Review

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Local versus global scales of organization in auditory cortex

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Topographic organization is a hallmark of sensory cortical organization. Topography is robust at spatial scales ranging from hundreds of microns to centimeters, but can dissolve at the level of neighboring neurons or subcellular compartments within a neuron. This dichotomous spatial organization is especially pronounced in the mouse auditory cortex, where an orderly tonotopic map can arise from heterogeneous frequency tuning between local neurons. Here, we address a debate surrounding the robustness of tonotopic organization in the auditory cortex that has persisted in some form for over 40 years. Drawing from various cortical areas, cortical layers, recording methodologies, and species, we describe how auditory cortical circuitry can simultaneously support a globally systematic, yet locally heterogeneous representation of this fundamental sound property.

A history of progress and controversy

The first evidence for a spatially organized representation of sound frequency at the level of the cerebral cortex (see Glossary) came from 19th century lesion experiments in dogs, in which specific behavioral deficits in discriminating low, middle, or high pitch sounds were attributed to the location of focal ablations along the posterior–anterior extent of perisylvian cortex [1,2]. A neurophysiological demonstration of cochleotopy was provided decades later by recording evoked potentials from the surface of the cortex while electrically stimulating a restricted set of auditory nerve fibers that innervated apical (low frequency) versus basal (high frequency) regions of the cochlea [3]. These experiments revealed an apical-to-basal organization along the posterior-to-anterior extent of the middle ectosylvian area of the cat that was subsequently matched to a tonotopic organization when electrical stimulation was replaced with airborne tone burst stimuli [4].

The advent of the microelectrode in the latter half of the 20th century (Box 1) ushered in a period of great productivity – as well as controversy – for early efforts to characterize the functional organization of auditory cortex. Most research laboratories gravitated towards an approach that involved systematic sampling of multiunit or single unit activity from the middle cortical layers of anesthetized animals at spatial densities ranging from 0.1 to 1 mm. These early efforts were successful in identifying the organization of multiple tonotopic and non-tonotopic cortical fields in the cat and primate and were also able to pinpoint locations of interest, such as boundaries

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Keywords: auditory cortex; frequency; homogeneity; maps; heterogeneity; layers; electrophysiology; imaging; calcium; resolution; scale.
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Glossary

Cortex: the cortex (latin 'bark', 'rind') is the thin (approximately 1–2 mm thick) layer of neurons that cover the mammalian forebrain. Most of the cortex, including auditory cortex, is composed of multiple layers (up to six) with different cellular morphology and connections. Cortical layers are grouped into the middle layer (the main thalamorecipient layer; often also called layer 4) that separates the supragranular and infragranular layers (above and below the thalamorecipient layer). The supragranular layers include layer 1, which is usually neuron-poor, and layers 2 and 3 which, in rodent auditory cortex, are often referred to together as layer 2/3. The infragranular layers (5 and 6) have major cortical projections to subcortical stations, including the thalamus.

Cortical fields, core and secondary areas: differences between different parts of the cortex include differences in architecture, connectivity, and function. The part of the cortex which is dominated by auditory responses is named 'auditory cortex'. The auditory cortex can be subdivided again based on a number of criteria (see main text) into core areas (e.g., A1 and AAF) surrounded by secondary areas. Core areas are densely interconnected with the lemniscal division of the auditory thalamus, although they also get inputs from other subdivisions of the auditory thalamus. Secondary areas receive their predominant input from non-lemniscal subdivisions of the auditory thalamus.

Topographic organization and tonotopy: in the cochlea, the auditory sensory organ of the inner ear, sound is mechanically filtered into narrow frequency bands by the basilar membrane. The resulting sensitivity to a narrow frequency band is inherited by the hair cells that sit on the basilar membrane and by the auditory nerve fibers that innervate them. Most of the brainstem auditory structures are composed of neurons that inherit the narrow tuning of the cochlear input. Furthermore, neurons that have similar frequency tuning are grouped together, and the progression of best frequencies of the neurons is continuous along one axis of the structure. This organization is referred to as tonotopic organization or tonotopy. Tonotopy is kept in the core ascending auditory pathway, including in particular the MGBv.

The thalamus and thalamocortical connections: the thalamus is a large forebrain nucleus with many subdivisions and is the main gateway to the cortex. Sensory nuclei of the thalamus process input from lower parts of the brain and project to the sensory cortices. The main thalamic input usually reaches the middle cortical layers, although auditory thalamic axons also branch in layer 6 (the 'thalamorecipient layers'). Neurons from all layers may receive thalamic input, as long as their dendrites reach the thalamorecipient layers. The cortex projects back to the thalamus, usually to the same subnuclei of the thalamus that project to it. The auditory thalamus is composed mostly of the MGB, which itself has three major subdivisions, the ventral, medial, and dorsal. The ventral subdivision (MGBv) is part of the core ascending auditory pathway and is the major input to A1.

