

Spatial map of frequency tuning-curve shapes in the mouse inferior colliculus

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Neurons in the central nucleus of the auditory midbrain inferior colliculus divide into four classes according to the shapes of their receptive fields. Neurons of two of these classes – sharply tuned, inhibition-dominated neurons of class II, and broadly tuned neurons of class III – show systematic gradients in their abundance on isofrequency contours. Sharp tuning is most prevalent in the center, broad tuning in the periphery of the ICC. This new map of tuning-

curve shape adds to the six previously described maps of neural response properties on isofrequency contours of the ICC and stresses the fact that very different sensitivities and selectivities to sound properties are combined in local clusters of collicular neurons. *NeuroReport* 14:1365–1369 © 2003 Lippincott Williams & Wilkins.

Key words: Auditory midbrain; Collicular maps; Frequency tuning; Inferior colliculus; Mapping; Mouse; Receptive field; Sound analysis

INTRODUCTION

Neurons in the 3D space of the main nucleus of the auditory midbrain, the central nucleus of the inferior colliculus (ICC), show response properties to sounds according to their spatial location. Besides the gradients of neural characteristic frequencies leading to the well-known tonotopy, tone response threshold, sharpness of tuning, tone response latency, best modulation frequency to amplitude-modulated tones, and best azimuth angle are mapped in the ICC [1,2]. Recently, excitatory and inhibitory frequency response areas of ICC neurons have been evaluated quantitatively [3,4]. Neurons were classified according to the shapes of their receptive fields (mainly by the shapes of excitatory tuning curves) into classes I–IV [4]. Basically, class I neurons had asymmetrical excitatory and inhibitory response areas with excitatory tuning often similar to that of auditory nerve fibers. Class II neurons had narrow symmetrical, tilted or closed excitatory response areas flanked by large and strong inhibitory areas. Class III neurons often had a V-shaped excitatory area with little inhibition at the sides. Class IV neurons had more than one excitatory characteristic frequency, i.e. often a double-peaked excitatory tuning curve and other specialties such as facilitatory areas.

In the present study, we recorded from single neurons at known locations in the ICC of the mouse, classified the neurons as described previously [4] according to the slopes of their excitatory tuning curves, and mapped them in the space of the ICC. Our goal was to determine whether

neurons are regularly distributed or clustered in the ICC according to the classification of receptive fields as described above.

MATERIALS AND METHODS

Recordings were taken from the left-side inferior colliculus of female house mice (*Mus musculus*) aged 8–15 weeks. The results are from two experimental series with differences in the strain of mice, recording electrodes and equipment for stimulus generation. In the first series (seven F1 hybrid mice of CBA and C57BL/6 inbred strains), glass pipettes filled with 3 M KCl (impedances 4–8 MΩ) were used as recording electrodes. Responses were amplified 10 000 times, band-pass-filtered (0.3–10 kHz, WPI DAM 80), and run through a window discriminator (WPI 120) to a computer for the assessment of frequency tuning curves of single units (software by Dr Schulze-Krüger, Germany). The computer synthesized tone bursts (50 ms duration, 5 ms rise and fall times, 300 ms intervals) with a 16-bit TMS 320 C30 D/A converter at 200 kHz conversion rate. Tones were sent to a dynamic speaker (EAS 10 TH400) via a power amplifier (Amphyton 25U-002C). The computer generated tone bursts of varying frequency (about three octaves around the characteristic frequency (CF) of a given neuron) and amplitude (a total range from –20 to 90 dB sound pressure level (SPL) could be scanned with an additional Kenwood RA 920A attenuator) in pseudo-random sequences of 675

different frequency–intensity combinations. Spike responses were automatically associated with the stimuli, resulting in plots of frequency tuning curves.

In the second series (13 mice of an outbred strain of hybrids of NMRI and feral mice), recordings were done with lacquer-insulated steel electrodes [7] of impedances of 2–4 M Ω . Responses were amplified 10 000 times, bandpass-filtered (0.4–4 kHz; Neuroamp 401) and run through a window discriminator (Heinecke) to a computer. The computer synthesized tone bursts (50 ms duration including 5 ms rise and fall times, 60 ms intervals) with a 16-bit TMS 320 C30 D/A converter at 120 kHz conversion rate. Tones were amplified (Denon, PMA 1060) and delivered by a dynamic speaker (CF-12HC-4) and went via a voltage amplifier and power supply to an electrostatic speaker [6]. The computer generated pseudo-random sequences of 675 different frequency–intensity combinations in a frequency range of three octaves around the CF of a given neuron and in an intensity range of –20 to 90 dB SPL (with an additional Kenwood RA 920 A attenuator). Spike responses of single units were automatically associated with the stimuli resulting in plots of frequency tuning curves.

