Organization and Trade-off of Spectro-Temporal Tuning Properties of Duration-Tuned Neurons in the Mammalian Inferior Colliculus

Running Head: Spectro-Temporal Tuning of Duration-Tuned Neurons

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Abbreviations: BD best duration; CF characteristic frequency; CNS central nervous system; DTN duration-tuned neuron; eDBW excitatory duration bandwidth; eDRA excitatory duration response area; eFBW excitatory frequency bandwidth; eFRA excitatory frequency response area; FM frequency modulated; FSL first-spike latency; IC inferior colliculus; I/O inside-out; O/I outside-in; Q₁₀ dB quality factor at 10 dB above threshold; SPL sound pressure level
Abstract

Morrison J A, Farzan F, Fremouw T, Sayegh R, Covey E, and Faure P A. Organization and trade-off of spectro-temporal tuning properties of duration-tuned neurons in the mammalian inferior colliculus. *J. Neurophysiol.* XX: YYYY-ZZZZ, 2014. First published Month DD, 2014; doi:WW.WWWW/jn.XXXX.2014. Neurons throughout the mammalian central auditory pathway respond selectively to stimulus frequency and amplitude, and some are also selective for stimulus duration. First found in the auditory midbrain or inferior colliculus (IC), these duration-tuned neurons (DTNs) provide a potential neural mechanism for encoding temporal features of sound. In this study, we investigated how having an additional neural response filter—one selective to the duration of an auditory stimulus—influences frequency tuning and neural organization by recording single-unit responses and measuring the dorsal-ventral position and spectral-temporal tuning properties of auditory DTNs from the IC of the awake big brown bat (*Eptesicus fuscus*). Like other IC neurons, DTNs were tonotopically organized and had either V-shaped, U-shaped or O-shaped frequency tuning curves (excitatory frequency response areas). We hypothesized there would be an interaction between frequency and duration tuning in DTNs, as electrical engineering theory for resonant filters dictates a trade-off in spectral-temporal resolution: sharp tuning in the frequency domain results in poorer resolution in the time domain, and vice versa. While the IC is a more complex signal analyzer than an electrical filter, a similar operational trade-off could exist in the responses of DTNs. Our data revealed two patterns of spectro-temporal sensitivity and spatial organization within the IC: DTNs with sharp frequency tuning and broad duration tuning were located in the dorsal IC, whereas cells with wide spectral tuning and narrow temporal tuning were found in the ventral IC.
INTRODUCTION

The detection and analysis of temporal features of sound is a fundamental property of auditory systems (Griffin et al., 1960; Simmons et al., 1979; Moiseff & Konishi, 1981; Faure & Hoy, 2000). The duration of an acoustic signal contains important species-specific information and neural mechanisms of duration selectivity exist across a variety of vertebrates (Aubie et al., 2009, 2012). For example, human speech contains vocal elements called phonemes that vary in multiple acoustic parameters, including signal duration (Denes, 1955). Temporal differences between phonemes can occur on timescales shorter than what is consciously processed (Liberman et al., 1967; Shannon et al., 1995), suggesting there must be a neural mechanism for computing and/or comparing auditory signal durations. In amphibians, signal duration is an important feature of mating calls (Narins & Capranica, 1980; Akre et al., 2011; Jones et al., 2013) and frogs possess midbrain neurons with responses selective to the duration of an auditory stimulus (Potter, 1965; Narins & Capranica, 1980; Gooler & Feng, 1992; Leary et al., 2008). Echolocating bats use acoustic information for orientation and prey detection, and they also possess duration selective neurons (Galazyuk & Feng, 1997; Fuzessery & Hall, 1999; Faure et al., 2003; Mora & Kössl, 2004; Luo et al., 2008). Indeed, neural tuning for sound duration appears to be a general feature of vertebrate hearing (Sayegh et al., 2011; Aubie et al., 2012). In this paper we investigate the relationship between spectral and temporal tuning in a subclass of midbrain auditory neurons selective to stimulus duration.

Duration-tuned neurons (DTNs) first appear at the level of the auditory midbrain (Casseday et al., 1994). The mammalian inferior colliculus (IC) is a complex integrative center that receives parallel synaptic inputs from lower brainstem auditory nuclei (Adams, 1979; Covey et al., 1991; Casseday et al., 2002) and descending modulatory inputs from the auditory thalamus and cortex (Coleman & Clerici, 1987; Yan & Suga, 1996; Zhang & Suga, 2000; Ma & Suga, 2001). Below the level of the IC, most auditory neurons respond to any sound, regardless of its duration, as
long as the signal amplitude is suprathreshold and the frequency falls within the cell’s excitatory
tuning curve (Galambos & Davis, 1943). In the same way that auditory neurons are tuned to
stimulus frequency and have a characteristic frequency (CF), DTNs are tuned to stimulus duration
and respond maximally at a characteristic or best duration (BD) and show diminished responses to
sounds shorter and/or longer than BD. First discovered from the torus semicircularis of bullfrogs
(Potter, 1965) and subsequently from the IC of bats (Jen & Schlegel, 1982; Casseday et al., 1994),
DTNs have now been described from the auditory midbrain of several animals including chinchillas
(Chen, 1990), mice (Brand et al., 2000; Xia et al., 2000), guinea pigs (Wang et al., 2006), rats
(Perez-Gonzalez et al., 2006), and a variety of echolocating bats (Pinheiro et al., 1991; Ehrlich
et al., 1997; Fuzessery & Hall, 1999; Faure et al., 2003; Mora & Kössl, 2004; Luo et al., 2008).
Auditory DTNs have also been described from the cortex of bats and cats (Galazyuk & Feng, 1997;
He et al., 1997; Ma & Suga, 2001), and visual DTNs have been found in the cortex of cats (Duysens
et al., 1996). The existence of DTNs in different classes of vertebrates and across different sensory
modalities indicates that duration tuning is a general feature of sensory systems (Faure et al., 2003).

To date, research on auditory DTNs has focused primarily on the neural mechanisms that create
their temporally selective responses (Casseday et al., 1994; Ehrlich et al., 1997; Fuzessery & Hall,
1999; Casseday et al., 2000; Hooper et al., 2002; Faure et al., 2003; Jen & Wu, 2006; Aubie et al.,
2009, 2012; Sayegh et al., 2012; Sayegh et al., 2014), and on the stability (tolerance) of duration
tuning with changes in signal amplitude (Zhou & Jen, 2001; Mora & Kössl, 2004; Fremouw et al.,
2005). We know that duration tuning is disrupted or abolished by blocking neural inhibition in the
IC, which also suggests that duration tuning originates there (Casseday et al., 1994, 2000; Faure
et al., 2003; Leary et al., 2008). Although detailed reports exist on the frequency tuning properties
of IC neurons in general (Wu & Jen, 1991; Casseday & Covey, 1992; Haplea et al., 1994; Zhang
& Suga, 2000; Wu et al., 2004), much less is known about the frequency tuning characteristics
of DTNs in particular. This report aims to fill this gap in our knowledge by describing the
topographical organization and spectral-temporal tuning properties of DTNs from the IC. It also
tests the hypothesis that DTNs experience an interaction or trade-off in their spectral-temporal resolution.