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Box 1. Methods of measuring brain activity

Extracellular electrophysiological recordings are conducted by inserting a microelectrode into the area of interest. The uninsulated contact measures currents that are produced by neuronal activity in its neighborhood. The signal recorded by such electrodes can be filtered to reveal relatively slow fluctuations, called local field potentials (LFPs) that represent mostly synaptic currents, and fast fluctuations (<1 ms) that are caused by spikes in nearby neurons. Electrodes can be engineered to record spikes from many neurons (multiunit activity) or only currents from very close neurons, in which case it corresponds to the activity of a single neuron. Although extracellular recordings are considered to be the 'ground truth' for understanding neuronal responses, they are limited by being blind – the experimenter cannot select the neurons from which to record and has relatively coarse information about the layer in which the neuronal activity is measured. In addition, microelectrode recordings are informative only about the activity in small extents of the cortical area, and in order to generate a tonotopic map in auditory cortex, multiple electrode penetrations are necessary (a few tens to hundreds of penetrations, depending on the species). Thus, optical imaging methods have been developed in order to either increase the cellular resolution (calcium imaging) or to gain information about large extents of the cortex (intrinsic signal imaging).

Calcium imaging is based on the fact that calcium concentration in neurons is extremely low, and that calcium entry invariably follows the generation of an action potential in neuronal cell bodies, through the activation of voltage-sensitive calcium channels. Calcium indicators are molecules that fluoresce in the presence of calcium. Two important technological advances underlie the use of calcium imaging *in vivo*. On the one hand, modern techniques make it possible to introduce calcium indicators into multiple neurons in the tissue. Such techniques include the use of the so-called acetoxymethyl (AM) ester dyes. Which are injected into the extracellular space and are taken by the cells in the tissue, and the use of genetically encoded calcium indicators, introduced through viral injection or by using transgenic techniques. The second advance is the use of two-photon scanning microscopy, which made it possible to spatially resolve calcium signals to subcellular levels. Using both methods in conjunction, it became possible to image tens to hundreds of neurons simultaneously in small fields. The main drawbacks of calcium imaging is the fact that calcium signals reflect electrical activity only indirectly, with relatively slow dynamics that make it difficult to resolve single spikes, and the limitations imposed by light scattering that currently limit calcium imaging to the superficial layers of the cortex.

On the other hand, at the other end of the range of useful spatial resolutions, intrinsic signal imaging uses the changes in reflectivity associated with the oxygenation level of the blood and its amount in the tissue. By shining a light of a particular wavelength (540–700 μm, depending on what component of the hemodynamic signal is emphasized in a particular experiment) on the cortex and imaging the reflection, it is possible to observe territories of the cortex that are active. Intrinsic signal imaging reflects neuronal activity even more indirectly than calcium imaging, because it depends on the changes in tissue oxygenation that accompany large cortical activations.

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between tonotopic and non-tonotopic fields or circumscribed modules with particular tuning properties, which were then used to guide the placement of neuroanatomical tracers [5–10]. These initial studies established the contemporary framework for how cortical fields are parceled, where they receive inputs from and send outputs to, and where they might sit within a distributed network of auditory information processing. By contrast, careful study of the same cortical regions by other laboratories during this period found only weak evidence for a tonotopic organization, arguing instead that frequency tuning at the level of the auditory cortex was heterogeneous or strongly modulated by cognitive factors such as attention [11–13].

The discrepancies in these early findings, which probably stemmed from basic differences in experimental methodologies, were never fully resolved at the time. The heterogeneous frequency organization reported by the minority of these early studies gradually faded from view as auditory cortex research in the latter years of the 20th century became increasingly reliant upon microelectrode recordings from the thalamorecipient layers of primary auditory areas in anesthetized animals, conditions that probably favor the appearance of precise tonotopy. However, the debate over the degree of tonotopic mapping precision has reappeared in recent years, perhaps reflecting a shift towards experimental approaches that enable measurements at finer spatial scales, from other cortical layers and other states of vigilance. The purpose of this review is to provide a foundation for understanding how this issue has been studied historically and then highlight very recent findings that may reconcile these differences and point the way towards new directions for auditory cortex research. This controversy in its different reincarnations also carries important lessons for the study of other cortical areas.

