Auditory Cortical Contrast Enhancing by Global Winner-Take-All Inhibitory Interactions

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Abstract

Brains decompose the world into discrete objects of perception, thereby facing the problem of how to segregate and selectively address similar objects that are concurrently present in a scene. Theoretical models propose that this could be achieved by neuronal implementations of so-called winner-take-all algorithms where neuronal representations of objects or feature elements interact in a competitive manner. Here we present evidence for the existence of such a mechanism in an animal species. We present electrophysiological, neuropharmacological and neuroanatomical data which suggest a novel view of the role of GABA\textsubscript{A}-mediated inhibition in primary auditory cortex (AI), where intracortical GABA\textsubscript{A}-mediated inhibition operates on a global scale within a circular map of sound periodicity representation in AI, with functionally inhibitory projections of similar effect from any location throughout the whole map. These interactions could underlie the proposed competitive “winner-take-all” algorithm to support object segregation, e.g., segregation of different speakers in cocktail-party situations.

Introduction

The parcellation of sensory input into perceptually distinct objects is a basic ability of fundamental importance for all higher animals (e.g. [1–3]). However, the neuronal mechanisms by which multiple and often similar objects that are concurrently present in a scene can be separated are presently not understood. It has been suggested (e.g. Ref. [4]) that this can in principle be accomplished by a so-called winner-take-all algorithm. In general, a winner-take-all computational algorithm describes a process where several active elements in a (neuronal) network compete for the resources of the whole network, resulting in a state where one element (the “winner”, which for example is the most active element) suppresses the activity of all other elements in the network and thereby remains as the only active element within the network while all other elements are inactive (“losers”). In the context of sensory scene analysis this means that the neuronal representation of one perceptual object suppresses that of other concurrent objects. Despite ample demonstration of the usefulness of the winner-take-all algorithm in theoretical work [3–8] physiological evidence for its existence in living brains is still lacking (e.g. Ref. [9]).

In central sensory systems perceptual objects are believed to be formed by binding together stimulus features that belong to the same object [10]. Such features are represented in functional maps, in which the parameter space of a feature is systematically analyzed by neuronal filters each selective for a certain range of the parameter space. Therefore, the physiological implementation of a winner-take-all algorithm poses specific constraints on the functional organization of neuronal interconnectivity patterns and their recruitment during stimulus processing. Here, we hypothesize that a winner-take-all process would require a neuronal interconnectivity pattern by which any location within a feature map is allowed to inhibit all other locations in a global fashion (cf. Fig. 1B,D). In the case of multiple sensory objects which differ in the feature that is represented within the map this would lead to a thalamic input to multiple areas within the cortical map resulting in an initial state with multiple active spots within the map. These active spots would then activate inhibitory interconnections between each other, and the strongest inhibitory input should be provided by the most active spot within the map. Consequently this spot might receive less inhibition than it would impose on other locations, resulting in an activity pattern within the feature map where only one spot which had the strongest activity in the beginning (the “winner”) would still be active while all other locations in the map would be silent. Note, that this slightly higher activity of the winner not necessarily has to result from stronger thalamic input but could as well be the result of some top-down influence of higher cortical areas, e.g. of those that control attentional demands [11,12].
Figure 1. Conceptual framework of the study. The concept of local cortical (lateral) inhibition (left) is contrasted with that of global cortical inhibition (right), as illustrated by examples of a tonotopic and a periodicity map obtained by optical imaging in gerbil AI (A,B, cf. Ref. [17]) and by schematic drawings of the interconnectivity pattern (C,D). A: Different colors within the tonotopic map depict representations of different pure tone frequencies from low (blue) to high (red). B: Different colors within the periodicity map depict representations of different AM tone periodicities from low (blue) to high (red). The concept of local (lateral) inhibition proposes an inhibitory interconnection pattern, whereby any given unit (or stimulus representation) inhibits only its immediate neighbors within the parameter space (A,C: red projections), resulting in local contrast enhancement. The concept of global inhibition proposes an inhibitory interconnection pattern, whereby any given unit (or stimulus representation) inhibits all other representation within the parameter space (B,D: blue projections, inhibitory interneurons are not shown), resulting in the implementation of a “winner-take-all” algorithm, i.e. global contrast enhancement. Note that since any BP representation in a circular map has an eccentric location, projections from any BP representation within the map to all other locations of the map (blue lines) result in an asymmetric geometry of projections (cf. Fig. 6B). Gray lines: inactive projections. Black arrows: Thalamic input. E,F: Stimulation paradigms (schemes) used to test local (red) and global (blue) inhibitory concepts, respectively. E: Isointensity frequency response functions (light red curve) are usually obtained by plotting pure tone (vertical dark red line) evoked discharge rates as a function of tone frequency. F: Stimulation paradigm used here in the competitive interaction experiment. Two AM tone complexes were presented simultaneously (duration: 200 ms, 65 dB SPL). Spectra of both AM tone complexes (vertical dark blue lines) were entirely outside the frequency receptive field (FRF; light blue curve; cf. Ref. [33]) of the unit, which in our experiments always meant above the FRF, because all recorded units showed responses to low frequency pure tones. One of the complexes had a fixed fm (f1/fm1) set to best periodicity of one of the units, the second had a different fc (fc2) and varied in fm (fm2). Note that fc2 could be higher or lower than fc1 in the experiments, but both AM spectra were always completely above the units’ FRF.