Knowledge of the interaction between spectral and temporal tuning allows for a more general understanding of how the mammalian central nervous system (CNS) encodes and analyzes acoustic information, and will be essential for understanding the functional role(s) of DTNs in normal hearing. Recent work suggests that the temporal selectivity of a neural filter influences its spectral selectivity (Wu & Jen, 2008a). For example, some neurons from the nuclei of the lateral lemniscus that are important for analyzing temporal features of sound have broader frequency tuning curves than other types of brainstem auditory neurons (Covey & Casseday, 1991), suggesting that neural specializations for temporal processing arise at the expense of spectral processing. In the case of a resonant electrical filter, this trade-off happens because sampling sounds over a long duration adds more cycles of the signal to increase the accuracy of frequency estimation at the expense of temporal precision. Conversely, increasing the accuracy estimate of the start and/or end of a signal, and thus its duration, requires sacrificing frequency resolution at those time points (Blanchard, 1941). Because the auditory system is also a signal analyzer, we hypothesized that its neural components may be subject to a time-frequency trade-off not unlike that of a resonant electrical filter. If true, then this hypothesis predicts that DTNs with narrow duration tuning will have broad frequency tuning, and cells with wide temporal tuning will have sharp spectral tuning. The purpose of this study was to test this hypothesis by investigating the frequency and duration tuning characteristics of DTNs from the mammalian IC.
MATERIALS AND METHODS

Electrophysiological Recordings

Single unit recordings were conducted at the University of Washington (UW) and McMaster University (MU). Procedures conducted in Seattle were approved by the UW Laboratory Animal Care and Use Committee, while procedures conducted in Hamilton were approved by the MU Animal Research Ethics Board and were in accordance with the Canadian Council on Animal Care. Animals at both institutions were housed in outdoor husbandry facilities where lighting and temperature corresponded to ambient conditions, and food and water were available ad libitum.

Surgical Preparation

Neural recordings were obtained from the IC of 39 awake big brown bats (Eptesicus fuscus) of both sexes (23 from UW, 16 from MU) that were brought into the laboratory to acclimatize 1-3 days before surgery. Bats were anesthetized either by a combination of Metofane® (methoxyflurane) inhalation (1-5 min) and a subcutaneous injection of a neuroleptic (0.3 mL 1:1 mixture of 0.025 mg/mL fentanyl citrate + 1.25 mg/mL Inapsine® (droperidol) 19.1 mg/kg), or by Isoflurane®:oxygen inhalation (mixture 1-5%; flow 1-5 L/min). Anesthetized bats were then placed in a foam-lined restraint, molded to the shape of the body to hold the bat firmly yet comfortably while still allowing access to the head, within a stereotaxic alignment system fitted with a custom bite bar/mask for gaseous inhalation (David Kopf Instruments: Model 1900). The hair covering the skull was shaved and the underlying skin was swabbed with 70-100% ethanol followed by Betadine® disinfectant. Local anesthetic (0.2 mL bupivicaine; 5 mg/mL) was injected subcutaneously prior to making a midline incision in the scalp. The temporal muscles were reflected, the skull was scraped clean and swabbed with 70-100% ethanol, and a stainless steel post was affixed to the skull to ensure that the position of the bat’s head could be precisely replicated between recording sessions. The post was glued to the skull overlying the dorsal surface of the cortex with cyanoacrylate gel adhesive.
(Zap Gel, Pacer Technology®) or superglue (Henkel Lockite Corporation) and instantly cured with liquid acrylic hardener (Jet Liquid, Lang Dental Mfg. Co.). One end of a chlorided silver wire attached to the head post was placed under the temporal musculature and served as the reference electrode.

**Stimulus Generation**

Sound pulses were digitally synthesized with custom software controlling two signal processing boards from Tucker Davis Technologies (TDT: Apos II sampling rate = 357 kHz) that were optically interfaced to two digital-to-analog (D/A) converters (TDT: DA3-2). The output of each D/A was fed through a lowpass anti-aliasing filter (TDT FT6-2; $f_c$=120 kHz) and one (TDT PA5; MU) or two programmable attenuators (TDT PA4; UW) before being mixed in a summer with equal weighting (TDT SM5) and fed through a manual attenuator (Leader LAT-45) prior to final amplification (Krohn-Hite Model 7500). All stimuli were presented monaurally, contralateral to the IC being recorded, with a Brüel & Kjær (B&K) ¼ inch condenser microphone (Type 4939; protective grid on) modified for use as a loudspeaker with a transmitting adaptor (B&K Type UA-9020) to correct for nonlinearities in the transfer function (Frederiksen, 1977). The diaphragm of the loudspeaker was positioned ca. 1 mm in front of the external auditory meatus. The output of the speaker, recorded with a B&K Type 4138 ½ inch condenser microphone (90° incidence; grid off) connected to a measuring amplifier (B&K Type 2606) and bandpass filter (Krohn-Hite Model 3500), was calibrated (B&K Type 4231) and expressed in decibels sound pressure level (dB SPL re 20 μPa) equivalent to the peak amplitude of continuous tones of the same frequency (Stapells et al., 1982). The loudspeaker transfer function was flat ±6 dB from 28 to 118 kHz, and there was at least 30 dB attenuation at the ear opposite the source (Ehrlich et al., 1997). All stimuli had rise/fall times of 0.4 or 0.5 ms shaped with a square cosine function, and were presented at a rate of 3 Hz.
Neural Recordings

Electrophysiological recordings began 1-4 days after surgery and took place in a double-walled sound-attenuating booth (Industrial Acoustics Co., Inc.). Each bat was used in 1-8 sessions lasting 4-8 hours each on separate days. Recordings were terminated if the animal showed signs of discomfort. Between sessions, the electrode penetration site was covered with a piece of contact lens and/or Gelfoam covered in topical antibiotic (Neosporin or Polysporin).

Bats were given a subcutaneous injection of a neuroleptic (0.3 mL 1:1 mixture of fentanyl citrate 0.025 mg/mL + Inapsine® (droperidol) 1.25 mg/mL; 19.1 mg/kg) before being placed in the foam-lined body restraint suspended within a custom stereotaxic frame (ASI Instruments) mounted atop a floating vibration table (TMC Micro-g). To prevent the bat from moving during recording, the head post was clamped with a micromanipulator (David Kopf Instruments) mounted on the stereotaxic frame. In the first recording session, a scalpel was used to make a small opening in the skull and dura mater overlying the IC for insertion of thin-wall borosilicate glass micropipette electrodes pulled to a tip diameter of $\sim 1.0 \, \mu m$ and filled with either 0.9% or 3 M NaCl. Electrode impedances ranged from 7-53 MΩ. Microelectrodes were visually aimed at the dorsal surface of the IC with a manual micromanipulator (ASI Instruments) and advanced into the brain with a stepping hydraulic micropositioner (David Kopf Instruments: Model 650). Action potentials were recorded with a Neuroprobe amplifier (A-M Systems: Model 1600) whose x10 output was further amplified (500-1000x) and bandpass filtered (TDT: PC1; highpass $f_c = 700 \, Hz$; lowpass $f_c = 3 \, kHz$). Spike times were logged to a computer by passing the PC1 output to a spike discriminator (TDT: SD1) and event timer (TDT: ET1) synchronized to a timing generator (TDT: TG6). Spikes were visualized on-line as dot raster displays with custom software. All recordings were assumed to be from the central nucleus of the IC.
**Data Analysis**