General principles of auditory cortex organization

Primary auditory areas are distinguished from secondary areas according to three criteria. First, they receive heavy input from the lemniscal, tonotopically organized subdivision of the auditory thalamus, named the ventral subdivision of the medial geniculate body (MGBv) based on its anatomical location in cats; second, they exhibit anatomical or neurochemical features consistent with primary sensory cortex such as koniocellular cytoarchitecture, dense myelination, and elevated expression levels of various molecules such as parvalbumin, cytochrome oxidase, and acetylcholinesterase; and lastly, they are tonotopically organized. Beginning with Woolsey's and Rose's seminal work in the cat [3,14,15], the existence and relative positioning of primary and secondary auditory areas have been identified according to one or more of these criteria in over 20 mammalian species (for a review, see [16]).

Three primary auditory areas have been identified in non-human primates, each separated from one another by mirror reversals in tonotopy: the primary auditory cortex (A1), the rostral area, and the rostrotemporal area (for a review, see [17]). Most rodents, carnivores, and bats also have three primary areas separated by frequency reversals in the tonotopic gradients: A1, the anterior auditory field (AAF), and a posterior auditory field. In the auditory pallidum of birds, field L exhibits many of the same features as A1, including a prominent input from nucleus ovoidalis, the presumed homolog of MGBv [18], and a tonotopic organization [19–21].

Although all researchers in the field are in agreement about the existence of a tonotopic organization in the primary fields of auditory cortex, how tight this organization is has been questioned in the past [12,22,23]. In the following sections, we will review the evidence for and against tonotopic order based on techniques that characterize the

auditory cortex using a variety of neural signal types at various spatial scales and cortical depths.

Low resolution optical imaging reveals tonotopic order
Imaging methods (Box 1) make it possible to visualize correlates of neural activity, such as hemodynamic responses, over large areas (many mm^2) of the brain and thus enable the investigation of the functional representation of relevant stimulus features.

Compared with the successful application of intrinsic signal imaging in the visual cortex, its successful application in the auditory cortex has proven more difficult possibly owing to the poor driven rates in superficial layers of the auditory cortex under deep barbiturate anesthesia [24–27], different hemodynamic responses in the auditory cortex because of different spatial layout of blood vessels [28], or owing to more variable response properties of neurons within cortical columns in the auditory cortex. Interestingly, these difficulties mirrored early investigations of auditory cortex organization with 2-deoxyglucose [29,30], which had been utilized to great effect in the visual cortex [31,32].

Several approaches have been used to improve intrinsic signal quality in the auditory cortex, and with these modifications optical imaging of the auditory cortex demonstrated the presence of large-scale tonotopic maps in cats and a variety of rodent species [33–42]. In particular, using tone sequences and analyzing the timing of the resulting activations (a technique pioneered in the visual cortex by Kalatsky *et al.* [38]) turned out to be a useful tool for delineating tonotopic organization in core auditory cortex [38,43].

Although intrinsic imaging has the advantage of revealing relatively quickly large-scale maps, the technique has several drawbacks. First, imaging hemodynamic responses biases the signal towards areas containing highly responsive cells that share similar tuning and that are located close to each other. Thus, areas where cells might be tuned very selectively but respond with only few spikes will not show strong optical activations using

hemodynamic responses. Moreover, because the hemodynamic signal integrates across a volume and is biased towards superficial layers, laminar differences in processing cannot be resolved. Nevertheless, intrinsic optical imaging studies to a large degree confirmed the presence of tonotopic maps, within their limited resolution (which is not better than that of electrophysiological mapping using electrode penetrations).

The case of the mouse
Compared with humans, the hearing range of the mouse is significantly higher and nearly half as wide (in octaves: approximately 3 kHz to 100 kHz, approximately 5 octaves as compared to 20 Hz to 16 kHz, approximately 10 octaves). Despite these differences, the mouse is becoming an increasingly popular model for studies of the auditory cortex. Many of the newer imaging and optogenetic techniques have been pioneered in the mouse, and the availability of genetically modified mouse strains makes it possible to apply a large arsenal of molecular manipulations.

