Temporal precision of the LSO neurons and their inputs

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1 Why do we need temporal precision in ILD coding?

Unlike other sensory systems, the auditory system lacks a direct spatial map and must compute sound location through interaural cues—specifically, interaural time differences (ITDs) and interaural level differences (ILDs). According to the duplex theory of sound localization, ITDs are primarily used to localize low-frequency sounds, whereas ILDs are employed for high-frequency sound localization. However, recent findings indicate that neurons in the lateral superior olive (LSO), a key nucleus in ILD processing, can also encode ITDs derived from the envelopes of amplitude-modulated high-frequency sounds (ITD_{ENV}).

LSO neurons exhibit a rate-based response, increasing firing with ipsilateral excitation and decreasing with contralateral inhibition, a mechanism that depends critically on the precise temporal alignment of bilateral inputs. In vitro current-clamp recordings have revealed that LSO neurons respond transiently to sustained depolarization and that their responsiveness is modulated by both the rate of depolarization and synaptic noise introduced by temporal jitter in converging inputs. In this study, we investigate these dynamics in vivo by presenting mice with transposed tone pulses (stimuli that allow manipulation of the envelope independently from the pulse rate) to sharpen the temporal features of the stimulus. Such stimuli have proven effective in human psychophysical and computational studies for probing ITD_{ENV} sensitivity with high-frequency carriers, suggesting that LSO neurons with appropriate membrane properties can collectively encode envelope ITDs. Our experiments aim to test these predictions in vivo and determine whether ITD_{ENV} encoding varies systematically along the LSO's tonotopic axis.

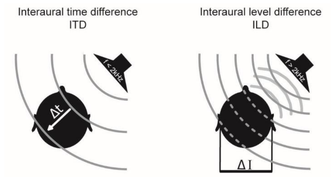

Fig. 1: Illustration of ITD & ILD (Grothe & Pecka 2014)

2 LSO-centred sound localization circuit

Neurons of the lateral superior olive (LSO), located within the superior olivary complex of the brainstem, are among the first auditory neurons to receive binaural input. Each LSO neuron integrates excitatory and inhibitory signals: glutamatergic excitation arises from spherical bushy cells in the ipsilateral ventral cochlear nucleus (VCN), while glycinergic inhibition is provided by neurons in the medial nucleus of the trapezoid body (MNTB).

The excitatory VCN input is driven by 1–3 auditory nerve fibers (ANFs) per spherical bushy cell (Doucet and Ryugo, 2003). In the inhibitory pathway, each principal neuron of the MNTB receives a large, fast synaptic input via the calyx of Held from a single globular bushy cell (GBC) in the contralateral VCN. Notably, each GBC is driven by a converging input of 9–70 ANFs (Glendenning et al., 1985), allowing for precise temporal encoding. The robust convergence onto globular bushy cells (GBCs), characterized by large-diameter, heavily myelinated axons and the presence of the giant calyx of Held terminal, ensures that inhibitory signals arrive with sufficient temporal precision to coincide with ipsilateral excitatory inputs, despite the presence of an additional synaptic relay in the inhibitory pathway.

Approximately 40 excitatory fibers from the ipsilateral VCN converge onto a single LSO neuron, while only about 4 strong glycinergic inputs from the MNTB provide inhibitory drive to each LSO cell (modified from Brughera et al., 2020). This asymmetry in convergence supports the precise temporal integration and spatial sensitivity required for interaural level difference (ILD) processing.

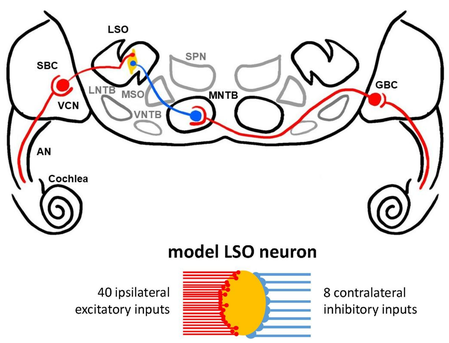

Fig. 2: LSO inputs and model EI neuron (from Brughera et al. 2020).

3 Method

Fig. 3: A) Extracellular single-cell recording during acoustic stimulation.
A mouse is placed on a heating pad (37°C) in a sound-proof chamber and fixed in a stereotaxic device. Sounds are presented unilaterally or bilaterally via earphones (TDT MF1). A cranial window allows insertion of the recording electrode into the brain of the MMF-anaesthetised mouse. This technique enables high quality recordings of single neurons with large signal-to-noise ratios.

