characteristics in different groups of cats. Descriptive comparisons
[medians ± quartile deviation (Q)] are used for cat K55 versus long-deaf groups
of cats.

Results

Given the many comparisons analyzed statistically in this study, the
Results focus on comparisons of temporal processing between groups of cats
that differ with respect to a single factor (passive stimulation or behavioral
training). For example the LDTrain and LDPass groups differ with respect to
only one factor (behavioral training), but the LDTrain and LDU groups differ with
respect to two factors (passive stimulation and behavioral training). Two factor
comparisons are confounded; statistical results of confounded comparisons are
indicated in illustrations by grey horizontal bars and asterisks.

The results have been structured as follows: Examples of basic
electrophysiological results for repetition rate transfer functions are presented
in Fig. 2, and phase-locked spike rate results are shown in Fig. 3. Quantitative
effects of passive versus behaviorally-relevant stimulation on neuronal
temporal processing in the ICC of short-deaf and long-deaf groups of cats are
illustrated in Figs. 4 and 5, respectively. In Fig. 5 we also present the ICC
results recorded in long-deaf cat K55, the unique single-case-study that
received behavioral-training alone (no passive stimulation). In short-deaf and
long-deaf groups of animals the effects of passive versus behaviorally-relevant
stimulation on neuronal temporal processing in the multisensory ICX are
presented in Fig. 6. To determine the effects of deafness duration and SGC
density on the efficacy of passively-induced or training-induced neuronal
temporal processing in the ICC, we compare long-deaf versus short-deaf
groups of animals with similar stimulation histories in Fig. 7.

Finally, to evaluate potential differences and transformations in neuronal
temporal plasticity in response to passive versus behaviorally-relevant
stimulation between the ICC and the AI, in the Discussion we illustrate the AI
results for the short-deaf groups, long-deaf groups and K55 in Fig. 8. Because
our published cortical papers (Beitel and Vollmer 2011; Vollmer et al. 2011) include data and statistical comparisons not relevant to the present work, the AI results have been statistically reanalyzed and adapted to allow for direct comparisons in the Discussion of the ICC and AI results obtained in the same animals.

**Basic characteristics of temporal processing in the IC: RRTFs and phase-locked spike rates**

RRTF FILTER CHARACTERISTICS. RRTFs provide the information required to relate the number of phase-locked spikes to the stimulus pulse repetition rate (Fig. 2B, E). From the shape or profile of a neuronal unit’s RRTF, two important parameters, the BRR and the CR, were measured and used to evaluate neuronal temporal plasticity in the ICC and the ICX. We obtained RRTFs for 418 neuronal units (ICC: n=342; ICX: n=76). In the examples illustrated in Fig. 2B and E, band-pass RRTFs are shown for two single neurons. Band-pass RRTFs are characterized by a decrease in phase-locked spikes to ≤50% of the maximum number of phase-locked spikes at pulse rates both below and above BRR. The band-pass RRTF of the ICC neuron (Fig. 2E) extends towards higher pulse rates resulting in a higher BRR and a higher CR (50 pps and 115 pps, respectively) compared with the band-pass RRTF of the ICX neuron (30 and 50 pps, respectively; Fig. 2B). RRTFs with band-pass filter characteristics were the most common profile in the ICC (all groups mean: 79.5%) and the ICX (all groups mean: 51.3%). This suggests that the general filter-characteristics of IC neurons provide a relatively stable framework for assessing the effects of passive and behaviorally-relevant ICES on collicular temporal processing in the profoundly deaf cat.

Low-pass and high-pass RRTFs are essentially monotonic functions that either decrease (low-pass) or increase (high-pass) in value as repetition rate increases. Across all groups, the proportions of low-pass RRTFs were higher in the ICX (21.1%) than in the ICC (5.6%; Chi-square test, P<0.001). In the ICX of LDU animals, the majority of neurons have low-pass filter characteristics. The proportions of high-pass RRTFs were <2.0% for all groups in the ICC. High-pass RRTFs were not observed in the ICX in any of the groups.
Several neuronal units recorded in the ICC (N=38) or the ICX (N=19) had
RRTFs that were not classified as band-pass because they exceeded the 50%
response criterion for pulse rates on the low side of BRR as required for
inclusion in the band-pass category. If the response criterion is suspended and
these units are included in the band-pass category, RRTFs with band-pass
filter characteristics would be even more dominant in the ICC (all groups mean:
90.6%) and the ICX (all groups mean: 78.9%).

In summary, RRTFs with band-pass filter characteristics are the most
common temporal response profile in the ICC and the ICX. The general filter-
characteristics of IC neurons were largely unaffected by differences in hearing
experience.

**PHASE-LOCKED SPIKE RATES.** To investigate whether phase-locked spike
rates in the IC could be modified by electric hearing experience, mean phase-
locked spike rates (sp/s) in the ICC (Fig. 3A, B) and the ICX (Fig. 3C, D) of
short-deaf and long-deaf animals were plotted as functions of electric stimulus
pulse rate (pps). In Fig. 3A the peak mean phase-locked spike rates in the two
short-deaf groups occur at a stimulus pulse rate of 60 pps, and the peak mean
spike rate in the SDtrain group is slightly higher [24.2±1.6 (SE) sp/s] than that
in the SDpass group [22.7±2.4 (SE) sp/s]. This ordinal sequence of spike rates
is similar to that reported in short-deaf groups of cats in the AI (Beitel et al.
2011).