The tonotopic organization of fields A1 or AAF as well as several secondary auditory fields have been identified in the mouse auditory cortex using conventional microelectrode mapping of multiunit spiking in the thalamorecipient layers [25,44–48] or low resolution optical imaging of a voltage-sensitive dye [49], flavoprotein autofluorescence [50,51], and intrinsic signals [52]. Tonotopic organization in the middle layers of mouse A1 probably arises from topographically organized feedforward projections from the MGBv [44] (Figure 1A). Point-to-point thalamocortical connectivity has also been demonstrated in an acute thalamocortical slice preparation that preserves synaptic connections between MGBv and A1 [53–56]. For example, by bathing the brain slice in a voltage-sensitive dye, it has also been possible to demonstrate a point-to-point functional mapping between a discrete stimulation site within the MGBv and a focus of activity within the tonotopically aligned region of A1 [44,57].
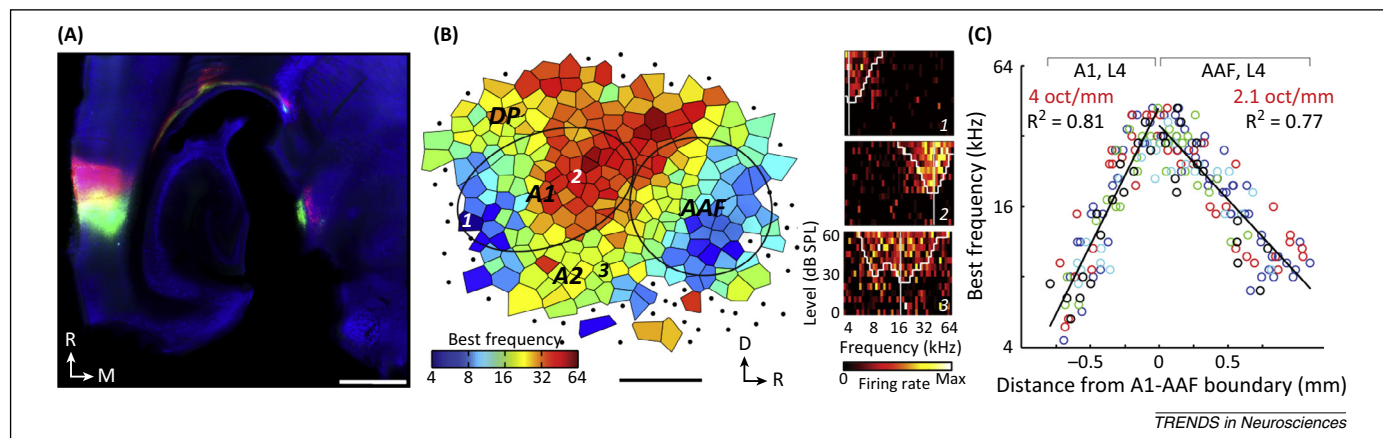

Figure 1. Evidence for order: large-scale tonotopy within the middle cortical layers of the mouse auditory cortex. **(A)** Auditory thalamocortical slice immunoreacted for parvalbumin (blue). Retrograde tracers (cholera toxin β subunit) conjugated to a green or red fluorophore were injected into a low- (7 kHz) or mid-frequency (22.6 kHz) region of the A1 map, respectively. The A1 injection sites appear at the left of the image, the labeled thalamocortical axons in the middle of the image, and the retrogradely labeled ventral subdivision of the medial geniculate body (MGBv) cell bodies to the right of the image. Scale bar = 0.25 mm. **(B)** A tessellated best frequency (frequency that elicits the most spikes across all levels) map delineated from 300 multiunit recording sites in the middle layers of the area identified in (A). Note the clear tonotopic gradient within the primary auditory cortex (A1) and the anterior auditory field (AAF) compared with the non-tonotopic organization of the remaining fields. Right, tonal receptive fields from A1 (top and middle) and secondary auditory cortex (A2; bottom) measured at the numbered locations shown on the tessellated map. **(C)** Best frequency distribution along the caudal-to-rostral axis through A1 and AAF. Distance is relative to the mirror reversal in best frequency that indicates the boundary between each field. Data from individual mice are represented by different colors. Unbroken black lines indicate the linear fit of the A1 and AAF data. For further details regarding data in (A–C), see [24].

Thus, recent efforts to characterize the functional organization of the thalamorecipient layers through *in vivo* mapping (Figure 1B,C), low resolution *in vivo* imaging, *in vitro* functional connectivity studies, and anatomical connectivity studies all point towards a precisely organized gradient of sound frequency in A1 that arises from tonotopically organized projections from MGBv. The feedforward thalamocortical connectivity and tonotopic organization of the mouse auditory cortex are in close agreement with extensively studied model systems such as the rat [58] and the cat [59]. However, it is critical to note that all of these techniques are limited to a relatively coarse spatial resolution of 0.1 mm or more. As such, they can be used to reconstruct the macroscopic organization of the auditory cortex but offer very little insight into the finer scales of spatial organization that may exist between neighboring neurons.

