Representation of species-specific vocalizations in the inferior colliculus of the guinea pig

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ABSTRACT

The responses of individual neurons to four typical guinea pig vocalization calls (purr, chutter, chirp and whistle) were recorded in the inferior colliculus (IC) of anesthetized guinea pigs. All calls elicited a response in approximately 80% of units. Unit selectivity for individual calls was low, as a majority of neurons (55% of 124 units) responded to all vocalizations and only a small portion of neurons (3%) responded to only one call or did not respond to any of the calls (3%). In 15% of units, the response to one call was at least 25% stronger than the response to any other sound (tone, noise and other calls); these neurons were selective for chirp or whistle, and no unit preferred chutter or purr. Neuronal activity provided information about the spectrotemporal patterns of the calls. Peristimulus time histograms (PSTHs) reflected the energy of the near-CF band, and the population PSTH reliably matched the sound envelope for calls characterized by one or more short impulses (chirp, purr and chutter) but did not exactly fit the envelope for whistle – a slow-modulated and relatively long call. Calculations based on firing rates indicated the approximate positions of the main spectral peaks but did not always reflect their relative magnitude. The time-reversed version of whistle elicited on average a weaker response than did the natural whistle (by 24%), but there were neurons with a significantly stronger response to the natural (‘forward-selective’, 30%) as well as to the time-reversed whistle (‘reverse-selective’, 15%). This study does not prove the existence of units selectively responding to animal calls, but it provides evidence for the encoding of the spectrotemporal acoustic patterns of vocalizations by IC units.
INTRODUCTION

Species-specific vocalizations are a prominent group of acoustic signals playing an essential role in social behavior. These calls are typically complex sounds characterized by time-varying amplitudes and spectral features. The behavioral relevance of vocalization sounds and their specific acoustic patterns have raised questions regarding the neuronal representation of these sounds.

A traditional concept in the perception of such signals is based on the existence of highly specific neurons, so-called “call detectors”. The first studies performed in the auditory cortex of awake squirrel monkeys suggested that neurons might exist in the cortex that extract specific features of calls, similarly to the visual cortex performing a feature extraction (Manley and Müller-Preuss 1978; Newman and Wollberg 1973; Newman and Symmes 1974; Newman 1978; Winter and Funkenstein 1973; Wollberg and Newman 1972). The existence of specific cells - “call detectors” - was not confirmed in these studies, and the authors later came to the conclusion that the pattern discrimination of a complex sound can be accomplished by a functional ensemble of neurons (Pelleg-Toiba and Wollberg 1991). Later, Rauschecker et al. (1995) found a preference for increasingly complex stimuli in neurons in the superior temporal gyrus of anaesthetized rhesus monkeys and suggested that the lateral belt areas of the monkey auditory cortex may form an important stage for the processing of communication sounds. Similarly, Wang et al. (1995) described a representation of behaviorally important and spectrotemporally complex species-specific vocalizations in the primary auditory cortex of anaesthetized common marmosets. In their view, the representation of vocalizations is carried out by dispersed and synchronized cortical cell assemblies that correspond to each individual vocalization in a specific and abstracted way.
In contrast to the large amount of studies that have investigated the role of individual cortical areas in the processing of animal vocalizations, the subcortical nuclei have attracted less attention in this regard. Creutzfeldt et al. (1980) studied the thalamocortical transformation of responses to complex auditory stimuli in non-anaesthetized guinea pigs. They concluded from their results that the responses of medial geniculate body (MGB) cells represent more components of a call than do cortical cells, even if the two cells are synaptically connected. The responses of MGB neurons to species-specific vocalizations in guinea pigs were also the subject of a study by Tanaka and Taniguchi (1991). These authors observed a low responsiveness of MGB neurons to vocalizations; however, the responses to vocalizations displayed discharge patterns that were not possible to predict from the properties of their responses to pure tones. Only a few studies have been designed to investigate the responses of neurons in the inferior colliculus to vocal stimuli (Aitkin et al. 1994; Poon and Chiu 1997). The aim of the study by Aitkin et al. (1994), performed in anaesthetized cats, was to gain information about the differential coding properties of neurons in three subdivisions of the inferior colliculus: the central (CNIC) and external (ENIC) nuclei and dorsal cortex (DCIC). Feline vocal stimuli were found to be more effective in terms of higher firing rates than white noise or pure tone stimuli at the characteristic frequency (CF) in the ENIC and DCIC in comparison with the CNIC. There were no units that responded exclusively to one vocal stimulus, but a high proportion of units in the ENIC responded strongly to broadband stimuli, and some of these showed a clear preference for one vocal stimulus over another.

In the present study we have focused on guinea pig communication sounds. The guinea pig has a large repertoire of vocal communication calls (11 distinct calls according to Harper 1976). These calls fundamentally differ in their spectrotemporal features (Syka et al. 1997), but each call is stereotyped from one to another vocalization and also from one to another animal. We
have analyzed the responses of IC neurons to four main types of guinea pig calls. Our goals were
to determine how species-specific vocalizations are represented in the IC and what information
about the spectral and temporal characteristics of these complex signals is provided by the IC
units to higher structures of the auditory pathway.

METHODS

Animal preparation

Experiments were performed on 24 adult, healthy, pigmented female guinea pigs
weighing 300-500 g. The care and use of animals reported in this study were approved by the
Ethics Committee of the Institute of Experimental Medicine and followed the guidelines of the
Declaration of Helsinki. Animals were anesthetized with an intramuscular injection of 1 ml/kg of
a mixture of ketamine (Narkamon 5%, Spofa) and xylazine (Rompun 2%, Bayer) at a ratio of 2:1,
which corresponds to a dose of 33 mg/kg of ketamine and 6.6 mg/kg of xylazine. Supplementary
injections of one half of the original dose of the ketamine-xylazine mixture were administered
every hour to maintain a sufficient level of anesthesia.