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It is notable that in contrast to this idea, intracortical inhibition in AI has so far been conceptualized predominately on a local scale, i.e. as lateral inhibition, whereby neurons in a given location of a map inhibit only their direct neighbors with a decline of inhibitory influence according to some space function (cf. Fig. 1A,C; e.g. Refs. [13–16]), and long-range inhibitory projections have only very rarely been demonstrated [17].

For the auditory cortex, local inhibition is supported by studies demonstrating that a stimulus within an inhibitory sideband of a neuron’s receptive field can suppress responses to a stimulus concurrently presented within the excitatory center [18–20], or that excitation and inhibition of neurons are co-tuned, i.e. they show approximately the same dependence on frequency and intensity of a pure tone stimulus [21]. Also neurons exhibiting multiple inhibitory areas can be considered as reflecting local inhibitory influences [22]. For these types of inhibitory action it is not clear, however, whether they occur in cortex or are transmitted from some subcortical level (cf. Ref. [23]). Microiontophoretic studies in auditory cortex with pharmacological blockade of GABA<sub>A</sub>-mediated inhibition can provide more direct evidence and have shown broadening of frequency tuning curves with bicuculline (BIC) [13–16]. But this effect is controversial as it is not seen with the more specific GABA<sub>A</sub>-antagonist gabazine [23] pointing to the possibility that at least part of the extensive GABAergic neuronal systems in auditory cortex serve other than local inhibitory functions. Based on these contradictory data, the whole concept of sensory neurons acting as feature detectors is currently under debate, and it has been suggested that neuronal activity within auditory cortical maps represents auditory objects rather than stimulus features [24,25].

A suitable substrate to test for more global mechanisms of inhibition under the winner-take-all concept is a recently described periodicity map in gerbil auditory cortex [4]. This map is functionally superimposed on the tonotopic map of the primary field AI. The almost circular functional gradient for different sound periodicities is a geometry that could support inhibitory connections of similar effect from any location throughout the map. An interesting applied aspect of competition between different sound periodicities is its implication for voice segregation in a cocktail-party situation [26].

Here we have used a combination of electrophysiological, neuropharmacological and neuroanatomical techniques to investigate whether a global inhibitory interconnectivity pattern for object segregation is indeed realized in the periodicity map of the auditory cortex. We specifically address the following questions: (1) Does the neuronal activity which represents a certain stimulus within the circular periodicity map suppress the neuronal response to a concurrently presented second stimulus which is represented somewhere else in the map? (2) If so, is this suppression mediated by GABA<sub>A</sub>-mediated inhibition? (3) Do direct projections within the map have the appropriate length and topography to interconnect different frequency domains of the cyclic periodicity map; and if so, do these projections terminate on inhibitory interneurons in order to provide an anatomical substrate for suppressive competitive interactions between representations of different periodicities?

**Results**

Simultaneous recordings demonstrate competitive interactions within AI

In a first set of experiments we tested the hypothesis that inhibition within AI acts globally, implementing a “winner-take-all” algorithm for sound object segregation on the basis of periodicity discrimination. In 6 anaesthetized animals, we made simultaneous recordings in two regions of AI representing different periodicity ranges. Recordings were made in situations where only one amplitude modulated (AM) tone (i.e. one auditory object with a particular periodicity) was presented as well as in situations where two AM tones were presented concurrently (see Fig 1F). Responses from a total of 24 simultaneously recorded pairs of units were obtained. All these units were located in the low frequency area of AI and had BFs ≤ 5 kHz. Of these, 19 showed a response behavior consistent with the global inhibition hypothesis.

An example of this type of behavior is shown in Fig. 2 which compares the responses of two units with different best periodicities (BP) for AM tones (A,B), which were recorded simultaneously from neighboring periodicity representations in AI (cf. scheme of periodicity map between left and middle column). The left column (A,B) shows the different responses of the units to AM tones with a common carrier frequency (fc) of 12 kHz and modulation frequencies (fm) which varied between 0 (unmodulated carrier) and 3 kHz. The rate modulation transfer functions of these responses (rMTF = plot of evoked spike rate as a function of the fm of the AM tone) revealed a BP of 600 Hz for the unit in A and a BP of 900 Hz for the unit in B. Similar recordings during stimulation with an AM carrier of 8 kHz revealed identical BP values in both units (not shown).

The middle column (C,D) shows the responses of the same units during stimulation with two simultaneously presented AM tones. In panel C the unit from (A) is always stimulated with its optimal AM tone (fc = 12 kHz; fm = 600 Hz) and in addition with an AM tone of 8 kHz fc and varying fm. This varying fm of the second AM tone is plotted on the ordinate in panels C to F. As can be seen from the response plane diagram, this unit responded only when the fm of both AM tones matched its AM tone receptive field, although its optimal AM tone was present throughout all stimulus conditions. The presence of the second AM tone therefore suppressed the response of this unit to its optimal AM tone as long as the periodicity of the second AM tone fell outside the AM tone receptive field of the unit. In contrast, the second unit (panel D) showed the reverse response behavior. As for the previous unit, the optimal AM tone complex was presented in all stimulus conditions (fc = 8 kHz, fm = 900 Hz) in addition to a second AM tone with a fc of 12 kHz and varying fm (cf. Fig. 1F). In this case, however, the unit responded to its optimal AM tone irrespective of the periodicity of the second AM tone. Note, that this type of response behavior does not reflect a loss of stimulus selectivity of this unit: This unit still responded highly selective to the AM tone with its optimal fm (= BP), simply “ignoring” other stimuli which were presented simultaneously.