In Fig. 3B peak mean phase-locked spike rates in the three long-deaf
groups occur at stimulus pulse rates of 40 or 60 pps. Peak phase-locked spike
rate is lowest in the long-deafened unstimulated animals [group LDU; 15.7±1.0
(SE) sp/s], intermediate after passive stimulation [LDpass group; 18.5±1.0 (SE)
sp/s] and highest in the trained animals [group LDtrain; 30.5±1.9 (SE) sp/s].
The results in the short-deaf and long-deaf groups, specifically that phase-
locked spike rates are higher in trained animals, intermediate following passive
stimulation and poorest in long-deaf unstimulated subjects, suggest that
electric hearing experience can differentially modify temporal coding properties
in the ICC. It should be noted that this ordinal sequence of spike rates for
animals with differing hearing histories is similar to that reported in same long-
deaf groups of cats in the AI (Vollmer and Beitel 2011).
Data from the ICX in the short-deaf animals (Fig. 3C) show that peak mean spike rate is similar for the SDpass and the SDtrain groups [17.8±5.5 (SE) sp/s and 17.2±2.3 (SE) sp/s, respectively]. However, the corresponding stimulus pulse rate for the SDpass group (60 pps) is higher than for the SDtrain group (20 pps). In the long-deaf cats (Fig. 3D), the peak mean spike rate in the ICX is highest in the LDtrain group [25.5±2.6 (SE) sp/s at 40 pps], intermediate in the LDU group [16.6±3.1 (SE) sp/s at 40 pps] and lowest in the LDpass group [9.8±1.3 (SE) sp/s at 20 pps]. In contrast to the ICC, these peak mean spike rate data in the multisensory ICX of both short-deaf and long-deaf animals suggest that phase-locked spike rates in the ICX are not coherently or consistently modified by electric hearing experience.

As shown in the insets in Figures 3A-D the proportions of phase-locked neurons decline as the pulse rate increases. The interpolated stimulus pulse rate at which about half of the neuronal population contributes to the mean phase-locked spike rate (50%-rate) is indicated by a dot on each function. For the two short-deaf groups SDpass and SDtrain, the 50%-rates were similar (~120 pps and 140 pps, respectively; Fig. 3A). Figure 3B illustrates that the two long-deaf groups that had electric hearing experience (LDpass and LDtrain groups) had similarly high 50%-rates of 160pps and 170 pps, respectively. The 50%-rate in the unstimulated LDU group is clearly lower (~90 pps). The results suggest that electric hearing experience, independent of its behavioral relevance, similarly affects the proportions of phase-locked neurons in the ICC of short-deaf and long-deaf animals.

Note that in the ICX (Fig. 3C, D), the 50%-rates (dots) in all five groups occur at lower stimulus pulse rates than in the ICC (SDpass: 100 pps, SDtrain: 70 pps; LDU: 65 pps, LDpass: 55 pps, LDtrain: 95 pps). Moreover, the nominal sequences of 50%-rates in the ICX (Fig. 3C and D) are different from the ordinal sequences in the ICC (Fig. 3A and B).

Passive stimulation and behavioral training effects on temporal plasticity in the ICC of short-deaf cats

In the ICC of short-deaf animals, exposure to ICES can enhance temporal processing, but the previous study (Vollmer et al., 1999) did not determine whether this effect is due to passive (i.e., behaviorally-meaningless)
stimulation or to training with behaviorally-relevant ICES. An important objective of the present study was, therefore, to evaluate whether passive stimulation or behaviorally-relevant ICES differentially affect neuronal temporal plasticity in the IC. In this section we first compare neuronal temporal processing in the ICC of passively stimulated short-deaf cats (SDpass) and short-deaf cats that also received behaviorally-relevant stimulation (SDtrain).

**Best repetition rate (BRR) in the ICC.** Figure 4A shows cumulative probability functions for BRRs in passively stimulated short-deaf cats (SDpass) and cats that also received behaviorally-relevant stimulation (SDtrain). The intersection of a function with the dashed horizontal line indicates that 80% of the ICC units in a group of animals have BRRs corresponding to values at or below the pulse rate on the abscissa that is aligned with the intersection. For example, the 80% intersections for BRRs in the two groups are located at 70 pps indicating that 80% of neural units have BRRs ≤70 pps, and only 20% of units have BRRs >70 pps (Fig. 4A). The inset graph in Fig. 4A illustrates the quantitative distribution of neurons as a function of BRR. Statistical analyses shows no difference in the cumulative distributions of BRR between the two groups of cats (two-sample Kolmogorov-Smirnov test, P>0.05; Fig. 4A). Also, the corresponding medians in BRR for groups SDpass [50±19.1 (Q) pps] and SDtrain [49.2±19.2 (Q) pps] are not different (Mann-Whitney Rank Sum Test, P>0.05; Fig. 4B). Thus, behavioral training elicits no apparent effect on BRRs in the short-deaf animals.

**Cutoff rate (CR) in the ICC.** Figure 4C shows cumulative probability functions for CRs in the ICC of SDpass and SDtrain animals. The 80% intersections are similar (118 and 120 pps, respectively), and the cumulative distributions of CR do not differ across groups (two-sample Kolmogorov-Smirnov test, P>0.05). Correspondingly, there is no difference in median CRs between the SDpass [86.9±34.1 (Q) pps] and SDtrain animals [86.9±18.7 (Q) pps] (P>0.05; Fig. 4D). Behavioral training has no effect on CRs in the ICC of short-deaf animals.

**Mean minimum latency and jitter in the ICC.** Figure 4E shows cumulative probability functions for ICC minimum latencies in the two groups of cats. The 80% intersections for minimum latencies are located at about 7.6 ms (SDtrain group) and 8.6 ms (SDpass group). In contrast to the results for BRR and CR,
the cumulative distributions of minimum latencies differ significantly across groups (two-sample Kolmogorov-Smirnov test, P<0.05), and minimum latency is significantly shorter in the SDtrain group [6.23±0.76 (Q) ms] compared to the SDpass group [6.73±1.21 (Q) ms] (Fig. 4F; P<0.01). The difference in minimum latencies between the SDtrain group and the SDpass group is the only statistical evidence in this study for a behavioral training effect in the ICC.

To analyze the temporal precision of ICC unit responses to ICES, minimum latency variability was determined for neuronal units stimulated by repetitions of a single electric pulse. Comparison of the median standard deviation (jitter) of minimum latencies found no difference in temporal precision between SDpass [0.64±0.18 (Q) ms] and SDtrain animals [0.56±0.17 (Q) ms] (P>0.05).

In summary, the results suggest that, with the exception of minimum latencies, behavioral training of short-deaf animals has no different or greater effect than passive stimulation on temporal processing in the ICC. Together with previously published findings demonstrating an increase in temporal processing following exposure to higher-rate stimulation (not separately determined for passive versus behaviorally-relevant ICES; Vollmer et al. 1999), these results provide evidence that changes in temporal processing in ICC neurons of short-deaf animals are due primarily to passive stimulation.