The skin and underlying muscles on the skull were retracted to expose the dorsal cranium
between points bregma and lambda. A small hole (of diameter ~5 mm) was made by a trephine in
one side of the skull above the IC and the dura mater was removed. The animal’s head was
rigidly held in a stereotaxic apparatus by a U-shaped holder, which is fixed at its base to the skull
by two screws and secured by acrylic resin. This type of fixation enabled the animal’s head to be
free for electrode penetration and for free-field acoustical stimulation. A DC-powered electric
heating pad maintained a rectal temperature of 37–38°C.
**Acoustic stimulation**

Animals were placed in a soundproof anechoic room and acoustical stimuli were delivered in free-field conditions from a two-way loudspeaker system (Tesla ARN 5614 and Motorola KSN-1005) placed 70 cm in front of the animal’s head. The acoustic system was calibrated with a B&K 4133 microphone, placed in the position of the animal’s head and facing the speakers. The frequency-response curve was relatively flat and varied by less than ±9 dB between 0.15 and 45 kHz.

Two types of simple stimuli were applied: pure tone pips or broadband noise (BBN) bursts (both of duration 100 ms with 3 ms rise/fall times presented with the repetition rate of 1 Hz). Sound stimuli were generated by a sound-wave generator (Hewlett-Packard HP33120A) and shaped with a custom-made electronic gate. This equipment was used to reduce the intensity of the stimuli from maximal output by minimal steps of 1 dB.

Four typical vocalization calls was chosen from the large variety of guinea pig natural calls (11 distinct calls according to Harper 1976). Calls were previously tape recorded from spontaneously vocalized female guinea pigs (age 2-24 months) in a sound-attenuated room. During the experiment calls were presented from a DAT recorder (SONY DTC-55ES) via the electronic gate. The temporal and spectral parameters of the calls and their variability have been previously described in Syka et al. (1997). One representative of each call, shown in Fig.1, was selected for this study.

Purr (Fig. 1-A) consists of a series of regular low-frequency impulses (fundamental frequency around 300 Hz). The most complex sound is whistle (Fig. 1-B), which is a long-lasting frequency- and amplitude-modulated sound consisting of many harmonics in a wide frequency range. Chutter (Fig. 1-C) is a sequence of irregular noise bursts and chirp (Fig. 1-D) is an isolated brief acoustic impulse with a harmonic structure. The stimuli were presented once every 2.9s for
chutter and purr, 2.5 s for chirp and 2.2 s for whistle. Unless specified, all vocalization responses reported in this paper were obtained at a maximal effective sound level of 75 dB SPL. The time-reversed whistle was generated by reversing the time course of the natural whistle call. All other parameters of the call, such as the sound level or the repetition rate, were preserved.

**Recording of neuronal activity**

Neuronal responses were recorded during a 30 s or 60 s period when either simple sounds or vocalization signals were presented. Extracellular unit activity was recorded with a glass micropipette filled with 3 M-KCl. The electrode was inserted into the IC in a dorso-ventral direction through the cortex, using an electronically controlled microdrive with 1µm steps. One single track or several parallel penetrations separated by 300–500 µm were made in one or two frontal or sagittal planes. As a search stimulus, BBN bursts or tone sweeps were used.

The signal from the electrode was amplified by a WPI DAM 60 differential amplifier and band-pass filtered in the range of 300 Hz to 10 kHz. Then the signal was transmitted via a CED 1401plus interface into a PC computer running the Spike2 program, where the activity was saved and later analyzed. Typically, the activity of only a single unit was recorded from one microelectrode, but in some experiments a unit pair was recorded from one electrode at the same time. In this case, the recorded neuronal activity was processed with Spike2 software to discriminate the individual units in the record according to their spike shapes. Only reliably discriminated units were included in the data set.

**Data analysis**

The following parameters of the response were evaluated in the process of data analysis:
The characteristic frequency (CF) was determined as the frequency of a pure tone stimulus that evokes a neuronal response at a minimal intensity.

The level of the spontaneous firing rate of each neuron was evaluated from 600 ms periods preceding the onset of a tone-burst stimulus at near-threshold intensity and was calculated as the total number of spikes normalized per 1 second. In several guinea pigs, a 5-min recording of spontaneous activity was performed and the rate was calculated in the same way. Spontaneous activities obtained from inter-stimulus periods and long-term recordings were not significantly different.

The frequency tuning was determined on the basis of the Q10 value calculated as the value of the CF divided by the bandwidth at 10 dB above the CF threshold.

The type of response was determined from peristimulus time histograms (PSTH; bin width 1 ms, 200 bins) computed for a stimulus intensity 20 dB above threshold at the CF. The PSTHs from responses to BBN stimulation were also evaluated.

The driven firing rate was expressed as the total number of spikes over the stimulus duration, shifted by the response latency and normalized per 1 stimulus and per 1 second with the spontaneous firing rate subtracted.

The vector strength (VS), describing the synchronization of the neuronal response to the periodicity of the stimulus, was calculated according to Goldberg and Brown (1969) and Rees and Palmer (1989) for two sounds consisting of several phrases: chutter and purr. The values of the VS were evaluated from the entire response to chutter (i.e., from the response to all 5 phrases of this sound) and from the response to the last 11 phrases of purr. The significance was evaluated using the Rayleigh statistics (Gaese and Ostwald 1995; Barbour and Wang 2002).

Population response maps were constructed as 3D plots (Wang et al. 1995; Nagarajan et al. 2002) by aligning individual PSTHs (bin width 5 ms) according to the CF (bin width 0.05
kHz) of individual unit (Y axis). If two or more units had the same CF, the values were dispersed into adjacent bins, but never changed by more than 0.1 kHz. Finally, the 3D plots were discriminated at the level of 80 spikes/s, i.e., only a driven rate over this value is represented by a black dot in the presented figures; all other bins are white.

A rate vs. CF profile was calculated as the average driven rate (Young and Sachs 1979) of all units having a CF within a 0.35 octave-wide frequency window moved in 1/8 octave steps. Only points calculated from three or more units were included into the profile. The response was calculated from a time window shifted by 10 ms relative to the call to compensate for the response latency.

All statistical tests were performed in Prism software.