The right column (E,F) shows the responses of both units that were recorded when the fm of the fixed AM tone complex was set to a value different from the BP of the units. As can be seen, the responses look qualitatively similar in E,F as in C,D, although the responses were a little weaker in E,F compared to C,D, respectively. Based on our model of competitive “winner-take-all” interactions in AI this is exactly what would be expected: In the cases where the fixed AM tone had a non-optimal fm, the fm was still in the AM tone receptive field of the unit (cf. A,B); that is, should elicit a weaker response than an AM tone at BP. In combination with the second AM tone, the loser-type unit (E) still responded only in cases, where both AM tones had periodicities in the AM tone receptive field of the unit, that is, under conditions where there was no competitive interaction in AI. Note, that in this experiment the second AM tone with varying fm sometimes had a fm at or close to BP. In contrast, the winner-type unit (F) always responded to the fixed (off-BP but within AM tone receptive field)
When both AM tone complexes had similar periodicities, that is, when both periodicities were within the receptive field of the unit, the strength of the response to the best AM tone complex could be modulated by the second AM tone complex: Strongest responses were typically observed when both AM tone complexes were presented at their respective best periodicities (the 'winner') always responded to its best AM tone complex irrespective of the periodicity of the concurrent AM tone complex. Note that the two AM tone complexes when the periodicities of both AM tone complexes fell within its periodicity receptive field. By contrast, the unit in Fig. 2C (the 'loser') responded only to the second AM tone, but the response was weaker that in D, except in those cases where the second AM tone hit the BP of the unit or was close to it (within the AM tone receptive field).

Winner-take-all competition is that the two AM tone complexes (which were recorded simultaneously during stimulation with either a single AM tone complex \(A_1, B_1\) or 2 concurrently presented AM tone complexes \(C-F\)) interact with each other. The result of the interaction depends on the periodicity of the concurrent AM tone complexes (in steps of 150 Hz) and the periodicity receptive field. To exclude this possibility we performed control experiments (not shown) where a single AM tone at BP was presented 200 times while the second AM tone was omitted. In the five units tested in this manner we did not observe any signs of adaptation.

Of 24 cell pairs tested with this paradigm, 10 showed the above type of behavior, with one unit behaving like a 'loser' and the other unit like a 'winner'. However, in nine pairs both units behaved like 'losers'. This is qualitatively what one might expect given that we were only able to record from two units simultaneously. If our hypothesis is correct, at any given time there should be many 'losers' within the map but only one 'winning' BP-representation. For the remaining 5 pairs both units behaved like 'winners' (see Discussion). It is also worth pointing out that two of the ten winner-loser-pairs recorded showed a 'switching' type of behavior, whereby one unit was the 'winner' during the measurements with the first set of two AM tone complexes (where the fm of the fixed AM tone was set to BP of the first unit), whereas the other unit was the 'winner' during measurements with the second set of stimuli (where the fm of the fixed AM tone was set to BP of the second unit). This change of a winner- to a loser-type behavior was also observed in five additional units that were recorded separately in single-electrode recordings. This switching behavior might be counterintuitive at first sight, but it has to be expected from a mechanism that is able to dynamically select and segregate an auditory object out of a combination of concurrent objects as the relevant object that has to be attended by a subject may vary over time. We will discuss this phenomenon in more detail below (Discussion section).

Quantitative comparison of winner- and loser-responses
As described above, a unit was considered a 'winner' when it responded to its optimal AM tone complex independent of the second tone complex presented in a certain experiment. A criterion
for this decision was a significant excitatory response to all AM tone complex combinations in response plane histograms (cf. Methods). All unit responses that did not fulfill this criterion were considered 'losers'. After this qualitative classification of responses we performed a quantitative comparison of winner- and loser-type responses on the basis of tuning properties or rate functions (Fig. 3).

Fig. 3A replicates the classification of response type on the basis of rate functions: Here, the response range of the units divided by the range of AM tone complex combinations presented is given for all units classified as either winner or loser. As expected, this value is 1 for all winners (blue), except in three units where the response to one AM tone complex combination dropped below significance although there was a significant response in the response plane histogram (an effect which is due to the different time windows which were used for the two types of analysis). In contrast, the values for the losers (red) vary over a wide range between 0 and 1. Mean values and standard deviations are given between the single data points (Winners: mean = 0.999, SD = 0.003; Losers: mean = 0.563, SD = 0.234).

Fig. 3B compares the sharpness of tuning as expressed by Q-values (cf. Methods) of winners (blue) and losers (red). Although winners responded to each pair of AM tone complexes (per definition) response strength could vary so that filter characteristics and BP could be defined for winner-type responses (the criterion for classification of filter type was set to 50% of maximal response, cf. Methods).

