Regularity of Firing of Neurons in the Inferior Colliculus

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Rees, Adrian, Ali Sarbaz, Manuel S. Malmierca, and Fiona E. N. Le Beau. Regularity of firing of neurons in the inferior colliculus. J. Neurophysiol. 77: 2945–2965, 1997. The spike discharge regularity of 254 tonically firing units in the inferior colliculus (IC) of the anesthetized guinea pig was studied in response to tones presented at best frequency (BF) to the ear contralateral to the recorded IC. Regularity of firing was measured by calculating the coefficient of variation (CV) as a function of time over the course of a unit’s response. Two hundred and fifteen units (56 under urethane and 159 under chloralose anesthesia) in the central nucleus of the IC (CNIC) were studied in detail. In response to tones at 15–25 dB above threshold, 80% of units in the urethane sample fired regularly (CV < 0.5) during their sustained response, and 46% were highly regular (CV ≈ 0.35). For chloralose the values were 68% and 23%, respectively. Units recorded under urethane were significantly more regular than those recorded under chloralose. For units in the sample with a measurable onset CV, 63% were regular and 44% highly regular under urethane, and 73% were regular and 54% highly regular under chloralose. The units’ peristimulus time histogram (PSTH) patterns were classified into subdivisions of four categories: choppers [9%: chop-supported (C.), chop-onset (C.)]; pausers [42%: pauser-chop-supported (P/C.), pauser-chop-onset (P/C.), pauser-no-chop]; on-sustained (43%: primary-type, L-type, h-type); and sustained (6%). The presence of chopping was a reliable predictor of regularity: C. and P/C. units were highly regular throughout their response, whereas C. and P/C. units were highly regular at onset and became less regular. Some units in the other PSTH categories were highly regular despite the absence of chopping, and units with virtually identical PSTHs showed very different sustained CVs. Regularity was measured as a function of firing rate in 71 units. In 23%, regularity remained constant when firing rate changed with stimulus level. Forty-six percent fired more regularly as firing rate increased, 8% fired less regularly, and 23% of units showed no consistent relationship between CV and firing rate. Regularity did not correlate with the neurons’ frequency response areas or BFs. Regular firing was also found in a smaller sample of units recorded in cortices surrounding the CNIC. We conclude that regular firing is a characteristic feature of most neurons in the IC. Regularity is a specific feature correlated with four PSTH types (C., C., P/C., and P/C.). Other PSTH types may or may not exhibit regularity.

INTRODUCTION

Despite detailed accounts of regular firing by neurons in the cochlear nuclei and other sites in the auditory brain stem (e.g., Blackburn and Sachs 1989, 1992; Goldberg and Greenwood 1966; Guinan et al. 1972; Parham and Kim 1992; Pfeiffer 1966; Tsuchitani 1982; Young et al. 1988a), little is known about the firing patterns of neurons in the inferior colliculus (IC), the main midbrain auditory nucleus. Evidence for the existence of regular firing in the IC has, however, been found. Units showing chopper peristimulus time histograms (PSTHs) have been reported in mouse (Willot and Urban 1978), rabbit (Syka et al. 1981), and guinea pig (Popelar and Syka 1982), while Langner and Schreiner (1988) found that the raster plots for 30% of units recorded in cat IC had regularly spaced action potentials at the onset of the response to a tone.

The study of regularity in IC neurons is important for several reasons. Oscillatory firing patterns depend on a neuron’s biophysical and morphological properties (Llinás 1988). Regularity may therefore help us to understand the functional organization of the IC by enabling the biophysical properties of neurons studied in vitro to be correlated with their responses to physiological stimuli in vivo.

This approach has been particularly successful in the anteroventral cochlear nucleus (AVCN) and posteroventral cochlear nucleus, where neuronal subtypes can be distinguished on the basis of their regular chopping patterns (Blackburn and Sachs 1989; Feng et al. 1994; Young et al. 1988a). Morphological identification of regularly firing neurons in the cochlear nucleus from intracellular studies shows regularity is chiefly, but not exclusively (Ostapoff et al. 1994), a property of stellate cells (Bourk 1976; Oertel 1983; Rhode et al. 1983; Rouillier and Ryugo 1984; Smith and Rhode 1989). The different chopping patterns seen in some regular neurons correlated with the branching patterns of their dendrites (Rhode et al. 1983), and the spatial pattern of synaptic inputs may also contribute to these differences (Blackburn and Sachs 1989; Cant 1981; Smith and Rhode 1989). In the dorsal cochlear nucleus (DCN), stellate cells and units showing the pause-build pattern associated with fusiform cells fire regularly (Parham and Kim 1992). Intracellular recordings in the IC (Peruzzi and Oliver 1994, 1995; Smith 1992; Wagner 1994) are not as advanced as in the cochlear nucleus, but as more information becomes available, detailed measurements of regularity in the IC will be needed to determine whether regularity helps to define physiological profiles that characterize different neuronal types.

The pattern and degree of regularity in some neurons reflects the nature of the inhibition they receive. In vitro studies in AVCN (Manis and Marx 1991; Oertel 1983) have demonstrated that regular firing is an intrinsic property of some neurons, initiated when their membrane conductances are subjected to sustained depolarization. But in some cases PSTH patterns and regularity are not invariant with cell type. Intracellular studies in DCN fusiform cells (Manis 1990; Manis et al. 1993; Rhode and Smith 1986) demonstrate that the PSTH and regularity of such units can change markedly when they are hyperpolarized to different degrees. Similar effects have now been reported in preliminary results for IC neurons in vitro (Peruzzi and Oliver 1994, 1995). In vivo,
such membrane hyperpolarizations would originate from inhibitory inputs. We have previously reported results that are consistent with the notion that inhibition influences temporal response patterns in the central nucleus of the IC (CNIC) by controlling the activation of intrinsic membrane conductances (Le Beau et al. 1995, 1996). Blockade of inhibition by the iontophoretic application of antagonists for γ-aminobutyric acid (GABA) or glycine resulted in systematic changes in the PSTHs of some neurons, and there was a shift toward a greater number of chopper responses, denoting more regular firing. Thus the regularity of discharge of neurons in the IC is influenced by the strength of the inhibition they receive (Le Beau et al. 1995, 1996). Quantitative information about the patterns of regularity in IC neurons is essential for the development of such pharmacological studies.

The temporal firing patterns of neurons are important in the transmission of information in sensory pathways. In the auditory pathway, regular firing may serve to enhance the signaling of intensity differences and amplitude modulation. Shofner and Dye (1989) showed that regular units in the AVCN signaled changes in intensity more reliably than irregular units, and Blackburn and Sachs (1990) found that the formant peaks of vowels were well represented at high stimulus levels in the discharges of regularly firing neurons in AVCN. The responses of units in the cochlear nucleus to pure tones were generated by a Hewlett-Packard 3325A Waveform Synthesizer (Farnell Oscillators, DAM 80). After window discrimination and further amplification, the spikes were converted to logic pulses that gated with rise/fall times of 5 ms. The phase of the tone was not locked to the tone burst envelope and therefore no phase-locked responses were apparent in the data. The signal was attenuated independently to the two ears by a pair of digitally controlled attenuators before being applied to the earphones.