We report acoustic thresholds from 149 DTNs and detailed spectral and temporal tuning measurements from 78 DTNs from a collective database. Single units were found by searching with short duration (1-10 ms) pure tones and/or downward frequency-modulated (FM) sweeps. Upon isolating a unit, the acoustic threshold (dB SPL), CF (kHz), frequency tuning characteristics (V-, U- or O-shaped frequency tuning), stimulus BD (ms), and duration-selective response class (shortpass or bandpass) were measured. Each cell was stimulated with a range of stimulus frequencies and durations spanning the CF and BD at +10, +20, and +30 dB above threshold, yielding 450 spike count functions (78 cells x 3 SPLs x 2 tuning parameters [frequency, duration] minus 18 cases [4 spectral, 14 temporal] where the cell was lost before data collection at +30 dB had completed). Spectral tuning was measured with BD tones randomly varied in frequency steps of 250-1000 Hz and spanning the excitatory spectral bandwidth of the cell. Temporal tuning was measured with CF tones randomly varied in duration steps of 1 ms and spanning the excitatory temporal bandwidth of the cell. Acoustic testing was conducted in blocks consisting of 10-20 stimulus repetitions per frequency or duration step. To minimize the effects of spontaneous activity, data were windowed so that spikes were counted only if they were evoked between stimulus onset and 100 ms after stimulus offset. Data analysis was automated with custom Matlab and Python scripts.

**Measuring CF, BD, FSL, 50% Tuning Bandwidth, and Q_{10 dB} Values**

The CF of each cell was defined as the stimulus frequency with the lowest acoustic threshold. The BD of each cell was defined as the stimulus duration evoking the highest spike count at +10 dB above threshold using a CF tone. Because stimulus durations were always presented as integer values varied in 1 ms steps, for the purposes of analysis and to remove the discretization of the data, we calculated BD to be the average of the shortest and longest stimulus durations with evoked spike counts \(\geq 90\%\) of the peak count at +10 dB re threshold (Fremouw et al., 2005). First spike latency
(FSL) was measured in response to CF and BD tones presented at +10 dB re threshold. Our study only included DTNs with an average response probability of $\geq 0.5$ spikes per stimulus, a criterion that ensured all neurons were healthy and responding consistently.

We measured the 50% excitatory frequency bandwidth (eFBW) and the 50% excitatory duration bandwidth (eDBW) from each cell’s frequency and duration tuning spike count functions, respectively, with a common technique (Fig. 1). First, we noted the stimulus value evoking the peak spike count, and then found the lowest and highest stimulus frequencies or durations where the function dropped to $\leq 50\%$ of the peak thus delimiting the lower and upper cutoffs of the 50% eFBW and 50% eDBW. Cutoffs were computed with two complementary methods (see Sayegh et al., 2012, for use of a similar technique). The inside-out method started at the peak of a tuning function and moved outward, toward the minimum and maximum stimulus values along the abscissa, noting the first data points where the function decreased to $\leq 50\%$ of the peak. The outside-in method started at the minimum and maximum stimulus values and moved inward, toward the median value along the abscissa, noting the first data points where the function increased to $\geq 50\%$ of the peak. Once the cut-offs of a tuning function were delineated, stimulus steps straddling the boundaries were interpolated to obtain a more precise estimate.

Comparing the Inside-Out and Outside-In Methods. The inside-out (I/O) and outside-in (O/I) methods yielded the same excitatory bandwidths in 388 of 450 spike count functions (230 eFBWs and 220 eDBWs). Overall, the O/I method yielded significantly larger spectral (paired $t=3.871, p<0.001$) and temporal bandwidths (paired $t=5.209, p<0.001$) than the I/O method. The O/I method also yielded more variable bandwidth measures (spectral: O/I SD=2904.2 kHz, I/O SD=2694.7 kHz; temporal: O/I SD=5.2 ms, I/O SD=2.8 ms). Because the I/O method yielded more conservative tuning bandwidth estimates, for the remainder of this manuscript we used it for measuring eFBWs and eDBWs and constructing excitatory response areas.

We also report spectral and temporal quality factors (Covey & Casseday, 1991; Haplea et al.,
The Q-value is a dimensionless measure of tuning sharpness, with higher values indicating that a filter is more sharply tuned (Capranica, 1992). The Q-value is calculated by dividing the center (resonant) frequency of a filter by its excitatory bandwidth at a given amplitude level. The $Q_{10 \text{ dB}}$ of a neuron’s frequency tuning curve was calculated as the CF divided by the eFBW at +10 dB re threshold, and the $Q_{10 \text{ dB}}$ of its duration tuning curve was calculated as the BD divided by the eDBW at +10 dB re threshold.

**Construction and Analysis of Excitatory Response Areas**

The excitatory response area (eRA) of a cell provides a visual depiction of how its excitatory bandwidth changes with increasing SPL, and can be used to categorize the shape of a tuning curve. We calculated an eRA for each DTN by measuring its excitatory bandwidth (see above) at +10, +20 and +30 dB re threshold (Kiang, 1965; Ehret & Schreiner, 2005). There is debate on how to define the boundaries of an eRA and a range of definitions have been employed, including the use of hand-scored boundaries (Jen & Kamada, 1982; Covey & Casseday, 1991; Casseday & Covey, 1992). Another approach defines eRA boundaries as the range of stimulus frequencies evoking spikes at the estimated spontaneous rate plus 20% of the peak rate (Sutter, 2000). Building on this quantitative approach, we measured spectral and temporal excitatory bandwidths in our population of DTNs in 5% response intervals, yielding four bandwidth measures (i.e. from 35-50% of the peak response) at three SPLs (i.e. at +10, +20 and +30 dB re threshold). To be included in the analysis, a cell was required to have an average response probability of $\geq 0.5$ spikes per stimulus at each suprathreshold level. This process yielded 74 excitatory frequency response areas (eFRAs) and 64 excitatory duration response areas (eDRAs) from 78 DTNs.

**Categorizing Frequency Tuning Classes.** There is debate on the existence of discrete frequency tuning classes and on how to quantify tuning curve shapes (e.g. Javel, 1994; Ramachandran et al., 1999; Casseday & Covey, 1992; Sutter, 2000; Palmer et al., 2013); however, there appears to
be consensus that frequency tuning curves of central auditory neurons can be shaped by neural inhibition (Javel, 1994; Palmer et al., 2013). Our study sought to investigate the relation between spectral and temporal tuning within the same cell, hence we sacrificed collecting high resolution frequency tuning curve data at high SPLs, where some of the more interesting and complicated frequency tuning curve shapes begin to emerge (e.g. slanted tuning curves; Sutter, 2000; Palmer et al., 2013), in order to collect medium resolution duration tuning curve data at the same SPLs within the typical recording time available for a cell. We used a scheme similar to Ramachandran et al. (1999) and Casseday & Covey (1992) to categorize frequency tuning curve shapes. Cells with spectral bandwidths that broadened with increasing SPL were categorized as having V-shaped frequency tuning curves, cells with spectral bandwidths that broadened slightly but then remained constant with increasing SPL were categorized as having U-shaped frequency tuning curves, and cells with spectral bandwidths that first broadened but then narrowed and closed with increasing SPL were categorized as having O-shaped (closed) frequency tuning curves. This classification scheme does not conflict with recent work by Palmer et al. (2013) who demonstrated that the eFRAs of IC neurons are sculpted by the integration of afferent inputs (i.e. neural excitation and inhibition), resulting in continuous rather than discrete frequency tuning curve classes. The V-, U- and O-shaped frequency tuning classes employed in our study seem equivalent to the V (including VN), N, and C classes used by Palmer et al. (2013).