Histological control

At the end of the experiments, the electrode was left in the deepest position of the last penetration within the IC and carefully broken just above the surface of the brain. The broken tip of the electrode clearly marked the electrode track when resting in the perfused brain for several days. The guinea pigs were sacrificed with an overdose of 1–2 ml of pentobarbital (Pentobarbital, Spofa, 50 mg/ml) and perfused with 10% formaldehyde. The brains were sectioned on a freezing Reichert microtome (slice thickness 40 µm) and stained with cresyl violet. In animals in which only one electrode penetration was made or in which multiple tracks were made in one or more frontal planes, the brain was cut in the frontal plane. Similarly, sections were cut in the sagittal plane in guinea pigs with several electrode penetrations in the sagittal planes. Individual electrode tracks within the IC were subsequently reconstructed from histological sections, and the position of individual neurons was assessed according to the coordinates indicated on the electronically driven microdrive.
RESULTS

Data were obtained from 153 neurons in 24 guinea pigs. Histological control of neuronal distribution revealed that 84 neurons were located in the CNIC, 15 in the DCIC and 54 neurons in the ECIC. Because the number of units recorded in the DCIC was relatively small and the responses of DCIC neurons were similar to the responses of ECIC neurons, neurons in the DCIC and ECIC are displayed together. In Fig. 2A, the average responses to vocal stimuli of neurons located in the CNIC are compared with those of neurons located in peripheral IC subdivisions (DCIC and ECIC). The average responses to stimulation with vocalization sounds do not show any significant differences between neurons recorded in the CNIC and in the peripheral IC subnuclei (DCIC and ECIC). Because the responses of neurons recorded in the central and peripheral parts of the IC to vocalizations did not differ significantly, we present the results of all neurons together irrespective of their localization in individual IC subdivisions.

Unit responsiveness to simple sounds

The majority of recorded neurons (92%) were spontaneously active with an average spontaneous firing rate of 12.3 ± 16.8 sp/s. All but four units responded to pure tone stimulation. CF was evaluated for all units responding to pure tones and ranged between 60 Hz and 25 kHz. The distribution of neurons according to CF is presented in Fig. 2B. All neurons not driven by pure tones responded to stimulation with BBN and/or with vocalization sounds. Based on the shape of the PSTH obtained for stimulation by a pure tone at the CF, two types of neuronal responses were distinguished: one-third of neurons (33%) responded only at the stimulus onset (onset units), whereas two-thirds of neurons (67%) responded during the whole stimulus presentation (sustained units).
Stimulation with BBN evoked an excitatory response in 81% of neurons. Five percent of neurons displayed an inhibition of their spontaneous activity during BBN presentation, and the remaining 14% of units did not respond to BBN at all (mostly low-CF neurons with a CF below 1 kHz). Neurons with excitatory responses to BBN were mostly sustained units (91%); only 9% of IC neurons responded to BBN stimulation with an onset response. The frequency tuning of individual IC neurons was characterized by the $Q_{10}$ value, which ranged from 0.1 to 16. In the majority of neurons (75%), $Q_{10}$ values were between 1 and 5. As a rule, the $Q_{10}$ value increased with increasing CF. These characteristics of the responses to simple stimuli are fully compatible with those reported in a previous extensive study of the response properties in the IC of the guinea pig (Syka et al. 2000).

Unit responsiveness to vocalization calls

One hundred and twenty-four IC neurons were held long enough to record their responses to all four calls. The distribution of IC neurons according to their responsiveness to vocalizations is demonstrated in Fig. 3-A. More than half of IC neurons (55%) responded to all four types of vocalization signals by either an excitatory or an inhibitory response; 23% responded to three vocalizations; 16% of neurons reacted to two vocalizations, and only a small portion of neurons responded to only one call (3%) or did not respond to any call (3%). All neurons that were driven by only one call or were not driven by vocalizations at all responded to stimulation with pure tone and/or with BBN. The responsiveness of IC neurons expressed as the percentage of units responding to individual calls (Fig. 3-B) ranged from the lowest responsiveness to purr (74% of neurons) to the most efficient, chutter (87% of neurons).

When the strength of the response, evaluated as the firing rate over the duration of the stimulus, was compared between the calls and simple sounds (tones, BBN), 37% of units gave a
stronger response to one or more calls than was the maximal response to tone or BBN. The majority of these units that preferred vocalization signals gave the strongest response to chirp (67%); whistle was the most efficient in 23% of neurons, chutter in 6% and purr in 4%. In 15% of all units, the response to the most efficient call was at least 25% stronger than the response to the second most efficient sound (either tone, BBN or another call); 80% of these units were selective for chirp, 20% for whistle and no units preferred chutter and purr. The stronger response to chirp and whistle corresponds to the results shown in Fig. 2A, which demonstrate that the average driven response was stronger in the case of chirp and whistle in comparison with chutter and purr.

The ability of a neuron to respond to an individual call was evaluated in relation to its response pattern to a pure tone, classified as either an onset or sustained response pattern (Fig. 3-C). The figure demonstrates that the efficacy of stimulation with vocalization calls did not depend on the pure tone response pattern, i.e., the portion of neurons responding to individual calls was similar for neurons with an onset or sustained PSTH response pattern.

The responsiveness to vocalization signals was also compared with the CF of individual neurons. The results of this analysis, which are presented in Fig. 4, showed that only a relatively small portion of low-CF neurons (with CF<1 kHz) responded to stimuli with a high-frequency content, such as whistle (28% is a significantly lower portion than in any other CF group, P<0.0001, chi-square test). On the other hand, high-CF neurons (CF > 4 kHz) responded significantly less frequently to purr (P<0.005, chi-square test), i.e., to a call with a predominantly low-frequency content. The agreement between a lower percentage of responding neurons in a particular CF-range and the lack of call energy in that frequency range suggests that the responsiveness of IC neurons to vocalizations is dependent on the relationship between the CF of a neuron and the frequency spectrum of the call.
**Spectrotemporal discharge pattern of the population response**

In order to evaluate the role of the spectrotemporal features of vocalization calls in the response of IC units, population response maps were constructed from the PSTHs of individual units and compared with the spectrograms of vocalization sounds (Fig. 5). A black dot in the population response map represents an elevated discharge of a unit with a given CF (ordinate) occurring during or after vocalization presentation (abscissa). Population response maps for all four tested calls are displayed in Fig. 5. All population response maps demonstrate the relationship between the spectrotemporal patterns of the stimuli and the neuronal responses: the areas of elevated neuronal activity match the areas of energy in the spectrograms. The main difference between the stimuli and the population responses is the wider CF range of discharging units than the corresponding frequency range in the stimulus, i.e., a unit with a CF equal to a frequency absent in the stimulus can also respond to a call if the stimulus spectrum extends into the unit’s receptive field.