Stimulus presentation

The animal was placed inside a sound-attenuating booth and stimuli were delivered dichotically through closed acoustic systems based on Sony MDR 464 earphones housed in an alloy enclosure and coupled to damped probe tubes (4 mm diam) that fitted into the Perspex speculum (Rees 1990). The output of the system at each ear was calibrated with the use of a Bruel & Kjaer 4134 microphone fitted with a 1-mm probe tube. The maximum output of the system was flat from 0.1 to 9 kHz (100 ± 5 dB SPL) and then fell with a slope of ~15 dB/octave. Second and third harmonic components in the signal were ≥60 dB below the level of the fundamental at the highest output level.

Single-unit recording

The activity of single units in the IC was recorded with glass-coated tungsten electrodes (Merrill and Ainsworth 1972). Vertical electrode penetrations were made stereotaxically through the cerebral cortex. The stereotaxic coordinates were derived with the use of the animal’s bregma as a reference. From the surface of the cortex the electrode was advanced manually for the first 2 mm and then by a stepping motor remotely controlled from outside the booth. Extracellularly recorded action potentials were amplified (×10,000) and filtered (0.3–3 kHz) by a preamplifier (World Precision Instruments, DAM 80). After window discrimination and further amplification, the spikes were converted to logic pulses that
were time stamped with an accuracy of 10 μs by a CED-1401 Laboratory Interface (Cambridge Electronic Design). This device also generated and recorded the occurrence of the pulses triggering each presentation of the tone sweep. The spike and synchronization times were passed to the computer for storage and further analysis. The positions of recorded neurons and electrode tracks were marked with electrolytic lesions for subsequent histological verification of the recording sites. These lesions were made by passing ~5 μA DC through the recording electrode (electrode negative) for 5 s.

Data collection and analysis

Single neurons were isolated with the use of 50-ms noise or tone bursts as search stimuli. For regularity analysis, spike times were collected in response to a 75-ms tone at best frequency (BF), at a repetition rate of 4 per second. To permit detailed regularity analysis with a resolution of 100 or 200 μs over 75 ms, our recording program collected the responses to 2,000 sweeps, or a maximum 8,000 spikes. The firing rates of neurons in the CNICs are not as high as those in the AVCN, and the large number of repetitions was required to ensure that sufficient spikes were collected to allow the analysis to be performed with fine resolution over the time course of the response. On-line, the neuronal responses were monitored audiovisually and displayed as a histogram on the computer screen. Further analysis of the data was performed off-line.

A PSTH (e.g., Fig. 2A) was generated from the spike times, and the beginning of the tone-driven response was selected by eye with a mouse-driven cursor. Several quantitative methods have been used to estimate spike discharge regularity (see Young et al. 1988b for review). The most useful method is that developed by Young et al. (1988a) on the basis of a technique described by Bourk (1976). The advantage of this method is that it takes account of changes in mean firing rate, and changes in the unit’s regularity can be tracked over the course of the response. This technique has been used extensively to study regularity in the AVCN and DCN and other auditory nuclei (e.g., Blackburn and Sachs 1989, 1992; Covey 1993; Feng et al. 1994; Parham and Kim 1992; Young et al. 1988a), and it is the method we have adopted here. Briefly, the time interval between each successive spike pair in the train was calculated and stored in a time bin corresponding to the time at which the first spike in the interval occurred. The binwidth for the regularity analysis was chosen as 100 or 200 μs. The mean and SD of the interspike intervals (ISIs) in each bin were calculated and the coefficient of variation (CV) was derived from the ratio of the SD:mean ISI (e.g., Fig. 2, B and C). To reduce end effects (see Young et al. 1988a), only the first 65 ms of the 75-ms response were included in the regularity analysis. Two CV values were extracted from these plots: 1) the onset CV, extending 2–5 ms from the beginning of the response (the precise value was chosen by visual inspection to give the longest window that captured the onset component before any rapid change in CV), and 2) the sustained CV, measured in a 10-ms window beginning 30 ms after response onset. An onset CV could not be obtained in all cases because the CV sometimes changed too rapidly to give a meaningful mean value. The analysis window for the sustained CV is the same as that chosen by Blackburn and Sachs (1992) for their study in the AVCN and includes part of the 20- to 39.9-ms window used by Parham and Kim (1992) to study DCN neurons, thus facilitating comparison with the data from these studies. The stimulus duration enabled us to calculate the sustained CV while meeting the criterion proposed by Young et al. (1988a) that the time between the end of the measurement window and the end of the response should be greater than the mean ISI plus twice the SD. Although the window values chosen were adequate for most units, the mean ISI in some recordings was occasionally very long (20–30 ms), particularly at low sound levels. In these cases the measurement was calculated in a window beginning earlier to minimize end effects. In this study we have adopted criteria like those used by Young et al. (1988a) to define different degrees of regularity. A regular response is defined as CV < 0.5, whereas CV ≤ 0.35 defines a highly regular response.

For clarity of presentation, all histograms and plots of mean ISI, SD, and CV are drawn with 200-μs bins, but 100-μs binwidths were used for the measurements reported. The regularity plot was smoothed with a three-point triangular function. Bins that contained fewer than three intervals were not plotted.

The response regularity at several intensity levels above threshold was measured for units that were sufficiently stable. To facilitate the selection of these points, a rate intensity function was generated for the unit on the basis of 20 presentations of a 75-ms tone at several sound levels.

For some units the relationship between CV and firing rate was investigated (see RESULTS). Not all units were amenable to this analysis, because data were available for some at only two levels, whereas for others the range of sound levels applied produced too little change in firing rate to allow confidence in a relationship with CV. Units were therefore included in this analysis if the following criteria were met: 1) measurements were available for at least three sound levels and 2) the unit’s firing rate changed by ≥50% with stimulus level. Each unit was then assigned to one of four groups according to whether 1) CV decreased as firing rate increased, 2) CV remained constant as firing rate increased, 3) CV increased with increasing firing rate, or 4) CV showed no consistent relationship with firing rate (i.e., the unit could not be assigned to groups 1–3). To qualify a unit for one of the first three groups, we arbitrarily required that 1) the CV followed the relevant trend, with an overall change in CV > 0.08 (except group 2, where CV was required to remain within 0.08 of its initial value), and 2) any change in CV in the direction opposite to that defining the group did not exceed 0.05. Units that were not assigned to one of groups 1–3 were deemed to show no consistent change in CV with firing rate and were assigned to group 4.

Raster plots of a unit’s responses were also generated from the spike times (e.g., Fig. 2D). The points in each horizontal line represent the time at which each spike occurred from the beginning of the sweep. Only the first 49 sweeps of the total response are shown in the raster plots.

Where possible, a unit’s frequency response area was determined to contralateral stimulation with the use of the method described by Evans (1979). In this analysis the response area is mapped by presenting 960 tone bursts (25 ms in duration) that vary in a pseudorandom sequence of frequency and attenuation over several octaves around the neuron’s BF. The spikes elicited by each tone were counted and the value was plotted as a bar centered on the appropriate frequency and intensity point of the response area diagram, with the bar’s length proportional to the number of spikes fired.

Statistical analysis

Some of the data samples were not normally distributed, and therefore nonparametric tests were used to determine statistical significance (Siegel and Castellan 1988). For two sample tests, the Wilcoxon matched-pairs test was used when pairs of samples based on measurements from the same units, and the Mann-Whitney U test was employed for two independent samples.