Passive stimulation and behavioral training effects on temporal plasticity in the ICC of long-deaf cats

Previous work has demonstrated that life-long deafness without electric hearing experience results in severe degradation in temporal processing in the ICC (Vollmer et al. 2005). Although exposure to ICES can enhance ICC temporal processing in long-deaf adult cats (Vollmer et al. 2005), prior studies have not determined whether temporal processing in the ICC of long-deaf cats is differentially affected by behaviorally-meaningless passive stimulation or by training with behaviorally-relevant ICES. Here we examine ICC neuronal temporal plasticity to both passive and behaviorally-relevant stimuli in long-deaf cats (Fig. 5). The qualitative descriptive results obtained in behaviorally trained cat K55 are also presented in Fig. 5B,D and F.
Best repetition rate (BRR) in the ICC. Figure 5A shows cumulative probability functions for BRRs in the long-deaf groups of cats. Similar to the inset in Fig. 4A, the inset in Fig. 5A illustrates the distribution of neurons as a function of BRR. In Fig. 5A the 80% intersection in the LDU group occurs at a BRR of about 48 pps indicating that 80% of units in this group have BRRs ≤48 pps, and only 20% of units have BRRs >48 pps. The 80% intersection for the LDpass group is at a BRR of about 60 pps, and for the LDtrain group the intersection occurs at about 76 pps. The two-sample Kolmogorov-Smirnov test revealed differences in the cumulative distributions of BRR in LDU animals when compared to the LDpass and LDtrain groups (both P<0.001). However, there were no differences in the distributions of BRR between the LDpass and the LDstim groups (P=0.3946).

Corresponding statistical analyses of the median BRRs show that passive ICES in the LDpass group significantly improves temporal following capacity [39.4±14.5 (Q) pps] when compared to LDU animals [29.6±12.8 (Q) pps] (Kruskal-Wallis, P<0.001; Dunn’s test, P<0.01; Fig. 5B). Importantly, there is no difference in BRRs between the LDpass group and the LDtrain group [49±14.4 (Q) pps] (Dunn’s test, P>0.05). Thus, similar to short-deaf animals the addition of behavioral training to passive stimulation has no apparent effect on BRRs in the ICC of long-deaf animals.

Cutoff rate (CR) in the ICC. Figure 5C shows cumulative probability functions for CRs in the long-deaf groups of cats. The 80% intersection for group LDU is located at a CR of 90 pps, and for groups LDpass and LDtrain group the 80% intersections are located at CRs of 108 pps and 124 pps, respectively. Similar to the results for BRR, the distributions of CR in LDU animals were significantly different from those in both the LDpass and LDtrain groups (two-sample Kolmogorov-Smirnov test, both P<0.001). But there were no differences in the distributions of CR between the LDpass and the LDtrain groups (P=0.0557).

The statistical comparisons of median CRs in Fig. 5D are consistent with the BRR results: Passive ICES elevates the degraded CRs in long-deaf animals [LDpass versus LDU; 83.3±21.1 (Q) pps vs. 53.8±26.8 (Q) pps, respectively; Kruskal-Wallis, P<0.001; Dunn’s test, P<0.01). However, the addition of behavioral training to passive ICES failed to enhance CRs in the
ICC of these long-deaf animals (LDtrain versus LDpass; LDtrain: 96.3±21 (Q) pps; Dunn’s test: P>0.05).

Mean minimum latency and jitter in the ICC. Figure 5E shows cumulative probability functions for minimum latencies in the three long-deaf groups of cats. In Figure 5E the 80% intersections for the three groups are located at minimum latencies of about 7.3 ms (LDpass), 8.3 ms (LDtrain) and 9.7 ms (LDU). Cumulative distribution differed across all three groups of long-deaf animals (two-sample Kolmogorov-Smirnov test, all P<0.02).

Corresponding statistical analysis of median minimum latencies shows that passive stimulation significantly decreases the long minimum latencies of neurons in long-deaf animals [LDpass group vs. LDU; 6.45±0.67 (Q) ms vs. 8.47±0.78 (Q) ms, respectively] Fig. 5F; Kruskal-Wallis, P<0.001; Dunn’s test, P<0.001). However, unlike the effects seen in BRRs and CRs, minimum latencies in the LDtrain group [6.88±0.96 (Q) ms] are significantly longer than in the LDpass group (Dunn’s test, P<0.05).

To analyze the temporal precision of ICC unit responses to ICES, the standard deviation (jitter) of minimum latencies was compared across the three groups of animals, and no differences in precision among the three groups of cats were found for either cumulative distribution (two-sample Kolmogorov-Smirnov test, all P>0.44) or median jitter (Kruskal-Wallis, P>0.05).

**K55: Effects of behaviorally-relevant ICES in the absence of passive stimulation**

As mentioned in the Methods, it is important to note that cats in the LDpass and LDtrain groups received passive ICES ~4 h/d, 5 d/wk, whereas cat K55 and the cats in the LDtrain group typically received less than 1-min of behaviorally-relevant ICES during a daily training session.

In this section we compare the neuronal temporal processing results recorded in the ICC in a unique long-deaf cat, K55, with the ICC results obtained in the LDU, LDpass and LDtrain groups described above.

Although K55 received behaviorally-meaningful ICES during psychophysical training, importantly, this cat never received behaviorally-meaningless, passive ICES. Thus, the electrophysiological data recorded in K55 are of interest to be assessed separately because these data address the
question of how behavioral training in the complete absence of passive ICES in a long-deaf animal may affect neuronal temporal processing in the deaf auditory system. Because K55 is a unique, single-case-study, only descriptive, qualitative comparisons of medians and quartile deviation (Q) values with long-deaf groups of cats are included in Results.

Comparisons of temporal processing in the ICC. In the ICC, median BRR in K55 [29.7±4.9 (Q) pps] and in the LDU group [29.6±12.8 (Q) pps] are similar (Fig. 5B), median CR is lower in K55 [39.5±10.2 (Q) pps] compared to the LDU group [53.8±26.8 (Q) pps] (Fig. 5D), and median minimum latency is longer in K55 [9.35±1.29 (Q) ms] than in the unstimulated LDU group [8.47±0.78 (Q) ms] (Fig. 5F).