For winners, Q-values were restricted to values between 0 (non-selective filter type where no BP could be determined) and 1 (BP equals upper border of BP presented). In contrast, Q-values of losers were never 0, varied over a wider range exceeding 1 and were significantly larger than those of winners (Winners: mean Q = 0.25, SD = 0.30; Losers: mean Q = 0.83, SD = 0.87; ANOVA P = 3.8^{-4}).

A comparison of tuning characteristics of the condition where only a single AM tone complex was presented with the condition, where two concurrent AM tone complexes were presented reveals another difference between winners and losers (Fig. 3C): Whereas the tuning for the winners was always sharper during the single-AM condition compared to the double-AM condition, that is, Q-values decreased from the single-AM to the double-AM condition, Q-values of losers could either decrease or increase from the single-AM to the double-AM condition. A paired t-test revealed
that these changes in Q-value were significant across the population of winners, but not significant across the losers (Winners: single-AM: mean Q = 1.04, SD = 0.50; double-AM: mean Q = 0.14, SD = 0.23, paired t-test: P = 2.6E-4; Losers: single-AM: mean Q = 0.96, SD = 0.36; double-AM: mean Q = 1.00, SD = 0.06, paired t-test: P = 0.44).

Finally, winners and losers showed largely different frequency distributions of filter types of rate functions recorded in response to two AM tone complexes (Fig. 3D). Whereas complex filter characteristics were found for both winners and losers, band-pass and low-pass filter characteristics were encountered only in loser-type responses. High-pass, non-selective and band suppression filter characteristics were found only in winners. Note the high percentage of non-selective tunings in losers.

**Iontophoretic application of BIC modulates competitive interactions within AI**

The experiments described above demonstrate the existence of suppression of neuronal representations of some periodicities within the periodicity map in AI during simultaneous stimulation with two concurrent periodic sounds. To test whether the suppressive interactions resulted from GABA\(_A\)-mediated inhibitory processes, we examined the effect of iontophoretic application of BIC on the responses of single AI units to stimulation with two simultaneously presented AM tone complexes in a total of 27 units. We hypothesized that if the suppression were GABA\(_A\)-mediated, a cell showing a ‘loser’-like response behavior might show a ‘winner’-like response behavior during blockade of GABA\(_A\)-receptors via iontophoresis of BIC at the recording site (see Method section). As illustrated in Fig. 4, we found that this was indeed the case.

Fig. 4A shows the response of a ‘loser’ in a stimulation situation with two concurrent AM tones. Similar to Fig. 2C, this unit responded to the combination of AM tones only if the periodicities of both sounds fell within its AM tone receptive field. The response to the AM tone with the BP of that unit (which was present in all stimulus combinations presented) was inhibited by the presence of any AM tone whose periodicity fell outside the unit’s AM tone receptive field. Fig. 4B shows the responses of the same unit to the same stimuli during iontophoretic application of BIC. The unit now responded to the AM tone with its BP irrespective of the periodicity of the second AM tone complex. This is indicative of a ‘winner’-like response behavior. The effect was reversible, with the unit again showing a ‘loser’-like response behavior to concurrent AM tones within 20 min of the termination of BIC application (Fig. 4C).

This type of behavior illustrated in Fig. 4 was observed in 10 of 22 units (the responses of 5 units were to weak to be analyzed quantitatively), (cf. Fig. 5A, blue). Another 6 units showed a widening of the periodicity range under BIC (cf. Fig. 5A, red, dots above diagonal). In the context of our model proposed here this would imply that the inhibition imposed on these 6 ‘losers’ by units activated by the second AM tone could not be blocked completely, but nevertheless – and in contrast to control conditions - they maintained responses to their BP when the competing AM tone had fallen in the vicinity of their AM receptive fields. One unit showed a shrinking of the periodicity range under BIC (cf. Fig. 5A, red, dot below diagonal). The remaining 5 units showed a winner-type response behavior before BIC-application and maintained this behavior during BIC-application (cf. Fig. 5A, pink). In summary, all units manipulated with BIC except for the one loser that showed a shrinking of the periodicity range under BIC ( = 21 out of 22) showed response behavior consistent with our model of competitive winner-take-all interactions.

Fig. 5B shows a quantitative analysis of the described effect of BIC on tuning properties of the responses to competing AM tone complexes in AI units: For all units except one the Q-values decreases during application of BIC, but the effect is stronger in units that changed their response behavior from a loser- to a winner-type behavior (blue) than in those that remained losers during the BIC condition [red] (ANOVA: Changes in Q from control to BIC condition in blue vs. red group: P = 0.05). The change in Q from control to drug condition was significant in the blue group [loser to winner, mean Q control = 1.12, SD = 0.63; mean Q BIC = 0.35, SD = 0.28, paired t-test: P = 0.005), but there was only a trend to smaller Q-values in the red group [loser to winner, mean Q control = 0.84, SD = 0.30; mean Q BIC = 0.71, SD = 0.21, paired t-test: P = 0.08). The pink group (winner to winner) also showed significantly smaller Q-values under the drug condition which is, as response range is unchanged (cf. Fig. 5A) due to smaller BP values under the drug condition (mean Q control = 0.6, SD = 0.18; mean Q BIC = 0.01, SD = 0.02, paired t-test: P = 8.9E-4).