This factor is more prominent in the responses of high-CF neurons to low-frequency sounds such as purr or chutter (Fig. 5-A, C) than in the responses of low-CF neurons to sounds of higher-frequencies (e.g., the weak response of neurons with CF < 1 kHz to whistle, see Fig. 4-B and Fig 5-B). This pattern is apparently associated with the shape of the tuning curves at higher intensities, where the low-frequency tail enables responses of high-CF neurons to low-frequency sound content, but the sharp slope of the high-frequency border of the tuning curve does not allow the response of low-CF units to sounds of higher frequencies. The spectral and temporal aspects of the encoding are analyzed in detail in the following paragraphs.
**Representation of temporal features**

Purr and chatter are characterized by a rhythmic repetition of several phrases. The frequency of the regular repetition of phrases, i.e. the vocalization frequency (Wang et al. 1995), is expressed in the neuronal response pattern. The spectrum of the population PSTH (Fig. 6) indicates the vocalization frequency of chirp as well as of chatter (Fig. 8). The synchronization of the neuronal firing to the vocalization frequency is also detectable from PSTHs of individual units, where 64% of units show significant synchronization to purr (Rayleigh statistics of the VS, p<0.001) and 61% units to chatter. The synchronization to the vocalization stimuli is more frequent in low-CF units: 86% of units with a CF below 4 kHz are significantly synchronized to chatter, but only 48% of units with a CF above 4 kHz are synchronized. In the case of purr, the statistics show that 75% of units with a CF below 4kHz are significantly synchronized to the stimulus, whereas only 54% of units with a CF above 4kHz follow the stimulus.

A comparison of population PSTHs (i.e., average PSTHs) and sound envelopes (Fig. 6) shows a good agreement in their shapes for sounds characterized by one or more short phrases, i.e., chirp, purr and chatter (Fig. 6-A, C and D). Neuronal responses to these calls follow the energy of the sound, and the magnitudes of the peaks in the population PSTHs were approximately proportional to the peak magnitudes in the sound envelope. The similarity between the population PSTH and the sound envelope can be quantified by the correlation coefficient, which was 0.93 for chirp, 0.81 for chatter and 0.71 for purr. These values, calculated for the average responses of the entire population of neurons, were below the maximal single neuron values for chirp and purr. The response pattern of the neuron showing the maximal correlation to the sound envelope of chirp had a correlation coefficient of 0.97. The maximum correlation coefficient for purr was 0.80. In the case of chatter, the highest single unit correlation coefficient (r=0.71) was lower that the correlation coefficient of the entire population. When the units having
the highest correlation coefficients to the sound envelope were grouped together, the correlation coefficient between the average response of the group and the sound envelope reached an even higher value than that of the best unit. The maximal value of the correlation coefficient was reached by a group of 5 neurons with the highest correlation coefficients in the case of chirp (r=0.98), 10 neurons in the case of purr (r=0.90) and 40 neurons in the case of chutter (r=0.88). As the values calculated for the best unit, the entire population and the optimal subpopulation were similar, it suggests that the information about the peaks in the envelope of the calls could be available at the level of single units or a small neuronal pool.

Different results were obtained for whistle. The correlation coefficient between the population PSTH and the sound envelope was 0.77, but there were some striking differences: the shape of the population PSTH fundamentally differed from the monotonically increasing envelope of the call (Fig. 6-B), as the population PSTH was multi-modal with two main peaks: one at the beginning of the sound (~150ms) and the second during the sound (~320 ms).

Subpopulation PSTHs (Fig. 9), calculated only for units from a limited CF range, demonstrate that there are different sources of these peaks. The early peak represents the onset activity of units with CFs lower than 7 kHz (Fig. 9A). These units have no enhanced activity at ~320 ms, and the sustained pattern is adequate for the sustained character of the frequency range <7 kHz in the sound. The monotonically increasing pattern of the sound energy does not exactly correspond to the response pattern (the correlation coefficient r = 0.48), because the neuronal activity decreases from the elevated onset part to a constant level in the second half of the response.

The second peak in the population PSTH reflects the onset activity of units with CFs above 8 kHz (Fig. 9B). These high-CF units weakly reacted or remained silent till ~320 ms, because there is almost no energy in the appropriate frequency component in whistle during this
time interval. They began responding at ~320 ms due to the extension of the stimulus spectrum above 8 kHz. There is one more peak in the last part of the response that corresponds with the higher energy content before the end of the stimulus. The correlation coefficient between the relevant frequency component and the response pattern was 0.80.

The differences in subpopulation PSTHs indicate the importance of energy in the near-CF frequency band for the neuronal response. The relevance of the near-CF frequency band is further demonstrated in individual neurons in Fig. 10, where various patterns of the neuronal responses (PSTHs) are compared to the sound obtained by the band-pass filtering of whistle for four units with different CFs. The correlation coefficient between the response and the near-CF component is higher than the correlation coefficient between the response and the entire vocalization (e.g. 0.66 versus 0.45 for the unit in Fig. 10C). These data clearly demonstrate the importance of the near-CF frequency for generating the unit response and suggest that the unit response to whistle stimulation is based on the relationship between the stimulus spectrum and the neuronal excitatory and inhibitory response fields.

The variability of the response patterns is further emphasized by the fact that units with close or identical CFs can respond in different ways, as shown in Fig. 11. The response of the unit shown in Fig 11A-top is positively correlated (r=0.83) with the envelope of the near-CF component, but the unit shown in Fig. 11A-centre displays a negative correlation (correlation coefficient –0.44). Similarly, the response patterns of units shown in Fig. 11B have non-zero correlation coefficients to the near-CF component: the top PSTH is positively correlated (r=0.61) and the bottom PSTH is negatively correlated (–0.06). For both neurons displaying a negative correlation, the shape of the response corresponds with the non-monotonic rate-level function observed when stimulated by a tone at the CF.
Responses to whistle and reversed whistle

In some experiments, whistle was presented in a temporally reversed form with the aim of evaluating the selectivity of neuronal responses for a given temporal pattern. This procedure changes the temporal features of the sound, but preserves the spectral characteristics.

In Fig.12, a comparison of the responsiveness measured by the driven rate to the natural and reversed calls is shown. Two of 47 units reacted in a different way to the stimuli, i.e., by an excitatory response to one stimulus and an inhibitory response to the other, but both these neurons gave only weak driven responses and the difference between them was not significant (Mann-Whitney test, p>0.05).