The overall shape of a neuron’s eFRA was classified by how it changed with frequency and SPL. A cell was scored as having a V-shaped eFRA if its 50% tuning bandwidth increased by >1 kHz at each amplitude level, U-shaped if its bandwidth did not increase or decrease by ≥1 kHz at each amplitude level, and O-shaped if its bandwidth first increased but then decreased so that the cell had an upper threshold beyond which spikes (≥0.5 spikes per stimulus) were no longer evoked (i.e. the cell had a closed tuning curve). The final shape of a cell’s frequency tuning curve was based on the consistency of its eFRA scores from 35% to 50% of the peak response. This automated process yielded 62 robust eFRAs for 74 cells; the shapes of the frequency tuning curves
for the remaining 12 cells were independently hand-scored by two investigators.

**Categorizing Duration Tuning Classes.** The shape of a cell’s eDRA was classified by how it changed with duration and SPL (Fig. 2). Shortpass DTNs fire maximally at BD and have spiking responses that eventually drop to ≤50% of the peak response for sounds longer but not shorter than BD. Bandpass DTNs also fire maximally at BD, but have spiking responses that eventually drop to ≤50% of the peak response for sounds both shorter and longer than BD. This process yielded 51 robust eDRAs for 64 cells; the shapes of the duration tuning curves for the remaining 13 cells were independently hand-scored by two investigators. Shortpass and bandpass cells were the only DTNs included because they are unambiguous examples of duration tuning (see Sayegh et al., 2011, for a review).

**Statistical Analysis**

All data are reported as the mean ± standard deviation (SD) and were measured from responses recorded at +10 dB re threshold. Cells were grouped by response class (i.e. V-, U-, and O-shaped frequency tuning; shortpass and bandpass duration tuning), except when testing for differences within tuning response classes. All statistical tests were calculated with IBM SPSS Statistics version 20. Independent samples t-tests were used to compare duration tuning response classes, unless Levene’s test for equality of variances was found to be significant in which case Welch’s t-test was used. Paired t-tests were used to compare excitatory bandwidths computed with the Inside-Out and Outside-In methods. Analysis of variance (ANOVA) tests were used to compare between eRA classes.
RESULTS

Topographical Organization of DTN Response Properties

Our first goal was to examine the spatial organization of DTNs within the IC. Tonotopy is well established in mammalian hearing and from the central auditory system of bats (Haplea et al., 1994; Covey & Casseday, 1991; Covey et al., 1991; Grothe et al., 2001). As with other types of central auditory neurons, DTNs from the IC of *E. fuscus* showed a clear tonotopic organization evidenced by a strong positive correlation between recording electrode depth and CF (Fig. 3A; $R^2=0.801$, $p<<0.001$). Careful inspection of the data shows a compressive non-linearity in the correlation function with an expanded representation of CFs between 25 and 35 kHz. This overrepresentation may not be surprising given the dominance of this frequency band in the search and approached echolocation calls of *E. fuscus* citep casseday 1992.

Acoustic thresholds of DTNs were also topographically organized, although the relation was not as strong as for tonotopy. There was a positive correlation between acoustic threshold and CF; DTNs with the lowest thresholds were found in the dorsal IC and were tuned to lower CFs than DTNs with higher thresholds located in the ventral IC and tuned to higher CFs (Fig. 3B; $R^2=0.3773$, $p<<0.001$).

A spatial organization of DTN response properties was less evident in the temporal domain. There was no correlation between recording electrode depth and neuronal FSL (Fig. 3C; $R^2<<0.001$, $p=0.982$), demonstrating that DTNs with similar response latencies were found across a wide range of spatial locations and CFs within the IC. There was a weak negative correlation between electrode depth and stimulus BD (Fig. 3D; $R^2=0.0906$, $p=0.008$), and a similarly weak negative correlation between CF and BD ($R^2=0.123$, $p=0.002$; Fig. 3E), indicating that cells tuned to longer durations were more likely to be found at shallower depths and were tuned to lower CFs.

A behavioural audiogram for *E. fuscus* measured by Koay et al. (1997) shows that hearing thresholds in the big brown bat were lowest in a narrow band of frequencies from 15 to 35 kHz,
with a second sensitivity peak between 55 and 70 kHz (Fig. 4). Plotting the acoustic thresholds of our sample of DTNs on the audiogram reveals that cells with the lowest thresholds mirrored the bat’s best behavioral sensitivity in the frequency range from 15 to 55 kHz. Interestingly, the majority of DTNs had CFs between 25 and 60 kHz—a spectral bandwidth important for target ranging and corresponding to the dominant frequencies in the fundamental FM element of the bat’s search and approach phase calls (Surlykke, 1992; Surlykke & Moss, 2000). Few DTNs in our database had CFs between 50 and 120 kHz, a frequency range corresponding to the second harmonic of the bat’s FM echolocation calls. Although we cannot exclude the possibility that we failed to record from DTNs tuned to higher CFs, our sample size suggests there are fewer DTNs tuned to the bat’s second harmonic.

**Frequency Tuning Classes of DTNs**

The eFRA shapes for DTNs from the IC of *E. fuscus* correspond to a subset of frequency tuning classes reported for other types of central auditory neurons (e.g. Wu & Jen, 1991; Casseday & Covey, 1992; Sutter, 2000; Palmer et al., 2013). The frequency tuning curves of DTNs were classified into one of three classes (Fig. ). The most common eFRA (n=37 cells; 50.0%) was from DTNs with V-shaped frequency tuning curves similar to primary afferents of the periphery auditory system (Kiang, 1965) and from some neurons located in lower brainstem auditory nuclei (Rhode & Smith, 1985; Covey & Casseday, 1991; Covey et al., 1991; Haplea et al., 1994; Ramachandran et al., 1999). Figure A shows an example of a bandpass DTN with a V-shaped frequency tuning curve (left column). Its spectral Q10 dB value was 8.483, hence the cell was not sharply tuned in frequency. Also shown are the cell’s spike count functions in response to 4 ms BD tones randomly varied in frequency (middle column) and to 41 kHz CF tones randomly varied in duration (right column) and presented at +10, +20, and +30 dB above threshold. Unlike the eFRAs, note the consistency of the eDRA functions over a 20 dB range of SPLs. The cell’s temporal Q10 dB value was 0.574, hence it was also not sharply duration tuned.
The second most common eFRA (n=30 cells; 40.5%) was from DTNs with U-shaped frequency tuning curves and nearly constant spectral bandwidths. Figure B (left column) shows an example of a shortpass DTN with level-tolerant frequency tuning. This cell responded with multiple spikes per stimulus but only over a narrow range of frequencies between 27 and 29 kHz (middle column). The cell’s sharp frequency tuning was confirmed by its large spectral Q_{10\,DB} value of 55.56. In contrast, the cell was less selective in the time domain (right column) as evidenced by a temporal Q_{10\,DB} value of 0.518. The eFRAs and eDRAs of this cell were tolerant over a 20 dB change in SPL.