Because of this dependence of the Q value from a potential change in BP, we performed the same analysis with a modified Q-value, where the response range under both conditions was set in relation to the BP of the control condition (Fig. 5C). This analysis led to similar results: \( Q_{rel BP \ controlling} \) – values again changed significantly stronger in the blue compared to the red group (ANOVA: P = 0.02). The change in \( Q_{rel BP \ controlling} \) from control to
drug condition was significant in the blue group (loser to winner, mean $Q_{rel}$ BP control control $= 1.12$, SD $= 0.63$; mean $Q_{rel}$ BP control BIC $= 0.45$, SD $= 0.26$, paired t-test: $P = 0.002$), but there was only a trend to smaller $Q_{rel}$ BP control-values in the red group (loser to loser, mean $Q_{rel}$ BP control control $= 0.84$, SD $= 0.30$; mean $Q_{rel}$ BP control BIC $= 0.73$, SD $= 0.20$, paired t-test: $P = 0.08$). The pink group (winner to winner) consequently showed no change in this analysis, as response range is unchanged in this group.

Finally, to exclude the possibility that the change of a loser-type response behavior to a winner-type response is not induced by the GABA$\alpha$-blocking effect of BIC but rather by secondary effects of BIC [23] we performed control experiments where we repeated the experiment presented in Fig. 4 in three units (not shown) with the GABA$\alpha$-antagonist Gabazine which is known not to have these side effects. In all cases, a change from a loser-type to a winner-type response behavior was observed.

Figure 5. Effect of blocking of GABA$\alpha$-mediated inhibition by BIC on winner and loser responses in two AM conditions. (A) Relationship between response range and range of presented stimuli whereas some units (blue) changed their response properties from loser to winner type, others stayed losers (red) but even then typically responded to a wider range of stimulus combinations (red dots above diagonal). Winners (pink) always stayed winners under drug conditions.
be expected for a projection that originates eccentrically in a circular map and then covers the whole map (cf. Fig. 1D).

Not only after large injections, but also following very small injections of biocytin (5 nl; injection site diameter 130 ± 10 μm, 137 ± 47 neurons labeled, cf. Tab 1), did we observe a similar axonal projection pattern, indicating that even individual neurons or small neuronal populations have that long-range and asymmetric projections across the tonotopic gradient. For example, approximately 400 of the 25,000 labeled boutons in Fig. 6B were located more than 1.8 mm away from the injection site.

The antibody against PV particularly stained somata and proximal aspects of dendrites of mainly non-pyramidal cells (as well as of few pyramidal neurons in layer VI and various non-classifiable punctae of the neuropil). All layers contained PV-positive neuronal elements, but staining was most intensive in layers III/IV. As illustrated in Figs. 6C–E, biocytin-labeled axons often terminated on various PV-positive GABAergic interneurons at the injection site (shown on microphotograph in the inset). Blue dots represent biocytin-filled neurons at the injection site, and blue lines represent outlines of traced sections. Green-shaded area corresponds to the auditory konicocortex (which comprises fields AI and AAF [14,28]). Blue stars represent biocytin-filled neurons at the injection site (shown on microphotograph in the inset). Blue dots represent biocytin-filled boutons. Note again the asymmetric distribution of labeled boutons of the non-tonotopic, long-range projections within AI (red arrows).

Taken together, these results suggest that the long-range excitatory projection of a pyramidal cell contacts a distant GABAergic interneuron which in turn inhibits the surrounding pyramidal cells. This interconnectivity pattern could form the anatomical substrate for the suppressive interactions we observed between representations of different periodicity ranges within AI (Fig. 2). The geometry of the described projections shows that...
connectivities emanating from a given point in AI asymmetrically cover large parts of the map and thereby provide indirect evidence that a given neuron or neuron ensemble can inhibit other ensembles throughout the map.

Discussion

In this study, we have presented electrophysiological, neuropharmacological and neuroanatomical evidence that GABAA-mediated inhibitory processes in AI mediate global suppressive interactions between representations of different AM-tone periodicities. These interactions may underlie a competitive “winner-take-all” algorithm which supports object segregation. That is, in our model the stimulus feature of sound periodicity is used to segregate sound objects that differ in their periodicity. Such a mechanism might be particularly useful in the so-called ‘cocktail-party phenomenon’, where voices of different concurrent speakers can selectively be attended to. Based on our model and consistent with the data presented in Fig. 2 (two AM paradigm), the segregation of two sounds should be easy when the periodicities of the sounds are considerably different (e.g. listening to a man talking while many children are talking simultaneously), but it should be very hard or even impossible if the two periodicities are very similar (e.g. listening to a particular child in a group of children) because here the responses of the “losers” in the winner-take-all interaction are not inhibited (cf. Fig. 2C,E). In this latter case, additional sound cues like sound source location in space will be crucially important for sound segregation (i.e. speaker separation) and the mechanism discussed here would be rather ineffective. But in all cases where sound objects differ in their periodicity – which is the typical situation for voices of different speakers – the winner-take-all mechanisms proposed in our model would be very powerful in speaker (= sound object) segregation. So based on our model it is not the sound feature (periodicity) per se what matters in auditory cortical processing, but what the cortex does with it, namely object segregation of periodic sounds.