In both series, only acute experiments were done. Surgery was performed under 120 mg/kg ketamine (Ketavet) and 5 mg/kg xylazine (Rompun) and the anesthesia continued with injections of 35 mg/kg ketamine (Ketavet) and 1 mg/kg xylazine (Rompun) about every 20–45 min. At the end of the measurements, the still anesthetized animals were killed by cervical dislocation. The experiments comply with the Principles of Animal Care, publication No 86-23, revised 1985, of the National Institute of Health and also with the respective current German and Russian laws. The experiments were approved by the appropriate authorities (Regierungspräsidium Tübingen, Germany; Sechenov Institute, Russia).

In the anesthetized animals, a metal bar was glued to the frontal–parietal bones of the skull and the head fixed in a horizontal position. The bone and dura over the left-side IC was removed and the animal placed on a feedback-controlled heating pad (rectal temperature at $37 \pm 1^\circ\text{C}$) in a sound-proof and anechoic room. Loudspeakers were placed in front of the animal, 45° to the right of the mouse sagittal plane and thus contralateral to the recorded IC. At the animal's right pinna, the speaker systems were flat within ± 5 dB between 3 and 80 kHz (first series) and within ± 6 dB between 1 and 50 kHz (second series). Harmonic distortions were > 35 dB (mostly more than 50 dB) below the level of the fundamental frequency. Calibration of the sounds was done with a 6.5 mm calibrated microphone (Bruel and Kjaer 4135 with preamplifier 2633 and measuring amplifier 2606 or 2636). Electrodes were placed on the brain surface stereotaxically with reference to the λ -point of the skull and advanced into the ICC perpendicularly to its surface by a remote-controlled microdrive (PM 10-1 piezo-stepper or SPI Nanostepper). Thus, for all recorded neurons, rostrocaudal, mediolateral, and dorsoventral coordinates in the same reference frame were available.

RESULTS

Data from the two experimental series were not significantly different, neither for the proportions of neuron observed in

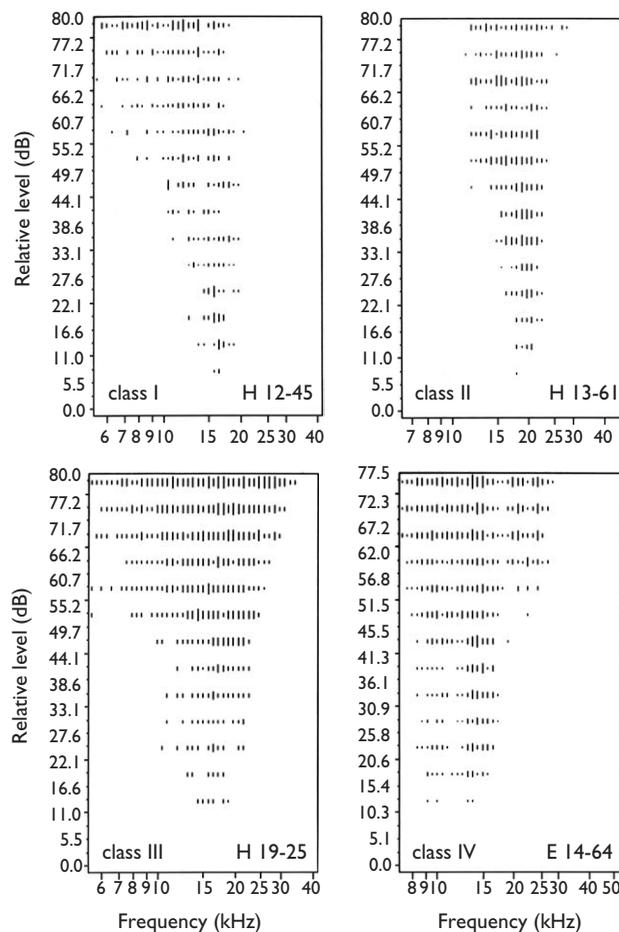


Fig. 1. An example tuning curve of one neuron of each of the four classes. The vertical dashes indicate spike numbers in response to the respective frequency–intensity combinations.

the tuning-curve classes, nor for the spatial distributions of neurons of the different classes in the ICC. Hence, all data have been combined. We determined the shapes of excitatory tuning curves of 119 neurons with CFs ranging between 12 and 31 kHz. Twelve (10%) of these neurons had two CFs and, therefore, were classified as class IV. The other neurons were classified on the basis of the low- and high-frequency slopes of their excitatory tuning curves exactly as described previously [4]. We analyzed 45 (38%) neurons of class I, 32 (27%) of class II, and 30 (25%) of class III. Figure 1 shows an example tuning curve of each class of neurons. Neurons of the four classes were recorded in the ICC at the rostrocaudal and mediolateral coordinates with reference to the λ -point of the skull as shown in Fig. 2. The depths of the recordings in the ICC were not considered in this figure, and all recording sites were projected to the shown horizontal plane.