High resolution imaging: beyond smooth tonotopy

Although the results surveyed up to this point seem to have settled the issue of the existence of a tonotopic map in the auditory cortex, the picture has been muddled again when Ca^{2+} indicators such as Oregon Green Bapta-1 (OGB-1) have been introduced into neurons in live animals and *in vivo* Ca^{2+} signals have been measured with two-photon imaging [60–63] (Box 1). The Ca^{2+} signals are due to voltage-activated currents, and when measured from neuronal somata they reflect action potentials generation. The Ca^{2+} signals are somewhat slow (with a rise time of a few tens of milliseconds and a decay of hundreds of milliseconds), although they are much faster than intrinsic signals. Although Ca^{2+} signals are typically too slow to document the occurrence of single action potentials, in the auditory cortex they are roughly proportional to the number of action potentials that occurred within a window with a duration of 50–100 ms [64], and can therefore be used to document frequency selectivity in auditory cortex neurons. The fluorescence evoked by Ca^{2+} entry into the neurons can be read in several different ways. The highest spatial resolution is achieved by using two-photon scanning microscopy, which sequentially illuminates only a small voxel (~1 fl, depending on illumination wavelength and the numerical aperture of the objective) of brain tissue. *In vivo*, the technique makes it possible to record transients from single neurons and even from subcellular structures (dendrites [65] and even spines [66]). Furthermore, the sparse illumination prevents bleaching of out-of-focus focal planes [60,61]. By sequentially imaging many voxels in one imaging plane rapidly, it is possible to sample the Ca^{2+} signals from many neurons essentially simultaneously and therefore create activity maps with single cell resolution. Because of light scattering in brain tissue, initial implementation of this technique was focused on imaging activity in supragranular layers [60–63].

A highly influential application of this technique to the primary visual cortex (V1) revealed species differences in its micro-organization. Whereas cats had orientation columns, rodents showed a salt and pepper organization of single neuron orientation preference [62,67] and substantial heterogeneity of spatial receptive fields both within a tangential imaging plane [68] and across a cortical column [69]. Similar studies in rodent primary somatosensory

cortex (S1) revealed that at the neuronal population level, whisker selectivity varied smoothly over the cortical surface but that whisker selectivity of neighboring neurons could differ considerably [70,71].

In vivo two-photon imaging in the supragranular layers of mouse A1 [64,72] demonstrated that sound evoked Ca^{2+} transients could be reliably measured from single neurons. Individual neurons responded to sounds and were frequency- and sound level-selective. Unexpectedly, the frequency selectivity of neighboring neurons was often very different (Figure 2A,B), and over spatial scales of <200 μm no reliable tonotopic gradient could be observed, despite the fact that in the best published maps of mouse auditory cortex [46] the frequency gradient of A1 is approximately 2–4 oct/mm. However, when multiple fields separated by more than approximately 200 μm were imaged in the same animal, the expected shift in average frequency preference was observed [64,72] (Figure 2A). Best frequency was the only response property that showed even approximate spatial order in these experiments. Other stimulus properties, such as bandwidth, produced highly heterogeneous distributions of preferences [72].

These data, representing integrated somatic responses, have received support from *in vivo* imaging experiments that measured the frequency tuning of single spines of layer 2/3 neurons using fast two-photon Ca^{2+} imaging. These experiments showed that individual spines on single layer 2/3 neurons could be tuned to very different frequencies [66] (Figure 2C,D). Moreover, these differences were observed even among neighboring spines on the same dendritic segment, suggesting a salt and pepper organization of synaptic tuning on single layer 2/3 neurons.