The results presented are consistent with our recent study of GABAA-mediated inhibition in gerbil auditory cortex [23] and with previous studies that have demonstrated co-tuning of excitation and inhibition in AI [21] and suggest a role of auditory cortex in object representation rather than or in addition to feature extraction [24,25].

Some of the units recorded did not show a response behavior as presented in Fig. 2: For example, in five pair recordings both units behaved like winners (cf. Results). This is not surprising, since one would not expect every single unit in the map to participate in the supposed ‘winner-take-all’-mechanism. At least some units in each BP-representation should respond to their preferred periodicity irrespective of concurrent sounds: In a cocktail-party situation, one has to be able to switch one’s attention to a new sound source, for example if somebody is calling one’s name. It is conceivable that some ‘base’ activation for every sound source has to be maintained within the map to allow some top-down mechanism to switch the attention to another sound source.

The data from the competitive interaction experiments reported here support our hypothesis of a competitive “winner-take-all” algorithm that might be used by the auditory system to segregate a sound with a particular periodicity, such as an animal vocalization or a speech sound, from a mixture of simultaneously presented, concurrent sounds. By recording simultaneously from two recording locations, we were able to demonstrate that winner- and loser-type responses can be observed at the same time in a competitive interaction experiment. This observation is compatible with the idea of a direct suppressive interaction between units, although it is not yet direct evidence of such an interaction. However, we recently found such evidence with a stimulation paradigm as simple as a mere pure tone stimulation [29]. We could demonstrate that iontophoretic manipulation of a unit’s response rate by either GABA (increased rate at the application site) or the GABAA-blocker gabazine (decreased rate at the application site) leads to opposite effects on response rate at recording sites remote from the application site. Therefore it is also conceivable that the effects observed in our competitive interaction experiment indeed result from such direct suppressive interactions.

In addition to the known spatial cues [3], the mechanism described in this report may be used by the auditory system to segregate the speech of different speakers in cocktail-party-situations [26]. If this is the case, one would expect to find a neuronal correlate in AI of a switch in the focus of attention from one object to another [30], e.g., from one speaker to another in a cocktail-party situation. Interestingly, two of the ten winner-loser-pairs we recorded (as well as 5 individually recorded units) showed labile responses to AM-tone complexes which were suggestive of
this type of behavior. In these cases, one unit was the ‘winner’ during measurements with the first set of two AM tone complexes, whereas the other unit was the ‘winner’ during measurements with the second set of stimuli. In addition, another five individually recorded units showed this type of ‘switching’ behavior. This suggests that different representations within the periodicity map may be ‘winners’ at different times. It should be emphasized that these experiments were performed on anesthetized animals, which might be an explanation for the low occurrence of this type of spontaneous ‘switching’ behavior. This phenomenon may be observed more frequently in awake animals. Indeed, it is conceivable that in the awake state there may never be a stable winner-loser-relationship between different representations in the periodicity map.

Using optical imaging of intrinsic signals, we could previously demonstrate the presence of a periodicity map with a circular topography in AI of the Mongolian gerbil [4]. The neuroanatomical data presented here show that intrinsic horizontal connections in AI have the appropriate topographical specificity and spatial extent to support the proposed competitive interaction mechanism within the cyclic periodicity map. Furthermore, these laterally projecting axons could indirectly mediate inhibitory interactions between different regions of the periodicity map given that a substantial fraction of their synaptic targets are inhibitory interneurons. We did not consider direct long-range inhibitory connections (for a review see Ref. [17], because in cortex they are reported to be extremely rare (e.g. 0.7–0.8% of callosal projecting neurons [31]). The pattern of interconnections necessary for a competitive interaction mechanism, where all representations within the parameter space are about equally interconnected with all other representations in the map (cf. Ref. [4]), is easily realized within a circular functional map, but is much more difficult to implement in a map with a linear functional gradient. Our data therefore may also explain the functional need for a circular topography of the periodicity map in AI.

From a functional point of view, it is not surprising that GABA_A-mediated inhibition in AI does not seem to shape frequency receptive fields by some local contrast enhancing mechanism such as lateral inhibition [23]. The cells providing the input to AI are already tuned for pure-tone frequency by virtue of lateral inhibitory mechanisms operating at lower levels of the auditory pathway. There is no need for the auditory system to solve the same task repeatedly at multiple levels. Rather the auditory cortex seems to use the same network elements (GABAergic inhibition) to accomplish the demands of auditory processing which are more sophisticated than the extraction of simple acoustic features, e.g. object recognition and segregation. In line with this view is the observation that the auditory cortex is not required for simple tasks such as pure tone discrimination, but is crucial for the discrimination of more complex sounds that possess a virtual pitch percept [32]. For all these higher processing tasks, mechanisms that influence the whole cortical map via globally effective interactions rather than local contrast enhancing inhibition seem to be required.