Compared to groups LDpass [BRR: 39.4±14.5 (Q) pps, CR: 83.3±21.1 (Q) pps, minimum latency: 6.45±0.67 (Q) ms] and LDtrain [BRR: 49±14.4 (Q) pps, CR: 96.3±21 (Q) pps, minimum latency: 6.88±0.96 (Q) ms], in K55 median BRR and CR are lower (Fig. 5B,D) and median minimum latency is longer (Fig. 5F).

In summary, the absence of passive stimulation appeared to severely compromise neuronal temporal processing in the ICC of K55. The results in the ICC suggest that a limited daily amount of behavioral-relevant ICES in the absence of passive ICES is not sufficient to enhance degraded temporal processing in a long-deaf cat (K55). Instead, a critical amount of ICES, independent of its behavioral relevance, may be required to induce neuronal temporal plasticity in the functionally degraded, unstimulated ICC.

Temporal processing in the ICX in short-deaf and long-deaf cats is not differentially modulated by passive or behaviorally-relevant ICES

To investigate the potential for auditory temporal plasticity in the multisensory ICX, we compared the effects of passive and behaviorally-relevant stimulation in long-deaf and short-deaf groups of animals.

Comparisons between the two short-deaf groups (Mann-Whitney Rank Sum Tests; Fig. 6A, C, E) and comparisons among the three long-deaf groups (Kruskal-Wallis ANOVA on Ranks; Fig. 6B, D, F) show no significant (P>0.05) or meaningful (Fig. 6C) differences in temporal processing after either passive
or behaviorally-relevant stimulation in the ICX. Temporal precision (jitter) effects were not observed in any of the groups.

The results in this section suggest that temporal processing in the ICX is not differentially modulated by passive or behaviorally-relevant ICES, independent of deafness duration or age at stimulation onset. Although neuronal sample sizes in the ICX of the five groups of cats are small, the results are consistent with previously reported ICX results (Vollmer et al. 1999; 2005) from larger cohorts of short-deaf and long-deaf animals that are reviewed in the Discussion.

**Temporal processing in long-deaf cats versus short-deaf cats:**

**ICC**

Compared to short-deaf cats, the duration of deafness is much longer (mean, >7 years) in the long-deaf cats and therefore the percentage survival of spiral ganglion cells is severely reduced to an average of about 6% of normal (see Table 1). To investigate the potential effects of deafness duration and SGC density on the efficacy of training-induced plasticity in the ICC, we compared neuronal temporal processing in long-deaf versus short-deaf groups of animals. All statistical comparisons in this section (Mann-Whitney Rank Sum Tests) are based on pairs of short-deaf and long-deaf groups of cats that were matched on the basis of their stimulation histories (passive or behaviorally-relevant ICES).

In the ICC, comparisons of median BRRs and CRs between the passively stimulated groups SDpass (50 pps and 86.9 pps, respectively) and LDpass (39.4 pps and 83.8 pps, respectively) revealed no differences (Fig. 7A; both P>0.05). Correspondingly, comparisons of BRRs and CRs between the two behaviorally trained groups SDtrain (49 pps and 86.9 pps, respectively) and LDtrain (49 pps and 96.3 pps, respectively) demonstrated no differences (Fig. 7B; both P>0.05). However, minimum latencies are significantly shorter in the SDtrain group versus the LDtrain group (P=0.001) and are significantly longer in the SDpass group versus the LDpass group (P=0.015). There are no significant jitter effects in any of the groups (P>0.05).

In summary, results in the ICC indicate that the effects of passive stimulation and behaviorally-relevant stimulation on temporal repetition rate
following (BRR and CR) and jitter are independent of the duration of deafness or the age at stimulation onset.

Discussion

This study compares the effects of passive versus behaviorally-relevant ICES on neuronal temporal processing in collicular (ICC and ICX) auditory structures in short-deaf and long-deaf cats. Earlier studies have shown that, in both the ICC and the AI, deafness and the absence of prosthetic ICES in long-deaf unstimulated animals resulted in severely degraded temporal processing (Vollmer and Beitel 2011; Vollmer et al. 2005). In the present study, exposure to passive ICES enhanced temporal processing in the ICC of long-deaf cats. With the exception of minimum latencies in short-deaf animals, adding limited amounts of behaviorally-relevant ICES had no significant effect on ICC temporal processing in passively stimulated short-deaf or long-deaf cats. In contrast, our prior studies of the AI in sequential recordings made in the same animals showed that passive stimulation had a weak effect on temporal processing, whereas behavioral relevance was highly effective at inducing temporal plasticity at the cortical level (Beitel et al. 2011; Vollmer and Beitel 2011; see final sections of Discussion, below). In the ICX neither passive nor behaviorally-relevant ICES affected neuronal temporal processing in the profoundly deaf animals.

Repetition rate transfer functions (RRTFs) with band-pass filter characteristics were the dominant profile in the ICC and the ICX. These findings are in agreement with dominant band-pass filter characteristics observed in the AI (Beitel et al., 2011; Vollmer and Beitel, 2011). Thus, across collicular and cortical auditory structures, the general filter-characteristics of neurons appear to be largely unaffected by differences in hearing experience. In contrast, the ordinal sequences of phase-locked spike rates in the ICC reflected systematic effects of different electric hearing experience in both short-deaf and long-deaf cats: spike rates were highest in trained animals, intermediate following passive stimulation and poorest in long-deaf unstimulated subjects. Similar ordinal sequences were observed in the AI (Beitel et al., 2011; Vollmer and Beitel 2011). The results in the short-deaf and long-deaf groups suggest that consistent modifications of phase-locked spike
rates by different electric hearing experience occurred in both the ICC and the AI. In the ICX, neither deafness history nor electric hearing experience produced a systematic or corresponding pattern of phase-locked spike rates. The temporal plasticity results summarized above are compatible with the hypotheses that 1) temporal plasticity in the deaf auditory system is not limited to critical periods during development, 2) temporal plasticity in the ICC depends on a critical amount of ICES, but not on the behavioral significance of the signal, whereas 3) behaviorally-driven temporal plasticity emerges as a pivotal cortical or forebrain attribute in the neonatally deafened cat.