This is not to say that there is no evidence for significant local order in the auditory cortex when using Ca^{2+} imaging. Three types of evidence to that effect emerged from these studies. First, although responses of simultaneously imaged cells were heterogeneous, neurons did show high noise correlation, suggesting that they might form interconnected networks [64,72,73]. These noise correlations decreased with distance between neurons, with a spatial scale of approximately 100 μm [64,74] (see also [75]), suggesting that neuronal interactions are organized within anatomical columns. Second, different Ca^{2+} dyes have different affinity for Ca^{2+} and thus can report different aspects of the neural response. The widely used indicator OGB-1 is a high affinity indicator and can report both subthreshold and suprathreshold signals, at least under *in vitro* conditions [72]. Thus, a fraction of the imaged fluorescence signal can reflect synaptic inputs to neurons, rather than their spiking activity. Moreover, because in the auditory cortex spike rates are relatively low compared with V1, the fraction of subthreshold responses in the OGB-1 signal is higher than in V1. By contrast, Fluo-4 has a low Ca^{2+} affinity and thus does not report subthreshold responses [71,72]. When the spatial distribution of frequency selectivity was studied by using either dye it was observed that the responses were more spatially homogeneous with OGB-1 than with Fluo-4, suggesting that the additional subthreshold contribution increased spatial homogeneity possibly due to the wide frequency range of synaptic inputs to layer 2/3 [66]. Because a large fraction of
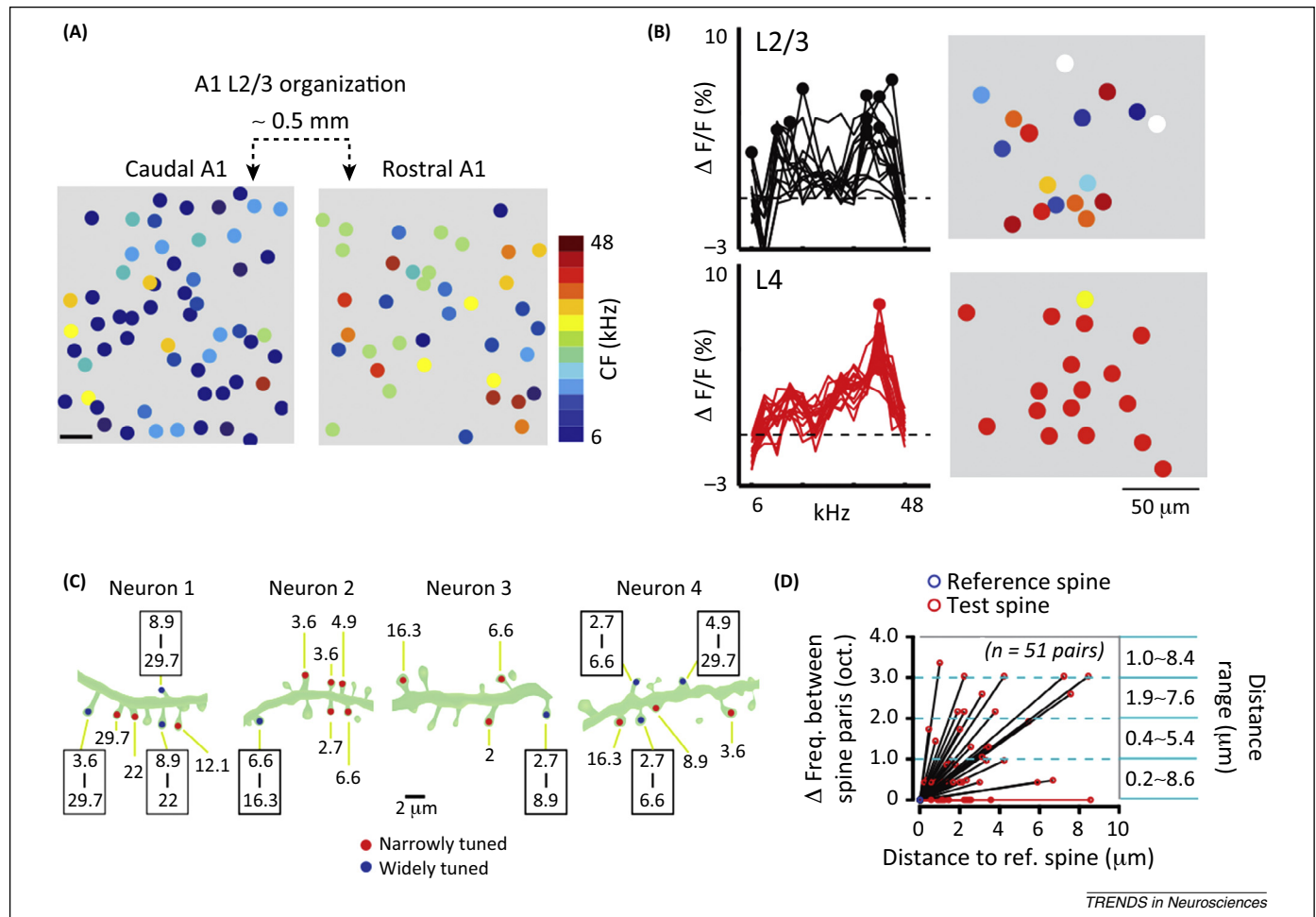
Figure 2. Evidence for disorder: single cell imaging shows heterogeneity in supragranular layers. **(A)** Reconstruction of two imaging sites from layer 2/3 in one mouse. Characteristic frequency (CF, frequency tuning at threshold) for each cell illustrates both local heterogeneity at local scales and a coarse tonotopic organization at larger spatial scales (for further details, see [7]). Scale bar = 10 μm. **(B)** Fractional changes in fluorescence measured from a single imaging site in layer 2/3 and a second imaging site from layer 4 of the same column illustrates the shift from homogeneous to heterogeneous frequency tuning between the thalamic input layers and superficial layers (for further details, see [74]). The precise low resolution tonotopy observed with microelectrode recordings from layer 4 (Figure 1C) is therefore well matched with the coarse tonotopy over large spatial scales in layer 2/3 (A) and the similar frequency tuning organization within local layer 4 ensembles (A, bottom). **(C)** The bandwidth and center frequency of Ca^{2+} response-based sound tuning between neighboring spines on a single dendrite are highly heterogeneous. Cartoons depict dendritic segments from four layer 2/3 neurons, with numbers indicating the effective range of tone frequencies for each spine. Narrowly tuned and widely tuned spines are indicated by red and blue dots, respectively. **(D)** Plot of the distance between neighboring sound-responsive spines versus their best frequency (for further details, see [66]).