Materials and Methods

Animal preparation

Animals were prepared under deep general (Halothane, Hoechst) and local anesthesia, according to procedures described in detail elsewhere [33]. Left auditory cortex was exposed by craniotomy, leaving the dura intact. For stereotaxic fixation during electrophysiological recordings a 2.5 cm long aluminum bar was fixed to the frontal bones with dental acrylic and served as a head anchor. Insect pins were inserted into the skull to improve the stability of the head anchor and to serve as reference electrodes. Animals were then transferred to an anechoic, sound-attenuating chamber. Anesthesia was maintained by ketamine (Ketavet, 50 mg/ml), xylazine (Rompun 2%) and isotonic sodium chloride solution (mixture 9:1:10) i.p. (0.06 ml/h). At the end of the recording session (after 20 to 24 h), animals were killed by an injection of T61 (Intervet), i.p. Experimental procedures were performed according to the federal regulations and were approved by the animal committee of the state of Saxony-Anhalt, Germany.

Electrophysiological recordings

Anaesthetized animals were placed on a 37°C heating blanket to maintain body temperature with only the head fixed. All recordings were performed in a shielded, sound-attenuating chamber. Neural responses were recorded from primary auditory cortex (AI) with tungsten microelectrodes (TM3B10, 1 MΩ, WPI Inc., Sarasota, USA). Tracks were guided tangentially such that electrodes had a long track in the middle layers of AI. Unit activity was recorded using a multi-channel recording system (MAP = Multichannel Acquisition Processor, Plexon Inc.: amplification (20,000×), band-pass filter (250 Hz 2-pole low-cut filter and 8 kHz 6-pole high-cut filter), 40 kHz sampling at 12-bit resolution per recording channel). Spike waveforms of single units were separated online using a spike sorting algorithm (template matching: Sort Client software, Plexon Inc.), which allows a separation of 1 to 4 waveforms from multi-unit recordings. In the dataset presented in this study, we generally extracted only one spike from the multi-unit recording and the spike waveform was used to ensure the stability of the recording over the course of the experiments. Data from different spike clusters were stored separately for off-line analysis.

Acoustic stimulation

Acoustic stimuli were delivered free field via an attenuator (PA4, Tucker Davies Inc.), an amplifier (STAX SRM-1/ MK-2) and an electrostatic headphone (STAX SR lambda professional) which was mounted approximately 2 cm in front of the animal’s head. The speaker’s output was measured prior to an experiment using a ½-inch condenser microphone (Bruel & Kjaer 4190) placed at the position of the animal’s head and facing the speaker using a measuring amplifier (Bruel & Kjaer 2610), and a signal analyzer (Bruel & Kjaer 2033). For frequencies between 0.5 and 20 kHz, the output of the speaker was found to be flat within ±5 dB and without distortion up to 90 dB SPL. Stimulus intensities higher than 90 dB SPL were not used.

To characterize basic neuronal response properties, pure tones (Fig. 1E) and AM tones (sinusoidally amplitude modulated pure tones) were produced by a computer-controlled multifunction generator (DD1, System 2, Tucker Davies Inc.). AM signals of 100% modulation depth were produced by adding three sine waves, viz. the carrier frequency fc and two sidebands with half the amplitude of fc (fc+modulation frequency (fm) and fc – fm). All components started at phase zero at stimulus onset. For the competitive interaction experiments, the best periodicity (BP; periodicity of the AM tone complex that elicited the highest response rate) for a given unit was determined from the responses to AM tones. Subsequently, the AM tone with the BP was presented simultaneously with a second AM tone complex with a different fc and varying fm (see Fig. 1F). In this case, both AM tone complexes had the same amplitude. All spectral components started at phase zero at stimulus onset. All stimuli were presented at a constant intensity of 65±5 dB SPL and had a duration of 200 ms with 5 ms rise and fall times. In some measurements
where microiontophoretical application of BIC was performed (cf. below), stimuli were presented with 500 ms duration (cf. Fig. 4), as sometimes prolongations of neuronal responses have been reported during BIC-application (e.g. Refs. [13–16,23]). Neuronal activity was also recorded during a 50 ms pre-stimulus and a 150 ms post-stimulus period. Stimuli were presented in random order with 15 repetitions of each stimulus, and were randomized separately for each repetition.

Microiontophoresis

Three-barrel glass pipettes (3BBL W/FIL 1.2 MM, WPI Inc., Sarasota, USA), broken to a total tip diameter of 10–18 μm, were used for microiontophoresis. One barrel contained BIC (10 mM, Sigma (-)bicuculline methiodide; Sigma), and the other two NaCl (3 M NaCl) for recording of neuronal activity and for current compensation. An Ionophor microiontophoresis system (Science Products) was used to generate and monitor ejection and retaining currents. To ensure that an adequate ejection current was used, we performed control experiments with 4-barrel glass pipettes in which we first applied GABA iontophoretically with a current which was sufficient to inhibit a unit’s response to its BF, and then ejected BIC with a current which antagonized the GABA-induced inhibition. These ejection currents (20 to 40 nA) were then used to study the effect of BIC on responses to AM-tone complexes. The use of such low ejection currents essentially excluded the possibility that iontophoresis of BIC would induce the well-documented side-effects of the drug which are not due to the blockade of GABAA receptors (cf. Ref. [23]). Retaining currents ranged from −15 to −20 nA. For all cells, recordings were made before (control), during (BIC) and after (recovery) the application of BIC. For each of these conditions, responses to at least one set of pure tone stimuli were recorded. Measurements during the recovery condition were repeated until responses returned to pre-drug levels.