**The statistics of prosthetic signals drive auditory temporal processing and plasticity in deaf cats**

The natural auditory environment (acoustic biotope) contains sounds produced by animals as well as innumerable environmental sounds (Aertsen et al. 1979; Müller-Preuss 1990). Most abiotic sounds (e.g., wind, rain) contribute primarily to environmental noise, whereas vocalizations and other sounds produced by con-specifics, predators or prey provide valuable information to resident animals. The temporal patterns of auditory inputs are critically important for the development of neuronal temporal processing in the immature auditory system as well as for modifications in temporal processing in the adult system (e.g., Kilgard et al., 2001; Kim and Bao 2009; Nelken et al., 1999; Shetake et al., 2012; Zhou and Merzenich, 2009). Animals adapt to their sensory environment, and the statistical structure of the auditory environment is reflected in the structure and function of the auditory system (Joris et al., 2004).

Research in hearing animals has shown that, compared to neurons in the IC, neurons in the MGB and the AI may be more sensitive to behaviorally relevant features of acoustic stimuli (Chechik and Nelkin 2012) and less susceptible to stimulus-induced redundancy (Chechik et al 2006). In cats deafened shortly after birth, cochlear prostheses can provide electric hearing for the animals and also provide a means for investigators to monitor, select and control the statistical structure of the animals’ auditory environment.

As mentioned in the Methods, the choice of the stimulus (SAM 300/30) used in the present study was based on the goal of providing temporally challenging stimulation: A modulation rate of 30 Hz is within the upper range of
the temporal following ability of AI neurons, and a carrier rate of 300 pps is
within the upper range of temporal following ability of ICC neurons. Similar to
findings in earlier studies (Beitel et al. 2011; Vollmer et al. 1999, 2005; Vollmer
and Beitel 2011), exposure to the SAM 300/30 stimulus was capable of driving
temporal processing in both AI and IC neurons. However unlike the ICES
applied as a warning signal during behavioral training, passive ICES was
entirely redundant and appeared to be ignored by the deaf animals.

The structure and function of the auditory system in profoundly deaf cats
is severely altered by loss of hair cells, SGCs and auditory nerve fibers (Leake
et al. 1991, 1999; Vollmer et al. 2007), by alterations in spontaneous neuronal
activity (Hancock et al. 2013; Shepherd et al. 1999) and structural and synaptic
morphological changes at levels from the auditory nerve to the auditory cortex
(Leake et al. 2008; Lustig et al. 1994; O’Neil et al. 2011; Shibata 2007). These
degenerative changes are especially severe after the extremely long durations
of deafness in the long-deaf cats. For profoundly deaf animals there is no
acoustic biotope. It is against this constellation of pathological factors and the
limitations of passive and behaviorally-relevant ICES that the effects of
deafness and electric auditory experience on neuronal temporal processing
must be evaluated.

**Collicular temporal processing and plasticity**

**ICC.** Developmental studies that investigated the effects of rearing in
acoustic environments or passive exposure to sound in the IC have focused on
alterations in frequency tuning or tonotopic organization in the ICC (Miyakawa
et al. 2013; Oliver et al., 2011; Poon and Chen, 1992; Yu et al., 2007). In
contrast, studies in neonatally deafened, mature cats have reported that
deafness and auditory experience differentially affect temporal signal
processing in the IC (Snyder et al., 1995; Vollmer et al., 1999, 2005). These
latter studies, however, did not independently investigate effects of
behaviorally-meaningless versus behaviorally-relevant ICES. In the current
study we report evidence that exposure of profoundly deaf cats to passive,
behaviorally-meaningless ICES generates robust temporal plasticity in the ICC.
This strongly suggests that passively-driven temporal plasticity is established at
or below the level of the auditory midbrain.
Neuronal temporal plasticity induced by additional behavioral training was not observed in the ICC of long-deaf cats (LD train vs. LDpass). In short-deaf cats we observed one positive effect of behavioral training on neuronal temporal plasticity in the ICC: minimum latency was significantly shorter in the SDtrain group compared to the SDpass group. Consistent with our results, there are both positive (Birt et al. 1979; Burcs et al. 2014; Gonzalez-Lima and Scheich 1984; Olds et al. 1978) and negative (Birt and Olds 1981) examples of associative learning and conditioning effects on neural activity in the IC of hearing animals.

It is possible that longer periods of behavioral training or a different behavioral training method could more effectively produce neuronal temporal plasticity in the ICC of deaf cats. However, findings to date strongly indicate that learning-based temporal plasticity in hearing and deaf animals originates primarily at levels above the auditory midbrain (Beitel et al., 2011; Vollmer and Beitel, 2011).

An additional issue in this study is whether behavioral training alone in the complete absence of passive ICES is sufficient to produce neuronal temporal plasticity in the ICC of profoundly deaf cats. Long-deaf cat K55 received a limited daily amount of behaviorally-relevant ICES but never received behaviorally-meaningless ICES. When compared to temporal processing parameters (BRR, CR, Minimum Latency) in the LDU group, neuronal temporal processing in the ICC of cat K55 was virtually identical to or poorer than neuronal temporal processing in the long-deaf unstimulated animals. In contrast, temporal processing in all three parameters was enhanced in passively-stimulated cats (LDpass group) compared to the LDU group and cat K55. These results suggest that a critical amount of ICES, independent of its behavioral relevance, may be necessary and sufficient to induce neuronal temporal plasticity in the functionally degraded, unstimulated ICC. Of course cat K55 is a unique single-case-study, and no definite conclusion can be based upon results in a single animal. Nevertheless, the results in K55 are consistent with the absence of behavioral training effects on temporal processing in short-deaf (SDtrain; Fig. 4) and long-deaf (LDtrain; Fig. 5) animals.

ICC Mechanisms. Deafness-induced degradations in ICC temporal
processing reflect changes in both inhibitory (reduced) and excitatory (increased) synaptic strength that produce an imbalance in synaptic drive (Caspary et al., 2002; Vale and Sanes, 2002). Chronic-passive electric stimulation of auditory nerve fibers via cochlear implants in congenitally and adult deaf animals reversed pathological alterations in auditory nerve synapses in the cochlear nucleus (O’Neil et al., 2010; Ryugo et al., 2005) and synaptic transmission in the ICC (Argence et al., 2008), potentially allowing rescue of synaptic dynamics and neuronal temporal processing at the midbrain level of the central auditory system.