The third and least common type of eFRA (n=7 cells; 9.46%) was from DTNs with O-shaped frequency tuning curves. These non-monotonic cells were interesting because they had both lower and upper acoustic thresholds, hence the neuron eventually ceased responding as the tone amplitude was increased (Casseday & Covey, 1992; Ehret & Schreiner, 2005). Figure C shows an example of a shortpass DTN with a closed frequency tuning curve (left column), along with its spectral (middle column) and temporal (right column) spike count functions at +10, +20, and +30 dB re threshold. The spectral and temporal Q_{10\,DB} values were 14.74 and 0.719, respectively, hence this DTN displayed intermediate tuning sharpness in the frequency domain but was more narrowly tuned in the time domain.

**Topographical Organization of Spectral and Temporal Tuning Classes**

The spatial organization of spectral and temporal response properties in the IC may provide clues into the role(s) that DTNs serve in normal hearing, including the processing of species-specific vocalizations (Wu & Jen, 2008b; Sayegh et al., 2011). Expanding on this notion, we expected that topographical trends in the IC may facilitate the processing and perception of echolocation calls by the bat. Search phase calls of *E. fuscus* consist of shallow FM chirps sweeping down in frequency from ca. 30-20 kHz over a duration of ca. 10-20 ms (Griffin et al., 1960; Simmons, 1987). Because neurons with low CFs are located in the dorsal IC, we expected to find DTNs with U-shaped eFRAs
(i.e. sharp frequency tuning) tuned to long BDs (i.e. broad duration tuning) at shallow electrode depths. As a hunting bat approaches an insect, it increases its maximum frequency and signal bandwidth while decreasing call duration. Therefore, in the ventral IC where cells are tuned to high CFs, we expected to find DTNs with V-shaped eFRAs (i.e. broad spectral tuning) tuned to short BDs (i.e. narrow temporal tuning).

To test these predictions, we grouped cells by their spectral and temporal response class and used factorial ANOVA to test for an effect of recording electrode depth, CF, acoustic threshold, and/or FSL. In the spectral domain, DTNs with U-shaped eFRAs were found in more dorsal areas of the IC compared to DTNs with V-shaped and O-shaped eFRAs (Fig. 6; \( F = 4.075, \ p = 0.021 \)), but there was no effect of any other neural parameter (CF, \( F = 2.284, \ p = 0.109 \); BD, \( F = 0.294, \ p = 0.746 \); acoustic threshold, \( F = 1.352, \ p = 0.265 \); FSL, \( F = 0.654, \ p = 0.523 \)). In the temporal domain, there was no difference in recording electrode depth between shortpass (n=44; Fig. 6A–C) and bandpass DTNs (n=30; Fig. 6D–F); however, there was a strong tendency for shortpass DTNs to be located deeper in the IC (t=1.940, \( p = 0.056 \)). Consistent with this trend, shortpass DTNs were tuned to higher CFs than bandpass DTNs (Welch’s t=2.408, \( p = 0.019 \)). Not surprisingly, shortpass DTNs also had shorter BDs than bandpass DTNs (Welch’s t=4.486, \( p \ll 0.001 \)), but there was no significant difference in acoustic threshold (t=0.200, \( p = 0.842 \)) or FSL (Welch’s t=1.195, \( p = 0.238 \)).

A plot of frequency and duration 50% bandwidths and tuning sharpness as a function of recording electrode depth suggests that DTNs experience a spectral-temporal trade-off in response sensitivity. In the frequency domain, there was a moderate-to-strong positive correlation between recording electrode depth and the 50% frequency tuning bandwidth (Fig. 7A; \( R^2 = 0.373, \ p \ll 0.001 \)); DTNs with narrow spectral tuning were found in more dorsal areas of the IC compared to cells with broadband frequency tuning. The significance of this effect held, although the relationship was weaker, when tuning sharpness was examined with spectral \( Q_{10\,\text{dB}} \) values that characterize the spectral bandwidth of a filter relative to its CF (Fig. 7B; \( R^2 = 0.055, \ p = 0.040 \)). Not surprisingly, DTNs with U-shaped frequency tuning curves had narrower 50% spectral bandwidths (Fig. 7A;
F=6.238, \( p=0.003 \) and larger spectral Q_{10\,\text{dB}} values (Fig. 7C; F=6.693, \( p=0.002 \)) than DTNs with V-shaped and O-shaped frequency tuning curves.

In the time domain, there was a moderate-to-weak negative correlation between recording electrode depth and the 50% duration tuning bandwidth (Fig. 7C; \( R^2=0.101, \ p=0.005 \)); shortpass DTNs were more narrowly duration tuned than bandpass DTNs (Fig. 7C; Welch’s \( t=4.432, \ p<0.001 \)). The relationship was no longer significant when the sharpness of duration tuning was examined with temporal Q_{10\,\text{dB}} values that characterize the temporal bandwidth of a filter relative to its BD (Fig. 7D; \( R^2=0.003, \ p=0.610 \)). Moreover, the temporal Q_{10\,\text{dB}} value of shortpass and bandpass DTNs were not significantly different (t=1.285, \( p=0.203 \)).

### Relation of CF and BD to Spectral and Temporal Tuning Bandwidths

Our second objective was to examine how the CF and BD of DTNs relates to their spectral and temporal bandwidths and tuning sharpness. In the frequency domain, there was a strong positive correlation between neuronal CF and the 50% frequency tuning bandwidth (Fig. 8A; \( R^2=0.400, \ p<0.001 \)); DTNs tuned to lower CFs were also more narrowly tuned in frequency. When tuning sharpness was examined with spectral Q_{10\,\text{dB}} values, there was a weak but non-significant trend (Fig. 8B; \( R^2=0.041, \ p=0.074 \)). Interestingly, DTNs with the sharpest frequency selectivity were located at shallower electrode depths within the IC and had CFs matching the dominant frequency range of the fundamental FM acoustic element of the echolocation calls of E. fuscus.

In the time domain, there was a strong positive correlation between the calculated BD and the 50% duration tuning bandwidth (Fig. 8C; \( R^2=0.518, \ p<0.001 \)); DTNs with long BDs had absolutely wider temporal bandwidths. When this relation was re-examined with temporal Q_{10\,\text{dB}} values, no correlation was observed (Fig. 8D; \( R^2=0.002, \ p=0.715 \)).
Relation between Spectral and Temporal Tuning

Our final objective was to examine the interaction between frequency and duration tuning to see if DTNs experience a spectro-temporal trade-off in resolution analogous to that of resonant electrical filters. A plot of the 50% frequency tuning bandwidth as a function of the 50% duration tuning bandwidth revealed a weak but significant negative correlation (Fig. 9A; $R^2=0.073$, $p=0.017$). Across the population of cells tested, DTNs with the narrowest temporal bandwidths were among those neurons with the widest spectral bandwidths, and vice versa. When this relationship was re-examined with $Q_{10\,\text{dB}}$ values, no correlation was observed (Fig. 9B; $R^2=0.001$, $p=0.424$).