In order to evaluate possible systematic changes in the composition of neurons from the four classes over the collicular space, we divided this space concentrically into three zones (Fig. 2): a central zone with a radius of 250 μm around the center of the ICC, a pericentral zone with a width of 250 μm around the central zone, and a peripheral zone with a width of 250 μm around the pericentral zone.

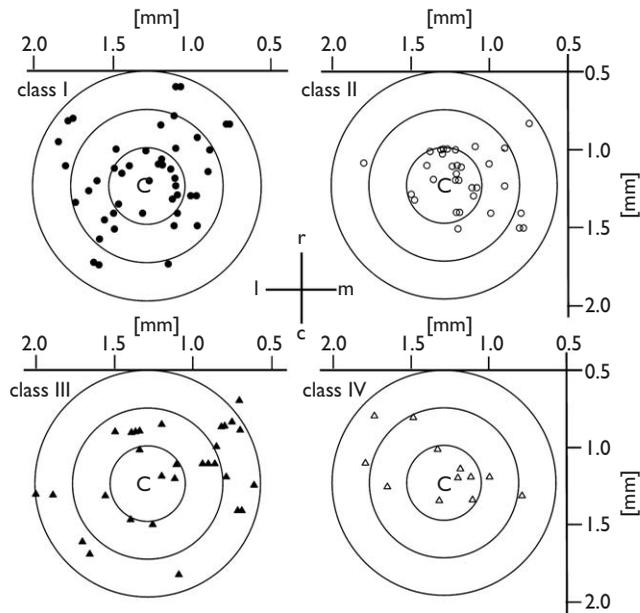


Fig. 2. Locations of the neurons in our sample plotted separately for the four classes in mediolateral and rostrocaudal coordinates with reference to the λ -point of the skull, which would be located at the origin (zero coordinates) of both axes. The dorsoventral location is not considered and all recording depths in the ICC are projected to the shown horizontal plane. C is the suggested center of the ICC. In each subfigure, three circles indicate the borders of the three zones, central, pericentral, peripheral, into which we divided the ICC for a quantitative evaluation of the spatial distribution of the neurons of the four classes.

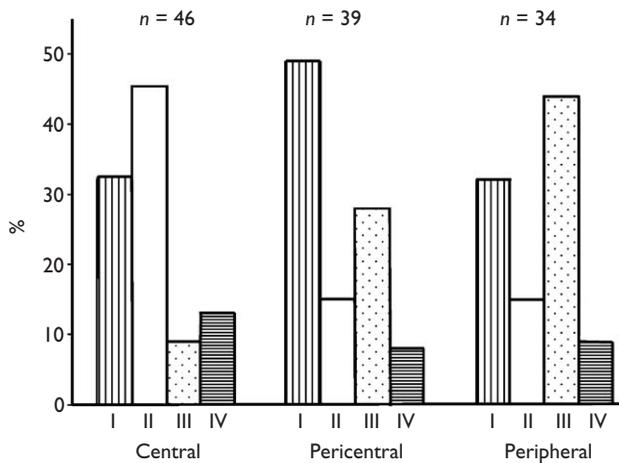


Fig. 3. Percentages of neurons of the four classes (I-IV) in the central, pericentral, and peripheral zones of the ICC (see Fig. 2 and text for the definition of the zones).

For the recorded CF-range, the center may be located at the coordinates of 1.3 mm caudal and 1.22 mm lateral of the λ -point of the skull [5]. With this position of the ICC center, the outer border of the peripheral zone roughly represents the lateral, medial and caudal margins of the ICC [5]. The distribution of neurons of the four classes in these three zones of the ICC is shown in Fig. 3. The one class III neuron