**ICX vs. ICC.** Our results in the ICX, based on relatively small samples of neurons (N=11-28), show that neuronal temporal processing in the ICX is 1) poorer than that in the ICC and 2) is not modulated by either passive or behaviorally-relevant electric stimulation of the auditory nerve. The current results are consistent with previously reported ICX results (Vollmer et al. 1999; 2005) from cohorts of short-deaf and long-deaf animals with larger samples of ICX neurons (N=55-72), indicating that the small sample size was not responsible for the non-significant statistical results in the ICX (Fig. 6).

The multisensory ICX in the cat receives input from both the auditory system and the somatosensory system (Aitkin et al. 1975, 1978, 1981; Andersen et al. 1980; Rockel and Jones 1973), and ICX neurons respond either to auditory stimuli or to tactile stimuli. There are very few bisensory neurons in the ICX. The ICX is the main target of the descending corticocollicular and thalamotectal systems, whereas the ICC is apparently a subsidiary target of these systems (Winer et al. 2002). The ICX also receives intrinsic input from the ICC and projects primarily to the non-tonotopic medial division of the MGB (Huffman and Henson 1990; Winer et al. 1998). There is apparently no auditory input to the ICX from either the lemniscal dorsal cochlear nucleus or the lateral lemnicus (Winer and Schreiner 2005). With the exception of the intrinsic projection from the ICC, the ICX appears to be essentially segregated from the ascending, lemniscal auditory system.

In the ICX we recorded neuronal temporal responses to ICES in the five groups of cats. Differences in neuronal temporal processing between groups were not significant or meaningful, and distinctive differences in electric hearing
experience in the groups of deaf animals had no apparent influence on the ICX results.

Related studies in humans. Several investigators have used scalp-recorded frequency-following responses to explore subcortical temporal plasticity after perceptual learning in humans (Carcagno and Plack, 2011; Song et al., 2012). Unfortunately, these important studies lacked control groups that were passively exposed to identical stimuli and could not unambiguously differentiate between a learning-induced versus a passive-induced stimulation effect. Furthermore, the brainstem or midbrain structures contributing to the recorded responses were not definitively identified (Chandrasekaran and Kraus, 2010).

Clinical relevance of passive stimulation effects. We have noted above that the temporal patterns of auditory inputs are critically important for the development of neuronal temporal processing in both the hearing and the deaf auditory systems. In our experiments with neonatally deafened cats, the statistics of the passive ICES (pulse and modulation rates, amplitude, stimulation duration) were designed so that the animals were able to ignore the stimuli and typically slept during a passive-stimulation session. The passive stimuli used in this study clearly produced neuronal temporal plasticity in both the ICC and the AI.

In contemporary clinical practice, a subject's cochlear implant is turned on during waking hours, exposing the patient's auditory system to arbitrary sources of sound. To the best of our knowledge, clinical routine does not include a protocol that shapes the statistics of the delivered stimuli in order to maximize beneficial effects, including neuronal temporal plasticity in the auditory system. Passive-stimulation with appropriate stimulus statistics could be applied conveniently to CI subjects during sleep and potentially could provide a means to overcome temporal processing deficits particularly in individuals with poor speech discrimination abilities.

Comparison of temporal processing in the AI versus the ICC

An important feature of this study is that we were able to compare, in the same animals, experience-induced neuronal temporal processing and plasticity at midbrain and forebrain levels of the auditory system in short-deaf and long-
deaf animals. Our objective was to compare the effects of passive exposure
versus learning-based exposure to ICES in the ICC and cortical field AI (Figs.
4, 5 and 8).

Short-Deaf cats: With the exception of shorter minimum latencies in the
SDtrain group, comparisons between SDpass and SDtrain animals revealed no
effect of behavioral training on neuronal temporal processing in the ICC (BRR,
CR, jitter; Fig. 4B,D; P>0.05). In contrast, recordings in the AI demonstrated
that behavioral training in the SDtrain group elicited significant increases in
both BRRs and CRs (Fig. 8A,C; P<0.001), significantly shorter minimum
latencies (Fig. 8E; P<0.05), and lower jitter compared to passive stimulation in
the SDpass group (Fig. 8P<0.05).

Long-Deaf cats: LDtrain animals showed a strong increase in phase-
locked spike rates in the ICC that was not observed in the ICC of SDtrain
animals (Fig. 3B and C, respectively). These findings are similar to results in
the AI (Vollmer and Beitel 2011). However, these changes in phase-locked
spike rates were not paralleled by stimulation-induced changes in the 50%
rates, BRR and CR in either the ICC or the AI.

Compared to the LDU group, passive ICES (LDpass group) increased
BRRs and CRs in the ICC and reduced minimum latencies (Fig. 5B, D, F;
P<0.01, P<0.01, and P<0.001, respectively). In the AI, however, the effects of
passive stimulation were inconsistent: passive ICES increased BRRs (Fig. 8B:
P<0.01), had a minor effect on CRs (Fig. 8D: P<0.05) and did not affect
minimum latencies (Fig. 8F: P>0.05) or jitter (P>0.05). The effects of passive
ICES on temporal processing in the ICC were statistically more robust than its
effects in the AI. In comparisons of the LDtrain and LDpass groups, the
significant effects of behavioral training in the AI (Fig. 8B: BRR, P<0.01; Fig.
8D: CR, P<0.05; Fig. 8F: minimum latency, P<0.001) contrast sharply with the
complete absence of behavioral training effects in the ICC of long-deaf cats
(Fig. 5B, D and F). Response jitter in the LDtrain and LDpass groups was not
significantly different in either the ICC or the AI (P>0.05).

In summary, in the ICC BRRs and CRs were not significantly different in
passively stimulated versus behaviorally trained short-deaf and long-deaf
groups of animals, whereas behavioral training significantly enhanced BRRs
and CRs in the AI of the profoundly deaf animals.
Moreover, similar to the ICC (Fig. 7), the AI effects of passive stimulation and behaviorally-relevant stimulation on temporal repetition rate following (BRR and CR) and jitter were independent of the duration of deafness or the age at stimulation onset (Vollmer and Beitel, 2011) supporting the hypothesis that temporal plasticity in both the auditory midbrain and auditory cortex is not limited to critical periods during development.