DISCUSSION

This is the first study to directly compare the spectral and temporal tuning properties of DTNs from the mammalian IC. Although frequency tuning and tonotopy are ubiquitous at all levels of the vertebrate central auditory pathway, DTNs are found only at and above the auditory midbrain (Casseday et al., 1994; Sayegh et al., 2011). While the responses of all auditory neurons, including DTNs, are selective for stimulus frequency and amplitude, DTNs have an additional neural filter for auditory processing—one that works in the time domain. Hence, the responses of DTNs can be viewed as level-dependent spectro-temporal filters. In echolocating bats, such filters could help to maximize the extraction of acoustic information from reflected echoes and/or trigger fixed action patterns associated with foraging (Casseday & Covey, 1996). Indeed, the range of emitted signal durations was a good predictor of the range of neuronal BDs for DTNs in the IC of a variety of echolocating bats such as *E. fuscus* (Ehrlich et al., 1997), *Myotis lucifugus* (Galazyuk & Feng, 1997), *Antrozous pallidus* (Fuzessery & Hall, 1999), and *Molossus molossus* (Mora & Kössl, 2004).
Topographical Organization of Spectral and Temporal Tuning

As expected from previous reports (e.g. Pinheiro et al., 1991; Haplea et al., 1994; Faure et al., 2003; Jen & Wu, 2006; Sayegh et al., 2012), DTNs exhibited a robust tonotopic organization and had neural thresholds that increased with recording electrode depth in the IC and that covered the dynamic range of hearing (Figs. 3–4). The cells were also tuned to the frequencies and durations of big brown bat echolocation calls (Simmons, 1987; Surlykke & Moss, 2000; Fenton et al., 2011). The frequency tuning bandwidths and spectral Q_{10\,\text{dB}} values of DTNs were similar to other central auditory neurons not selective for stimulus duration in \textit{E. fuscus} and other species of FM bats (Fig. 7A,B; Grinnell, 1963; Suga, 1964; Jen & Kamada, 1982; Casseday & Covey, 1992; Haplea et al., 1994). The range of spectral Q_{10\,\text{dB}} values for DTNs from the IC of \textit{E. fuscus} was higher than the range for DTNs reported from the IC of the mouse (Brand et al., 2000), suggesting the ability to echolocate has naturally selected for sharper frequency tuning in bats. This idea is further supported by the evolution of an auditory fovea in bats that employ Doppler-shift compensation (Pollak & Casseday, 1989), where peripheral and central auditory neurons within the fovea can have Q_{10\,\text{dB}} values \( \leq 400 \), compared to cells outside the fovea with Q_{10\,\text{dB}} values \( \leq 20 \) (Suga et al., 1976; Pollak et al., 1986). While many DTNs in the IC of \textit{E. fuscus} had small Q-values, we noted that cells tuned to the frequencies of the fundamental FM acoustic element were those with the highest Q_{10\,\text{dB}} values (Fig. 8B).

Our study found mixed evidence for the hypothesis that the temporal properties of DTNs are spatially organized within the IC. The negative correlations between electrode depth and BD (Fig. 3D) and CF and BD (Fig. 3E) are at odds with some previous reports, including by the present authors, that did not find evidence for a topographical organization of DTN temporal response properties within the IC of the bat (Pinheiro et al., 1991; Ehrlich et al., 1997; Faure et al., 2003; Luo et al., 2008). In contrast, two studies found a positive relation between CF and BD (Jen & Wu, 2006; Wu & Jen, 2006, 2008b), suggestive of a spatial map of duration tuning but organized in a direction opposite to the relation reported here (see Fig. 3D) and by Sayegh et al. (2012).
The inconsistency of this finding, both within and across laboratories, is puzzling. Perhaps DTNs are topographically organized along the medial-lateral and/or rostral-caudal axes of the IC? In the auditory midbrain of rats, Perez-Gonzalez et al. (2006) found that DTNs were more abundant in the external cortex and lateral areas of the IC. Unfortunately, their study did not report electrode depths so it was not possible to make direct comparisons with our results. Future studies employing three dimensional stereotaxic measures and/or intracellular recording with staining are needed to resolve the question of whether the mammalian IC contains an organized map of duration selectivity and/or if particular subdivisions of the IC are specialized for processing signal duration.

**Frequency Tuning and Implications for Auditory Processing**

Notwithstanding the eloquent paper by Palmer et al. (2013), we found three classes of eFRAs for DTNs from the IC of *E. fuscus*. V-shaped frequency tuning curves were most common among DTNs. Neurons with V-shaped spectral tuning are typical of primary auditory afferents (Kiang, 1965) and neurons in the cochlear nucleus (Rhode & Smith, 1985), superior olivary complex (Covey et al., 1991), lateral lemniscus (Covey & Casseday, 1991), and to a lesser extent the IC (Haplea et al., 1994). Primary afferents inherit their V-shaped tuning from the vibratory mechanics of the cochlea: as sound amplitude increases, this causes larger displacements of the basilar membrane and stronger shearing forces on the haircell stereocilia within the Organ of Corti, resulting in receptor depolarization, the generation of action potentials in a wide array of afferents, and a decrease in tuning sharpness (Robles & Ruggero, 2001). Therefore, it is not surprising that many auditory neurons have V-shaped frequency tuning curves.

Duration-tuned neurons with U-shaped eFRAs were the second most common spectral tuning class. In FM bats and other mammals, U-shaped tuning originates within the central auditory system through sideband inhibition that causes a narrowing of frequency selectivity (Galazyuk & Feng, 1997; Ramachandran et al., 1999; Palmer et al., 2013). Level tolerant cells have also been described as "filter" neurons because they have narrow (selective) frequency tuning with a constant
bandwidth over a large range of SPLs (in some cases up to +90 dB re: threshold; Casseday & Covey, 1992). Although not tested for duration selectivity, the filter neurons reported by Casseday & Covey (1992) from the IC of E. fuscus were clustered in isofrequency lamina tuned to the fundamental FM acoustic element of the bat’s calls, suggesting their importance in processing echolocation information. Similar clustering patterns have been reported for filter neurons from the auditory cortex of M. molossus and Macrotus waterhousii (Macías et al., 2013). Cells with U-shaped frequency tuning curves may play a role in the constancy of auditory perception during changes in stimulus amplitude (Comalli & Altshuler, 1976; Suga & Tsuzuki, 1985; Casseday & Covey, 1996). For example, in human speech sound amplitude can convey affective information (e.g. yelling indicates anger/excitement), whereas semantic information is generally amplitude independent (Breazeal & Aryananda, 2002). Echolocating animals in particular may benefit from level tolerant perception because echo amplitude decreases rapidly with target distance (Kick & Simmons, 1984; Macías et al., 2013).