Electrophysiological data analysis

Neuronal responses were visualized as rate functions and response plane histograms (cf. Figs. 2,4). Spontaneous activity was calculated from activity measured prior to stimulus onset and given in spikes/s (for rate functions) and spikes/bin for response plane histograms. The criterion for excitation was defined as spike activity significantly above spontaneous activity (spontaneous activity ±3 standard deviations [SD], under the assumption that spike activity is Poisson-distributed). From the evoked responses (spike rate minus spontaneous activity) to AM tones we determined the best periodicity (BP; AM tone periodicity that evoked the highest discharge rate) and the evoked spike rate at the BP. The authors are aware of the fact that a number of stimulus properties co-vary with stimulus periodicity, like envelope rise time, pause duration, or spectral content. We nevertheless refer to best stimuli as ‘best periodicity’ considering that other stimulus features may influence response properties.

To describe tuning sharpness of responses to AM tones (in both single and double AM experiments) we defined a Q-value as BP divided by the bandwidth of the evoked response (that is, the response that is significantly above spontaneous activity in rate functions, as defined above).

A unit was defined as ‘winner’ if there was significant excitation visible in response plane histograms to all stimulus combinations in a competitive interaction experiment. Units where the response to at least one stimulus combination was below the criterion for significant excitation were defined as ‘losers’.

From rate functions filter types to AM tone complexes were defined as follows: The criterion for the classification of filter types was the number of crossings of the rate function with a line at 50% maximal response, and the location of these crossings relative to the BP. The filter function was defined as band-pass, if the rate function crossed the 50% criterion twice and started and ended below the criterion. The inverse case (2 crossings, start and end above criterion) was defined as band-suppression. The low-pass filter characteristic was defined by only one crossing, where the rate function started above and ended below the criterion. The inverse case (1 crossing, start below and end above criterion) was defined as high-pass. If there were more than two crossings of the 50% criterion, the filter characteristic of the rate function was defined as ‘complex’. Finally, if there was no crossing and the rate function was completely above the criterion, the response was defined as non-selective. Note that in the latter case no BP was determined.

Neuroanatomy: Tracing and immunohistology

In order to access AI, gerbils (N = 10) were prepared as described above. Injections of 100 nl (N = 2), 20 nl (N = 2), or 5 nl (N = 6) of 5% biocytin (SIGMA-Aldrich Chemicals, Germany), dissolved in 0.05 M TRIS buffer (pH 7.6), were made by pressure (one injection per animal) over a period of two minutes using fine glass micropipettes (tip diameter 20 μm) and an oil hydraulic nanoliter delivery system (WPI, Germany) (Table 1). The injections were targeted at particular frequency regions of AI using best frequencies maps obtained in preceding electrophysiological experiments or using stereotaxic coordinates and features of the scull and cortical vasculature (for details see Ref. [28,34]). Following the injections, the animals recovered and they were allowed to survive for 24 hours. They were then re-anesthetized (0.5 mg ketamine/100 g body weight and 0.3 mg xylazine/100 g body weight, ip.) and perfused transcardially with 20 ml phosphate buffered saline (pH 7.4), followed by 200 ml of 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (pH 7.4). The brains were removed, stored overnight in 4% paraformaldehyde at 4°C and then cut into 50 μm-thick sagittal sections using a vibratome (Leica Microsystems, Germany).

To visualize the transport of biocytin, sections were processed using the avidin-biotin-peroxidase reaction (ABC-kit, VECTOR Laboratories, USA) with diaminobenzidine as the chromogen. Then, consecutive sections were either not counterstained (for optimal visualization of traced connections), counterstained with methylgreen (to determine laminar and areal boundaries), or processed for parvalbumin (PV) (to identify putative inhibitory target cells of the traced connections). For PV staining, sections were first incubated in a solution of a PV-antibody (SIGMA-Aldrich, dilution of 1:4000, 0.1% Triton) for 48 hours, then in a solution of a secondary antibody (biotinylated anti-mouse, SIGMA Aldrich, 1:200) for two hours and visualized using the ABC method with 2-chloronaphtol as the chromogen. After microscopic inspection (Leica Microsystems, Germany), digital photographs were taken (Finepix S2, Fuji, Japan) of the regions of interest (Fig. 5). For an appropriate illustration of small biocytin injections (5 nl), in two cases (G166, G171) the distribution of labeled boutons was reconstructed 3-dimensionally over several sections using NeuroLucida software (MicroBrightField Europe) (Fig. 6B).

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Author Contributions
Conceived and designed the experiments: HSchulze. Performed the experiments: EB SK AD JC CM. Analyzed the data: HSchulze FO.

Contributed reagents/materials/analysis tools: EB HSchulze FO HScheich. Wrote the paper: EB SK HSchulze FO JC HScheich.

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