inputs to supragranular layer 2/3 neurons originates in layer 4, these results suggested that layer 4 might be more homogeneously organized in frequency than layer 2/3. The third type of evidence for local order in A1 comes from recent *in vivo* two-photon Ca^{2+} imaging of layer 4 and layer 2/3 neurons in the same animal. This study directly demonstrated that the representation of frequency is much more homogeneous in layer 4 than in layer 2/3 [74] (Figure 2B).

Comparing different methodologies
Which picture of the tonotopic organization of auditory cortex is the valid one? Is it the smooth tonotopic organization that emerges from low resolution imaging and microelectrode mapping or is it the heterogeneous organization that emerges from two-photon imaging? Or may both pictures be different approximations to the same reality?

Many of the differences between the different methodologies are probably due to increased spatial resolution of two-photon imaging over electrophysiological methods as well as different sampling biases. Two-photon imaging has a spatial resolution of ~1 μm, whereas electrophysiological mapping experiments sample at 50–100 μm and intrinsic optical signals represent activity on even coarser spatial scales. Thus, much of the heterogeneity that is seen on very small spatial scales is not readily accessible to electrophysiological methods. The cortical layer from which the neurons have been recorded is difficult to identify from electrode depth readings, and essentially no modern mapping study in auditory cortex attempted to precisely localize the recording sites using lesions. This factor is of concern, especially in small rodents, as evidence of layer specificity of the neuronal responses in auditory cortex accumulates fast [74,76–81]. Of particular relevance is the observation that sound-evoked spiking responses of auditory cortex are sparse [78,82,83], and that responses of layer 2/3 neurons are weaker driven and sparser than layer 4 neurons [74,78], and are more likely to have irregular tuning for pure tone bursts, the stimuli

used to characterize tonotopic organization [25]. Thus, electrophysiological mapping is presumably biased towards the responses of layer 4 neurons, with this bias possibly increasing due to anesthesia or state changes [83].

Comparing the biases of the different methods is more difficult. Electrophysiological mapping experiments are most often done using multiunit recordings, without special attempt to separate the activity of single neurons. They are therefore biased towards the most robustly driven neurons, which dominate the multiunit signal. As a consequence, weakly driven cells or neurons that do not generate large extracellular potentials will be less represented. Two-photon imaging, by contrast, introduces other biases. Because inhibitory cells buffer Ca^{2+}, these cells will not generate large Ca^{2+} transients. Moreover, when using synthetic dyes there can be loading differences over an imaged area as well as intermingled loading of neurons and astrocytes. To compensate for the difference in baseline fluorescence, typically the fractional change in fluorescence is often used. However, potential differences in loading could lead to different recording qualities in different cells. Imaging data have to be interpreted carefully, as calcium transients reliably reported action potentials only when the optical plane intersected with the center of the soma [64,71]. These factors could artificially increase the variability of the neuronal response areas measured with calcium imaging techniques.

The olive branch
Because of these methodological issues, we currently favor a view that integrates both sets of results into a common framework. This framework should be considered as a working hypothesis to guide and be refined by future experiments. In this framework, tonotopy is the major organizational principle of the input to A1, even in mice.

There is clear evidence for a tonotopically organized forebrain region in mammals and birds, in which the auditory transduction organ converts sound frequency into a cochleotopic gradient of electrical activity. In this regard, tonotopy may be an epiphenomenon of an ancient organizing principle that predates the evolutionary split between mammals and birds; namely, neurons make topographic projections to other neurons. In the case of tonotopy, the spatial frequency gradient constructed by the auditory periphery is largely preserved throughout the lemniscal divisions of the ascending central auditory pathways, ultimately culminating in tonotopically organized MGv projections to middle layers of the primary auditory areas, where it can be reconstructed through microelectrode recordings.

However, as in other cortices, notably somatosensory cortex, the projections from layer 4 to layer 2/3 are divergent, and the same neuron may receive very different frequency-specific inputs [66] (Figure 2C,D). Under the appropriate conditions, such neurons may still be reasonably narrowly tuned to frequency (see [84] for orientation selectivity in visual cortex under similar conditions), but now neighboring neurons may show very different frequency tunings, even though they share substantial amount of input (as indexed by their noise correlations) [64,73,74]. This diversity is specifically reflected in the two-photon studies of neuronal responses in the supragranular layers [74].