The least common frequency tuning class was DTNs with O-shaped eFRAs. One explanation for their low number is that they are simply more difficult to find owing to their restricted range of acoustic excitability. Alternatively, if DTNs with closed frequency tuning curves were more common in IC regions other than the central nucleus, then it is possible that our electrode sampling regime underestimated their true proportion. A recent study by Palmer et al. (2013) summarizing >20 years of recording from the IC of the guinea pig found that neurons with closed tuning curves comprised <6% of the sampled population, a proportion that closely agrees with our results for DTNs. Echolocating animals may benefit from DTNs with closed frequency tuning curves because these cells could facilitate the analysis of auditory scenes (Moss & Surlykke, 2010). For example, echolocating bats face the challenge of detecting faint echoes that are similar in frequency and duration to their emitted vocalizations (Simmons & Stein, 1980). A typical auditory neuron with appropriate frequency tuning would respond to both the pulse and echo if its acoustic threshold was lower than the amplitude of the received echo; however, the responses of a DTN with appropriate
spectro-temporal tuning and an O-shaped eFRA would be highly selective for the echo if the cell’s lower and upper acoustic thresholds were below than the amplitude of the emitted call but bracketed the amplitude of the received echo. In other words, populations of DTNs with closed frequency tuning curves could be highly selective and respond only to the loud outgoing call or the fainter returning echo, but not both. These cells could also be helpful in discriminating echoes of the bat’s own call from those of other bats, and in highly cluttered acoustic environments (Moss & Surlykke, 2010).

**Spectral and Temporal Tuning Bandwidths and Sharpness**

Studies investigating the selectivity of auditory neurons typically report tuning bandwidths or quality factors. To characterize the spectral-temporal selectivity of DTNs, we reported both. The 50% response bandwidth is a reliable and unbiased estimate of the lower and upper cut-off values of a spectral or temporal tuning curve. Moreover, its value is independent of differences in the maximum firing rate between cells.

The quality factor (Q-value) is commonly used to report spectral tuning sharpness (Covey & Casseday, 1991; Capranica, 1992; Haplea et al., 1994; Brand et al., 2000). This measure works well because tuning in the frequency domain is based on the accepted notion that, owing to the vibratory mechanics of the cochlea and the asymmetry in the envelope of the basilar membrane traveling wave, spectral bandwidths of primary auditory afferents increase with SPL and CF (Oxenham & Shera, 2003; Ruggero & Temchin, 2005; Temchin et al., 2008). To the best of our knowledge, our study is the first to use a Q-value in the temporal domain. We originally thought Q-values would be useful in quantifying temporal tuning sharpness because the 50% temporal bandwidth increased with neuronal BD (but not SPL) in a manner analogous to the increase in 50% spectral bandwidth with CF (Fig. 8; Fremouw et al., 2005; Jen & Wu, 2006). Although we found a positive correlation between neuronal BD and the 50% duration tuning bandwidth, there was no relation between BD and temporal $Q_{10\,\text{dB}}$. Moreover, the range of temporal $Q_{10\,\text{dB}}$ values in our
population of DTNs was quite narrow. In hindsight, these findings were not surprising. Primary afferents inherit their frequency selectivity from the mechanics of the Organ of Corti, but there is no mechanism linking the sharpness of duration tuning to the mechanics of the cochlea, nor is there a compelling reason to characterize the BD of a neural temporal filter relative to its bandwidth. We conclude that frequency tuning bandwidths and spectral Q-values are useful and appropriate for characterizing neural filter properties in the frequency domain, and that duration tuning bandwidths but not temporal Q-values are useful for characterizing neural filter properties in the time domain.

Relation between Spectral and Temporal Tuning

Our study found that the spectral and temporal tuning bandwidths of DTNs from the IC of the bat showed opposite patterns of spatial organization: cells with the widest spectral bandwidths tended to be found in the ventral IC, whereas cells with widest temporal bandwidths tended to be found in the dorsal IC (Fig. 7A, C). The organization of duration tuning classes mirrored these trends: bandpass DTNs with wide temporal tuning curves were located in the dorsal IC, whilst shortpass DTNs with narrow temporal tuning curves were distributed more in the ventral IC (Fig. 6; see also Fuzessery & Hall, 1999). A direct comparison of spectral and temporal tuning properties revealed that DTNs with the smallest temporal bandwidths were among those cells with the widest spectral bandwidths, and vice versa (Fig. 9). Altogether, these data suggest that DTNs in the IC of the bat experience a spectral-temporal trade-off in tuning bandwidth resolution.

Any trade-off that exists is suggestive of a physical constraint on the capabilities of the auditory system as a signal analyzer. A resonant electrical circuit is a closed system and the duration of ringing in the filter’s response depends on its center (resonant) frequency and spectral bandwidth (Blanchard, 1941). Frequency tuning in the peripheral auditory system results directly from the mechano-electrochemical transduction processes but can also be inherited and modified by neurons located in higher auditory centers; however, duration selectivity is an emergent neural property that first appears at the level of the IC. There is evidence that auditory brainstem neurons have
response properties that effectively facilitate independent processing of spectral and temporal information. For example, neurons in the columnar region of the ventral nucleus of the lateral lemniscus (VNLLc) are broadly tuned in frequency and always respond with a single spike tightly time-locked to stimulus onset (Covey & Casseday, 1991). It would seem that frequency analysis by VNLLc neurons has been abandoned to create a robust representation of signal timing—an important aspect of temporal processing and a vital part of the computational circuits proposed for creating duration selective responses (Fuzessery & Hall, 1999; Casseday et al., 2000; Faure et al., 2003; Aubie et al., 2009, 2012). While our study found evidence of a trade-off in the resolution of DTN spectral and temporal tuning bandwidths (Fig. 9A), future studies are needed to determine its underlying cause.
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DISCLOSURES

Conflict of Interest Statement: The authors declare that this work was conducted absent of any financial or commercial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

E.C. and P.A.F conceived and designed research; J.A.M., T.F., R.S. and P.A.F. collected data; J.A.M., F.F., R.S. and P.A.F analyzed data; all authors interpreted results; J.A.M. and P.A.F. prepared figures and drafted paper; all authors edited, revised, and approved submitted manuscript.
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Figure 1

Measuring spectral and temporal excitatory bandwidths. Each panel shows the mean ± SD spikes per stimulus at +10 dB above threshold in response to pure tones that were randomly varied in frequency (left column, stimulus duration = 1 ms) or randomly varied in duration (right column, stimulus frequency = 56 kHz). The spike count data within each column are identical. In each panel, the 50% excitatory bandwidth (BW) is illustrated with a gray box and the cut-off frequencies/durations are the edges of the box. (A, B) Spike count functions illustrating how the 50% spectral BW was measured with the (A) inside-out and (B) outside-in methods. (A) Using the inside-out (I/O) method, the lower cut-off frequency where the spike count function first crossed the 50% criterion (dotted line) was 42.2 kHz, and the upper cut-off frequency was 50.5 kHz, yielding a spectral BW = 8.3 kHz. (B) Using the O/I method on the same data, the lower cut-off frequency was 36.8 kHz and the upper cut-off frequency was 50.5 kHz, yielding a spectral BW = 13.7 kHz. (C, D) Spike count functions illustrating how the 50% temporal BW was measured with the (A) I/O and (B) O/I methods. (C) Using the I/O method, the lower cut-off duration of the shortpass DTN was arbitrarily set to 1 ms (i.e. the shortest duration tested) and the upper cut-off duration where the spike count function first crossed the 50% criterion (dotted line) was 3.9 ms, yielding a temporal BW = 2.9 ms. (D) Using the O/I method on the same data, the lower cut-off duration was again arbitrarily set to 1 ms (i.e. the shortest duration tested) and the upper cut-off duration was 5.9 ms, yielding a temporal BW = 4.9 ms. For calculation details, see Materials and Methods.