Histological processing and analysis

At the end of each experiment the animal was given an overdose of anesthetic and perfused transcranially, first with 200 ml of rinse solution (0.05 M NaNO₃ in 0.1 M phosphate buffer, pH 7.4),
followed by 800 ml of fixative (1.25% glutaraldehyde and 1% paraformaldehyde freshly depolymerized in 0.1 M phosphate buffer, pH 7.4). After ≥2 h of in situ fixation, the brain was removed and subjected to a further period of fixation (∼20 h at 4°C) and cryoprotection (30% sucrose in 0.1 ml phosphate buffer for 1–3 days at 4°C). Tranverse or sagittal sections were cut at 70 μm on a freezing microtome and mounted on gelatin subbed slides. The sections were stained with cresyl violet and coverslipped.

The subdivisions of the IC [CNIC, dorsal cortex (DCIC), and external cortex (ECIC)] were recognized on the basis of cytoarchitectural criteria we have previously defined for the guinea pig (Malmierca et al. 1995). The ECIC could be further subdivided into its lateral and rostral portions. The electrolytic lesions were identified in the sections, enabling the positions of the recorded units to be assigned to one of the subdivisions of the IC.

RESULTS

The data in this paper are based on the responses of 254 tonically firing neurons. The locations of all neurons in the sample were verified histologically by reconstructing electrode tracks with the use of electrolytic lesions placed in the same or adjacent tracks. Eighty-five percent (215 of 254) of units were located in the CNIC. Of the remainder, 30 units were found in the ECIC and 9 in the DCIC. Although the number recorded in subdivisions outside the central nucleus is small (39 units), the data from these neurons are described for comparison. The BFs of the units were in the range of 0.14–22 kHz.

PSTH and regularity patterns in CNIC

To facilitate comparison with our recent study of the IC with iontophoresis (Le Beau et al. 1996), the units were first classified by PSTH alone into the groups used there: chopper, pauser, on-sustained, and sustained. In the present study we have further subdivided these groups, because the PSTHs described here contain more sweeps, thus permitting analysis at higher resolution, and because we have used regularity to identify characteristics not apparent in the PSTH alone.

The classification is based on the PSTH taken at 15–25 dB above threshold. This range was chosen because characteristic features of the PSTH (e.g., pausing) were not always present at lower levels. In the main, PSTH type did not change above 25 dB, although the firing rate in the sustained component of the response was markedly reduced in units with strongly nonmonotonic rate level functions. Some of the terms used to define the units are similar to those used to categorize units in the cochlear nucleus and elsewhere. It is emphasized, however, that this terminology is used for convenience. Its usage does not imply that the responses described are identical to those in other locations, or that the mechanisms through which they arise are the same. Figure 1 summarizes the classification and shows the percentage of each subtype in the samples for the two anesthetics.

CHOPPER UNITS (9%, 19 OF 215). These units showed the most obviously regular firing in the CNIC. They are defined by a PSTH that has either three or more well defined peaks close to response onset or that showed evidence of regularly spaced peaks over the duration of the response. Regularity analysis enabled units in this group to be subdivided into chopper-sustained (C_s; 4%, 8 of 215) units that fired regularly throughout their response (Fig. 2), and chopper-onset (C_o; 5%, 11 of 215) units that fired regularly near response onset but were more irregular over the remainder of their response (Fig. 3). Each figure shows analyses of the unit’s firing pattern at two intensity levels above threshold. In each figure, A and E depict the PSTH, and B and F show the mean ISI together with its SD. The CV calculated from these values is shown in C and G, D and H show raster plots of the times at which spikes occurred during the first 60 ms of each response for the first 49 tone presentations.

For the C_s unit, the PSTH at the lower sound level (Fig. 2A) shows several distinct chopping peaks over approximately the first 30 ms of the response to the tone. This chopping is more distinct at the higher stimulus level (Fig. 2E), where peaks are apparent for a longer period after the beginning of the response. The unit’s firing rate adapts over the first 20 ms, as shown by an increase in the mean ISI from 4 to ~8 ms in Fig. 2B and from 3 to 7 ms in Fig. 2F. The first peak in the PSTH of Fig. 2E is taller and narrower than that in Fig. 2A, denoting the greater precision with which the first spike in the response occurs. The raster plots (Fig. 2, D and H) further illustrate that the timing of the first two spikes in the response is relatively constant from sweep to sweep, but there is considerable jitter in the timing of the subsequent spikes, which accounts for the broadening and eventual absence of the peaks later in the PSTH. The CV in Fig. 2, C and F, is virtually constant at 0.2, indicating highly regular firing, apart from a small fluctuation at the beginning of the response in Fig. 2C.

The C_o unit (Fig. 3) has a PSTH showing several narrow chopping peaks over the first 15 ms of the response. The equally spaced spikes are also evident in the dot raster for this unit (Fig. 3, D and H). The response shows rate adaptation over the first 35 ms (Fig. 3B), and the CV also increases over the first 30 ms of the response, from close to 0.1 at onset to ~0.5 (Fig. 3C). The absence of the chopping peaks in the PSTH after the first 15 ms does, in this case, reflect the gradual decrease in regularity that occurs after onset. At the higher stimulus level (50 dB above threshold) a similar increase in CV occurs (Fig. 3G) but the transition occurs more rapidly. This is also apparent from the presence of fewer peaks in the PSTH (Fig. 3E) and the poorer alignment of the spike times in the raster plot (Fig. 3H). The unit’s firing rate changes nonmonotonically with stimulus level, as evidenced by the lower firing rate in the sustained response of Fig. 3E compared with Fig. 3A, and by the longer mean ISI in the regularity plot (Fig. 3F) and raster (Fig. 3H) at the higher stimulus level.

PAUSERS (42%, 91 OF 215). The PSTHs of these units were characterized by a marked reduction or cessation of firing shortly after response onset. On the basis of PSTH and regularity we have divided this group into three subgroups: pauser-no-chop (P_nc), pauser/sustained-choppers (P/C_s), and pauser-onset-choppers (P/C_o).

The most numerous of the pauser group are the P_nc units (34%, 37 of 215). These units exhibit a variety of different PSTH shapes, but were similar in not showing chopping at any time in the response. Examples of two such units are
shown in Fig. 4. The sustained CVs for both units are similar, with a value of ~0.4, but the two units differ in their CV close to onset. The unit in Fig. 4, A–D, has a high CV near onset. Inspection of the raster plot shows that this is because the spike completing the first interval occurs before the pause on some sweeps and after the pause on others. Thus the pause has the consequence of making some of these intervals much longer than the others, leading to a large SD near onset (Fig. 4B). In contrast, for the other unit (Fig. 4, E–H), the second spike in the first interval always occurs after the pause (Fig. 4H). The ISI of the first interval is long (Fig. 4F), but has a low SD, which in turn results in a low CV near onset. The reduction in mean ISI that is apparent at the end of the response in both units (Fig. 4, B and F) is a manifestation of the end effect (see METHODS). Because the units did not fire spontaneously, a bin cannot contain an interval longer than the time between the bin and the end of the response; thus the mean ISI falls progressively toward the end of the response.

The data for the two P_{nc} units illustrated in Fig. 5 make two points. First, the absence of chopping does not mean that a unit did not fire regularly. Except where the pause disrupts the pattern of firing close to onset, the unit illustrated in Fig. 5, A–D, has a constant CV of 0.29, indicating highly regular firing. Chopping is not evident in the histogram because spikes do not occur at identical times in the response from one sweep to the next. The second point is that units second spike in the first interval always occurs after the pause (Fig. 4H). The ISI of the first interval is long (Fig. 4F), but has a low SD, which in turn results in a low CV near onset. The reduction in mean ISI that is apparent at the end of the response in both units (Fig. 4, B and F) is a manifestation of the end effect (see METHODS). Because the units did not fire spontaneously, a bin cannot contain an interval longer than the time between the bin and the end of the response; thus the mean ISI falls progressively toward the end of the response.

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The remaining units in the pauser group form two small but distinctive subgroups that are characterized by the presence of chopping. The P/Cs units (4%, 8 of 215) have a clear pause, but show evidence of sustained chopping over the duration of the response (Fig. 6, A–D). In the example illustrated, the chopping peaks are widely spaced, reflecting the unit’s long ISI, but these intervals are highly regular, as shown by the low CV that remains constant over the response. Units in the second subgroup (P/Co: 5%, 10 of 215) chop only at the onset of the response. In the example shown (Fig. 6, E–H), three clear peaks are visible in the PSTH immediately after onset and a high degree of regularity is confirmed by a CV of ~0.1 at response onset (Fig. 6G). After onset, the CV increases to a maximum of 0.5, decreases, and then increases again to reach a constant value for the remainder of the response. The maximum CV corresponds to the interval that begins with the third spike. As can be seen from the raster plot, the spike completing this interval sometimes occurs before the pause and sometimes after it. This variability results in an increase in the SD relative to the ISI.

ON-SUSTAINED UNITS (43%, 93 OF 215). These units had a clear onset component followed by a lower level of sustained activity that was maintained for the duration of the stimulus. They did not show any evidence of a pause or of multiple peaks in their PSTHs. We have identified three subtypes within this group. The first shows a gradual transition between the onset response and the sustained activity (Fig. 7, A–D); these are called the on-sustained primary-type (OSp; 16%, 35 of 215). Units in the second group show a marked reduction in firing immediately after the onset peaks, and then achieve a relatively constant level of firing over the remainder of the response. The maximum CV corresponds to the interval that begins with the third spike. As can be seen from the raster plot, the spike completing this interval sometimes occurs before the pause and sometimes after it. This variability results in an increase in the SD relative to the ISI.

Fig. 2. Temporal response data for a Cs unit, best frequency (BF) = 0.5 kHz, that fires regularly throughout its response. Data are shown for 2 stimulus levels above threshold. A and E: PSTHs. Black bar between histograms: onset and duration of the 75-ms tone stimulus. B and F: mean interspike interval (ISI) and SD measured over the 1st 65 ms of the response. C and G: coefficient of variation (CV) (SD/mean ISI). D and H: raster plots showing the neuron’s firing times during the 1st 80 ms of the 1st 49 sweeps collected.
of regularity. The OS₈ group in particular often showed irregular firing close to onset, as shown in Fig. 8, A–D.

SUSTAINED UNITS (6%, 12 OF 215). The responses of these units, as illustrated in Fig. 8, E–H, were characterized by the absence of a pronounced onset peak; thus the firing rate near onset and in the sustained part of the response are comparable. At levels close to threshold, units of other PSTH types may show a similar firing pattern, but sustained units maintain this pattern at higher levels above threshold, where units of other types exhibit a well-defined peak at onset. Although the sustained units in this sample were characterized at 15–25 dB, histograms obtained at higher levels were in many instances used to confirm their classification as sustained units. In the example shown, the unit shows a relatively constant level of regularity with a CV of slightly <0.5.

ONSET UNITS. Because regularity was our primary interest, we did not routinely collect data from units with purely onset responses. We did, however, record a few onset units whose PSTHs had several peaks (e.g., Fig. 9, A and B). These units had onset CVs that ranged between 0.25 and 0.39, and some of these onset units were capable of eliciting brief periods of highly regular firing. Because our sampling of onset units was selective, they are not included in the sample of 215 CNIC units used for detailed analysis.

Onset and sustained regularity

The patterns of regular and irregular discharge that we observed for units in the CNIC (Figs. 2–8) clearly demonstrate that the regularity of many units varies over the course of the response to a tone. To quantify these observations, we measured the units’ regularities at two places in their responses, at onset and in the region of sustained firing (see METHODS). The data in Figs. 2–8 show that the 30- to 40-ms window over which the sustained CV was measured is appropriate because it begins after any rate adaptation has finished and the CV has reached a plateau. Onset values were obtained only if a representative mean over 2–5 ms could be measured (see METHODS). The onset and sustained values for each unit were measured at the same sound level. In all cases this level was within 15–25 dB of pure tone threshold, with the majority of measurements taken at 20 dB above threshold. For most units this would be a point on the rising limb of the rate-level function, avoiding both
the highest firing rates, where the function plateaus, or any nonmonotonic regions of the rate-level function.

Figure 10 shows the individual onset and sustained CVs subdivided according to PSTH type. The plots for units that showed some degree of chopping are similar (Fig. 10, A and B). The points for the \( C_o \) and \( P/C_o \) types fall close to the line of identity, demonstrating the similarity in their onset and sustained CVs. These types also show highly regular firing in both measurement intervals. The \( C_o \) and the \( P/C_o \) units all show highly regular firing at onset, but a wider distribution of sustained CVs. This is particularly noticeable for the \( P/C_o \) units; three units are extremely regular at onset with CVs close to 0.1 but sustained CVs that are well in excess of 0.5, denoting a high degree of irregularity. The other unit types, \( P/C_o \) units, exhibited a wider spread of CVs. The points for some units in all these groups fall in the bottom left quadrant of the graph, demonstrating that they fire regularly in both parts of the response. In contrast, other units of the same PSTH types were not regular. Most of the \( P_{nc} \) units fired regularly during the sustained part of their responses, but some were highly irregular at onset. This is expected given the particularly large SDs often found for intervals beginning just before the pause. Despite the differences in the PSTHs of the on-sustained subgroups, there is considerable overlap in the CV values, although the \( OS_{b} \) units tended to have high onset CVs. In summary, the data emphasize that for units whose PSTHs do not show chopping there is no consistent pattern of regularity for units within a single subgroup.

Anesthetic differences

Two different anesthetics were used for these experiments as detailed in METHODS. The 215 units recorded in the CNIC comprised 56 units from urethan- and 159 from chloralose-anesthetized animals. All of the PSTH subtypes described above were recorded under both anesthetic regimes, and all of the changes in CV detailed in the results were observed with both anesthetics. There were differences in the proportions of the different PSTH subtypes obtained under the two conditions, but in many cases the sample sizes were too small for meaningful comparison. The biggest difference between the anesthetics is the higher incidence of pausers, particularly \( P_{nc} \) units, and the lower incidence of \( OS_{b} \) units with chloralose. We found no significant difference between
the sustained CV values under the different anesthetics when comparing between subtypes, but again the small samples limit the analysis. When we compared the sustained CV of the samples obtained with the two anesthetics, irrespective of PSTH subtype, we found that sustained CVs of the urethan sample were significantly lower than those of the chloralose sample ($P < 0.01$) for measurements made at 15–25 dB above threshold. Under chloralose, 68% (108 of 159) of units fired regularly (CV < 0.5) over their sustained response, compared with 80% (45 of 56) for urethan. The proportion of highly regular (CV ≈ 0.35) units was 25% (39 of 159) with chloralose and 46% (26 of 56) with urethan. Onset CVs were obtained for 52 units with urethan, of which 63% (33 of 52) were regular and 44% (23 of 52) highly regular. The equivalent values for 84 units obtained under chloralose were 73% (61 of 84) regular and 54% (45 of 84) highly regular. There was no significant difference between the minimum CV values (see below and Fig. 15) obtained with the two anesthetics. Because the data were qualitatively similar, we have not subdivided the data by anesthetic in the figures. But so that possible quantitative differences between the anesthetics are not obscured, and are available for comparison with future studies, the sustained CV values for the two anesthetics are given separately in Table 1.

Patterns of regularity change

In addition to categorizing the units on the basis of their PSTH pattern, we also categorized units according to how their regularity changed over the course of the response to a tone. The following groups were identified. The names of the groups express the change in regularity, although they are defined by CV changes that are the inverse of this. Constant: the CV changed by <0.1 over the response. Decrease: the CV was lower at onset than over the sustained response. Increase: the CV was higher at onset than over the sustained response. Transient: a low CV at onset is followed by a transient increase in CV before it decreases to a lower value that is maintained for the remainder of the response. The distribution of these responses is shown in Fig. 11. C₀ and P/C units showed constant regularity, and, as one would expect, most C₀ units showed the increasing pattern. Most of the other pauser units showed transient regularity patterns for the reasons described above. For the on-sustained and sustained units all of the different response patterns were in
A neuron’s regularity was often influenced by stimulus level. To quantify these changes, we measured CV at several levels above threshold. Figure 12 shows examples of Pnc and Co units where CV changes with sound level. Figure 12, A and D, shows the units’ rate level functions on the basis of the mean rate of firing over the whole of the 75-ms tone burst. The data depicted in Fig. 12A show a monotonically saturating response, whereas the response in Fig. 12D is marked by 10.220.33.3 on August 27, 2017 http://jn.physiology.org/ Downloaded from

Fig. 12F the sustained firing rate is conspicuously nonmonotonic and the CV varies inversely with this rate response. Thus both units whose responses are depicted in Fig. 12 fired more regularly as their firing rate increased.

Other units did not show a direct relationship between firing rate and regularity. Figure 13 shows data from six units in which sustained CV (●) and mean firing rate (○) are plotted as a function of sound level. Despite the changes in firing rate that occur as level increases, the CV values for these units remained almost constant. This was the case when the firing rate was relatively low (Fig. 13C), or relatively high (Fig. 13A), for different relationships between firing rate and sound level, including nonmonotonicity (Fig. 13F), and for different PSTH types, as indicated in the legend.
The different monotonic and nonmonotonic rate-level functions previously described in the CNIC (Rees and Palmer 1988) were all observed in this study. Each rate-level function type occurred in association with more than one PSTH subtype, and there was no obvious correspondence between CV and rate-level type. This issue merits more attention in future studies, however, because the level above threshold at which PSTH and regularity were defined was below that at which nonmonotonicity would be apparent in most cases.

To explore the relationship between CV and firing rate across the unit sample, we plotted the sustained CV for each unit as a function of firing rate, where the latter was again measured over the same time window as the CV. Data were included in this analysis if the criteria described in METHODS were fulfilled. Each unit was then assigned to one of four groups according to whether 1) CV decreased as firing rate increased, 2) CV remained constant as firing rate increased, and 3) CV increased with increasing firing rate, or 4) CV showed no consistent relationship with firing rate (i.e., the unit could not be assigned to groups 1–3). Of the 71 units that met the criteria, 46% (33 of 71) became more regular (CV decreased) as firing rate increased (Fig. 14A), 23% (16 of 71) showed no change in CV (Fig. 14B), and 8% (6 of 71) fired less regularly (CV increased) as their firing rate increased (Fig. 14C). The remainder of the sample 23% (16 of 71) showed no consistent relationship between CV and firing rate (Fig. 14D). The majority of the latter group were among the less regular units in the sample; only four of the group had CVs that fell below 0.35.

**Maximum regularities of units in the CNIC**

Because the regularity of some units changes as function of firing rate, we also measured the maximum degree of regularity exhibited by each unit. For this analysis we took the lowest onset and sustained CVs measured for each unit, irrespective of the sound level at which the measurements were obtained. Units were included in this analysis only if CV values were measured at three or more sound levels, but we may nevertheless have underestimated the lowest CV for some units. At onset, 84% (56 of 67) of units were regular and 69% (46 of 67) were highly regular, whereas the corresponding values for sustained CV were 85% (76 of 89) and 51% (45 of 89). The data are plotted as a histogram in Fig. 15. The onset CV distribution (median = 0.29, n = 67) extends to lower CVs than the sustained values (median = 0.35, n = 89). The sustained data are skewed toward the
Regularity of units in other subdivisions of the IC

We recorded 39 units under urethan anesthesia in the subdivisions surrounding the CNIC. Most (77%, 30 of 39) were located in the ECIC (26 in the rostral division and 4 in the lateral divisions), with 23% (9 of 39) in the DCIC. The regularity of units in these subdivisions is shown in Table 2. The corresponding values for CNIC units recorded under urethan are included for comparison. The number of units recorded in the cortical subdivisions was small, but some units in both subdivisions showed regular and highly regular firing. Two DCIC units were choppers and three regular units recorded in this subdivision had a sustained response. As noted above, most ECIC units were recorded in the rostral part of ECIC. No chopper units were found in the rostral sample, all the units being either PNC or ON-sustained types. The PSTHs of units in the lateral part of ECIC were more difficult to classify according to our scheme, and only one unit fired regularly during its sustained response. We obtained onset and sustained CVs at 15–25 dB above threshold for units in the cortical subdivisions, and these are shown in Fig. 16. In this figure we have plotted the values for CNIC (○) units for comparison. The distribution for ECIC units, which lower CVs and therefore the Mann-Whitney U test was used to compare the two distributions. The test confirms that the onset CVs are significantly lower than the sustained CVs (P < 0.001, adjusted for ties). When the urethan and chloralose samples were compared for this measure, they did not differ significantly.

Regularity and frequency response properties

We did not find any relationship between regularity and unit BF for either the onset or the sustained CVs measured at 15–25 dB above threshold. For 82 units we were able to obtain a frequency response area to contralateral stimulation. Most of the units had V-shaped response areas, accounting for ~80% of the sample, with 11% (9 of 82) having a narrow response area that did not broaden noticeably at high intensities (Le Beau et al. 1995). The remaining units could not be classified into one of the above groups. The small number of narrow response areas precludes any analysis of the relationship between PSTH or regularity and response area type, but each group contained some regular and highly regular neurons as well as irregularly firing units. Regular firing is thus not exclusively associated with one particular type of frequency response area.
tained CVs and their regularity remained almost constant over the response. The regularity of \(C_s\) and \(P/C_s\) units was also predictable, with all showing a highly regular onset CV that became less regular or, in the case of some \(P/C_s\) units, irregular as the response proceeded. Equally, however, other units types showed highly regular firing despite the absence of any chopping in their response. These included some units in the \(P_{nc}\) and \(ON\)-sustained subgroups. In some cases units with very similar PSTH patterns, like the two \(P_{nc}\) units in Fig. 5, showed quite different degrees of regularity. Furthermore, measurement of CV in some cases showed that a unit was firing irregularly at onset despite the presence of two distinct peaks in its PSTH. In such cases the peaks represent different latencies for the first spike rather than consecutive intervals. For this reason our definition of chopping required evidence of three peaks. Thus, for units that do not chop, PSTH subgroup is not a reliable predictor of regularity. Detailed analysis of the CV data for CNIC units (Figs. 10 and 15) shows that regularity varies along a continuum. There is no evidence of a bi- or multimodal distribution corresponding to clearly differentiated subtypes within the CNIC.

Reasons why regular firing occurs in the absence of chopping in the PSTH are discussed in RESULTS. Chopping is most likely to be evident near onset because jitter in the times at which spikes occur from sweep to sweep accumulates with time and prevents peaks from building up in the latter part of the histogram. In pauser units, however, any regularity at onset may be disrupted by the pause, and the combination of these two effects reduces the likelihood of chopping. Chopping is present in \(P/C_s\) units, because the pause occurs after several intervals have been completed.

We cannot tell from our data whether the different PSTH subgroups represent cell types with distinct morphological and biophysical properties or whether they reveal differences in the activation of synaptic inputs and membrane conductances in morphologically similar neurons. Conversely, it is not clear whether neurons with similar PSTHs but different regularities are alike. Evidence from iontophoretic studies from our laboratory shows that the firing patterns of neurons can change markedly when GABA and glycinenergic inhibitory inputs are blocked (Le Beau et al. 1996). We found that units belonging to several PSTH types became choppers when inhibitory antagonists were applied. Other changes in PSTH class were also noted; for example, a unit with a sustained response became a pauser with GABAergic blockade. These iontophoretic results show that, under certain experimental conditions, many IC neurons can fire with more than one PSTH type, even at stimulus levels at which the PSTH is normally relatively constant.

Our results show that the majority of units in the CNIC of the guinea pig fire regularly when monaurally driven by a pure tone stimulus at BF. Depending on the anesthetic used, between 60 and 80% of units fired regularly, and 23–46% percent of units were defined as highly regular (sustained CV \(\leq 0.35\)) at 15–25 dB above threshold. A higher proportion of regular units was recorded under urethan than chloralose anesthesia. Units were classified into four main categories, chopper, pauser, \(ON\)-sustained, and sustained, in accordance with Le Beau et al. (1996), and then further subdivided into subtypes recognized within these groups.

Only when there was chopping present in a unit’s PSTH was the degree and pattern of regularity predictable. With few exceptions, \(C_s\) and \(P/C_s\) units had highly regular sustained CVs and their regularity remained almost constant over the response. The regularity of \(C_s\) and \(P/C_s\) units was also predictable, with all showing a highly regular onset CV that became less regular or, in the case of some \(P/C_s\) units, irregular as the response proceeded. Equally, however, other units types showed highly regular firing despite the absence of any chopping in their response. These included some units in the \(P_{nc}\) and \(ON\)-sustained subgroups. In some cases units with very similar PSTH patterns, like the two \(P_{nc}\) units in Fig. 5, showed quite different degrees of regularity. Furthermore, measurement of CV in some cases showed that a unit was firing irregularly at onset despite the presence of two distinct peaks in its PSTH. In such cases the peaks represent different latencies for the first spike rather than consecutive intervals. For this reason our definition of chopping required evidence of three peaks. Thus, for units that do not chop, PSTH subgroup is not a reliable predictor of regularity. Detailed analysis of the CV data for CNIC units (Figs. 10 and 15) shows that regularity varies along a continuum. There is no evidence of a bi- or multimodal distribution corresponding to clearly differentiated subtypes within the CNIC.

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The conclusion of our iontophoretic study that the tendency to chop is reduced by inhibition is indirectly supported by the differences in regularity observed with different anesthetics. Although we found no qualitative differences between the PSTH types or patterns of regularity change seen with urethan or chloralose, a significantly lower percentage of highly regular units was recorded under chloralose anesthesia. The difference could be explained if inhibition were less affected by chloralose. This suggestion is supported by Evans and Nelson (1973), who found that, compared with other anesthetics, chloralose had the least effect on inhibition.
in the DCN, and gave responses most like those in the unanesthetized decerebrate preparation.

**Comparison with previous studies**

Regularity analysis has not previously been used in studies in the IC, and, as our results demonstrate, regular firing cannot be identified on the basis of PSTH pattern alone except where there is chopping. Chopper responses have been previously described in the IC (Popelar and Syka 1982; Syka et al. 1981; Willot and Urban 1978), where, as here, they constitute a small proportion of the sample. Chopper PSTHs have not been reported in cat. This is surprising in view of the observation by Langner and Schreiner (1988).

**TABLE 1. Sustained CV of neurons in CNIC by PSTH subgroup and anesthetic**

<table>
<thead>
<tr>
<th>PSTH Subgroup</th>
<th>Subgroup</th>
<th>U</th>
<th>C</th>
<th>U</th>
<th>C</th>
<th>U</th>
<th>C</th>
<th>U</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chopper</td>
<td>Sustained chop</td>
<td>3 (5)</td>
<td>5 (3)</td>
<td>3 (5)</td>
<td>3 (2)</td>
<td>2 (1)</td>
<td>1 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Onset chop</td>
<td>3 (5)</td>
<td>8 (5)</td>
<td>1 (2)</td>
<td>2 (1)</td>
<td>6 (4)</td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pauser</td>
<td>P/Cs</td>
<td>5 (9)</td>
<td>3 (2)</td>
<td>4 (2)</td>
<td>1 (1)</td>
<td>2 (1)</td>
<td>3 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P/Co</td>
<td>4 (7)</td>
<td>6 (4)</td>
<td>4 (7)</td>
<td>1 (1)</td>
<td>4 (7)</td>
<td>4 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pnc</td>
<td>11 (20)</td>
<td>62 (39)</td>
<td>14 (25)</td>
<td>21 (13)</td>
<td>6 (11)</td>
<td>4 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OsP</td>
<td>14 (25)</td>
<td>21 (13)</td>
<td>4 (7)</td>
<td>1 (1)</td>
<td>4 (7)</td>
<td>4 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OsL</td>
<td>10 (18)</td>
<td>30 (19)</td>
<td>4 (7)</td>
<td>1 (1)</td>
<td>4 (7)</td>
<td>4 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OsH</td>
<td>3 (5)</td>
<td>15 (9)</td>
<td>2 (4)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>5 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained</td>
<td></td>
<td>3 (5)</td>
<td>9 (6)</td>
<td>3 (2)</td>
<td>3 (2)</td>
<td>2 (4)</td>
<td>3 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>56</td>
<td>159</td>
<td>26 (46)</td>
<td>27 (23)</td>
<td>19 (34)</td>
<td>71 (45)</td>
<td>11 (20)</td>
<td>51 (32)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages of total. N, number of neurons; CV, coefficient of variation; CNIC, central nucleus of inferior colliculus; PSTH, peristimulus time histogram; U, urethan; C, chloralose; P/Cs, pauser-chop-sustained; P/Co, pauser-chop-onset; Pnc, pauser-no-chop; OsP, on-sustained primary; OsL, on-sustained L-shaped; OsH, on-sustained h-type.
that the raster plots of 30% of units in cat CNIC showed regular patterns of action potentials at the onset of the response to tones. Thus one might at least expect reports of Cs units. It seems unlikely that the effects of different anesthetics could be responsible for the apparent lack of choppers in the IC of cat. Although in the present study and that by Popelar and Syka (1982) nonbarbiturate anesthesia was used, Willot and Urban (1978) used pentobarbital sodium anesthesia for their study in mouse and the data of Langner and Schreiner (1988) in cat CNIC were also recorded under barbiturate anesthesia. At some levels in the pathway, barbiturate may even enhance regularity, perhaps by reducing inhibition. In the lateral superior olivary nucleus (LSO) the majority of units in the pentobarbital-anesthetized cat responded with a chopping pattern to ipsilateral stimuli (Guinan et al. 1972; Tsuchitani 1982), but chopping was much less in evidence in a study in decerebrate cat (Brownell et al. 1979). In addition, these authors found that some units became choppers when pentobarbital was administered.

Extensive measurements of regularity have been made for neurons in several nuclei peripheral to the IC, including the ventral cochlear nucleus (Blackburn and Sachs 1989, 1992; Winter and Palmer 1990; Young et al. 1988), DCN (Parham and Kim 1992; Stabler et al. 1996), LSO (Goldberg et al. 1964; Tsuchitani 1988), and dorsal nucleus of the lateral lemniscus (DNLL), the latter in bat (Covey 1993), where chopping is reported to be a common response (Markovitz and Pollak 1994).

Some of the PSTH and regularity patterns reported in the present study are similar to those described in more peripheral nuclei in the pathway. For example, the responses of Cs units (Fig. 2) whose CV remains constant are similar to those of sustained chopper units described in the cochlear nucleus (Blackburn and Sachs 1989; Young et al. 1988). In contrast, the response pattern illustrated in Fig. 3 is like the slowly adapting chopper pattern, a variation of the chop-T type reported in the AVCN (Blackburn and Sachs 1992). These sustained and transient chopping patterns have also been reported in LSO and DNLL (Covey 1993; Markovitz and Pollak 1994; Yang and Pollak 1994). The patterns of many Pnc units that were irregular at onset but became regular later (e.g., Figs. 4A and 5B) are qualitatively similar to the patterns of regular firing reported for pause-build units in the DCN (Parham and Kim 1992).

Caution should be exercised in drawing parallels too closely between the regularity in the CNIC and that at other sites. Even the most regular chopper units recorded in the CNIC were not as regular as many chopper units in the AVCN, where Cs units with CVs of ≤0.15 are not unusual (see Blackburn and Sachs 1989, their Fig. 6), or in the LSO (Tsuchitani 1988). Even when comparing units with similar regularities, except near onset where fast chopping sometimes occurs, the chopping peaks in the responses of CNIC units are not as pronounced as those of AVCN choppers.

Although CNIC units are not as regular as many chopper units in the AVCN, the LSO, or the DNLL, there is no population of CNIC units as irregular as the primary-like units. The CNIC receives direct projections from several nuclei in the auditory pathway that contain regularly firing neurons (Adams 1979). It seems unlikely that regular firing in the

FIG. 11. Histograms showing distributions of regularity patterns seen over the course of the response of CNIC units, classified according to PSTH type. PSTH subtypes shown in key.
CNIC simply reflects the activity of these inputs, because highly regular firing has been reported in IC slices (Peruzzi and Oliver 1995). Convergence of inputs, together with spatial and temporal integration, would also limit the fidelity with which rapid regular firing could be transmitted from one nucleus to another. Thus the regularity of collicular neurons must probably be generated de novo in the CNIC.

In view of the fact that 46% of units in our sample became more regular as their firing rate increased (mean ISI decreased), it is important to discount the possibility that the regularity we observe in such cases occurs because the ISI of an otherwise irregular process approaches its refractory period. This effect is discussed in detail by Goldberg et al. (1964) and by Young et al. (1988a). These authors model the spike train as a Poisson process whose rate is limited by a refractory period or deadtime ($t_D$) during which a spike cannot occur (Eq. 1, taken from Goldberg et al. 1964). CV can be calculated by rearranging this expression (Eq. 2).

As the mean ISI approaches $t_D$, the SD of the mean interval decreases, as does the CV

$$SD = ISI - t_D$$  \hspace{1cm} (1)

$$CV = \frac{SD}{ISI} = 1 - \frac{t_D}{ISI}$$  \hspace{1cm} (2)

The relationship between mean ISI and SD derived from Eq. 1 is depicted in Fig. 17. The solid line has a slope of 1 and intersects the abscissa when the mean ISI = $t_D$ (1.5 ms). We chose this value because it represents the shortest ISI seen in our data. The CV derived from the ratio of the SD:ISI values approaches 0 as ISI approaches $t_D$. The data points in Fig. 17 show the ISI and SD values for units that fired more regularly as their rate increased (Fig. 14A). Most of the data do not match the slope of the model derived from Eq. 1, even though extrapolation of the real data suggests that the $t_D$ chosen for the solid line fit is a good estimate for the deadtime of these units. The difference between the data and the model is particularly clear at longer intervals (lower firing rates), but at intervals close to the deadtime the two converge as expected. For this reason it is important, as we have in this study, to define the regularity of units at rates below their maximum.

The above analysis, together with the fact that the regularity of the other units in the sample was constant, decreased, or showed no consistent change in regularity with firing rate, demonstrates that the refractory effect is not the only determinant of the sustained regularity in CNIC neurons. It is possible that the transiently low CV measured for the first couple of intervals in the response of some otherwise irregular units might be due to spikes occurring close to their maximum rates. For most such units the effect would be too brief for them to be included in measurements of onset regularity. Other mechanisms must determine the regularity of CNIC units as well.

Evidence from intracellular studies suggests that regular firing by neurons in the AVCN and DCN reflects the behavior of their membrane conductances (Manis 1990; Manis and Marx 1991; Oertel 1983; Oertel et al. 1988). In the AVCN, stellate cells have characteristically linear current-voltage relationships and they fire regularly when activated by a sustained depolarizing current (Oertel et al. 1988). The sustained depolarization activating the intrinsic membrane oscillations in stellate cells is probably generated by low-pass filtering and temporal summation of synaptic currents. Calculations suggest that there is significant low-pass filtering of the synaptic inputs to stellate cells, even when their dendritic contacts are close to the soma (White et al. 1994). Explanations for the different patterns of regular firing observed (e.g., sustained and transient chopping) variously include different dendritic branching patterns (Rhode et al. 1983), the proportion of inputs going to soma and dendrites (Blackburn and Sachs 1989; Cant 1981; Smith and Rhode 1989; Young et al. 1989), the interplay of excitatory and inhibitory inputs (Arle and Kim 1991; Banks and Sachs 1991), and membrane firing threshold (Hewitt and Meddis 1993).

Information about the membrane properties of neurons in the IC is still very limited, but two studies have used intracellular recording and labeling techniques to study the responses of CNIC neurons in tissue slices (Peruzzi and Oliver 1994, 1995; Wagner 1994). Both report neurons in the IC with linear current-voltage relationships; Peruzzi and Oliver (1994, 1995) also measured the regularity of the neurons they impaled in the IC of young rats. Of 30 tonically firing neurons that increased their firing rate with depolarizing current, 43% fired regularly.

As discussed earlier, the firing patterns and regularity of some neuron types in the CNIC are similar to those of DCN fusiform cells. Furthermore, the appearance of chopping when inhibition is blocked in some CNIC units (Le Beau et al. 1996) and the changes in firing pattern reported with the manipulation of membrane potential in intracellular studies (Peruzzi and Oliver 1995) suggests that similar mechanisms may operate to those described in fusiform cells. Manis has suggested that the chopper, build up and pauser firing patterns observed in fusiform cells (Manis 1990; Manis et al. 1993; Rhode and Smith 1986) reflect the degree of inactivation of transient potassium (Kₐ) channels (Connor and Stevens 1971; see Hille 1992 for review). Immunocytochemical evidence shows that fusiform cells express a fast inactivating potassium channel (Juiz et al. 1996), but no labeling for this channel (Kᵥ1.4) was seen in the IC. This suggests that another variant of the channel is present in the IC because cells in the cortex of the IC are sensitive to 4-aminopyridine, an antagonist of Kₐ channels (Smith 1992).

This discussion has focused on the role of intrinsic membrane properties in the generation of regularity in the IC, but, given the variety of response patterns that occur, there may be more than one mechanism for regular firing. In an elegant study, Covey et al. (1996) have recently described the complex currents recorded by whole cell patching of neurons in the IC.
of the bat in vivo. Complex patterns of excitatory and inhibitory currents were observed including oscillatory potentials that continue after stimulus offset. It is unlikely that synaptically mediated activity could account for fast chopper responses, but some form of synaptically generated oscillatory activity might provide an alternative explanation for the long interval regularity exhibited by some neurons (e.g., Fig. 6A).

The current data do not permit us to comment directly on the relationship between regular firing and neuronal morphology in the IC. Regularly firing units were, however, found in the cortices of the IC, which previous studies have shown contain no (Malmierca 1991; Malmierca et al. 1993) or few (Oliver et al. 1991) neurons with flat (disk-shaped) morphologies. Thus there do not appear to be sharp borders between the subdivisions of the IC with respect to regularity. These findings suggest that regular firing in the IC may be associated with several morphological cell types, but intracellular recording and labeling are required to address this question directly.

In the present study the regularity of CNIC neurons was measured from responses obtained with contralateral monaural stimuli. Although most neurons in the IC receive binaural inputs, we chose the simplest means of physiological stimulation so that the neurons were activated with a restricted subset of their possible inputs. Our aim is to develop a profile of the responses of CNIC neurons that can be used as a basis for comparison with other in vivo studies and data gathered in vitro where neurons are studied in the absence of their main
TABLE 2. Sustained CV of neurons in the IC by subdivision

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>CV ≤ 0.35</th>
<th>0.35 &lt; CV &lt; 0.5</th>
<th>CV ≥ 0.5</th>
<th>n</th>
<th>CV ≤ 0.35</th>
<th>0.35 &lt; CV &lt; 0.5</th>
<th>CV ≥ 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNIC</td>
<td>52</td>
<td>23 (44)</td>
<td>10 (19)</td>
<td>19 (37)</td>
<td>56</td>
<td>26 (46)</td>
<td>19 (33)</td>
<td>11 (19)</td>
</tr>
<tr>
<td>ECIC</td>
<td>30</td>
<td>6 (20)</td>
<td>6 (20)</td>
<td>17 (55)</td>
<td>30</td>
<td>3 (10)</td>
<td>18 (60)</td>
<td>9 (30)</td>
</tr>
<tr>
<td>DCIC</td>
<td>4</td>
<td>2 (50)</td>
<td>1 (25)</td>
<td>1 (25)</td>
<td>9</td>
<td>2 (22)</td>
<td>5 (56)</td>
<td>2 (22)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages. n, number of neurons. IC, inferior colliculus; ECIC, external cortex of IC; DCIC, dorsal cortex of IC. For other abbreviations, see Table 1.

afferent inputs. Although such a situation can never be achieved with physiological stimulation in vivo, it is useful progressively to build up the complexity of the stimulus to determine how responses change with each new level. The effect of binaural activation is particularly important in this respect. Binaural stimulation might influence regularity by virtue of its effects on firing rate or via synaptic effects on the membrane properties that also influence the regularity of a neuron. For example, in the LSO, Tsuchitani (1988) showed that, compared with ipsilateral stimulation alone, binaural stimulation can reduce a neuron’s regularity, even when the neuron’s firing rate was unchanged. We are currently addressing the issue of how binaural stimulation modifies regularity in the CNIC.

Significance of regular firing in CNIC for auditory processing

The significance of regularity in auditory processing is not yet understood, but it is of sufficient importance that it is established at several different levels of the auditory pathway. In the auditory pathway regular firing is often viewed as a process that enhances the signaling of changes in intensity (Blackburn and Sachs 1990; Shofner and Dye 1989; Sullivan and Konishi 1984). Regular firing reduces the variance of the unit’s response, and differences in intensity are therefore signaled more reliably. Thus the temporal patterns of highly regular units may exist to enhance the encoding of information by firing rate.

In the AVCN and posterventral cochlear nucleus, the strongly periodic firing of some regular neurons has been shown to influence the neurons’ responses to amplitude modulation, with the best modulation frequencies for such cells correlating closely with the frequency of this intrinsic periodic firing (Kim et al. 1990). This tendency to fire with a particular frequency also governs the selectivity of the response to modulation. Recently we reported preliminary data that reveals correlations between regularity and responses to modulation in the CNIC (Sarbaz and Rees 1996a,b). Regularly firing neurons are also a prerequisite in some models of temporal pitch perception and may serve as neural oscillators (Langner 1983, 1992).

![FIG. 16. Scatter plot showing onset vs. sustained CV measured at 15–25 dB above threshold for units in the external cortex of the inferior colliculus (ECIC) and dorsal cortex of the inferior colliculus (DCIC; ●, △). Data for CNIC units (○) shown for comparison. Onset and sustained values for individual units were measured at the same sound level. Horizontal and vertical dashed lines: boundaries of regular firing (CV = 0.5). Dotted line: line of identity.](image1)

![FIG. 17. Solid line: SD vs. ISI calculated from Eq. 1 (see text), which models neural firing as a Poisson process limited by a deadtime (1.5 ms). The relationship has unity slope and intercepts the abscissa at the deadtime. Data for 23 CNIC neurons in which CV increased with mean firing rate (cf. Fig. 14A) are shown on the same axes. Each symbol and line type represent data for a single unit. The model fails to predict most of the regularity present in the experimental data.](image2)
The neurons we investigated, however, vary their firing frequency and often their regularity with stimulus intensity and therefore would be of limited use as constant oscillators. The pattern of neural intervals may also be of significance in the representation of information in the IC. The fact that a unit has a low CV and therefore fires regularly does not necessarily imply that it fires with a constant periodic discharge throughout its response. A low CV demonstrates that intervals occurring at particular times in the response tend to be constant from sweep to sweep, not that all intervals in the response are the same length. Thus there may be distinct firing patterns among different CNIC units that are not visible in the PSTH because of temporal jitter. Further analysis is required to explore this issue. In the present results we have only analyzed the relationship between consecutive spikes. It is possible that higher-order patterns might emerge if intervals cluster into particular patterns.

The coding of sensory information by temporal response patterns is a well-established concept in the auditory system for the representation of information such as space and frequency. At higher levels in the pathway, further transformation of information into temporal codes may occur (Middlebrooks et al. 1994). More generally, there is renewed discussion concerning the utility of temporal patterns of activity for stimulus coding and the representation of contextual information (e.g., Hopfield 1995; Sejnowski 1995). The present results clearly demonstrate that the firing patterns of neurons in the CNIC are not random, but the significance of this temporal activity remains to be addressed.

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