A transition from precise, homogeneous frequency organization in layer 4 to coarse, diffuse organization in layer 2/3 has also been described in recent low resolution imaging and microelectrode recording studies. In the thalamocortical slice preparation, moving a stimulating electrode from low to high frequency areas of the MGBv reveals an orderly march of voltage-sensitive dye response peaks across the low-to-high frequency extent of A1 in layer 4, yet the topography is significantly degraded in layer 2/3 [44]. Moreover, the precisely organized frequency gradient commonly observed with microelectrode mapping from layer 4 (Figure 1C) is substantially degraded when tonotopy is reconstructed from layer 2/3 recording sites [25]. Thus, approaches to characterize functional organization at low and high spatial resolution have converged on a laminar transformation from homogeneous frequency tuning in the thalamorecipient layers to distributed, heterogeneous frequency tuning in superficial layers.

Lessons to other sensory systems
The rapidly increasing information about fine structure of the representations in a number of sensory cortices suggests that all sensory cortices share many similarities, but also show significant differences. Studies in mouse V1 showed that although retinotopy was rather robust on large scales, it was heterogeneous on small scales [85]. This heterogeneity with respect to the organization of the periphery receptor might be an organizing feature of at least mouse layer 2/3 [86]. Nevertheless, the functional heterogeneity in layer 2/3 of auditory cortex seems to be more pronounced than in V1. This could be due to the fact that in contrast to visual objects, auditory objects often co-activate distant frequency channels and are thus less likely to be adequately represented by narrowly tuned, tonotopically organized sheets. This difference between the physics of auditory objects on the one hand and visual objects on the other hand may be crucial for understanding A1, as well as in directing our attempt to elucidate its function. It has been suggested that the relatively short intracortical connectivity length is an important organizational principle of the brain [87,88]. One possible consequence of this principle is that locally interconnected neurons code for the ethologically relevant entities ('auditory objects') that arise from auditory processing. Thus, elucidating the functional properties of neighboring neurons and of the interactions between them is a way to identify what features A1 encodes. For example, neighboring interconnected neurons may be individually activated by sound frequencies with certain frequency relationships; as an ensemble, they could then encode a complex sound feature. Thus, by investigating the relationship of tuning properties of local populations, taking into account both order and heterogeneity of these properties, we might be able to infer what A1 can encode.

Lessons to other species
Much of the tonotopy controversy in its most recent reincarnation was centered around the mouse model of

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Box 2. Outstanding questions

- What circuits give rise to the heterogeneous organization in layer 2/3?
- What are the functional relationships between neighboring cells in layer 2/3?
- What is encoded by layer 2/3 neurons?
- What is the relationship between tuning properties and local circuit connectivity?
- What are the specific roles of identified classes of neurons in shaping order and disorder in A1?
- Are there species-specific differences in A1 organization?

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auditory cortex. It could be that the small brain size of mice does not support homogeneous organization by sensory maps. Although the cortical micro-organization of small carnivores has not been examined, both small and large rodents lack orientation maps in V1 [62,89], suggesting that rodents and carnivores might have evolved different cortical processing strategies. However, local diversity in V1 response properties may not be restricted to smaller brains. Paired extracellular or intracellular recordings in cat V1 have also shown considerable receptive field heterogeneity between neighboring neurons [90,91].

The picture in the auditory cortex is less clear. Early electrophysiological evidence in cats [12,22] in addition to more recent data in ferrets [92] also suggest the presence of local disorder in carnivore auditory cortex. By contrast, a recent study of micro-organization based in A1 of cats observed that neighboring neurons, particularly in the supragranular layers, were precisely synchronized with highly similar receptive field properties for stimulus features related to sound frequency [76]. Thus, more work is required to determine whether the mixture of homogeneity and heterogeneity in early sensory cortices is a general principle of mammalian processing or might be exaggerated in small rodents either as an evolutionary adaptation or as a byproduct of cortical wiring constraints in a physically smaller brain (Box 2).

Concluding remarks

As spatial resolution of experimental techniques allow us to observe more neurons in small areas of the brain, a level of heterogeneity becomes obvious that has not been appreciated with traditional low resolution techniques. Although the smooth cortical organization uncovered at low resolution scales has provided an essential framework for understanding the organization and plasticity of primary sensory cortex, dynamic interactions between local cortical assemblies await discovery with approaches that reconstruct cortical circuits with cellular resolution. In particular, the interplay between homogeneity and heterogeneity in the organization of primary auditory cortex may give rise to a combined picture that demonstrate how the two can co-exist, and how the interplay between the two is crucial for understanding hearing, sensory processing in general, and possibly other brain functions as well.

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