**Cat K55:** The results in the ICC (Fig. 5B,D,F) suggest that a limited daily amount of behavioral-relevant ICES in the absence of passive ICES (K55) may not be sufficient to enhance temporal processing, as compared to the LDU group (Fig. 5B,D,F and jitter). Instead, a *critical amount of ICES* independent of its behavioral relevance may be required to induce neuronal temporal plasticity in the functionally degraded, unstimulated ICC. In contrast, in the AI a limited amount of behaviorally-relevant stimulation (K55) is sufficient to improve performance compared to the LDU group and reaches performance that is similar to the LDpass group (Fig. 8B,D,F and jitter). These results in K55 underscore the potential of behaviorally-relevant stimulation to improve temporal processing in the AI even in the complete absence of passive ICES.

In summary, the preceding analysis is unique in that it directly compares, in the same deaf animals, experience-induced temporal plasticity at different levels in the auditory system. The major findings are two-fold. First, passive ICES had a significant but weaker effect on temporal plasticity in the AI than in the ICC. This finding is compatible with the hypothesis that passively induced temporal plasticity at the level of the AI reflects modulations of temporal input patterns originating at subcortical levels including the ICC (Bartlett and Wang, 2005; Creutzfeldt et al. 1980; Langer and Schreiner 1988; Müller-Preuss 1990). The differences in the strength of passively-induced plasticity between the ICC and the AI are consistent with evidence that repetitious, meaningless sounds typically do not have long-term effects on AI plasticity in mature animals (Bao et al., 2004; Beitel et al., 2003; Polley et al., 2006).

Second, independent of the duration of deafness and age at stimulation onset, behaviorally-relevant ICES elicits robust temporal plasticity at the level of the AI but has little or no effect at the level of the ICC (SDtrain group, LDtrain group and cat K55). This finding supports the hypothesis that behaviorally-
driven neuronal temporal plasticity is essentially a cortical or forebrain attribute.

**Learning-induced neuronal temporal plasticity: Midbrain, thalamus, cortex?**

In the thalamic forebrain (MGB) auditory neuronal plasticity has been studied extensively (Weinberger, 2011; Weinberger and Bakin, 1998). However the MGB has not been a preferred site for research on *temporal* plasticity. We are aware of only one project that reported learning-induced neuronal temporal processing effects in the MGB (Gabriel et al., 1975, 1976). Whether the temporal plasticity observed in the AI is a forebrain phenomenon that requires an auditory thalamic component has not been fully established.

A study of the short-term effects of acetylcholine release has shown strong influences on receptive field plasticity in cortical neurons (Froemke et al. 2007). The release of acetylcholine has been linked as one component neuromodulator involved in learning induced plasticity. In Froemke et al. (2007) plasticity was mediated by cortico-cortical inputs and not by thalamo-cortical inputs. However the results do not exclude the possibility of thalamic-based learning induced plasticity, given that the MGB is also the target of neuromodulators. Further research is required to determine the role of the MGB in learning-induced plasticity.

Recently, Atencio and colleagues (2012) used ripple stimulation and receptive field analysis to show that a fundamental transformation occurs in spectrotetmal stimulus encoding from the ICC to the AI. Multidimensional receptive fields emerge in the AI, whereas a single filter is largely sufficient to account for neuronal processing in the ICC. Recent observations indicate that receptive field properties of the MGB are more similar to the ICC than to the AI (J. Y. Shih, personal communication) suggesting that cortex is the dominant site for generating receptive field complexity and plasticity (Sharpee et al. 2011; Schreiner and Polley 2014). Regardless of the midbrain and thalamic roles, behaviorally-driven temporal plasticity in auditory cortex is shaped essentially by an animal’s motivated attention to reinforced sensory signals, modulated by networks of top-down inputs and behaviorally-induced neuromodulator release (Engel et al. 2001; Harris et al. 2013; Jaramillo and Zador 2011; Polley et al. 2006; Schreiner and Polley 2014; Winkler et al. 2009).
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Endnote

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at the institutional website of one of the authors, which at the time of publication they indicate is: http://www.hno.ukw.de/fileadmin/uk/hno-klinik/Publikationen/2016-08-29_URL_Data_set.pdf. These materials are not a part of this manuscript, and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the website address, or for any links to or from it.
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Figure 1. Basic procedures for acquisition of electrophysiological data. A: Schematic coronal section through cat IC with recording electrode. Abbreviations of labeled major subdivisions: ICC, central nucleus of the IC; ICX, external nucleus of the IC; ICD, dorsal cortex of the IC; DNLL, dorsal nucleus of the lateral lemniscus; cic, commisure of the IC; pag, periaqueductal grey. B: Threshold vs. depth functions in the IC or spatial tuning curves (STCs) in response to biphasic electrical pulses (0.2 ms/phase) delivered on apical electrode pair 1,2 (open symbols) and on basal electrode pair 3,4 (closed symbols). The locations of minimum thresholds in the external (ICX) and central (ICC) nuclei of the IC for stimulation of each electrode pair provide a relative measure of characteristic frequencies. The high-threshold region between the two locations of minimum threshold indicates the border between the 2 nuclei (vertical - - -) and allows neurons in a given penetration to be assigned to either ICX or ICC (modified from Vollmer et al. 2007).

Fig. 2. Procedures for neuronal analysis. A, D: Examples of poststimulus time histograms (PSTHs) for responses from single neurons to pulse trains of increasing rates (ordinates) in the ICX (A) and ICC (D) of a LDtrain cat (CH618). Onset responses to the first pulse are excluded from vector strength analysis (grey area). Vector strength (VS) and total number of spikes (sp) are shown for each histogram. B, E: Corresponding band-pass repetition rate transfer functions (RRTFs) for the same two neurons as shown in A and D, respectively. Normalized numbers of phase-locked spikes are plotted versus stimulus pulse rate (pps). Two parameters are derived from the RRTFs: best repetition rate, BRR (pulse rate that evoked maximum number of phase-locked spikes) and cutoff rate, CR (higher pulse rate at which the number of spikes was equal to or just below 50% of the number at BRR). C, F: Raster plots of responses to 20 repetitions of a single electric pulse (2 pps, sweep duration 500 ms) for the same neurons as in A, B and D, E, respectively. Electric stimulus pulse onset occurs at 0 ms. First spike (minimum) latency for each stimulus repetition is represented. Mean minimum latency is shorter and standard deviation of latency (jitter) is smaller in the ICC (F) than in the ICX.
neuron (C).