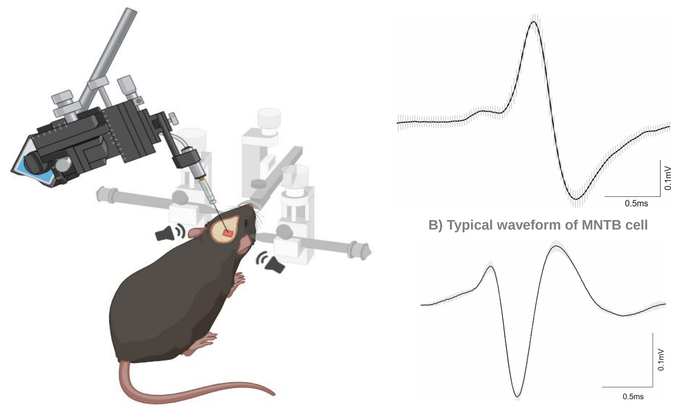

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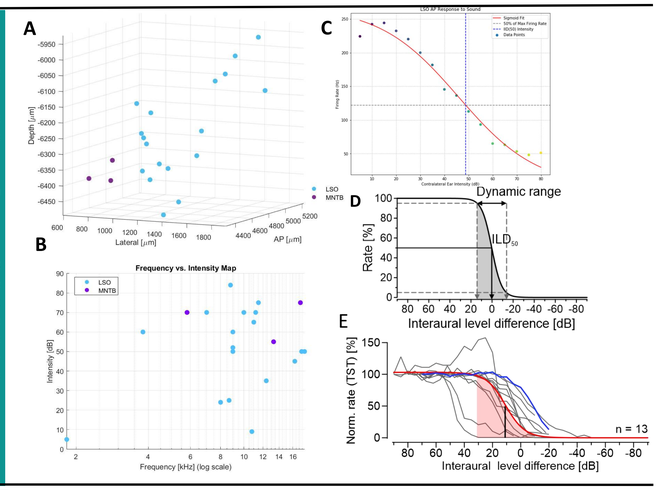
Fig. 4: Physiological properties for the analysed cells

A) location of the recorded MNTB and LSO cells. The MNTB ranges from approximately 250 to 750 µm. The LSO ranges from approximately 1100 to 1800 µm.

B) Frequency vs threshold for MNTB (violet) and LSO (blue) cells

C): ILD response from a LSO neuron.
LSO neuron's response to fixed ipsilateral sound level (20dB above threshold) and varying sound levels at the contralateral ear. The firing rate is plotted against contralateral input. As contralateral intensity increases, the firing rate decreases. The blue sigmoid fit models this response, with ILD(50)—marked by a vertical dashed line—indicating the ILD at which firing rate falls to 50% of its maximum.
D) ILD curve from 1 cell
E) ILD curves from 13 cells



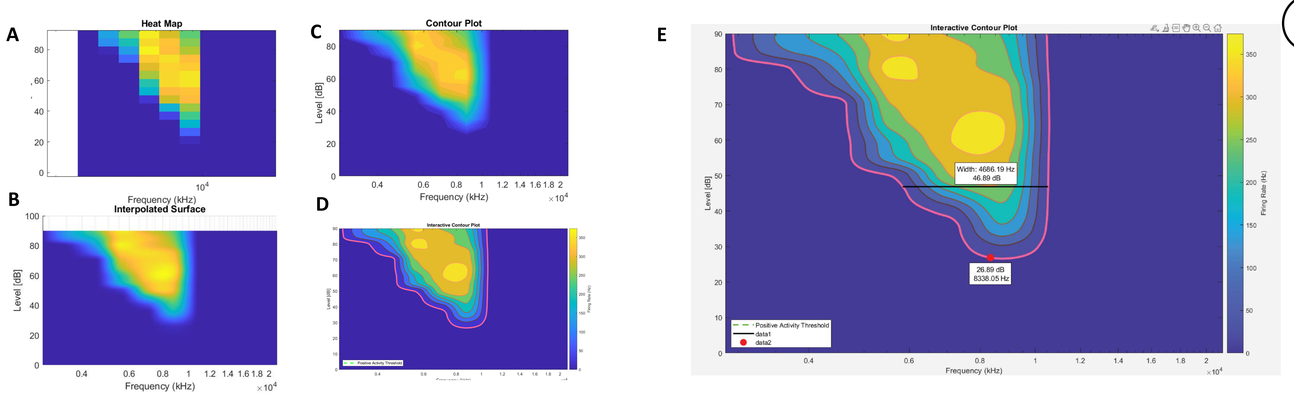


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Fig. 5: Frequency Response Map

A-D: Heatmap of the evoked response firing rate of a MNTB neuron.

The X-axis represents the frequency of the auditory stimulus in Hertz (Hz), ranging from 3.333 Hz to 25.000 Hz on a logarithmic scale. The Y-axis shows stimulus intensity in decibels (dB), increasing from 15 dB to 90 dB.

Color intensity within the heatmap reflects the neuron's firing rate: cooler colors (e.g., blue) indicate lower firing rates, while warmer colors (e.g., yellow) indicate higher firing rates.

Evoked responses were identified as neural activity exceeding a threshold of three standard deviations above the mean spontaneous firing rate, ensuring that the measured activity reflects stimulus-driven responses rather than spontaneous activity.