The driven firing rates obtained for whistle and reversed whistle in the same unit were positively correlated ($R^2=0.69$). The slope of the regression line (0.76) was significantly different from one (p<0.003), and the slope below a value of one indicates a weaker average response to the temporally-reversed stimulus. The responses of 21 units (45% of evaluated neurons) differed significantly (Mann-Whitney test, p<0.01): 14 units gave a significantly stronger response to whistle (the slope of the regression line was 0.59 for these units), and 7 reacted with a significantly stronger response to the reversed whistle (the slope of the regression line was 1.41 for these units).

Representation of spectral features

A comparison of the spectrotemporal acoustic and response patterns demonstrated a strong dependence of the firing pattern of the units on the spectral composition of the call. To understand what information about the spectral features is coded by neuronal firing, the neuronal representation of the spectral characteristics was analyzed using a rate vs. CF profile, which was compared to the short-term sound spectrum.
In Fig. 13, rate vs. CF profiles are shown for the whistle at three different times (A-C) and for purr (D), chirp (E) and chutter (F). Fig. 13-A shows the situation at the beginning of the whistle (150-260 ms). The correlation coefficient between the short-term sound spectrum and the appropriate rate vs. CF profile is 0.38. The peaks in the rate vs. CF profile indicate the positions of the two main spectral peaks. The magnitudes of the rate peaks do not exactly fit the spectrum of the sound as the rates of units with a CF corresponding to the fundamental frequency are slightly lower than that of the 1st harmonic; this is apparently caused by inhibition, i.e., by negative driven rates of a part of the low-CF units (Fig. 10-A). The range of the 2nd and 3rd harmonics is represented by one peak, and there is a near-zero level of driven activity in the high-CF region (>7kHz), which corresponds to the absence of any high-frequency component in the sound at this time.

Fig. 13-B shows a different situation, which is constructed for the middle part of whistle (260-370 ms). Although spectral peaks are reflected in the rate-CF profile, the magnitudes of the rate peaks do not correspond to the magnitudes of the spectral peaks. Even though the dominant frequencies are the fundamental, 1st and 2nd harmonics, the strongest response is at the frequency of the 3rd harmonic (~8 kHz), and the high-CF area is also elevated more than would be expected from the short-term spectrum of the sound; the correlation coefficient is only 0.09. This discrepancy could be explained by the onset reaction of high-CF neurons, which exceeds the sustained activity of lower-CF neurons.

Fig. 13-C is constructed for the latest phase of whistle (430-540 ms) when the maximal peak in the rate-CF profile indicates the dominant spectral peak of the fundamental frequency in the short-term sound spectrum; the correlation coefficient is 0.35.

The rate-CF profile for chirp (Fig. 13-E) also indicates the positions of the main spectral peaks, with isolated peaks of the fundamental and 1st harmonics frequency and one peak for the
2\textsuperscript{nd} and 3\textsuperscript{rd} harmonics; the correlation coefficient is 0.42. A somewhat different situation is seen for the other two sounds (Fig. 13-D, F), where some local spectral peaks are intensified in the rate-CF profile and create dominant elements. In both cases, a low level of high-CF driving rates reflects the weak high-frequency component of the calls with correlation coefficients of 0.54 for purr and 0.69 for chutter.

DISCUSSION

The inferior colliculus represents an important integrative structure in the central auditory system providing the main input to the auditory thalamo-cortical structures (Aitkin 1986). Since it is known that in the auditory cortex of many mammals, particularly primates and bats, specialized areas exist devoted to the processing of vocalizations (Rauschecker et al. 1995; Ohlemiller et al. 1996), it remains to be elucidated to what extent the specialized processing of species-specific vocalizations has a subcortical participation.

The representation of guinea pig communication sounds in the neuronal population within the IC was examined in the present study. The CF range of the recorded neurons covered the entire spectral range of the communication sounds that were employed. The set of units used in this study was comparable to the extensive mapping of IC neurons done by Syka et al. (2000), under the same anesthetics, in terms of the basic parameters of unit responses such as latency, threshold, spontaneous activity, \( Q_{10} \) etc.

The major findings can be summarized in six points. First, each of the guinea pig vocalizations evoked responses in \(~80\%\) of units. Second, the selectivity of IC units for individual calls was very low as the majority of units responded to all calls. Third, the spectrotemporal discharge pattern of the IC neuronal response was qualitatively similar to the spectrotemporal pattern of the species-specific vocalization. Fourth, the temporal envelope of the
call was coded by the firing rate, except for the slow modulation of whistle. Fifth, the main
spectral peaks of the call spectrum were expressed in the firing rate of IC units. Sixth, the average
response of IC neurons was significantly stronger to the natural than to the reversed whistle, but
there were also neurons that significantly preferred the time-reversed whistle.

**Coding of spectrotemporal features**

The results of the study demonstrate that the presence of highly specialized neurons in the
IC with the ability to detect specific features of a call is unlikely. Although the existence of such
highly specific units cannot be conclusively ruled out, it is more likely that a population of IC
neurons represents the spectrotemporal features of a call.

The population of neurons in the IC codes the acoustical pattern of a call in such a way
that the presence or absence of neural responses is a consequence of the tuning properties of the
IC units and of the spectrotemporal acoustical pattern of the sound.

The PSTHs of individual IC units demonstrated a close relationship to the energy of the
near-CF frequency component of the call. This observation suggests a dominant role for the near-
CF frequency band. Individual PSTHs (and especially average PSTHs) reliably copy the envelope
of calls characterized by one or more short phrases, i.e., chirp, chutter and purr. In calls
containing more than one component (phrase), such as purr and chutter, the acoustical patterns of
these components are very stereotypic. Also, the individual peaks in the response pattern are very
stereotypic with the exception of the first (onset) peak in the response to purr, which is enhanced
more than would be expected from the acoustical pattern. Some variability in the peak amplitude
of the sound is reflected in the response, as a stronger response is equivalent to a greater intensity
of the phrase.
The response seems to omit slow modulation of the sound envelope. This phenomenon is present mainly in the response to whistle, in which the sustained character of the response indicates just the presence of energy, but the slow changes in the sound envelope are not reflected in the modulations of the firing rate. This inability of units to follow the slow fluctuations in the envelope corresponds well with the weak synchronization between neuronal discharge and sound envelope as seen for sinusoidal amplitude-modulated tones at low modulation frequencies. The modulation transfer function of IC units typically has a band-pass character as shown by Rees and Moller (1983) in rat and later by Rees and Palmer (1989) in guinea pig. Our findings also correlate with results obtained in the auditory cortex of the marmoset (Nagarajan et al. 2002), where degradations in the temporal envelope performed by low-pass filtering of the temporal envelope at 4Hz and 10Hz dramatically diminished the synchronized response.

The rate vs. CF plots identified some of the major spectral components of the sound. The spectral profile calculated from the response did not always exactly fit the short term spectrum, but it clearly marked the position of the main spectral peaks. There were two main discrepancies: in the relative magnitudes of the spectral peaks and in the representation of the higher harmonics. In some cases, at first the magnitudes of the sound spectral peaks were not exactly matched by the magnitudes of the peaks in the response profile. The magnitudes of the peaks in the low-CF region are lowered by inhibition, which predominantly occurred in low-CF units, and also the pattern of temporal modulation may affect the spectral profile because an onset firing of units within a particular CF range can be greater than a sustained firing within another CF range, irrespective of the magnitudes present in the sound spectrum (Fig. 13-B).

Later, higher harmonics are not represented by several individual peaks but rather by a complex peak. This could be caused by fairly broad tuning curves at the sound intensity used for vocalization to produce the responses and also by the calculation method employed, the
averaging procedure of which used a 0.35 octave-wide window and thus limited the spectral resolution.

Although there are limits to the rate representation of fine detail in the sound spectrum, Nagaranjan et al. (2002) showed in the auditory cortex of anaesthetized marmosets that response magnitudes are relatively insensitive to the fidelity of the spectral envelope characteristics of a call, and Shannon et al. (1995) demonstrated that speech intelligibility is preserved as long as temporal envelope cues are present, despite spectral degradation to as few as four broad frequency bands.

*Whistle vs. reversed whistle*

The importance of the temporal structure of the call is demonstrated by the fact that whistle evoked a stronger response than did the artificial, time-reversed whistle. Not every unit responded in this manner; some units reacted stronger to the reversed sound, but the average response calculated over the population was significantly weaker for the reversed sound. The preference of neuronal responses for natural communication sounds over artificial, time-reversed sounds has been reported in cat (Gehr et al. 2000), primates (Wang et al. 1995; Wang and Kadia 2001), songbirds (Doupe and Konishi 1991; Margoliash 1983) and bat (Esser et al. 1997).

Our data demonstrate that a preference for natural calls in comparison to reversed calls is present in the guinea pig already at the sub-cortical level, but this preference is weaker than in the case of the auditory cortex in the marmoset (Wang et al. 1995). These authors characterized about 76% of neurons in the A1 cortex of the anaesthetized marmoset as “forward selective”, i.e., responding more strongly (measured by the synchronized discharge rate) to a natural call than to its time-reversed form. Weaker selectivity was reported by Gehr et al. (2000), who analyzed the responses of A1 neurons in the anaesthetized cat to meow and time-reversed meow. They divided
the response into two parts: an onset part, occurring within 50 ms after beginning of the call, and a sustained part, represented by the activity occurring later. They reported a larger onset response to the natural meow than to the time-reversed meow (approximately in a ratio of 2:1), but no significant differences between the natural and artificial calls in the sustained part.

The importance of sub-cortical processing of behaviorally relevant sounds has been reported in the mustached bat, where it is assumed that pulse+echo combination-sensitive responses originate in the MGB (Wenstrup and Grose 1995) or even in the inferior colliculus or below (Wenstrup et al. 1999). Also significant are the results of Aitkin et al. (1994) and Tanaka and Taniguchi (1991) showing that responses to vocalizations are not always predictable from the responses to pure-tone stimuli.

The possibility that the weaker response to temporally manipulated sounds is due to their lack of behavioral relevancy is supported by the study of Wang and Kadia (2001) showing that cortical neurons in cat responded similarly to marmoset natural and time-reversed communication sounds but marmoset cortical units preferred the natural call over the time-reversed call. The origin of the stronger response to whistle may be determined by the call features. There are two main features of the whistle: an amplitude modulation of the sound and also a frequency modulation determined by the increasing frequencies of all harmonics. There is a possibility that the preference for natural whistle could be based on the preference for rising amplitude vs. falling amplitude, for rising frequency vs. falling frequency or for a combination of these. This idea is supported by studies of neuronal responses to amplitude or frequency modulated sounds. Chiu and Poon (2000) reported that more than half of the cells in the IC of urethane-anaesthetized rats could be considered as “amplitude modulation-sensitive” with a preference for the rising phase of the amplitude modulation. Neuert et al. (2001) reported asymmetry in the responses to damped and ramped sinusoids in the IC of anaesthetized guinea pigs, where all units displayed significant
asymmetry in the discharge rate for at least one time constant of the amplitude modulation. The relevance of frequency modulation was demonstrated by the study of Kao et al. (1997), who made a reliable prediction of the response to a rat vocalization call based on the response to an FM tone.

*Diversity of calls*

The repertoire of guinea pig communication sounds is a discrete set of calls, but it is a very diverse group as regards the acoustic patterns of individual calls (Syka et al. 1997). In this study, four typical guinea pig calls were used. They differ in duration, number of phrases, frequency range and the harmonic vs. noisy character.

The different call features are reflected in the neural responses. From the results, a distributed representation of whistle by a population of neurons is evident, in which neurons with different CFs responded with fundamentally different response patterns and, on the other hand, quite identical response patterns were obtained irrespective of unit CF for the other three calls, chirp, purr and chutter. As demonstrated by this example, the use of more than just one call can provide a more detailed view of the coding schema, which can be masked by some effect resulting from the acoustic pattern of a particular call.

*Subnuclei and unit diversity within the IC*

The IC is not a homogeneous structure; three subnuclei have been recognized (Aitkin 1986), and also several unit types were described according to neuronal morphology and/or response properties (e.g. Oliver and Most 1984; Ramachandran et al. 1999; Rees et al. 1997; Syka et al. 2000).
Syka et al. (2000) evaluated the responses evoked by tonal stimulation at the CF of guinea pig IC neurons and demonstrated that CNIC neurons were characterized by lower thresholds, a shorter latency, a higher rate of spontaneous and driven activity and a sharper frequency tuning (as expressed by higher $Q_{10}$ values) in comparison with DCIC and ECIC neurons. The authors concluded that the CNIC belongs to the fast, frequency-tuned, low threshold and intensity-sensitive ascending pathway, whereas the other two IC subdivisions are involved in additional processing of information that includes feedback loops and polysensory pathways. It may be expected that the poorer frequency selectivity (lower $Q_{10}$ values) found in the ECIC and DCIC in comparison with the CNIC will result in larger responses to broad-band stimuli, such as noise or vocalizations, in the ECIC and DCIC. This assumption is confirmed by the fact that tonal stimuli evoked a significantly stronger average maximal response in the CNIC than in the ECIC, but the response to noise was slightly, but not significantly, lower in the CNIC than in the DCIC and ECIC. The efficacy of complex sounds was demonstrated by Aitkin et al. (1994) in the IC of anesthetized cats. Vocal stimuli were more efficient in terms of higher firing rates than noise or CF stimuli in 27% of the units in the CNIC, 82% in the ECIC and 72% in the DCIC.

In the present study, the average response to individual vocalization signals was not significantly different in central and peripheral IC subnuclei, which confirms a relative enhancement of responses to spectrally rich sounds in the peripheral subnuclei suggested by Syka et al. (2000), who found weaker responses to tonal stimuli in external nuclei relative to the central nucleus of the IC, but similar responses to broad-band noise in all nuclei. On the other hand, our study does not show stronger response to vocalizations in the external nuclei relative to the central nucleus of the IC as found in cat by Aitkin et al. (1994).

Also, neurons within the IC are not a homogeneous population. Two groups of units in the CNIC have been distinguished according to unit morphology (Oliver and Morest 1984): disc-
shaped and stellate. Units are usually divided into at least two groups according to their PSTH patterns, onset and sustained, but often even more categories of PSTHs have been recognized (Rees et al. 1997; Syka et al. 2000). The heterogeneity within the IC is expressed also in various rate-level functions (Syka et al. 2000), more general tone response maps (Davis et al. 1999) and also binaural interactions (Ramachandran et al. 1999).

Fig. 11 shows fundamentally different firing patterns in units with similar CFs. The fact that both patterns showed a non-zero correlation to the near-CF energy (one is positive, the other is negative) suggests that different coding schemes are employed by each of the units. The response patterns of the units with positive correlation coefficients correspond with the monotonic rate-level functions for tonal stimuli, whereas the units with negative correlation coefficients had non-monotonic rate-level functions. Variability in the rate level function (monotonic, non-monotonic and saturated type) has been previously reported in the IC of ketamine-xylazine anaesthetized guinea pigs by Syka et al. (2000).

The methods employed clearly showed coding of the spectrotemporal acoustic pattern in the IC, but the heterogeneity of the IC seems to be a limitation for averaging procedures. For example, averaging the different PSTHs shown in Fig. 11 could lead to information being erased rather than provided. This implies that the neurons in the IC could carry more information about the call features that seems to be the case from the presented results. As possible solutions to this problem, more sophisticated methods of analysis could be employed rather than just averaging the driven rate (Rieke et al. 1997), or neurons could be grouped into many subpopulations (Wang et al. 1995) according to their response properties. The second approach is also supported by the existence of different coding schemes employed by neurons that have been reported at various levels of the auditory pathway ranging from the brainstem to the cortex. The analysis of individual neuronal types revealed separate rate and temporal representation schemes in the
cochlear nucleus of cats (Blackburn and Sachs 1990); other studies of mammalian auditory
brainstem circuitry have revealed parallel projections important for both sound localization and
general acoustic feature extraction (Young 1998a,b). Recently, distinct subpopulations of neurons
were described on the basis of the representation of spectral and temporal features of a sound also
in the auditory cortex. Lu et al. (2001) reported two distinct populations of neurons with temporal
and rate codes of time-varying signals in the auditory cortex of awake primates. In the study of
Barbour and Wang (2003), the authors evaluated neurons on the basis of their sensitivity to the
spectral contrast of acoustical stimuli and described low-contrast vs. high-contrast preferring
neurons in the auditory cortex of awake primates.

Effect of anesthesia

The fact that the presented data were recorded in anesthetized guinea pigs raises the
question as to how representative are the data for awake animals. Studies performed in awake and
anaesthetized animals demonstrated an effect of anesthesia on the basic response properties of
central auditory neurons (e.g. Kisley and Gerstein 1999, Gaese and Ostwald 2001).

The spontaneous activity of neurons reported in this paper (12.3±16.8 spikes/s) is similar
to the average spontaneous activity of 13.3 spikes/s reported in an extensive study of the IC under
identical anesthesia by Syka et al. (2000), and a similar value (17.8 spikes/s) was reported by
Terterolo et al. (2002) in awake guinea pigs. When compared to pentobarbital, pentobarbital
reduces the spontaneous activity relativel to the awake state (Terterolo et al., 2002) as well as in
comparison to ketamine-xylazine anesthesia (Astl et al. 1996).

Also, similar latencies and the sustained pattern as the most frequent type of response
pattern to tone bursts were found in both awake (Terterolo et al., 2002) and ketamine-xylazine
anesthetized animals (Syka et al. 2000). The frequency tuning in the IC evaluated on the basis of
Q_{10} values was reported by Astl et al. (1996) to be similar, irrespective of the type of anesthesia (pentobarbital, urethane, ketamin-xylazine).

In summary, this study does not prove the existence of highly specific units in the IC, but provides evidence for the representation of the spectral and temporal features of complex sounds in a distributed way by a population of IC units. We can assume that relatively detailed information about sound features is provided by the IC neurons to higher, thalamo-cortical structures of the auditory pathway.
ACKNOWLEDGEMENTS

The authors are grateful to Dr. Lindsay Aitkin from the Department of Physiology, Monash University, Clayton, Victoria for his help in the initial part of the experiments.

The study was supported by Grant Agency of the Czech Republic (GA CR No. 309/01/1063) and Internal Grant Agency of the Ministry of Health (NK/6454-3).
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FIGURE LEGENDS

Fig. 1. Examples of the four typical guinea pig calls used in the study: purr (A), whistle (B), chutter (C) and chirp (D). Each call is represented by the spectrogram (top) and the waveform (bottom). The smaller right panel shows a detail of the spectrogram for the part of the call marked by a box. Several phrases are clearly recognizable in the waveforms of the purr (A) and chutter (C).