Figure 3. Mean phase-locked spike rate as a function of electric stimulus pulse rate for ICC (A, B) and ICX (C, D) neurons in short-deaf (A, C) and long-deaf (B, D) animals. Mean spike rates are calculated as proportional values, i.e., mean phase-locked spike rate x proportion of contributing neurons. Circles on the functions identify 50%-rate. Error bars: standard error of the mean (SE). Error bars are slightly offset to improve visualization. Inserts: Proportions of neurons phase-locked to a given pulse rate. Horizontal dashed lines intersect functions at the pulse rate (pps) at which 50% of units contribute to mean spike rate (50%-rate). Bin size=20 pps.

Figure 4. A, C and E: Cumulative probabilities (integrated data) of BRRs (A), CRs (C) and minimum latencies (E) of ICC neurons in two groups of short-deaf animals. Dashed horizontal lines indicate that 80% of the units have a BRR, CR or minimum latency equal to or smaller than the corresponding value on the abscissa. Bin sizes: 20 pps (BRR, CR) and 1 ms (minimum latency). Inserts show the distributions of neurons versus the parameters (BRR, CR, minimum latency). B, D and F: The box plots in each panel show the corresponding statistical comparisons of BRRs (B), CRs (D) and minimum latencies (F) in the ICC. Vertical boxes display the group medians, and the 25th and 75th percentile values; ‘whiskers’ illustrate the 10th and 90th percentiles. Brackets: number of units. Mann-Whitney Rank Sum Tests, **P<0.01.

Figure 5. A, C and E: Cumulative probabilities of BRRs (A), CRs (C) and minimum latencies (E) of ICC neurons in three groups of long-deaf animals. Dashed horizontal lines indicate that 80% of the units have a BRR, CR or minimum latency equal to or smaller than the corresponding value on the abscissa. Bin sizes: 20 pps (BRR, CR) and 1 ms (minimum latency). Inserts show the distributions of neurons versus the parameters (BRR, CR, minimum latency). B, D and F: Corresponding statistical comparisons of BRRs (B), CRs (D) and minimum latencies (F) in the ICC. Brackets: number of units. Kruskal-Wallis one-way ANOVA on ranks, all P<0.001; Dunn pairwise multiple comparison, *P<0.05, **P<0.01, ***P<0.001. Comparisons that differ with
respect to more than a single factor have grey statistical asterisks and are not reported in the text. Qualitative descriptive results obtained in behaviorally trained cat K55 are presented in B, D and F.

Figure 6. A, C and E: Statistical comparisons of BRRs (A), CRs (C) and minimum latencies (E) of ICX neurons in short-deaf animals. Brackets: number of units. Mann-Whitney Rank Sum Test, *P<0.05. B, D and F: Statistical comparisons of BRR (B), CR (D) and minimum latencies (F) of ICX neurons in long-deaf animals. Brackets: number of units. Kruskal-Wallis one-way ANOVA on ranks, n.s. The only significant statistical comparison in the ICX (Fig. 6C) is inconsistent with all other stimulation effects observed in the ICX and is, therefore, likely a false positive and meaningless statistical effect.

Figure 7. The effects of passive stimulation (A) and behaviorally-relevant stimulation (B) on BRR and CR in ICC neurons are independent of deafness duration (short-deaf vs. long-deaf cats). Mann-Whitney Rank Sum Tests; n.s., non-significant. Brackets: number of units.

Figure 8. A, C and E: Statistical comparisons of BRRs (A), CRs (C) and minimum latencies (E) in AI neurons of short-deaf cats. Mann-Whitney Rank Sum Tests, *P<0.05, **P<0.001. B, D and F: Statistical comparisons of BRRs (B), CRs (D) and minimum latencies (F) in AI neurons of long-deaf cats. Kruskal-Wallis one-way ANOVA on ranks, all P<0.001; Dunn pairwise multiple comparison, *P<0.05, **P<0.01, ***P<0.001. Brackets: number of units.

(Modified and reanalyzed from Beitel et al. 2011 and Vollmer and Beitel 2011.)
Threshold (0 dB = 1 mApp)

Minimum ICC threshold (1,2)
Minimum ICC threshold (3,4)
Border

Figure 1
Figure 4: Short-Deaf, ICC

A. Cumulative Probability vs. BRR (pps)

B. BRR (pps)

C. Cumulative Probability vs. CR (pps)

D. CR (pps)

E. Cumulative Probability vs. Minimum Latency (ms)

F. Minimum Latency (ms)
Figure 6

A. BRR (pps) comparison between SDpass (11) and SDtrain (14).

B. BRR (pps) comparison between LDU (11), LDpass (12), and LDtrain (28).

C. CR (pps) comparison between SDpass (11) and SDtrain (14).

D. CR (pps) comparison between LDU (11), LDpass (12), and LDtrain (28).

E. Minimum Latency (ms) comparison between SDpass (12) and SDtrain (14).

F. Minimum Latency (ms) comparison between LDU (11), LDpass (12), and LDtrain (28).

n.s. indicates no significant difference.
Figure 8

**Short-Deaf, CTX**

**A**
- BRR (pps)
- SDpass (75)
- SDtrain (123)

**C**
- CR (pps)
- SDpass (69)
- SDtrain (123)

**E**
- Minimum Latency (ms)
- SDpass (35)
- SDtrain (132)

**Long-Deaf, CTX**

**B**
- BRR (pps)
- LDU (69)
- LDpass (22)
- LDtrain (41)
- K55 (36)

**D**
- CR (pps)
- LDU (64)
- LDpass (16)
- LDtrain (28)
- K55 (29)

**F**
- Minimum Latency (ms)
- LDU (67)
- LDpass (22)
- LDtrain (42)
- K55 (36)
Table 1. Deafness, passive ICES and behavioral training histories.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cat</th>
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<th>Behavioral Training</th>
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<td>SGC (% normal)</td>
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SP, speech processor; SAM, 300 pulses/s modulated (100%) at 30 Hz.