6 Stimulus transience increases temporal precision but decreases LSO neurons' firing

Fig. 6 Overview of tested sinusoidal stimulus conditions.

A) The table lists 15 combinations of pulse frequency (F_{pulse}) and envelope frequency (F_{env}) that were presented in an ipsilateral stimulation. The sinusoidal stimulations used a broadband noise carrier.

B) Example waveforms for two representative conditions illustrating differences in pulse and modulation structure.

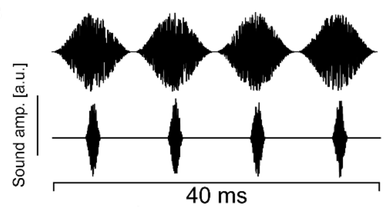
top: 100 Hz (F_{pulse}) – 100 Hz (F_{env})

bottom: 100 Hz (F_{pulse}) – 500 Hz (F_{env})),

A

F_{pulse} [Hz]	F_{env} [Hz]
20	20, 60, 100, 200, 500, 1000
50	50, 100, 200, 500, 1000
100	100, 200, 500, 1000, 2000

B

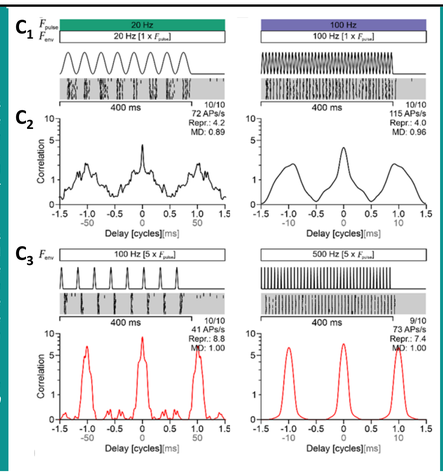


C) Effect of increasing F_{env} on LSO neuron firing

C_1) The top panel presents the stimulus conditions. The middle panel illustrates the stimulus envelope. The bottom panel shows a spike raster plot for stimulation with e.g. F_{pulse}= 20 Hz and F_{env}= 20 Hz (hereafter referred to as *P20_E20*) of ten repeats and similarly for *P100_E100*.

C_2) Auto-correlogramm of the spike trains depicted above. The y-axis is scaled logarithmically to enhance the illustration of the sub-correlating space (correlation <1), given that correlation can increase infinitely. The number of repeats (10/10), the AP rate, the reproducibility (repr.) and the modulation depth (MD) are presented in the top right corner.

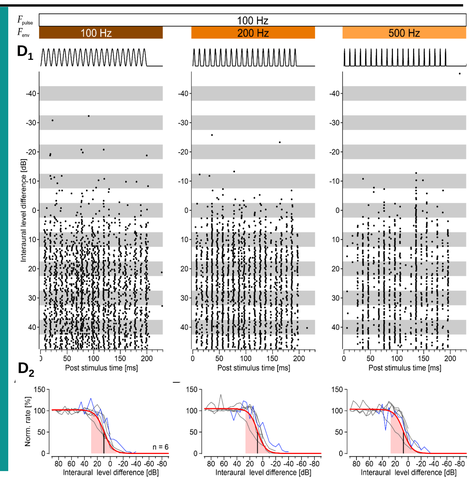
C_3) follows the structure of C_1 but for a stimulation with 5-fold increased F_{env} (*P20_E100* and *P100_E500*).



D) ILD coding is maintained with increasing stimulus transience (*P100_E100-500*).

D_1) raster plots at 19 different ILDs, each condition presented ten times. Ipsilateral intensity fixed to 20dB re threshold, contralateral intensity range: 0-90dB.

D_2) ILD functions for n=6 neurons (grey) and the mean ILD function (red). The blue curve depicts the data from the examples in D_1.



7 SUMMARY

We recorded sound-evoked activity from 19 LSO neurons. The LSO neurons were identified by their location in relation to Bregma, the dorsal surface of the brain and also to the location of the neighbouring MNTB (3 cells); and more importantly by their excitation in response to ipsilateral acoustic stimulation and inhibition in response to contralateral stimulation (EI cells). All EI LSO cells were responsive to classical ILD with differing ILD_{50} values. In addition to this rate-encoding of sound level, we also asked whether LSO cells are suited for temporal processing. Since after detailed measurements of the frequency-response areas we found neurons tuned to low and high sound frequencies ranging from 1.8 – 17,9 kHz. While the low CF cells may respond to the fine structure of pure tones, the high CF cell will not. Therefore we tested a novel stimulus paradigm that allows to change the frequency of the pulse train independently of the envelope and found that LSO neurons responed with lower firing rates but higher temporal precision to steeper envelopes. This suggests that LSO cells independently of their CF tuning and independently of the base pulse stimulus of the stimulation train respond with increasing temporal precision to steeper envelopes.

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