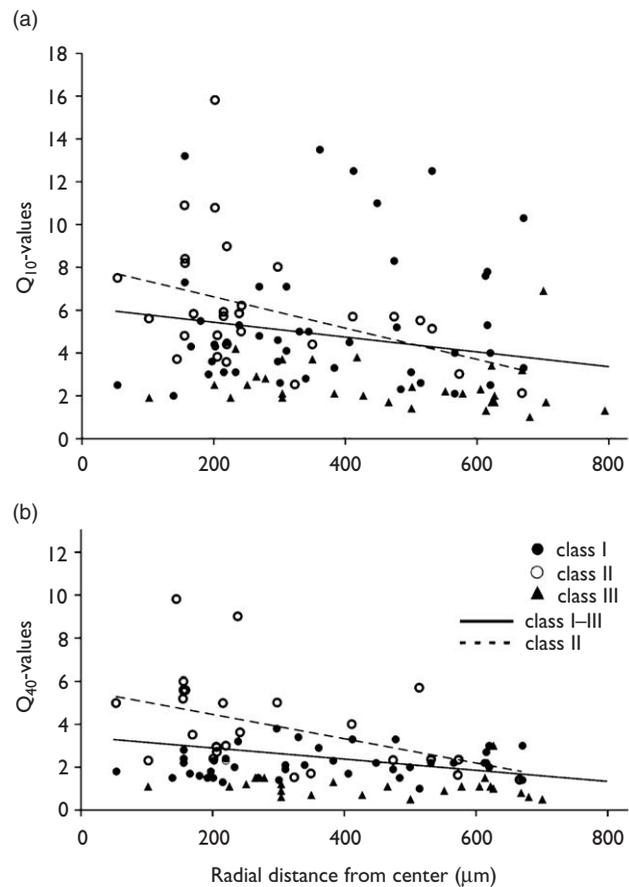


Fig. 4. Q_{10} and Q_{40} values (measure of sharpness of tuning, see text) of the neurons in classes I-III as a function of their location plotted as the radial distance from the center of the ICC (compare Fig. 2). Solid lines and dashed lines show statistically significant linear regressions for all neurons and class II neurons, respectively.

located just outside the peripheral zone (Fig. 2) is included in this zone. Class I neurons are present in all three zones in high percentages (32-49%). Almost half of the neurons recorded in the central zone (45.5%) are of class II, while neurons of class II are present with only 15% in the two more peripheral zones of the ICC. Neurons of class III are rarely encountered in the central zone (9%). They increase in abundance in the pericentral zone (28%) and are the dominating class in the peripheral zone (44%). Class IV neurons are rare in all three zones (8-13%). The distribution of neurons from the four classes is significantly inhomogeneous ($\chi^2 = 24.3$, $df = 6$, $p < 0.001$) across the ICC space, mainly because class II neurons dominate in the center and class III neurons the periphery.

The change in the presence of neurons in class II and class III going from the center of the ICC to its periphery has consequences for the average sharpness of frequency tuning across the ICC. In Fig. 4a,b Q_{10} and Q_{40} values, respectively ($Q = CF$ divided by the bandwidth of the excitatory tuning curve 10 dB or 40 dB above threshold at CF), of class I, II, and III neurons are plotted against the distance of their location from the center of the ICC. The regression lines related to all neurons (solid lines in Fig. 4a,b) demonstrate a

significant decrease of Q_{10} values ($r = -0.242$, $df = 105$, $p < 0.02$) and Q_{40} values ($r = -0.317$, $df = 94$, $p < 0.005$) with increasing distance from the center of the ICC. This average decrease in the sharpness of tuning from the central to the peripheral ICC may be the result of two different factors: first, the Q -values of class II neurons are significantly larger than those of class III neurons at all levels except 10 dB above threshold [4]. Since class II neurons decrease and class III neurons increase in number from the ICC center to its periphery, at least Q_{40} values should and actually do decrease in parallel. Second, the dashed regression lines in Fig. 4a,b indicate a significant decrease of Q_{10} values ($r = -0.425$, $df = 30$, $p < 0.03$) and Q_{40} values ($r = -0.417$, $df = 25$, $p < 0.05$) of class II neurons with increasing distance from the ICC center. Such a significant change in sharpness of tuning over collicular space is not present in neurons of class I and class III. Thus, mainly class II neurons are responsible for the decrease of Q_{10} values from the center to the periphery of the ICC.

DISCUSSION

All neurons recorded were placed in one of the four classes as before [4] with very similar proportions obtained in this study to the previous one, namely: class I: 38% vs 41.5%; class II: 27% vs 28%; class III: 25% vs 24.5%; class IV: 10% vs 6%. This supports the relevance of the classification method used. Since the results are from mice of very different genetic backgrounds, we assume that our results refer to mice without hearing loss in general.