Figure 2

Shortpass and bandpass duration-tuned neurons (DTNs) from the inferior colliculus (IC) of the bat. (A, B) Shortpass DTN. (C, D) Bandpass DTN. (Top row) Post-stimulus rastergrams illustrating the timing of action potentials in response to CF tone pulses randomly varied in duration and presented at +10 dB above threshold. Stimulus duration illustrated with black bars. (Bottom row) Duration tuning curves showing the mean ± SD spikes per stimulus in response to CF tone
pulses that were randomly varied in duration and presented at three suprathreshold levels. Note how the duration tuning curves remain tolerant (stable) despite large changes in SPL. The CF and acoustic threshold for each cell are given in the legends. The calculated BDs for UW223.02.07 and UW235.01.07 were 2.01 ms and 3.61 ms, respectively, and were derived from the mid-point duration value bracketing 90% of the peak spike count. The interpolated 50% temporal bandwidths measured at +10 dB (re threshold) were 1.97 ms and 3.39 ms, respectively. For calculation details, see Materials and Methods.

**Figure 3**

Topographical organization of DTN electrophysiological response properties. (A) There was a strong positive correlation between recording electrode depth and neural CF demonstrating that DTNs were tonotopically organized within the IC. (B) There was also a positive correlation between electrode depth and acoustic threshold. (C) There was no correlation between electrode depth and first spike latency. (D) There was a weak negative correlation between electrode depth and the calculated BD, and (E) a similar weak negative correlation between CF and BD across the population of cells tested. n=78 in all panels.

**Figure 4** Comparison of neural and behavioural threshold tuning. Acoustic spiking thresholds in a large population of DTNs plotted as a function of CF and compared to the mean behavioural audiogram of *E. fuscus* (*solid line*) measured by Koay et al. (1997). Note the marked drop in hearing sensitivity around 45 kHz in the audiogram. In the frequency range from 13 to 55 kHz, the most sensitive DTNs mirrored the bat’s best behavioural sensitivity. The range of neural thresholds across the population of DTNs covers most of the dynamic range of hearing.

**Figure 5** Frequency and duration tuning in the IC of the bat. Shown are three DTNs with different frequency tuning curves, CFs and duration tuning curves. (*Left column*) Threshold frequency tuning curves, (*middle column*) eFRA functions showing the mean ± SD spikes per stimulus in response to BD tones randomly varied in frequency, and (*right column*) duration tuning curves showing the mean ± SD spikes per stimulus in response to CF tones randomly varied in duration.
and presented at three suprathreshold levels.  

(A) Bandpass DTN with a broadband V-shaped frequency tuning curve.  
(B) Shortpass DTN with a narrowband U-shaped frequency tuning curve.  
(C) Shortpass DTN with an intermediate bandwidth O-shaped (closed) frequency tuning curve.

To highlight differences in spectral and temporal tuning, the frequency (left column) and duration tuning curves (right column) of the three neurons were plotted on a common abscissa. Unlike frequency tuning, duration tuning was highly tolerant over a 20 dB change in SPL. Panel legends show the CF, acoustic threshold (Thresh), stimulus duration (Dur), calculated BD, and spectral and temporal Q_{10 dB} values of each cell.

**Figure 6** Topographical organization of DTN spectral and temporal tuning classes.  

(Top row) Histograms illustrating the distribution of recording electrode depths for the population of shortpass and (bottom row) bandpass DTNs.  
(Left column) Histograms illustrating the distribution of recording electrode depths for DTNs with V-shaped, (middle column) U-shaped, and (right column) O-shaped (closed) frequency tuning curves.  
Cells with U-shaped tuning curves were located in more dorsal areas of the IC compared to DTNs with V-shaped and O-shaped tuning.

There was no significant difference in electrode depth between shortpass and bandpass DTNs, but there was a trend for shortpass DTNs to be found more ventrally in the IC.  
Cells with the narrowest spectral tuning (i.e. U-shaped frequency tuning curves) and broadest temporal tuning (i.e. bandpass DTNs) were located in more dorsal regions of the IC.  
The distribution of spectral and temporal response classes echoes the organizational trends of spectral and temporal bandwidths and tuning sharpness (see Figs. 7–9).

**Figure 7** Topographical organization of DTN spectral and temporal tuning bandwidths and quality factors.  

(A) Relation between recording electrode depth and the 50% bandwidth of frequency tuning; DTNs located in the ventral IC were more broadly tuned in frequency than cells in the dorsal IC.  
(B) There was a significant negative correlation between electrode depth and spectral Q_{10 dB} illustrating that DTNs in the dorsal IC had sharper frequency tuning curves.  
(C) In contrast, there was a significant negative correlation between electrode depth and the 50% bandwidth of
duration tuning; DTNs located in the dorsal IC had broader temporal tuning than cells in the ventral IC. (D) When this relationship was re-examined by calculating the temporal Q_{10 \, \text{dB}}, no correlation was observed. Legends in A and C apply to B and D, respectively. n=74 in all panels.

**Figure 8** Relation of CF and BD to spectral and temporal tuning bandwidths and quality factors. (A) There was a strong positive correlation between CF and the 50% bandwidth of frequency tuning, and (B) a weak negative trend between CF and spectral Q_{10 \, \text{dB}}. (C) There was a strong positive correlation between the calculated BD and the 50% bandwidth of duration tuning. (D) When this relationship was re-examined with temporal Q_{10 \, \text{dB}} values, no correlation was observed. n=78 in all panels.

**Figure 9** Trade-off between spectral and temporal tuning in DTNs. The data within each row are identical but the columns use different markers to highlight different spectral (left column) or temporal (right column) tuning classes. (A) There was a negative correlation between the 50% bandwidth of duration tuning and the 50% bandwidth of frequency tuning, suggesting that DTNs experience a trade-off in spectral-temporal resolution. (A, left panel) Cells with U-shaped frequency tuning curves had smaller spectral bandwidths than DTNs with V-shaped and O-shaped tuning curves, but there was no difference in temporal bandwidth between the three frequency tuning classes. (A, right panel) Shortpass DTNs had narrower 50% temporal bandwidths than bandpass DTNs, but there was no difference in spectral bandwidth between the two duration tuning classes. (B) There was no correlation between temporal Q_{10 \, \text{dB}} and spectral Q_{10 \, \text{dB}} values. (B, left panel) Cells with U-shaped frequency tuning curves had larger spectral Q_{10 \, \text{dB}} values than DTNs with V-shaped and O-shaped tuning curves, but there was no difference in temporal Q_{10 \, \text{dB}} values between the three frequency tuning classes. (B, right panel) There were no difference in temporal or spectral Q_{10 \, \text{dB}} values between shortpass and bandpass DTNs. For statistical details, see Results section *Topographical Organization of Spectral and Temporal Tuning Classes* and Figure 7. n=74 in all panels.
Figure 1:
Figure 2:
Figure 3:
Figure 4:
Figure 5:
Figure 6:
Figure 7:
Figure 8:
Figure 9: