Differentially responsive adjacent auditory cortical cells maintained coordinated firing

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Temporal relationships between adjacent single cells were studied in the auditory cortex of the waking guinea pig during silence and pure tone stimulation. One cell of each pair was responsive while the other was completely unresponsive Coordinated discharge was found for spontaneous activity in 14/17 (82%) pairs, generally at and near the origin of cross correlation histograms (CCHs, 5 ms bins). These relationships, involving the same temporal intervals, were also maintained during tone driven discharges of the responsive cell. Thus, responsive neurons may participate simultaneously in specific sensory processing tasks while also responding to a presumptive common modulatory influence within a local network, without the two processes necessarily being linked. Therefore, responsive cells may have greater information processing capacity than realized. *NeuroReport* 11:3467–3471 © 2000 Lippincott Williams & Wilkins.

Key words: Cross-correlation; Guinea pig; Multiple processing; Spontaneous activity; Wakefulness

INTRODUCTION

Many cells in sensory cortex respond simultaneously to the same stimulus. Although the responses of individual cells can be highly specific, suggesting that single cells serve as feature detectors [1], it is widely agreed that the processing of sensory stimuli critically involves the coordinated action of simultaneously active cells. Functional relationships between two or more cells may be studied by determining the temporal correlation between their spike trains [2]. Within the auditory cortex, many neurons exhibit shared input [3]. Temporal coordination can provide a representation of cortical responses to tone; in the barbiturate anesthetized marmoset, correlations between pairs of neurons can increase in the presence of tones in the absence of overall changes in discharge rate [4]. This coordination of spike trains has also been found for noise stimuli in the cat under ketamine anesthesia [5], indicating that such temporal relationships can be obtained for a variety of sounds.

Parameters of cross-correlograms appear to be related to receptive field properties. For example, studies of cluster (multi-unit) activity within the ketamine anesthetized cat have shown that the more similar the receptive field properties, the greater the degree of cross-correlation [6]. Such temporal relationships can be found across distances of millimeters in the primary auditory cortex [6] and even between different fields of the auditory cortex [7]. Such findings support the hypothesis that sensory objects are represented by distributed cells which may respond differentially to various aspects of a stimulus but whose discharges are temporally linked [8].

Previous studies have focused on the interactions between pairs of cells, both of which were responsive to acoustic stimulation. This is highly appropriate given the issue of how stimulus features are represented in auditory cortex. However, investigators have also been long familiar with cells that may be unresponsive to the wide variety of acoustic stimuli employed in a given experiment [9,10]. Such cells have been relatively unstudied. While cells unresponsive for a given set of sounds might be expected to exhibit coordinated discharges for spontaneous activity, they would seem to be poor candidates to maintain such relationships during acoustic stimulation. The current report concerns relationships between responsive and unresponsive cells in the auditory cortex of the waking guinea pig.

MATERIALS AND METHODS

Adult male Hartley guinea pigs ($n=7$; 375–660 g) were pre-medicated with atropine sulfate (0.6 mg/kg, i.p.) and diazepam (9 mg/kg, i.p.) and then anesthetized with sodium pentobarbital (25 mg/kg, i.p.). After placement in a stereotaxic frame (Kopf Instruments, Tujunga, CA) using blunt earbars, the calvaria was cleared, stainless steel screws were threaded into several burr holes and a pedestal of dental acrylic was anchored to the skull. Threaded spacers were embedded in the pedestal, which was bolted to a fixed support, and the ear bars were removed. Core temperature was monitored throughout the surgery and maintained at 37°C. A craniotomy was performed over the left auditory cortex, which was located by mapping potentials evoked by clicks delivered to the contralateral ear using a roving epidural Teflon coated

tungsten wire (A-M Systems, Inc., Carlsborg, WA; Halltronics, Irvine, CA; bandpass 1–300 Hz, 10 000 gain) A stainless steel chamber was fixed over the cortex, filled with sterile agar and sealed. After suturing the skin and applying an antibiotic (neomycin sulfate, E.R. Squibb and Sons, Inc., Princeton, NJ), the subject was permitted to recover in an incubator before being returned to its home cage. All procedures were performed in accordance with the University of California Irvine Animal Research Committee and the NIH Animal Welfare guidelines.

The guinea pig auditory cortex contains two principal tonotopically organized mirror image anterior and dorsocaudal fields and a smaller high frequency field [11]. Based on the known physiology and anatomy, craniotomies were limited to areas caudal to the Sylvian fissure and the chambers were implanted over cortical areas that yielded large amplitude ($>300\,\mu V$), short latency (10–15 ms) evoked potentials during surgery. Each subject was restrained in a hammock within an acoustic chamber (I.A.C. Bronx, New York). Guinea pigs adapt to hammock restraint, in which they can be routinely studied for periods of 2–3 h [12]. Recording sessions were terminated if the subject exhibited signs of discomfort, such as body movement or vocalization. The pedestal was bolted to a support and the hammock adjusted to provide a comfortable posture. A speaker (Aiwa, calibrated with a Bruel and Kjaer type 4134 condenser microphone, type 2204 sound level preamplifier and a Hewlett-Packard 3581A wave analyzer) was placed near the external auditory meatus of the ear contralateral to the recording chamber. Stimulus levels refer to the speaker and so were lower at the tympanic membrane. Extracellular discharges were recorded (Halltronics, Irvine, CA; 300–3000 Hz, 10 000 gain) with a stainless steel microelectrode (F.H.C., Brunswick, ME, 3–8 MΩ, tip = 5 μm) in penetrations perpendicular to the cortical surface using a motorized hydraulic stepping microdrive (F.H.C., Brunswick, ME). Recordings were limited to discharges having at least a 3:1 signal-to-noise ratio.

Receptive fields were obtained after insuring that recordings were stable for a period of 5–10 min. Tone bursts (100 ms, rise/fall 10 ms, intertone interval 1200 ms) were presented in an ascending frequency sequence consisting of 15 isointensity stimuli (20 repetitions). The sequence was presented in decreasing 10 dB steps from 90 to 0 dB. The total time required to present the complete stimulation protocol was ~1 h. Click evoked potentials were then obtained to provide physiological evidence that recordings were obtained below layer III. The short latency (~10–20 ms) component of click evoked potentials has a positive polarity on the surface of the brain, which reverses to a negativity near the border of layers III–IV [13,14]. Marking lesions were not made because that would have required changing microelectrodes before the next penetration, losing precious time during recording sessions that were limited to 2–3 h. Estimates of depth based on micrometer readings, as customarily provided [5] were ~900–1400, consistent with infragranular layers.

Multi-unit (cluster) recordings were analyzed off-line with commercial waveform sorting algorithms (DataWave Technologies, Longmont, CO) Waveforms were sorted according to the following parameters: peak, valley and peak-to-valley amplitudes, time to valley nadir and peak maximum, and spike width (their sum). Boundaries were set on two or more parameters in order to isolate waveforms, as is conventionally done. Peristimulus time histograms (PSTHs) and isointesity tuning curves were constructed for each cell based on spikes occurring during a time window of 10-60 ms after tone onset. The spontaneous rate during the 200 ms immediately preceding each tone sequence was subtracted from the rate of discharge during the response window of each tone.

Cross correlation histograms (CCHs) were constructed for each pair of cells for a total of 200 ms, centered on zero time lag, using bins of 5 ms (40 bins total). For each pair, a CCH was determined over the entire session, both during tone presentation and during inter-stimulus intervals. Separate CCHs were also computed for the tone presentations (evoked activity) and non-tone intervals (spontaneous activity). To assess the statistical significance of values within bins of the CCH, we used the method of Brosch and Schreiner [6] in their study of the auditory cortex. Briefly, Brosch and Schreiner determined the chance correlations of two statistically independent spike trains that otherwise had the same statistical properties as the spike trains under consideration. The chance correlation has an expectancy $E = (N_a \times N_b)/N$, where N_a and N_b are the number of spikes for cells a and b and N is the number of bins in the CCH. Assuming that spike times are distributed in a Poisson fashion, the s.d. $= \sqrt{E}$. Two spike trains were considered to be positively correlated if the value within one or more bins was >2 s.d. from the value of E. As negative correlations were not studied (nor ever observed), this level of deviation has a one-tailed probability of occurring by chance of 0.02. Within a CCH of 40 bins, and a probability of 0.02, there is a high probability of obtaining a single significant bin by chance. However, most CCHs contained more than one significant bin and almost all significant bins were ± 4 bins of the zero time point. All CCHs were also assessed at a deviation level of 3 s.d. ($p = 0.001$). Finally, CCHs from two separate time epochs of each recording, tone and inter-stimulus interval, contained identical significant bins, making it extremely improbable that such an outcome could occur by chance. No correction needed to be made for synchronous discharge due to common response to acoustic stimuli [3] because one cell of each pair was completely unresponsive.

RESULTS

Data were obtained from 13 recording sites in seven guinea pigs; recordings were obtained at three sites in three subjects and one site in the remainder. A total of 30 cells were isolated: 14 exhibited excitatory responses to tone while 16 exhibited no excitatory response to any of the tones at any stimulus level. Best frequencies (peaks of tuning curves) ranged from 0.624 to 42.0 kHz (median 12.0 kHz).

The discharges of 17 pairs of cells were studied. There were four more pairs than recording sites because two sites yielded simultaneous data from three cells and one site from four cells, at least one of which was unresponsive. Spontaneous rates were not significantly different for responsive *vs* unresponsive cells (1.60 ± 2.70 *vs* 0.66 ± 0.67 spikes/s, respectively, $t(25) = 1.19$, $p = 0.25$).

Figure 1 presents data for a typical pair of cells. Cells A and B had approximately equivalent spontaneous firing rates (cell A = 1.64 spikes/s, cell B = 1.43 spikes/s). Cell A responded to frequencies covering a range of up to three octaves with a BF of 0.75 kHz to 2.0 kHz depending on intensity. These types of frequency receptive fields were typical for responsive cells and are consistent with recording sites within primary auditory fields [15]. In contrast, cell B was completely unresponsive.

CCHs obtained for each pair of cells across the entire recording period indicated that 14/17 pairs (82%) exhibited significant relationships at the level of 2 s.d., and that all also reached significance at 3 s.d. Significant bins were generally clustered near the origin (Fig. 2a). These relationships could have reflected coordinated discharge during periods of tone or periods of intertone silence or both. Computation of CCHs for silent periods showed that the correlations were present for spontaneous activity in all 14 cases, at both 2 s.d. and 3 s.d. (Fig. 2b). Note the similarity of CCHs during the total recording period (Fig. 2a) compared with inter-stimulus periods of silence (Fig. 2b).

However, of particular interest, despite the fact that non-responsive cells exhibit no discharges to tones, significant correlations are still present during tone presentation (Fig. 2c) A total of 12/14 (86%) pairs exhibited clear peaks during tone presentation; 11/14 (79%) attained statistical significance at the 2 s.d. criterion and 9/14 (64%) did so at the level of 3 s.d. Thus, most pairs of cells exhibited significant discharge relationships during both silence and presentation of tone. Figure 2 also shows CCHs for one of the three pairs that do not have this relationship.

Some bins that were significant during silence were not significant during tone. Thus, the width of correlated discharge in the CCHs was narrower during tone stimulation than during interstimulus intervals (Fig. 2b,c); but much of the coordinated discharge during silence was maintained during tone presentation. Thus, across the group, bins that were significant during tone presentation were also significant during silence, in all 11 pairs at the 2 s.d. criterion and all nine pairs at the 3 s.d. criterion. Virtually all bins (22/24, 92%) that were significant during tone presentation (3 s.d. criterion) were also significant during silence. During tone presentation, the 5 ms bin near the CCH origin exhibited the highest level of coordinated discharge. This same bin was equally prominent during periods of silence. This consistency of relationship suggests that the cells maintained a particular aspect of their coordinated discharge during tone stimulation, specifically that which had minimal time lag.

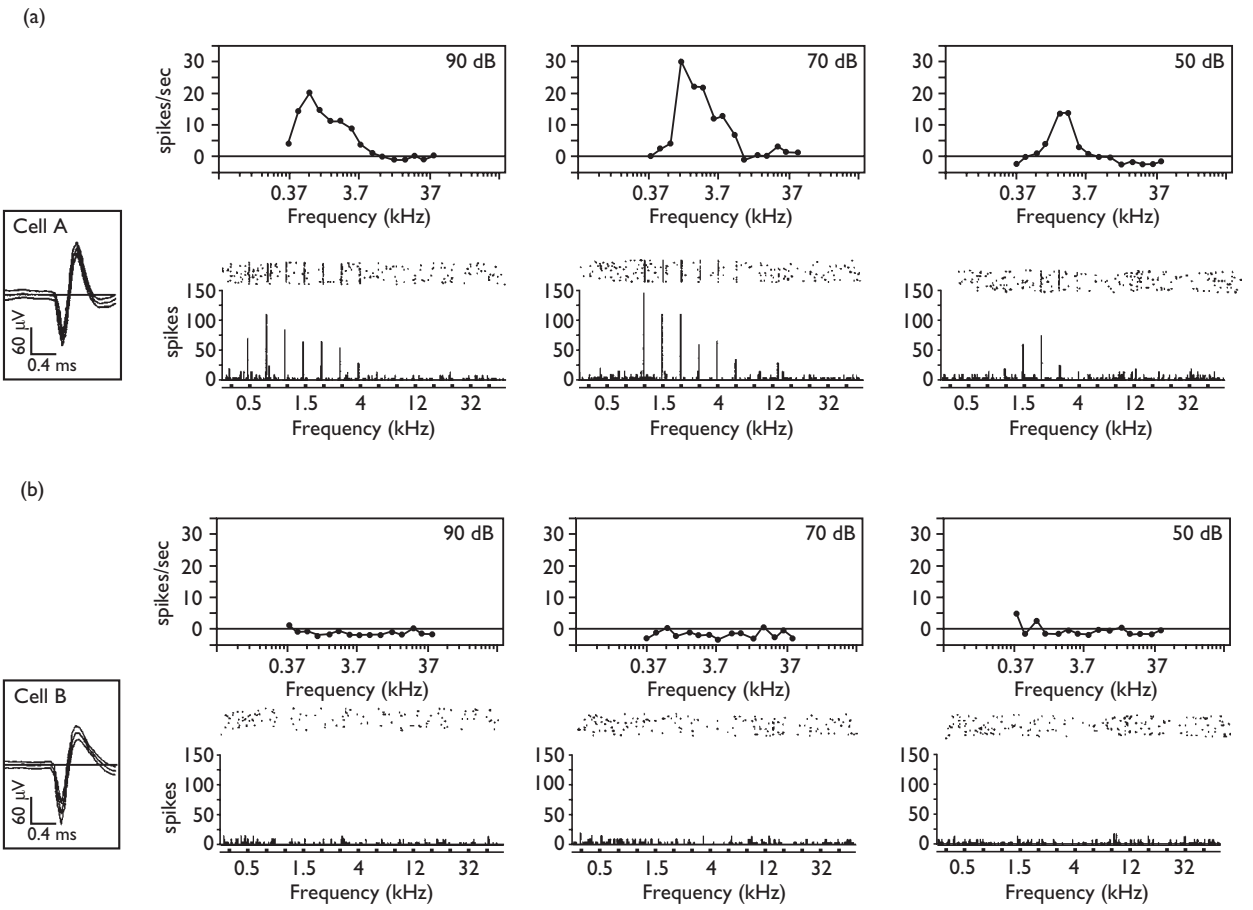

Fig. 1. Examples of data from two simultaneously recorded cells (average waveforms ± s.d.) and also tuning curves and PSTHs at three intensities. Cell A was responsive to many frequencies across intensity Cell B, however, was unresponsive to pure tones at any intensity.

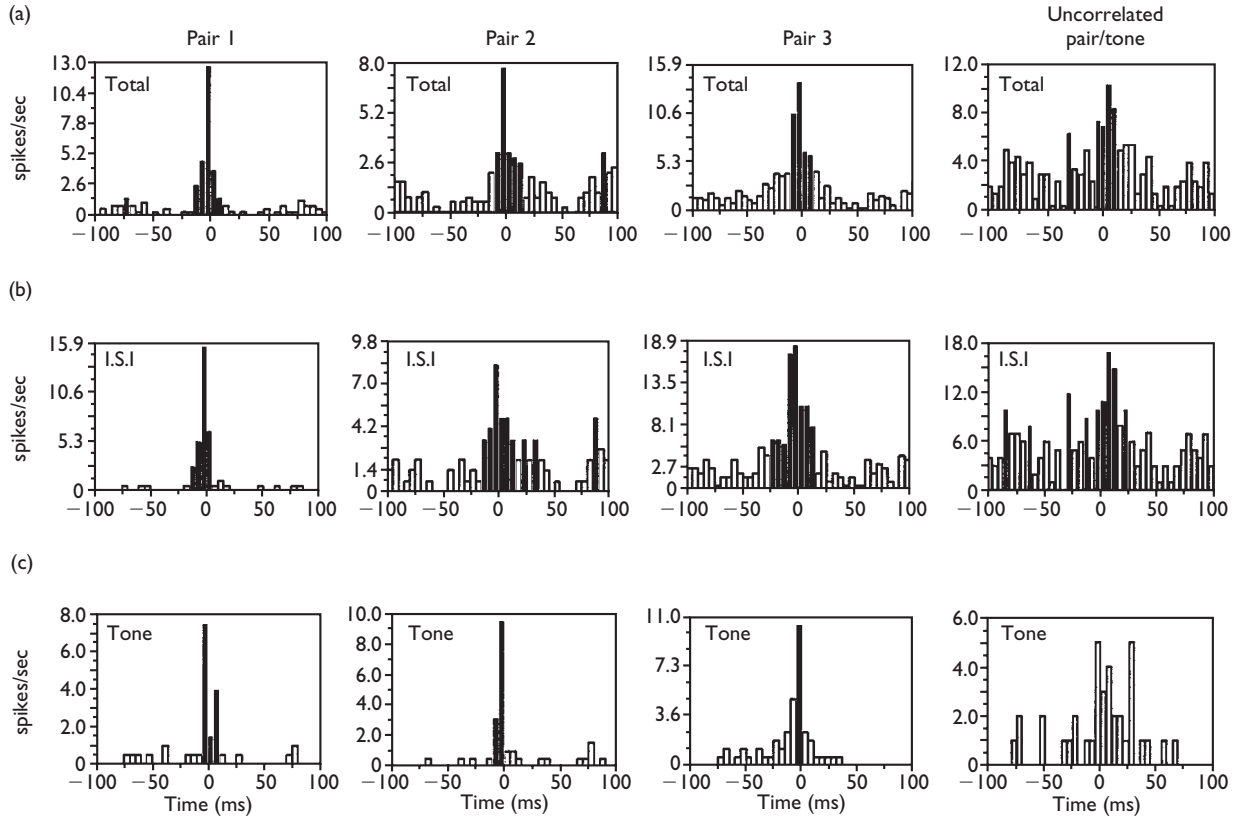

Fig. 2. CCHs for four pairs of cells (pair 1, 2, 3, uncorrelated pair/tone). For all pairs, one cell was responsive to tones, the other was completely unresponsive. Cross-correlations were obtained for three epochs: **a**: (Total), for all discharges during the recording session, i.e., both during presentation of tones and during interstimulus intervals; **b**: (ISI), for discharges occurring exclusively during the intervals between tones; **c**: (Tone), for discharges occurring only during tones. Note for pairs 1, 2, and 3 the significant bins present during tones are also present during silent interstimulus intervals, showing maintenance of a relationship independent of acoustic context. The uncorrelated pair/tone CCH showed no significant correlations during tone. Bins shown in black attained the 3 s.d. criterion of significance. Bin size = 5 ms.

DISCUSSION

The population of adjacent responsive and unresponsive cells studied may consist of pyramidal cells in infragranular layers. Similar recordings from adjacent responsive cells using the same type of microelectrode appear to be comprised of discharges from pyramidal cells [16]. In the present case, stable individual waveforms were obtained for prolonged periods in waking, undrugged animals; such stability is consistent with recordings from cells with large somas. Infragranular location is consistent with depth micrometer readings obtained during recording and a click evoked potential with an initial negativity [13].

The presence of unresponsive cells in the auditory cortex of awake mammals has been noted in prior reports. For example, 18% in the waking, muscle-relaxed cat [17], 46% in waking cat [18] and 28% in awake monkey [10]. Nonresponsive cells have been reported to be adjacent to responsive cells, constituting 28% of all recorded cells in the waking guinea pig [19]. The present results extend to the findings of adjacency and seem to provide the first evidence that the two types of cells are temporally related.

Unresponsive cells may play a role in the processing of acoustic stimuli other than those used in the present study. Cells unresponsive to pure tones may be driven by more complex stimuli, such as FM tones [10,20] or animal vocalizations [21,22]. Another possibility is that the unresponsive cells belong to a class that is only recruited under specific circumstances. For example, it has been reported that about 30% of the cells recorded in the primary auditory cortex of awake cats could be consistently driven only if the attention of the subject was attracted to the sound source itself [18].

Nonetheless, the present findings indicate that unresponsive cells can participate in coordinated discharges with responsive cells. The current results therefore extend the prior findings of deCharms and Merzenich [4] and Eggermont [5] that pairs of responsive cells can exhibit coordinated discharges independent of gross changes in ongoing rate of discharge. Maximal coordinated discharge occurred with minimal time lag between the cells of a pair, suggesting that the correlation reflects common influences on both cells [3,23]. The current results are noteworthy because they reveal that responsive neurons can simultaneously process two sources of input without mutual interaction.

CONCLUSION

Cells completely unresponsive to acoustic stimulation are found adjacent to cells that exhibit standard frequency receptive fields to pure tones. Their discharges are corre-

lated during periods of silence but this coordinated activity is also present while one cell responds to sound. Therefore, responsive cells can be simultaneously involved in the independent processing of an exogenous sensory input while responding to a modulatory influence within local networks. Thus, responsive cells may have greater information processing capacity than realized.

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