So far, tuning-curve shapes of single neurons have not been mapped in the ICC. Multi-unit mappings of excitatory tuning-curve shapes of units with CFs around 10, 20, and 30 kHz in the ICC of the mouse [8] showed that units in the center of the ICC had, on average, the steepest tuning-curve slopes (narrow tuning). Towards the periphery of the ICC, tuning-curve slopes progressively decreased (tuning widened), especially going from the center to more rostral and lateral locations. These data [8] can be understood on the basis of our present measurements. Multi-unit tuning curves are determined by the most broadly tuned neurons in the recorded cluster of units. Thus, in the center of the ICC, the dominance of class II neurons (present study) led to high probabilities of sharp and narrow tuning curves in Stiebler's study [8]. The decrease in abundance of class II and increase of class III neurons (present study) going from the ICC center to its periphery was the reason for the general increase of the tuning-curve bandwidths observed before [8]. Similarly, a rather concentric decrease of Q_{10} values from the center to the periphery of the ICC of the cat as measured by combining multi- and single-unit recordings [9,10] can be explained mainly by the decrease in number of class II neurons from the ICC center to its periphery. It has to be stated, however, that Q_{10} values are insufficient predictors of tuning-curve shape and sharpness of tuning, at least at the ICC level, because they do not significantly differ among neurons with different shapes of receptive fields (classes I–III) [4]. Only Q values measured at 20 dB or better at ≥ 30 dB above threshold at CF, do, on average, discriminate between class I and class III neurons [4]. This should be considered in further studies, trying to map

sharpness of frequency tuning in the auditory midbrain via calculation of Q -values.

The spatial distribution of neurons in the ICC characterized by four different shapes of excitatory tuning curves (classes I–IV) that is demonstrated here has important implications with regard to local patterns of excitatory and inhibitory convergence of input to ICC neurons and resulting sound-coding properties.

First, the dominance of class II neurons in the ICC center (Fig. 3) means a dominance of ample and strong inhibition there, because class II neurons have large, strong, and often symmetrical inhibitory areas at the sides of and also inside their excitatory receptive fields [4]. Inhibitory input cumulating in the center of the ICC and decreasing towards its periphery is not obvious from numerous studies on the distribution of ascending projections to the ICC (reviewed in [1,11,12]). The dominance of broader tuning (class III) with little influence of inhibition (small inhibitory areas with weak inhibitory effects [4]) in the periphery of ICC (Fig. 3) cannot be predicted from the ICC input neither [1,11,12]. Hence, we infer a gradient intrinsic to the neural network of the ICC, generating strong converging inhibition in its center and converging excitation in its periphery. This gradient could be due to respective gradients in the local mixture of and connections between excitatory and inhibitory neurons that have already been described in the ICC [13].

Second, the presence of class I neurons over all isofrequency contours of the ICC in proportions of about one-third or more of the local populations (Fig. 2, Fig. 3) shows that properties of excitatory tuning similar to those of auditory nerve fibers [4] are widely distributed in the ICC. This may be due to input from multipolar and stellate cells of the ventral cochlear nucleus with primary-like excitatory tuning and terminal fields over the whole tonotopy of the ICC [11,12,14,15].

Third, the projections of the ipsilateral medial superior olive mainly to the lateral ICC [11,12] coincide with a high proportion of class I and III neurons and a low proportion of class II neurons there (Fig. 2). At the same time, most neurons at this location have intrinsic onset-responding properties [16]. Thus, binaural analysis in the time domain appears to be associated with the presence of broader tuning, rather little inhibition, and onset responses in the ICC.

Finally, sharp frequency tuning and strong inhibitory influences in the central ICC by the high number of class II and low number of class III neurons (Fig. 3) provide high spectral resolution capabilities to this part of the ICC. These properties seem to be favorable for coding the bandwidths of frequency resolution of the auditory system, the critical bandwidths, which receive perceptual characteristics, such as intensity-independence of bandwidths, by inhibitory influences [17].

CONCLUSIONS

Taken together, our data indicate an anisotropy of neurons with regard to the sharpness of frequency tuning in the main nucleus of the inferior colliculus. Almost 50% of the collicular neurons distributed rather uniformly over isofrequency contours show excitatory tuning curves either

similar in shape to those of auditory nerve fibers or tuning curves with two sensitivity peaks and complex shapes. The other 50% or more are characterized by either very narrow tuning curves (sharp tuning) or broad tuning. The proportions of neurons with sharp or broad tuning change systematically from the collicular center (predominantly sharp tuning) to the periphery (predominantly broad tuning). Thus, analyses of collicular functions in auditory processing have to consider this map of frequency selectivity produced by more than half of collicular neurons. This map together with the previously described maps [1] contributes to supply different local clusters of neurons with place-specific combinations of analytical properties.

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