Fig. 2 A: Comparison of the occurrence of excitatory (white columns) and inhibitory (black columns) responses of neurons in the central IC nucleus (CNIC – the left column in a pair) and in two peripheral parts of the IC (ECIC and DCIC – the right column) to four guinea pig vocalizations. B: Distribution of the characteristic frequencies of 136 recorded IC units responding to a pure tone stimulus. The sampling of neuronal CFs covers the frequency range of all calls – see Fig. 1.

Fig. 3 A: Unit selectivity expressed as the number of calls evoking either an excitatory or inhibitory response of the unit. A majority of neurons responded to all four calls B: Efficacy of individual calls in evoking an excitatory (black column) or inhibitory (gray column) response is expressed as a percentage of the total number of neurons examined. C: Efficacy of individual calls in evoking an excitatory (black column) or inhibitory (gray column) response in neurons with a sustained response pattern (left column in a pair) or an onset response pattern (right column).
Fig. 4. Responsiveness expressed as the percentage of units responding to individual calls in neurons grouped according to their characteristic frequency range (CF range). Responsiveness to purr in units with a higher CF (columns marked by an asterisk) is significantly lower than in units with a lower CF (columns without an asterisk). Responsiveness to whistle is, in contrast, significantly lower in units with a lower CF (column marked by an asterisk).

Fig. 5 Population response maps (top) displayed for all four tested calls and accompanied by a call waveform (bottom). Each dot represents a discharge greater than 80 spikes/s at the indicated time (abscissa) from a neuron with the CF shown on the ordinate. No averaging or interpolation was used. Bin width: 5 ms (abscissa) and 0.05 kHz (ordinate).

Fig. 6 Relationship between the temporal pattern of calls and the response of IC neurons. A comparison of the sound envelopes (bottom) and population PSTHs (top) is shown for all four calls: purr (A), whistle (B), chutter (C) and chirp (D). Each population PSTH is calculated as the average PSTH of all recorded units. Bin width: 5 ms

Fig. 7 Examples of typical responses, expressed as PSTHs, to purr (A), chutter (B) and chirp (C) for a neuron (unit 21a) with a CF=1.2 kHz. Bin width: 5 ms.

Fig. 8. Coding of the vocalization frequency. The average inter-phrase frequency (arrow), which is expressed as a local maximum in the spectrum of the sound envelope (top), can be detected in the frequency spectrum of the population PSTH (bottom) for purr (A) and chutter (B).
Fig. 9. Relationship between the temporal pattern of whistle and the response of IC neurons. A: The subpopulation response of units with a low-CF (line) to whistle is compared to the population PSTH (gray fill). The lower part shows the waveform of whistle filtered by a low-pass filter at 7kHz. B: The subpopulation response of units with a high CF (line) is compared to the population PSTH (gray fill). The lower part shows the waveform of whistle filtered by a high-pass filter at 8 kHz.

Fig. 10. Examples of unit responses to whistle stimulation, demonstrating the variability of the response pattern. Unit CF is displayed above each PSTH (top). Bin width: 5 ms. The near-CF component of whistle obtained by band-pass filtering is shown below each PSTH. Parameters of the band-pass filter used are displayed above every waveform.

Fig. 11. Examples of different responses to whistle in units with a similar CF. Top and middle graphs show PSTHs of two different units; the bottom plot shows the near-CF component of whistle obtained by band-pass filtering. The unit CF is displayed above each PSTH, and the band-pass filter parameters are indicated above the waveform. Bin width: 5 ms.

Fig. 12. A scatter plot showing the relationship between the firing rates of units responding to whistle and inverted whistle. Each symbol represents one unit: open circles represent units with similar responses to the natural and time-reversed whistle, filled circles neurons with a significantly stronger response to the natural whistle (‘forward-selective’ neurons) and filled triangles neurons with a significantly stronger response to the time-reversed whistle (‘reverse-selective’ neurons). The slope of the regression line (solid line) is significantly different from one (dashed line).
Fig. 13. Comparison of rate-CF profiles (top) and call short-term spectra (bottom) for three periods of whistle (A, B, C – the appropriate time interval is indicated above every rate-CF profile), for purr (D – data calculated over the first phase containing four elementary phrases), for chirp (E) and for chutter (F - calculated over the first phrase of the sound).
Fig. 1

A  Purr

B  Whistle

C  Chutter

D  Chirp
Fig. 3

A

No. of effective calls

% of units

4 3 2 1 none

n = 124

B

% of units

noise chirp chutter purr whistle

C

% of units

noise chirp chutter purr whistle

- excitation - inhibition - no response
Fig. 4

- **Purr**
  - 0.1-1: 80%
  - 1-4: 20%
  - 4-16: 0%
  - 16-32: 0%

- **Whistle**
  - 0.1-1: 60%
  - 1-4: 40%
  - 4-16: 0%
  - 16-32: 40%

- **Chirp**
  - 0.1-1: 60%
  - 1-4: 40%
  - 4-16: 0%
  - 16-32: 0%

- **Chatter**
  - 0.1-1: 0%
  - 1-4: 0%
  - 4-16: 0%
  - 16-32: 100%

Legend:
- White: no response
- Light gray: inhibition
- Black: excitation
Fig. 5

A  Purr

B  Whistle

C  Chutter

D  Chirp
Fig. 6

(A) *purr*

(B) *whistle*

(C) *chutter*

(D) *chirp*
Fig. 7

A  purr

B  chutter

C  chirp
Fig. 8

A  purr  sound

B  chutter  sound

rel. amplitude

response

frequency [Hz]

frequency [Hz]
Fig. 9
Fig. 10

A  unit 21c  CF = 1.1kHz

B  unit 19a  CF = 4.5kHz

C  unit 7-6  CF = 6.1kHz

D  unit 19b  CF = 16.0kHz

filter: 0.1-2.6kHz

filter: 2.0-5.5kHz

filter: 5.6-6.6kHz

filter: 12.0-24.0kHz
Fig. 11

A  unit 11g5  CF = 2.0kHz

unit 18c  CF = 2.3kHz

B  unit 20a  CF = 5.0kHz

unit 9-4  CF = 5.0kHz

filter: 1.5-2.5 kHz

filter: 4.0-6.0 kHz

arbitary unit
Fig. 13

(A) Whistle 150-260 ms
(B) Whistle 260-370 ms
(C) Whistle 430-540 ms
(D) Purr
(E) Chirp
(F) Chitter